Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

[Melanie B. Pincus] Date

Impact of Stress, Pubertal Tempo, and Diet on Neurobehavioral Development in Female Rhesus Macaques

By

Melanie B. Pincus

Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences

Neuroscience

_____

Mar M. Sanchez, Ph.D.
Advisor

_____

Mark E. Wilson, Ph.D.
Committee Member

_____

Jocelyne Bachevalier, Ph.D.
Committee Member

_____

Shella Keilholz, Ph.D.
Committee Member

_____

Tanja Jovanovic, Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

Impact of Stress, Pubertal Tempo, and Diet on Neurobehavioral Development in Female Rhesus Macaques

By

Melanie B. Pincus

B.S., Stanford University, 2004

Advisor: Mar M. Sanchez, Ph.D.

An abstract of

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in Neuroscience

2017

Abstract

Impact of Stress, Pubertal Tempo, and Diet on Neurobehavioral Development in Female Rhesus Macaques

By

Melanie B. Pincus

.

Early life stress is one of the strongest risk factors for the emergence of psychopathology, particularly in women; yet, the mechanisms explaining how stress becomes biologically embedded remain poorly understood. Social subordination in rhesus macaques provides a naturalistic model for studying the impacts of chronic psychosocial stress on neurobehavioral development. This dissertation aims to investigate how chronic subordination stress in female macaques interacts with experiential factors, including pubertal timing and diet, to alter the development of corticolimbic circuitry and behavior. Puberty-induced increases in gonadal hormones are involved in shaping the brain during adolescence, and may modulate the effects of stress on brain development during this critical period. We examined the effects of social subordination and pharmacological delay of puberty in macaques at a postpubertal time point in adolescence and found that subordination was associated with decreased functional connectivity (FC) between dorsolateral prefrontal cortex (PFC) and amygdala (AMYG). Pharmacological delay of puberty resulted in increased FC between AMYG and orbitofrontal cortex (OFC), and both positive and negative effects on socioemotional behavior, but no interactions between experimentally-induced pubertal delay and subordination were detected. Given that stress is a cumulative risk factor for obesity in children and consuming an obesogenic diet impacts the corticolimbic circuits affected by stress, we then used this same social subordination model to study how social subordination and consumption of a high-calorie diet (HCD) interact to affect neurobehavioral development during infancy. Subordination in infancy was associated with higher AMYG-PFC FC, and higher FC between the insula (INS) and anterior cingulate cortex (ACC) at 6 months of age, and nursing from mothers consuming a HCD, obesogenic diet decreased AMYG-OFC FC at 2 weeks of age. Effects of subordination stress on corticolimbic FC, stress physiology, and inflammation were modified by diet during infancy. These results provide evidence that chronic social subordination stress, pubertal timing, and consumption of an obesogenic diet alters physiology, behavior, and the maturation of corticolimbic circuitry important to emotion regulation, reward processing, and interoception during primate development.

Impact of Stress, Pubertal Tempo, and Diet on Neurobehavioral Development in Female Rhesus Macaques

By

Melanie B. Pincus

B.S., Stanford University, 2004

Advisor: Mar M. Sanchez, Ph.D.

A dissertation submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in Neuroscience

2017

Acknowledgements

I am extremely grateful to Dr. Mar Sanchez for her support, wisdom, and guidance during this journey. I could have never completed this dissertation without her incredible help along the way. Having never studied nonhuman primates before, Drs. Sanchez, Wilson, and Ethun were very patient and provided a wonderful education on primate neurobiology and behavior. I'm especially appreciative of all the help Drs. Wilson and Ethun offered with data analysis. Thank you to my thesis committee members, Dr. Mark Wilson, Dr. Jocelyne Bachevalier, Dr. Shella Keilholz, and Dr. Tanja Jovanovic, for the time and energy they devoted to the development of my dissertation. Thank you to Dr. Melinda Higgins for her indefatigable assistance with all of my statistics questions.

Many, many thanks to Manuel Bautista, Patrice Harrington, Sara Dicker, Casey Lyon, Natalie Brutto, Kelly Bailey, Brandon Hughes, Jessica Johnson, without whom these studies would not be possible. I'm also very grateful to Dr. Jodi Godfrey, Dr. Jordan Kohn, Dr. Zsofia Kovacs Balint, Dr. Brittany Howell, Dr. Vasiliki Michopoulos, Desiree de Leon, and Elyse Morin for their amazing support. Special thanks to our collaborators, Dr. Martin Styner at the University of North Carolina, Chapel Hill and Dr. Eric Feczko, Eric Earl and Dr. Damien Fair at Oregon Health & Science University, Portland.

I would also like to thank the Yerkes National Primate Research Center (YNPRC) MRI Core for their technical contributions, and the YNPRC animal husbandry and veterinary staff for all their hard work! The studies were supported by the following grants: NIMH grants MH079100 and MH078105-01S1, NICHD grant 055255, OD P51OD011132, NIH/NICHD R03 HD082534, MH096773, K99/R00 MH091238, Oregon Clinical and Translational Institute/CTSA UL1TR000128, NIH grants HD077623, MH078105-04S1, MH091645, MH086633, U54 HD079124, MIH grant MH100029, HD079969

Table of Contents

List of Tables and Figures

Chapter 1. Introduction

Acknowledgement of Reproduction

The following chapter reviews the effects of chronic stress on physiology and brain structure and function. Some passages in the following chapter, with minor edits, were reprinted by permission from Godfrey, J. R., Pincus, M., & Sanchez, M. M. (2016). Effects of Social Subordination on Macaque Neurobehavioral Outcomes: Focus on Neurodevelopment. In Social Inequalities in Health in Nonhuman Primates (pp. 25-47). Springer, Cham.

1.1 Chronic Stress in Development and Human Health

The social and physical environment has profound effects on the body and brain through orchestrated responses of the neuroendocrine, autonomic, and immune systems[1–3]. Stressors are frequently encountered in the environment (both external and internal), engaging the brain as the central organ of stress and adaptation[4]. Actual or perceived challenges to the organism, including systemic stressors, like hypoxia and psychogenic stressors (e.g. threat of aggression), elicit a coordinated set of autonomic, neuroendocrine, metabolic, cognitive, and behavioral stress responses. The complex set of responses set in motion are adaptive, working to attenuate or avoid the stressor and maintain homeostasis[5,6]. Under conditions of chronic stress, however, repeated activation of the stress response systems inflicts wear and tear on the systems involved, and contributes to the etiology of various maladies[7,8]. With many Americans reporting extreme stress (22% of the adult population in 2011[9]), chronic stress is recognized as a significant public health concern. Adults reporting chronic stress are at increased risk for a number of diseases, including cardiovascular disease[10], obstructive pulmonary disease[11], diabetes and obesity[12], immune compromise[13], and psychopathology[8]. Mounting evidence from studies in humans and animal models suggests that the severity and trajectory of these outcomes is influenced by

developmental timing; chronic stress early in life has been shown to increase vulnerability to psycho- and physiopathology and lead to more severe outcomes[14,15]. Many of the brain regions that play a role in regulating the stress response are themselves impacted by chronic early life stress, and alterations to these regions are believed to contribute to the etiology of psycho- and physiopathology. An understanding of how chronic stress becomes 'biologically embedded', particularly early in life, is still incomplete; further research is needed to elucidate the mechanisms by which chronic stress alters the development of the brain and stress response systems and increases the risk for psychopathology and other adverse health outcomes. The main goal of this dissertation is to investigate how the social stress of subordination in infant and juvenile female rhesus macaques interacts with other experiential factors (i.e. puberty-related increase in gonadal hormones; highly caloric diet) to impact neurobehavioral and physiological development.

1.2 Stress Response and Chronic Activation of the Hypothalamic-Pituitary-Adrenal (HPA) Axis

1.2.1 Overview of the Autonomic and Endocrine Responses to Stress

Stress is defined as a state of bodily, emotional or mental tension produced by a perceived or actual threat to the organism's homeostasis, whereas a stressor is the stimulus that disrupts homeostasis. When a stressor is acute, the stress response is effective at meeting the challenges of the stressor while maintaining homeostasis; the stress response systems achieve this by mobilizing energy and allocating it to systems important for immediate survival, at the same time that it inhibits systems that are not critical. The autonomic nervous system participates in the fight-or-flight response to a stressor through activation of the sympathetic nervous system, and

oftentimes reciprocal inhibition of the parasympathetic nervous system. The hypothalamus and brainstem structures receive signals of substantial homeostatic perturbations (e.g. hypoxia, hemorrhage), and reflex arcs carry this information to preganglionic sympathetic neurons in the spinal cord, activating the sympatho-adrenomedullary (SAM) response[16]. The SAM response involves the release of adrenaline and noradrenaline from the adrenal glands and these catecholamines act rapidly throughout the body to facilitate the stress response via effects on heart rate and pupil dilation, dilation of blood vessels in the lungs and skeletal muscles, and vasoconstriction in nonessential organs[17]. The preganglionic sympathetic neurons also directly innervate target organs of the sympathetic nervous system, including the heart, lungs, and muscles, and stress-induced activation of the sympathetic neurons quickly increases heart rate, peripheral vasoconstriction, energy mobilization, and other outcomes[16].

Often in tandem with the SAM response, the HPA axis is activated and orchestrates a generally slower, and longer-lasting response to a stressor. When a stressor is perceived, limbic pathways activate parvocellular neurons in the paraventricular nucleus of the hypothalamus (PVN), causing them to release corticotropin-releasing hormone (CRH) into the vasculature of the median eminence[6,18]. CRH binds to receptors in the anterior pituitary, inducing the release of adrenocorticotropin hormone (ACTH) into systemic circulation. Upon reaching the adrenal glands, ACTH binds to receptors on adrenal cells, triggering the synthesis and release of glucocorticoids (GCs; e.g. corticosterone in rodents and cortisol in primates)[16,19]. GCs are highly catabolic steroid hormones that act throughout the body to stimulate gluconeogenesis, mobilize stored energy, and potentiate effects of the SAM response[16]. GCs are generally slow-acting and bind to two types of receptors: GC receptors (GRs) with a low affinity for GCs, and

mineralocorticoid receptors (MR) with a high affinity for GCs. GRs mediate the negative feedback mechanism of the HPA axis; GCs bind to GRs at the level of the hypothalamus and the pituitary, inhibiting ACTH and CRH synthesis, respectively[16,20]. GRs are also widely distributed in the brain[21,22], and GC binding to GRs in limbic regulatory regions play a role in inhibition of the HPA axis[16,23]. MRs are responsible for maintaining basal HPA axis activity[21], which oscillates in synchrony with circadian and ultradian rhythms independent of stressors[24,25]. GRs and MRs are intracellular receptors that translocate to the nucleus and work as transcription factors, regulating gene expression[16,20]. Due to their action as transcription factors when complexed with the GR or MR, GCs have broad acting and long-lasting effects on cellular function.

Under conditions of chronic stress, the negative feedback mechanism of the HPA axis is compromised, leading to excessive and prolonged GC release[26–28]. Elevated GC levels, in turn, can cause neurobehavioral changes owing to its direct effects on gene expression in many systems of the brain, including corticolimbic circuits involved in cognitive and emotional processes. Increased GR binding in the central nucleus of the amygdala (AMYG), for example, results in higher expression of CRH and enhanced fear and anxiety responses in rodent models[29,30]. Stress-induced changes to CRH levels may indirectly affect neurotransmitter concentrations, including through binding of CRH to receptors in serotonergic neurons of the dorsal raphe nucleus which has been found to both enhance startle response and increase synthesis and release of serotonin, suggesting CRH may be an important mediator of stress on serotonin neurotransmission[31]. Given that GCs interact with and regulate the expression of a number of neurotransmitters[32], social stress-induced changes in levels of both GCs and GRs may

impact other neurochemical systems, contributing to further emotional and behavioral changes in individuals experiencing chronic social stress.

Many limbic and higher-order cortical structures play a role in regulating the stress response, particularly when the stressor is social in nature[23,33]. The AMYG activates a HPA stress response through indirect projections of the central nucleus (CeA) to the PVN through the BNST, and a SMA response through direct innervation of brainstem nuclei[16,34]. The basolateral AMYG (BLA) and medial AMYG (MeA) also play a role in the stress response through indirect projections to the PVN through the BNST, and projections to the CeA, MeA, and BNST[35,36], respectively. By integrating information about stressors from primary sensory and association cortices and modulating activity in subcortical structures, the prefrontal cortex (PFC) is also involved in the initiation of a stress response[37,38]. The orbitofrontal cortex (OFC) and medial PFC (mPFC) have indirect connections to the PVN and direct connections with the AMYG[39,40], enabling regulation over the stress response. The posterior OFC has inhibitory projections to the intercalated masses of the AMYG, and via these projections, facilitate disinhibition of the CeA by the IM and initiation of a HPA axis response[41]. The OFC and mPFC also send excitatory projections to the CeA, and inhibit the HPA axis by activating the CeA[34,42,43]. In this way, the PFC plays a role in negative feedback of the stress response, along with the hippocampus.

1.2.2 Effects of Chronic Stress on the HPA axis and Regulatory Limbic Regions

Chronic stress alters the HPA axis and regulatory circuits as a result of allostatic overload on these systems[33]. Dysregulation of the HPA axis is evidenced as alterations of basal cortisol levels (either hypocortisolemia[44] or hypercortisolemia[45–49] have been reported), increased

reactivity[50,51], and decreased GC negative feedback of the HPA axis[45,46,52–54]. Chronic stress also compromises the structure and function of regulatory limbic regions. Chronic stress results in increased dendritic arborization of the AMYG and BNST[55,56], and stress-related disorders have been associated with increased volume of the AMYG[33,57,58], although findings are mixed[59]. Stress-induced elevation in GCs increases CRH expression in the CeA and induces long-lasting increases in fear and anxiety behavior[29,30]. It has been proposed that increased activity in the efferent pathways projecting from the AMYG and BNST to the hypothalamus and brain stem are a tractable mechanism by which chronic early life stress increases reactivity to potential threats[60,61]. In fact, the AMYG has been reported to be hyperactive in neuroimaging studies of humans with anxiety disorders[62], and stress-induced plasticity in this region is believed to be an important underlying causal factor[63]. The insula (INS) is densely connected with the AMYG and is also believed to play a role in processing emotional stimuli[64] and modulating neuroendocrine responses[65]. Chronic early life stress has been associated with reduced INS volume, suggesting vulnerability of this region to chronic stress[66]. Effects of chronic stress on limbic regions that process reward have also been reported. Chronic stress results in sustained dopamine (DA) release in the nucleus accumbens (NACC) and a compensatory down-regulation of D2 dopamine receptors (D2R)[67,68], with implications for neurotransmission in mesocorticolimbic circuits connecting the striatum and PFC. Subcortical regions are not unique in their vulnerability to the effects of chronic stress; a large body of research demonstrates the PFC is adversely impacted by chronic stress[69–76]. In adult rats, chronic stress causes spine loss and reduced dendritic branching in mPFC neurons[69,70] as well as impairments in working memory[69]—an executive function highly dependent on the PFC. In contrast, the OFC shows expansion of dendrites after chronic stress[70], suggesting region-specific alterations to the PFC. In humans, reductions in PFC gray

matter volume and functional activity have been linked with stress-related disorders such as PTSD and depression[71–73], and cumulative life adversity is associated with smaller gray matter volume in medial prefrontal and anterior cingulate prefrontal cortex[74]. Adults who experienced childhood maltreatment, a form of chronic early life stress, demonstrate regional volume loss in the AMYG and PFC[75,76], paralleled by decreased structural integrity of WM tracts critical for emotion regulation, e.g. the cingulum bundle, arcuate fasciculus, and fornix[77].

Although data are limited, studies of chronic stress during development also detect alterations to the HPA axis and corticolimbic circuits responsible for regulating emotional and stress responses. Deprived caregiving is an example of a highly potent stressor in early life, and the early maternal and social deprivation experienced by post-institutionalized children is linked with alterations to the HPA axis, e.g. higher basal cortisol levels[78,79]. Post-institutionalized children also have increased AMYG volume and reactivity compared to healthy controls[80,81], and decreased structural integrity of tracts connecting the PFC with AMYG and striatum[82,83]. Children from low socioeconomic status (SES) families have higher baseline salivary cortisol[84], suggesting that the higher incidence of stressors experienced by low SES children alters the HPA axis. More recently, low socioeconomic status was associated with decreased brain surface area, and increased hippocampal and AMYG volumes[85,86]. In rhesus macaques, peer-rearing is an established model of early life adversity and has been shown to increase the volume of region-specific areas of the PFC, including the dorsomedial PFC and the dorsal Anterior Cingulate Cortex (ACC) in juveniles[87].

1.2.3 Chronic Stress and Functional Connectivity of Corticolimbic Circuits

Our brains are organized by functionally interconnected networks[88,89] of cortical and subcortical regions. Functional connectivity (FC) analysis of neuroimaging data allows examination of large-scale networks and interactions both between and within networks. FC is a measure of the temporal coherence of neurophysiological events that can be measured during task or at rest and suggests communication between brain regions. Resting-state fMRI measures low frequency fluctuations in blood oxygen level-dependent (BOLD) signal in a task-free context, with local fluctuations corresponding to changes in the firing and metabolic rate of the neurons[90]. Resting-state FC provides insight into how regions are functionally coupled together and often recapitulate cotemporaneous functional networks elicited in experimental task[91–93]. Resting-state FC studies of chronic early life stress and chronic stress have informed a greater understanding of how corticolimbic circuits are impacted by stress. Chronic early life stress has been shown to reduce AMYG-PFC FC[94,95], and resting-state FC studies have noted aberrant AMYG-PFC and NACC-PFC FC in stress-related neuropsychiatric disorders[96,97] Chronic work-related stress in humans was also associated with stronger resting-state FC between INS and AMYG compared to healthy controls[98], suggesting INS connectivity may adversely impacted by chronic stress. Based on findings from task-based FC studies, regulation of anxious responsiveness may also depend on robust INS-PFC connectivity[99,100]; though it's unclear whether these patterns would hold for resting-state FC. Although understudied in children, the few resting-state FC studies reported that chronic early life stress was associated with alterations in cognitive control and default mode (DMN) networks. For example, chronic early life stress was associated with negative correlations between the left lateral frontal cortex and regions in the temporal cortex and hippocampus[101]. Higher interparental conflict—a common form of early life stress—was

correlated with stronger FC between the posterior cingulate cortex (PCC) and mPFC, and between the PCC and AMYG[102].

1.3 Macaque Model of Social Subordination

1.3.1 Subordination Status and Chronic Stress

Social subordination in rhesus and other macaques is a useful translational model for studying the effects of chronic social stress[46,52,103–109]. Rhesus macaques are an ideal animal model for studying social stress because of their biological and behavioral similarities to humans, reliance on social relationships, repertoire of complex social behaviors, and their similar central nervous system function[110,111]. In both humans and macaques, the PFC has reciprocal connections with the AMYG[39], NACC, and INS[112] and undergoes protracted development[113]. It has been proposed that the slow and extended development of the PFC may render it more vulnerable to insults of early life stress[4,114]. If so, the slow development of the PFC in the macaque makes it an especially valuable model for studying how chronic stress early in life impacts brain development. The reproductive system function of female macaques (*Macaca fascicularis* and *Macaca mulatta*) is also similar to that of women, proving macaques to be a useful model for understanding how gonadal hormones interact with social stress to influence brain development during puberty.

Social status hierarchies are a prominent organizing feature of macaque societies[115]. The groups are structured by matrilineal dominance hierarchies, with matrilines ranked relative to one another and members of matrilines having similar ranks. The dominance hierarchy functions to maintain stability within the group, and is evident in both free-ranging and captive groups[116].

Dominance hierarchies within groups are relatively stable for long periods of time, enabling researchers to investigate the effects of subordination as a social gradient[117]. Subordinate monkeys respond to aggressive threats by emitting submissive behavior, and submission is considered a defining feature of subordination in macaques[104,118,119]. Subordinate monkeys receive significantly more aggression from dominant-ranking monkeys, at the same time that they receive less affiliation from group mates particularly in the absence of family members [109,120,121]. Increased receipt of aggression and decreased receipt of social affiliation leads to elevations in the stress hormone cortisol in nonhuman primate (NHP) species, including macaques[109,121]. The aggressive behavior of dominant-ranking animals is unpredictable and frequently unprovoked[121]; unpredictability may add to the stressful experience of being a subordinate monkey. Due to the unpredictability of the aggression, it has been proposed that subordinates maintain a state of physiological readiness that prepares them to respond[120]. As a consequence, the HPA axis is repeatedly activated, leading to a number of stress-induced phenotypes characteristic of subordinate NHPs[46,103,105,122–128].

Subordination in adult macaques has been validated as a well-established model for studying the effects of chronic psychosocial stress on various adverse health outcomes. Subordinate status has been associated with hypercortisolemia[46], cardiovascular disease[122], compromised immune system[123,124], reproductive dysfunction[125–127], psychostimulant self-administration[128], emotional feeding[105], and alterations in emotion regulation[103]. In response to an acute stressor, subordinate macaques exhibit higher rates of anxiety-like behaviors than dominant monkeys[103,108], providing further evidence of dysregulated emotional responses in subordinates. Although there is relatively little research on subordination stress during development, the few

studies that exist corroborate findings reported in subordinate adults. Infants assume the same ranks as their mothers[129] and are initially buffered from the effects of social subordination by their mothers in the first few weeks of life. Although weaning is a gradual process, around three months of age infants spend increasing amounts of time exploring away from their mothers, playing with peers and interacting with non-kin, and these interactions inform them of their place in the social hierarchy[130–132]. As juveniles, subordinate macaques receive more aggression from dominant-ranking animals and emit submissive behaviors in response to agonistic behaviors[133]. The limited work that has been done suggests that subordination impacts development by inducing hypercortisolemia and altering white matter (WM) microstructure in juveniles[134], and delaying pubertal onset in adolescents[108].

1.3.2 Social Subordination and Alterations of the HPA axis

Dysregulation of the HPA axis is consistently reported as a consequence of social subordination in female macaques. Dysregulation of the HPA axis often manifests as hypercortisolemia and impaired GG negative feedback in adult subordinate females[45–49]. Because impaired negative feedback in macaques is associated with a decrease in GR expression in the hippocampus[135], altered limbic regulation of the HPA axis may play a role in the impaired negative feedback seen in subordinate adults. A recent experiment demonstrated causal social status effects on HPA axis function by manipulating social status and demonstrating that improvements in dominant rank increased dexamethasone-induced acute cortisol suppression and GC negative feedback[136]. These findings are consistent with evidence of down-regulation of GRs in other animal models of social subordination[137]. The adrenal response to ACTH has also been shown to be impaired in subordinate macaques, with some reporting reduced GC response[52] and others reporting a

heightened GC response[45]. Given that GCs interact with and regulate the expression of a number of other neurotransmitters[32], social stress-induced changes in levels of both GCs and GRs may impact other neurochemical systems, contributing to further emotional and behavioral changes in individuals experiencing chronic stress.

1.3.3 Chronic Stress and Immune Interactions

Acute and chronic psychosocial stress can activate the inflammatory response[138]. In response to an acute stressor (no background of chronic stress), catecholamine actions on α - and β-adrenergic receptors increase cytokine expression in the brain and periphery of rats[139], and has been shown to directly activate NF-κB *in vitro*[140]. The slower-acting GCs are considered primary regulators of the inflammatory response, suppressing production of key inflammatory proteins, including cytokines and chemokines, and increasing transcription of anti-inflammatory cytokines[141,142]. The immune response is altered, however, under conditions of chronic stress. In individuals exposed to chronic early-life stressors, acute psychosocial stress augments the inflammatory response, heightening NF-κB DNA binding in peripheral blood mononuclear cells and increasing IL-6 levels in plasma, compared with healthy controls[143,144]. Chronic stress also results in innate immune cells developing GC resistance, such that the efficacy of the GR complex in suppressing proinflammatory cytokines is reduced[145,146]. Decreases in GR expression and impaired GR signaling and function have all been found to contribute to the development of GC resistance by the innate immune cells[147–149]. In adult female rhesus macaques, genes involved in chemokine and cytokine inflammation were more highly expressed in subordinate-ranking individuals[124,150]. This is consistent with reports of higher C-reactive protein (CRP) and interleukin-6 (IL-6) levels in humans of low socioeconomic status[151,152]. Importantly, there is

evidence that peripheral inflammation can trigger neuroinflammation and impact the brain and behavior[138]. When cytokines access the brain parenchyma they trigger further local inflammation by different mechanisms, one of them through activation of microglia that also produce pro-inflammatory cytokines, which can affect brain structure (e.g. myelination[153]) and function (e.g. synthesis and release of neurotransmitters, such as serotonin[154]). Expression of IL-1 and IL-6 receptors is enriched in the hippocampus, and the overexpression of these pro-inflammatory cytokines in the context of chronic inflammation impairs hippocampal circuitry and cognition[155]. Microglia are activated by stress[156], and microgliosis in the PFC (including the ACC), and INS has been linked with stress-related psychopathology, including schizophrenia, depression, and suicidality[157,158].

1.3.4 Normative Non-Human Primate Brain Development

To understand how chronic early life stress alters the way the brain develops, it is imperative to compare the way the chronic exposure to stressors shape the brain with normative brain development. Brain regions mature at different rates, with phylogenetically newer cortical regions like the PFC and inferior temporal cortex maturing later than evolutionary older regions such as the piriform and entorhinal cortex[159–161]. Normative development of gray matter volume in the PFC follows an inverted U-shaped curve, increasing until an inflection point is reached in the peripubertal period, when gray matter volume begins to decline[159,162,163], though this decline may be unique to humans[113]. Increases in gray matter volume during infancy and childhood are likely driven by 'exuberant synaptogenesis', a developmental period characterized by an overproduction of dendrites, dendritic spines, and axons[164,165]. In parallel, WM volume in the brain increases linearly during childhood and adolescence[159,161,162]. Cortical myelination in both

humans and NHPs begins prenatally in some regions (e.g., motor cortex, to support some level of infant motoric independence right after birth) and occurs postnatally in other cortical regions, continuing into early adulthood in association areas such as the prefrontal, temporal, and parietal cortices[166]. For example, the uncinate fasciculus, which connects the PFC with temporal regions including AMYG and hippocampus, and is believed to relay bottom-up information to the PFC and enable ventromedial PFC (vmPFC) top-down control over AMYG reactivity to threat[167], only reaches adult levels of myelination at 3.5 years in monkeys and around 20 years in humans[166].

The AMYG appears to be functional at birth[168–170] but continues to develop postnatally, following rapid regional-specific structural development patterns that makes some nuclei, and the functions they underlie, more vulnerable than others to social experience. Most of this evidence comes from studies in rhesus monkeys (*Macaca mulatta*), that have indicated that the lateral and basal nuclei, involved in emotional processing and learning, show rapid volume increases from birth through 3 months of age, whereas the volume of the central nucleus, with direct connections to the autonomic nervous system that modulate stress responses, continues to increase during the juvenile period[171]. These nuclei-specific changes in monkeys result in an overall increase in total AMYG volume, with the highest rates evident during the first 4 postnatal months[172]. The increase in AMYG volume results from structural changes in both neurons and glia, although after 3 months of age it is mostly due to increased number of oligodendrocytes and myelination, while neuronal size/number and astrocyte number do not change[171]. Social experience in the beginning of life may impact the maturation of AMYG WM tracts/circuits in

the formative stages of their development, including AMYG projections to orbital PFC, which do not mature until 2 months when curiosity and frustration emerge in the infant[173].

1.3.5 Effects of Social Subordination Stress on Brain Structure and Connectivity in Adult NHPs

Recent neuroimaging work demonstrates that social subordination alters brain structure and FC, particularly of corticolimbic circuits and social brain networks. In a magnetic resonance imaging (MRI) study of adult, mostly male, rhesus macaques, the volumetric extent of gray matter (GM) in bilateral AMYG had a positive association with the social rank of the monkey (i.e., more subordinate monkeys had smaller AMYG volume)[174]. A positive relationship between GM volume and rank was also found for a region that included the 5HT-containing raphe nucleus, and the hypothalamus. The researchers also found evidence of social status effects on FC between the AMYG and raphe nucleus, and between AMYG and hypothalamus, with more positive coupling for subordinate monkeys and more negative coupling for dominant monkeys. In numerous other studies, chronic stress has been linked with increased AMYG volume[33,80,85,175], though findings are inconsistent[59,176], which may be explained by differences in subject populations, or type, duration, and severity of stressor[177]. In the study with adult macaque males, the finding of reduced AMYG volume in the subordinates may be attributable to the gender (male) and life stage (adult) of the macaques. Rank-related differences in AMYG structure, function, and connectivity with other regions may reflect the effects of social rank on the perception and salience of social stimuli or the impact of stress-induced allostatic load on this region in subordinate ranking monkeys.

Noonan et al. (2014) also found structural and functional differences in the basal ganglia associated with social rank[174]. Extent of GM in the basal ganglia significantly decreased as rank increased, and positive intrinsic coupling was observed between subregions of the basal ganglia for subordinate monkeys, whereas greater negative coupling was found among dominant monkeys. Given that the basal ganglia participates in DA neurotransmission in the mesocorticolimbic systems, these findings are consistent with studies demonstrating differences in DA D2 receptor expression and binding in the striatum for subordinate compared to dominant monkeys[128,178], and suggest that social stress in rhesus macaques may alter corticolimbic reward circuitry and motor pathways.

The PFC is a brain region that is critical for regulating socioemotional behavior[179] and mediating social cognition[180] and is also structurally and functionally affected by social rank and social group size in rhesus macaques[174,181] and by chronic social stress[182,183]. In a study of mostly male, adult rhesus macaques, GM volume in the rostral PFC and superior temporal sulcus (STS) was found to increase with social rank and social network size[174]. Given that PFC and STS have been implicated in social cognition in humans and NHPs, rank-related structural and functional changes in these regions may underlie differences in social experience, complexity, and cognition between dominant and subordinate monkeys. Alternatively, they may be indicative of social subordination stress-related changes, as evidence from primate and rodent models demonstrates that chronic stress leads to structural changes in the PFC. In rodents, chronic stress is associated with decreased dendritic arborization in the mPFC[184], and expansion of the OFC impairments[70], as well as evidence of impaired PFC function including deficits in working memory[69]. In rhesus macaques, peer-rearing--an established model of early life adversity--

increases the volume of region-specific areas of the PFC, including the dorsomedial PFC and the dorsal ACC in juveniles[87]. These findings suggest that the structural and functional changes in the cortex associated with social rank in rhesus monkeys could reflect differences in social stress experienced by the two groups.

1.3.6 Biobehavioral Effects of Social Subordination in Juvenile NHPs

Despite the extensive literature described above investigating the adverse effects of social stress on various health outcomes, it is surprising that few studies have taken a developmental approach to investigate the developmental outcomes of social subordination in macaques during infancy, adolescence, and throughout development. Recent studies are trying to fill that gap in our understanding of the emergence and developmental consequences of this social experience. One such study investigated the effects of several factors, including social subordination status, on the pubertal timing of female rhesus macaques[108]. Relative rank within the social group (calculated as the ratio of a subject's rank to the total number of adult animals in the group) was significantly correlated with age at menarche and age at first ovulation, with more subordinate animals reaching menarche and first ovulation later than more dominant animals. Other factors such as increased emotional reactivity and slower weight gain were also strong predictors of delayed puberty. This study did not investigate the relationship between social stress and neurobiological developmental outcomes, however.

To our knowledge, only two recent studies have focused on subordination effects on neurobehavioral and physiological development in female rhesus macaques. One study investigated the effects of social subordination on the development of the HPA axis, WM, and

socioemotional behavior in juvenile prepubertal female macaques, and tested whether social status effects were modified by polymorphisms in the serotonin transporter[134]. Briefly, diffusion tensor imaging (DTI) is an *in vivo* neuroimaging technique that measures water diffusion in the brain and provides measures of WM tract integrity and connectivity. Due to the hydrophobic nature of the myelin sheath surrounding axons, water diffusion is restricted and thus preferential along, and not perpendicular to, the axon[185]. Fractional anisotropy (FA) quantifies the preferential water diffusion along the axon and is used to infer information about WM structural integrity, including level of myelination and axonal packing density or organization, with higher myelination and axon packing density/organization reflected as higher FA. Prepubertal subordinate females had greater FA than dominant females in three clusters of brain WM tracts, including one cluster in left mPFC and two clusters along the left dorsal medial wall of the brain. Increased FA in the mPFC cluster was correlated with heightened fearful and submissive behaviors in the subjects' social groups, and increased fearful behavior in the Human Intruder paradigm, an ethologically relevant paradigm that measures fear and anxiety responses in response to threatening stimuli of varying intensity[108,186–188]. Increased FA in the dorsal medial wall clusters was also positively correlated with submissive behavior in the social groups. More subordinate status predicted higher baseline cortisol concentrations and a more blunted cortisol stress response to an acute stressor. Altogether, the authors interpreted these findings as evidence of neural adaptations to facilitate social behaviors suited for a high-pressure environment, given that the regions identified as having altered WM integrity in subordinates— frontal motor, sensorimotor, and mPFC—are heavily involved in emotional and sensorimotor processing and motor output.

In another study exploring the effects of social subordination stress on neural development, researchers investigated how social status and serotonin transporter (5HTT) polymorphisms impact the development of brain 5HT systems longitudinally during the pubertal transition in female rhesus macaques[189]. To accomplish this, the authors employed PET imaging techniques to determine binding potential of both the 5HTT and the 5HT1A receptors. This study revealed that subordinate juvenile macaques had higher 5HTT binding potential in OFC, as well as higher 5HT1A binding potential in hypothalamus, when compared to dominant macaques. These differences have to be interpreted in the context of global normative developmental increases in both 5HT1A receptors and 5HTTs in the regions studied from pre- to peripuberty, as the animals also develop more adult-like patterns of social and emotional behaviors, with increased receptor and transporter availability being associated with increased levels of emotional reactivity.

To our knowledge, studies by Howell et al.[134] and Embree et al.[189] are the only two examining the relationship between social subordination stress and brain development in female macaques during peripuberty. The findings reported in these studies are important because they reveal that social rank differences in brain neurotransmission and circuitry are already present in juvenile macaques.

1.4 Experiential Factors that Modify Effects of Stress on Brain and Behavior

1.4.1 Estradiol and Puberty

Estradiol (E2) interfaces with many neurotransmitter, neuropeptide, and hormone systems and influences structural and neurochemical changes in corticolimbic systems[190–197]. E2 is a gonadal hormone regulated by the hypothalamic-pituitary-gonadal (HPG) axis. In the primate HPG axis, gonadotropin-releasing hormone (GnRH) neurons in the basal hypothalamus release

gonadotropin-releasing hormone, which binds to gonadotropic cells of the anterior pituitary. The gonadotropes release luteinizing hormone (LH) and follicle-stimulating hormone (FSH) into systemic circulation, and these hormones travel to the gonads (ovaries in females, testes in males), and stimulate the synthesis and secretion of gonadal steroid hormones, including estrogens, progestins, and androgens. During prenatal and early postnatal development, gonadal hormones have an 'organizational' effect on neural circuits; these organizational effects refer to irreversible changes in nervous system structure and the programming of adult responses to hormones[198]. These organizational effects include a a transient rise in testosterone having a masculinizing effect on neural circuits in males, while the absence of testosterone in females contributes toward a female neural phenotype[198]. E2 also plays a role in programming the female brain early in development through a number of mechanisms, including region-specific suppression and promotion of apoptosis and synaptogenesis, and altering morphometry of neurons and astrocytes[199]. After the early postnatal period, the HPG axis is relatively quiet until puberty; nonetheless, pre-pubertal ovaries have been shown to secrete low concentrations of E2 in humans[200], and macaques[108,201], which have a regulatory action on LH secretion[201–203], insulin-like growth factor 1, and growth hormone[204].

Puberty refers to the developmental transition from a non-reproductive state into a reproductive one, and is marked by the reactivation of the HPG axis and rising levels of gonadal hormones, including E2 in females, and testosterone in males. Adolescence is a broader term capturing the period of social and cognitive maturation that is associated with and results from the hormonal changes of puberty[205]. The incidence of psychopathology markedly increases during adolescence[206], and the adolescent period is characterized by heightened emotional and stress

reactivity[207], including increased anxiety[208], negative affect and depressed mood[209,210]. Adolescents face varied psychosocial stressors arising from increased self-awareness, perceived expectations from family and peers, and maturational changes in their bodies, and these factors are possible triggers for the emergence of psychopathology in adolescence[211–213]. Women are twice as likely to develop stress-related mood disorders than men[214], and this gender difference arises after pubertal onset in adolescence and persists until menopause, when it disappears again[215], suggesting that developmental increases in E2 or other gonadal hormones may confer vulnerability to stress-related mood disorders. Pubertal stage is a stronger predictor of the onset of depression than is chronological age[216], and E2 (and testosterone) levels have been found to mediate the relationship between pubertal stage and depression in adolescence[217], further implicating developmental increases of E2 in the emergence of mood disorders.

Alternatively, it has been proposed that rapid fluctuations in E2, or low levels of E2, may underlie dysregulated emotion and the rise of mood disorders in adolescence.[195] In naturally cycling women, risk for developing depressed mood is highest in the mid to late-luteal phase when E2 levels are declining and P4 levels are peaking[218]. The sharp declines in E2 and P4 in the postpartum period have been associated with the development of major depressive disorder in a subset of women[219], and perimenopausal women tend to experience increased depression and irritability when E2 levels are declining at the onset of menopause, compared to postmenopausal time points[220]. During adolescence, negative mood increases during pubertal periods with the most dramatic increases in hormone levels[221], indicating that dramatic fluctuations in E2, rather than increases in E2 *per se*, may contribute to the emergence of mood disorders during the pubertal transition. It is still unclear whether developmental increases in E2, rapid fluctuations of

E2, or declining E2 levels during the menstrual cycle play a role in the emergence of mood disorders during adolescence.

The effects of E2 on mood are complex and multi-factorial, and other factors like stressor exposure appear to be important determinants of E2's impact on mood. Early life stress itself is one of the strongest predictors of developing psychopathology[60,222], and research suggest that early life stress may interact with pubertal onset to exacerbate the rise in depressed mood during adolescence[223]. Although there are little data on this question, one study found that stressful life events was associated with stronger depression scores in girls who had started puberty compared to prepubertal girls with similar life histories[223]. This finding appears to be consistent with other studies showing the stress modulates the impact of E2 on mood.

1.4.2 Effects of E2 on Emotion and Mood

E2 influences emotionality in animal models[224] and humans[225] and these changes are typically considered activational effects of E2. Broadly, activational effects of E2 produce changes in behavior that are distinct from those produced by a hypoestrogenic condition, and typically include increases in reproductive[226,227] and social behaviors[228,229], and decreases in emotional reactivity, including anxiety-like behavior, and improved mood[230–242]. Ovariectomy increases anxiety levels in rats and mice[230–232], and replacement of E2 and P4 decreases levels of anxiety-like behavior in female rats[231–235]. Intact rats and mice both display increased anxiety-like behaviors during diestrus, when E2 levels are low, and reduced anxiety-like behaviors when E2 levels are elevated during proestrus and estrus[231–236], suggesting that E2 may reduce anxiety-like behavior. However, administration of E2 or E2 benzoate has been shown to produce mixed

results in rodents, with some studies finding increased anxiety, and others finding decreased anxiety[243–245]. One proposed hypothesis to account for these discrepant findings is that E2 generally promotes arousal, and its actions on fear are context-dependent[243]. ER subtypes may also play a role in determining E2's effects on anxiety; agonists for ERβ have been shown to be anxiolytic[246], while those for ERα have either no effect or are anxiogenic[244]. The dose of E2 may be another important determinant of anxiolytic vs. anxiogenic outcomes, with low doses of E2 having an anxiolytic effect[247], while high doses have an anxiogenic effect in ovariectomized mice[247,248].

Notably, stressors appear to be an important factor in determining E2's actions and modulate the activational effects of E2 on anxiety. For example, a history of stressor exposure in rodents has been shown to attenuate E2's anxiolytic effects in the open field and elevated plus maze[245]. Oleson et al. found that ovariectomized adult mice that received treatment of E2 benzoate and P4 displayed expected decreases in anxiety-like behavior in various paradigms (included elevated plus maze and light-dark box), but an immune challenge stressor during the sensitive period of puberty either blocked the anxiolytic effect of the E2 and P4 hormone treatment in adulthood, or produced an anxiogenic effect[249]. In rhesus macaques, E2 administration reduced anxiety-like behavior in females with high baseline anxiety levels, but socially subordinate females with the short promoter allele of the serotonin transporter did not demonstrate a similar anxiolytic response to the E2 treatment[228], suggesting that the chronic stress of social subordination attenuated E2's anxiolytic effects.

Evidence suggests that E2 may also have anti-depressive effects[237–240,242,250]. Ovariectomy

increases, and E2 replacement decreases, duration of immobility in the tail suspension test and the forced-swim test[237–239], indicating that E2 reduces depressive behavior. Female transgenic mice who are deficient in E2—as a result of knocking out the aromatase gene--show greater depression-like behaviors compared to wild-type mice[240]. However, exposure to a stressor during puberty may reverse the relationship between E2 and depressive behavior; in mice exposed to an immune system stressor during puberty, E2 treatment actually increased the duration of immobility in the forced swim test and the tail suspension test, as compared to control mice who showed the expected decrease in depression-like behaviors[250]. Studies in naturally cycling and perimenopausal women suggest that E2 can improve mood and affect[241,242], though no studies to date have examined interactions between E2 and stress on mood in women. Acute treatment with E2 reduces depressive symptoms in perimenopausal women[241], and a meta-analysis of hormone replacement therapy in postmenopausal women found that E2 and conjugated equine estrogen decreases depressive symptoms[242]. Not all women experience depressed symptoms during the menopause transition, and for those who do, depressed mood can persist regardless of type of hormonal replacement[251]; history of stress may be an important determinant of response to E2 in women, but this topic has been understudied.

1.4.3 Interactions between E2 and HPA Axis

E2 has been shown to directly interact with the HPA axis, for example, by potentiating GC secretion to an acute stressor and diminishing GC negative feedback in ovariectomized rodents given E2 replacement[252,253]. E2 increases ACTH secretion in female baboons[254], and increases CRH expression in the hypothalamus of female macaques[255]. In female adult macaques receiving Lupron drug treatment to suppress the HPG axis, E2 replacement resulted in decreased GC

negative feedback to a dexamethasone suppression test, and increased GC response to a combined dexamethasone-suppression-CRF stimulation test[48]. In this experiment, a significant and earlier escape from the dexamethasone suppression test, as well as a heightened GC response to CRF stimulation, was detected in socially subordinate females compared to dominant females receiving E2 replacement, suggesting that E2 actions on the HPA axis are modulated by the effects of chronic psychosocial stress. Other factors, in addition to history of stress, also influence E2's effects on the HPA axis, including ER subtype and P4 levels. In rodents, ERα acts to decrease GC negative feedback to restraint stress, while ERβ had no effect; additionally, activation of ERα increased, while ERβ activation decreased, the peak corticosterone response to an acute stressor[256]. P4 has been shown to interact with E2, enhancing its potentiation of the corticosterone response to a stressor in rats[253], and diminishing E2's attenuating effect on negative GC feedback in macaques[48]. Hence, there are complex relationships between E2, ER subtype, P4, and the HPA axis that influence reactivity to stressors and negative feedback. During puberty, it has been theorized that E2 modulates the HPA axis and increases sensitivity to stress and the risk of developing mood disorders, e.g. depression[195]. Changes in neuroendocrine function are a hallmark of adolescence[257,258], including developmental increases in basal cortisol levels and reduced negative feedback[258,259], and these changes may play a role in enhancing sensitivity to stress during adolescence.

1.4.4 E2 and Neural Remodeling During Adolescence

During puberty and adolescence, E2 and other gonadal hormones have been shown to have a second phase of organizational effects on the brain, influencing structural and neurochemical changes, including changes to corticolimbic circuits important to emotion regulation[190,191,195,260–

[264.] E2's effects on the brain are achieved through a number of mechanisms, including influencing neurite outgrowth, cell survival and growth, apoptosis, synapse number, dendritic branching, and myelination through its interactions with glial cells[265–268]. Some of the neurodevelopmental changes that take place during the pubertal transition have been linked to the changing hormonal milieu of puberty[269,270]. In rats, for example, the number of ventral mPFC neurons is similar in male and females early in adolescence, but cell death in females leads them to have significantly fewer neurons than males by postnatal day 90[271]. Frontal cortex white matter volume linearly increases during adolescence in rats, but more so in males than females[272]. Prepubertal gonadectomy prevents these sexual dimorphisms from emerging in females, indicating that these changes in the mPFC and frontal cortex are driven by organizational effects of ovarian hormones[273]. While it has become increasingly clear that gonadal hormones have organizational effects during adolescence, it's not well understood how these effects are modified by exposure to chronic stressors. Research with adult animal models suggests that stress and E2 interact to promote dendritic remodeling of the mPFC in females; stress increases the dendritic length of BLA-projecting neurons in ovariectomized females, but only with E2 replacement, as no remodeling is seen without E2 replacement[184]. These results raise the possibility that rising levels of E2 during puberty could interact with stress to alter the maturation and development of the brain, but this question has yet to be explored.

Although limited, a few studies have started to examine how adolescent changes in human brain development are linked with hormonal maturation. In females, higher E2 levels have been associated with lower GM density in frontal and parietal lobes, and lower GM volume in the ACC, during the pubertal transition. In a study of white matter changes in female and male

adolescents, LH levels were correlated with overall WM volume in the brain in early puberty[274]. Functional activity and connectivity between corticolimbic regions also demonstrate changes during the adolescent transition[275–277], and while no study to date has addressed whether these changes are driven by gonadal hormones, evidence from naturally cycling women and exogenous administration of E2 and P4 in postmenopausal women suggests gonadal hormones could play a role. Corticolimbic FC changes across the menstrual cycle[278], and in response to exogenous treatment with E2 and progesterone (P4) in postmenopausal females[279], suggesting that a pubertal rise in E2 and fluctuations in P4 during the menstrual cycle could influence FC.

E2 also interfaces with many neurotransmitter systems, including glutamate, serotonin, DA, and GABA, and may influence maturational changes in these systems during pubertal development. Notably, E2 modulates the responsiveness of postsynaptic receptors[280,281], and the presynaptic release of neurotransmitters[282]. For example, E2 has an overall facilitating effect on dopaminergic neurotransmission; it enhances pre- and postsynaptic DA function in rodent models by augmenting DA release, increasing D1 and D2 receptor number by slowing degradation of these receptors, and enhancing DAT production[283–288]. Gonadal hormones also appear to affect the morphology of dopaminergic neurons, as ovariectomy in female NHPs resulted in a permanent decrease in DA cell number in the substantia nigra that was rescued by E2 replacement[289]. In primates, E2 and P4 both increase the density of terminal arborization of dopaminergic neurons in the dorsolateral prefrontal cortex[290]. E2 is also known to facilitate central serotonin (5HT) activity[291], which plays an important role in cognitive, emotional, and neuroendocrine processes[292]. In some studies, for example, E2 was shown to increase serotonin

concentration in the brain (though E2 and P4 together decreases serotonin concentration)[293–295]. In ovariectomized monkeys, E2 replacement increases tryptophan hydroxylase protein and mRNA expression in the dorsal raphe nucleus[296]. There is some evidence that stress and E2 interact to influence serotonergic transmission; cessation of ovarian hormones with surgical menopause in combination with the chronic stress of social subordination in macaques results in down-regulation of serotonergic neurotransmission in the AMYG[297,298].

Although understudied, some neurotransmitter systems, including DA and serotonin, appear to undergo maturational changes during adolescence[299–304]. For example, adolescence in rats is characterized by 4-fold lower serotonin turnover in the NACC, compared to adulthood and childhood[305], and low serotonin activity in adolescence is thought to contribute to adolescent sensitivity to mild stressors and increased anxiety[306]. Serotonin receptor expression also changes during adolescence; for example, 5-HT2A receptors reach peak expression in the cortex right before adolescence and then decline progressively over adolescence to adult levels, and this change is correlated with increased innervation and pruning of serotonin axons in rats and monkeys[307]. The dopaminergic system also matures during adolescence, with DA content, tyrosine hydroxylase and dopaminergic innervation of the frontal cortex increasing to a peak just before adolescence, and then subsiding in non-human primates[300–303]. In humans, striatal DA levels increases through adolescence, but other synaptic markers of DA contact peak at the beginning of adolescence, as in non-human primates[308]. Although these changes have not been linked directly with gonadal hormones, indirect evidence suggests that the changing hormonal milieu of puberty may play a role in maturation of neurotransmitter systems. For example, sensation seeking is highest during mid/late puberty in boys and girls[309], and studies in female

adults have linked high levels of E2 in adult females with lower levels of sensation seeking[310]. It's possible that rising levels of gonadal hormones have an organizational influence on the maturation of neurotransmitter systems in the brain—a relationship that could be modulated by the experience of chronic stress—but these questions have yet to be explored.

1.4.5 Pubertal Timing and Neurobehavioral Development

As reviewed above, chronic activation of the HPA axis modulates the impact of E2 on mood, GC reactivity and feedback, brain structure, function, and neurochemical systems. The cross-talk between the HPA and HGP systems appears to be bidirectional, as chronic activation of the HPA axis attenuates HPG activity via suppression of hypothalamic GnRH release, pituitary LH and FSH pulsatile release, and E2 synthesis in the ovary[311,312]. Subordination in adult female macaques is associated with impaired ovarian function, including reductions in E2 and P4, and subordinate status has been linked with increased incidence of anovulation[313] and delayed timing of menarche and and first ovulation [108,313]. Although pubertal onset has been implicated in the emergence of mood disorders and psychopathology, it is not well understood how the timing of puberty contributes to the etiology of mood disorders, or how it interacts with stressors to influence neurobehavioral development. Chronic stress and concomitant delayed puberty in NHPs may disrupt both steroid-independent maturational changes and organizational effects of E2 on brain structure, neurotransmitter systems, and the HPA axis, leading to compromised development of circuits and neurotransmitter systems important for emotion regulation. On the other hand, a number of studies report an association between early puberty in females and emotional and behavioral problems during adolescence and early adulthood[314–317], suggesting that earlier pubertal timing may increase the risk for mood disorders.

The association between earlier puberty in females and detrimental psychological outcomes have been explained by different models, including psychosocial and biological explanatory models. According to some psychosocial theories, early maturity increases exposure to stressors by placing girls in situations for which they are not cognitively or emotionally "developmentally ready"[318]. Biological theories propose that early maturers experience a rise in gonadal hormones at a time when the brain is more sensitive to organizing effects, leading to altered development[314,319]; these hypotheses are grounded in work by Schulz and Sisk demonstrating that sensitivity to gonadal hormones decreases over time[320]. Because early maturers would experience the greatest impact of gonadal hormones at a time when subcortical structures are maturing and the cognitive control systems are the least developed, top-down and bottom-up communication between these regions may develop in such a way that ultimately leads to imbalanced communication and emotional dysregulation. In contrast, on time and late maturers would experience the impact of gonadal hormones on the limbic system when the PFC and its top-down projections were more developed, which may promote better emotional regulation outcomes. Thus, it is possible that delayed puberty in individuals experiencing chronic stress may be protective, though it is unclear how pubertal timing interacts with chronic stress to impact development. In summary, pubertal onset, pubertal timing, and stress influence neurobehavioral development, but it is not clear how these factors interact with each other and impact the circuits important for emotional and neuroendocrine regulation during the adolescent period.

1.4.6 Dietary Environment and Neural Circuits Implicated in Nonhomeostatic Eating

Diet is another important experiential factor that may interact with stressor exposure to influence neurobehavioral development[321–324], and even the tempo of puberty[325–327]. The availability of palatable and calorically dense diets has contributed to an obesity epidemic. In 2013, the global prevalence of overweight and obesity was estimated at 2.1 billion people—a dramatic rise from 921 million in 1980[328]. Childhood obesity is also a serious public health concern internationally and domestically. In developed countries, prevalence of childhood obesity has also increased considerably with 23.8% of boys and 22.6% of girls reported to be overweight or obese in 2013[328]. Childhood obesity has more than doubled in children and quadrupled in adolescence in the U.S. in the past 30 years[329]. Obese children are more likely to be obese as adults[330]. They also develop obesity-related diseases[331–333] at earlier ages[334,335] and the health outcomes are more severe[331–333]. These health outcomes include high cholesterol and blood pressure, type 2 diabetes, bone and joint problems, cancer, and psychological health problems[336–338], making obesity not only a public health concern but also an economic burden[339,340]. Chronic stress is a cumulative risk factor for obesity in children[341], especially among girls[342–344]. It is believed that stress promotes emotional eating as a coping mechanism and emotional eating has been linked with reported stress in children[345], with higher rates of obesity among children in families reporting significant stressors in their lives, including poverty, domestic violence, drug abuse and childhood maltreatment[346–349]. Emerging research suggests that consumption of an obesogenic diet and resulting obesity impacts many of the same circuits compromised by stress, but it is not well understood how consumption of an obesogenic diet modifies stress-induced alterations to the brain, particularly during development.

Food intake is regulated by a homeostatic pathway that governs energy balance by monitoring energy stores and increasing the drive to eat when energy is low[350,351]. This pathway relies on circulating hormones like leptin and ghrelin transmitting information about peripheral energy levels to the brain. The arcuate nucleus (Arc) of the hypothalamus expresses leptin receptors and ghrelin receptors on distinct subsets of orexigenic neurons, and activation of leptin receptors suppresses food intake, whereas activation of ghrelin receptors promotes feeding behavior. While satiety and hunger signals are critical determinants of feeding behavior, food intake is also influenced by a non-homeostatic hedonic pathway that motivates food consumption even at times of energy abundance. The NACC, AMYG, INS, and PFC/ACC have all been proposed to contribute to non-homeostatic eating due to their role in appetitive behavior and reward processing[352–356]. The NACC plays a significant role in reward processing, with the VTA releasing DA in the NACC in response to natural reinforces including food and food cues[357]; this release of DA is thought to coordinate the affective, cognitive, and motor responses necessary to obtain food rewards[350]. The AMYG is involved in evaluating the salience of food rewards[358], learning stimulus-reward associations, and responding to the taste and sight of food[359]. The INS integrates olfacto-gustatory, affective, interoceptive, and sensorimotor information[360], and is the site of the primary gustatory cortex. The PFC consists of several distinct sub-regions, many of which may be important for processing the incentive salience of food rewards and providing top-down control of impulses underlying non-homeostatic eating. The mPFC is involved in emotional and motivational salience assessment, and emotion regulation via top-down control of limbic regions[361,362]. The ACC supports emotional processing, reward-based learning, error detection, and cognitive control[363,364]. Grabenhorst and Rolls also found a hedonic map in this region, with pleasant tastes activating more ventral areas and less pleasant tastes activating more

dorsal areas[365]. The OFC contributes to goal-directed behavior, reward processing, and impulse control[366–368]. It also processes the salience and rewarding properties of food and food cues; there are taste and flavor reward neurons in the OFC that change their firing rate in response to the quality and amount of food delivered[359,368,369]. The dorsolateral prefrontal cortex (dlPFC) is another PFC region that is important for guiding goal-directed behavior and, as a pivotal member of both the salience network and executive control network, it evaluates salient stimuli and allocates working memory to the evaluation and selection of incentive-based behavioral responses[354,370,371]. The PFC/ACC have direct reciprocal anatomical connections with the striatum and AMYG[352,354], with the densest projections from the PFC to the AMYG originating in ACC (BA24 and BA25), and the densest projections from the AMYG back to the PFC terminating in the OFC. The NACC and AMYG are also reciprocally connected with one another[354]. The INS is reciprocally connected with dlPFC (BA46), OFC (BA11, BA13), ACC (BA24), and the AMYG, and the NACC[112,372,373]. Alterations of structure, function, and connectivity within and between these regions have been documented as a result of consuming an obesogenic diet.

1.4.7 Effects of Diet and Diet-induced Obesity on Brain Structure and Function

Animal models have proven useful for understanding how the consumption of a high-calorie diet (HCD) impacts the brain. In parallel with addiction research in humans, in-depth cellular molecular work with animal models has given rise to the theory that consumption of a HCD has similar effects on the brain to drugs of abuse. Common drugs of abuse initially increase DA neurotransmission in the mesocorticolimbic pathway, but chronic drug use is associated with a reduction in basal DA secretion within the ventral tegmental area (VTA), and with increased

levels of the transcription factors cyclic AMP response element binding protein (CREB) and deltaFosB in the striatum[374–376]. These transcription factors alter neuronal responsiveness to DA release, and are believed to play a role in increasing the incentive motivation to obtain drugs[376]. Mice on a high-fat diet for 4 weeks showed significant increases in the level of deltaFosB in the NACC[377], a finding similar to that reported following exposure to drugs of abuse. Higher deltaFosB concentration in the NACC enhances food-reinforced operant responding[378], providing evidence that deltaFosB is instrumental in motivating food consumption. In adult rats, chronic consumption of a high-fat diet and a high-fat/high-sucrose diet have been associated with downregulation of the striatal D2 and D1 receptors[379,380]. Moreover, knockdown of D2Rs accelerated the onset of compulsive-like food seeking and addiction-like reward deficits in rats with access to a high-fat diet[379]. As with drugs of abuse, rats who became obese after consumption of a high-fat cafeteria-style diet showed reduced basal levels of DA in the NACC, and lower DA response to a laboratory chow meal[381]. Opioids and μ-opioid receptors are also implicated in encoding the rewarding properties of food and feeding behavior[382–384]. Direct stimulation of μ-opioid receptors promotes the consumption of high-fat foods[384], and sustained consumption of a high-fat diet downregulates the expression of μ-opioid receptors in reward circuits[385].

Research examining the effects of diet on development have focused on maternal consumption of a high-fat diet during the prenatal and early postnatal period. Maternal consumption of a high-fat diet has been associated with alterations to the CNS, including decreased dendritic arborization in the AMYG[386], increased neurogenesis of orexigenic cells in the hypothalamus[387], and differential expression of genes related to serotonergic and dopaminergic

neurotransmission[388,389], inflammation[390], and neuropeptides that regulate food intake[387,391]. With respect to homeostatic regulation of food intake, maternal consumption of a high-fat diet in the perinatal period was linked with higher expression of orexigenic peptides in the lateral hypothalamus[387], suggestive of increased neurogenesis of orexigenic neurons. Accumulating evidence also indicates the maternal consumption of a high-fat diet alters reward circuitry in offspring, possibly mediating an increased drive for non-homeostatic eating. Maternal consumption of a "junk-food" diet (high-fat and high-sugar) programs a greater preference for fatty, salty, and sugary foods as an adult[392,393]. Maternal consumption of junk food in rats also changes the expression of mRNA for μ-opioid receptors and DA transporter in offspring, with direction depending on age of offspring[393]. Maternal consumption of cafeteria-style diet in the postnatal period caused changes in DA and serotonin levels in rat offspring, presumably through lactational programming[394]. In a NHP model, the offspring of obese dams who consumed a high-fat diet during the perinatal period (including lactation) demonstrated preference for food high in fat and sugar compared to offspring from lean mothers fed a control diet[389]. Maternal obesity and high-fat diet consumption also decreased the abundance of DA fiber projections to the PFC, and reduced D2R and D1R protein levels in the PFC[389].

While a large body of work has focused on the mesocorticolimbic circuitry, research has begun to examine dietary programming effects on the HPA axis and its regulation by limbic regions. Consumption of a high-fat diet during pre-weaning development in rats was linked with increased expression of both corticosterone receptors in AMYG in adulthood, and pro-inflammatory and anti-inflammatory genes in the AMYG and hippocampus that are known to be regulated by GRs[395]. Importantly, these alterations predicted increased anxiety behavior,

decreased basal corticosterone levels, and a prolonged stress response following an acute stress challenge. As mentioned earlier, maternal high-fat diet has also been shown to result in dendritic atrophy of pyramidal neurons in the hippocampus and basolateral AMYG and impaired fear conditioning—a learning process that relies on a functioning basolateral AMYG[386]. These alterations suggest that consumption of a HCD in development may impact stress responses, fear, and anxiety. Indeed, NHPs exposed to a high-fat diet in the prenatal environment demonstrate enhanced fear responses in a novel task[388]. Similarly, maternal high-fat diet in mice increases the expression of anxiety behavior in the Open Field and Elevated Plus Maze tasks[390,396]. The bulk of the developmental studies that have been carried out in animal models have focused on maternal consumption, with very little research examining how infant consumption of a HCD affects early development. The few studies that have been done have found that early life consumption of a high-fat diet in the 3rd postnatal week of life in rodent models altered DA-related gene expression in the NACC suggestive of reduced DA transduction, and increased a preference for fat as an adult[397].

Consumption of a HCD and resulting BMI increases in humans have also been associated with alterations to brain structure, function, and connectivity. It is arguably more challenging to discern whether changes in brain circuitry observed in overweight and obese individuals contributed to over-eating, are the consequence of high BMI and fat mass, or both. Nevertheless, many of the diet-induced changes observed in animal models bear resemblance to the obesity-induced alterations evident in humans. As in rodent models, obese humans demonstrate lower striatal availability of D2R [398–400] and μ-opioid receptors[401], though some studies report unaltered D2R availability[401,402]. Lower striatal D2R availability was associated with decreased activation

in the dlPFC, ACC, OFC, and somatosensory cortex in obesity[403], suggestive of altered striatal inputs to the PFC in obese individuals. In response to visual cues of high-calorie foods and tastes of food, obese adults show greater activation in the NACC, dorsal striatum, medial and lateral OFC, INS, AMYG, mPFC, ACC compared to lean adults[404,405], indicating that obesity is associated with heightened responses to food cues in a broad set of brain networks involved in reward, motivation, emotion, and memory. Studies in obese children and teens report similar results. Compared to lean children, obese children between 8-12 years old showed increased activation in the AMYG and INS in response to taste, but no functional differences in the ventral striatum[406]. Research conducted with adolescents finds that obese vs. lean girls show higher activity in the INS and somatosensory regions to conditioned visual cues of food and to appetitive tastes, but decreased caudate activation to taste[407,408]. These results suggest that obese children demonstrate greater responses to food cues, but diminished response to food ingestion in reward and motivation areas. Diet-induced obesity has also been linked with reductions in total brain volume[409–411], and with reduced volume or cortical thickness of GM of PFC, INS, AMYG[412–417]. Studies of adolescents tend to report similar results[418,419], however one study of children reported no association between cortical thickness and BMI in their pediatric sample[420]. Another found a negative association between the gray matter volume in newborn INS and the subsequent accretion of fat mass in the first six months of postnatal life[421], suggesting a role for the INS in the etiology of childhood obesity.

1.4.8 Effects of Diet and Diet-induced Obesity on Functional Connectivity of Corticolimbic Circuits

In the past decade, researchers have used resting-state fMRI to probe how connectivity between the PFC/ACC and limbic regions—including the AMYG, INS, NACC—are impacted by diet-induced obesity. The bulk of these studies have been conducted with adults, but recent studies have also examined connectivity changes associated with obesity in children and adolescents. High BMI was correlated with stronger NACC-ACC and NACC-vmPFC connectivity in women[422], and stronger connectivity between the NACC and mPFC was reported in adults with excess body weight[423], which was interpreted as evidence of overactive reward circuitry. An effective connectivity study found relatively decreased modulation of the OFC and the NACC by the AMYG, possibly indicating impaired flexibility in updating the reward value of food, and increased modulation of the NACC by the OFC in obese adults compared to lean adults, which might contribute to a heightened drive to eat in response to a food cue[424]. Opposite patterns of AMYG-INS connectivity were reported for obese and lean individuals during a fast, with obese adults showing stronger connectivity between these regions and lean subjects showing weaker connectivity[425], suggesting obesity-related alterations in interoceptive processing. In children, obesity was associated with greater FC between the left middle frontal gyrus (which includes dlPFC – BA46) and left lateral OFC, and a region spanning the left vmPFC and right medial OFC[426]. In another resting-state FC study conducted with children, the authors computed an 'imbalance measure' by computing the difference between Frontal Pole-NACC FC (putatively measuring impulsivity) and inferior parietal lobe-NACC FC (putatively measuring response inhibition). The researchers found that as the imbalance measure more heavily favored impulsivity-related FC, self-reported food approach behaviors increased and food avoidance behaviors decreased[427]. The differences in connectivity evident between obese and lean adults and children may reflect alterations in top-down inhibitory control and overactive reward

circuitry. As with theories of drug addiction, non-homeostatic eating of a HCD is believed to contribute to an over-active reward pathway that enhances the incentive salience of food, coupled with impaired connectivity in top-down PFC-based control circuits that typically manage impulses[399]. These alterations, in turn, may play a role in sustaining non-homeostatic eating, though it is difficult to study how the pathophysiology of obesity unfolds in humans given the challenges in carrying out prospective studies with children. It is unclear whether the obesity-related differences reported in humans are the cause of consuming a HCD, the consequence of this feeding pattern and resulting obese phenotype, or both. Studying the effects of diet on brain development longitudinally before frank obesity emerges would help elucidate the complex mechanisms involved in the etiology of this disease.

In summary, consumption of a HCD in animal models alters the circuitry of the reward system in similar ways as drugs of abuse, leading to long-term reductions in basal DA secretion and lower striatal availability of D2Rs[379–381], among other changes. Maternal consumption of a high-fat diet changes DA-related gene expression in the NACC of their offspring, suggestive of reduced DA transduction as well, and increases a preference for fat as an adult[397], demonstrating that a HCD environment early in life leads to long term changes to reward circuitry. Because D2Rs in the striatum mediate neurotransmission in PFC-striatal pathways and modulate PFC activity, D2R down-regulation has consequences for PFC-striatal pathways and has been associated with decreased activity of the PFC[428]. The PFC, in turn, exerts an inhibitory influence on subcortical DA transmission[429], and diet-induced alterations to the PFC may impair top-down inhibitory processes and emotion regulation. This hypothesis is supported by decreases in PFC volume[412–417], heightened responses to food cues in regions that process reward and emotion[404,405],

including the NACC, AMYG, and INS, and increased FC in circuits that process reward[422,423], e.g. circuits connecting the NACC with the ACC and vmPFC, in obese or overweight versus lean individuals. In addition, obesity is associated with deactivation of the INS in response to gastric distention[430], and stronger FC between the AMYG and INS[425], suggestive of impaired interoception—another process believed to contribute to nonhomeostatic or emotional eating. There are several gaps in the literature that stand out, including a limited understanding of how consumption of a HCD alters connections between corticolimbic regions early in development and before frank obesity emerges, how these corticolimbic alterations influence eating and other behaviors and program the HPA axis, and how the dietary environment interacts with early life stress to shape development.

1.4.9 Impacts of Diet on Emotional Behavior

Accumulating evidence suggests that diet-induced obesity compromises brain structure, function, and connectivity[389,399,431–435]. These alterations may contribute to emotion dysregulation and underlie the well-established association between obesity and mood disorders in humans[323,436,437]. Developmental consumption of a high-fat diet enhances fear response to threatening objects in macaques[388], and increased anxiety behavior in the Open Field and Elevated plus maze tasks in rodent models[390,396]. In adults, obesity is often co-morbid with mood disorders and anxiety[323,436,437], especially in females[438,439]. Childhood obesity has also been linked with externalizing and internalizing behavioral and emotional problems, including depression and axiety[439,440]. These findings underscore the likelihood that hypercaloric diets play a role in compromised neurobehavioral function and dysregulated emotional outcomes.

1.4.10 Signals Linking Diet to Neurobehavioral Outcomes

Sustained intake of a HCD is associated with dysfunction of the HPA axis, including hypercortisolemia[441] and heightened GC secretion in response to a stressor[105,441,442]. GCs may be an important biological signal mediating diet-induced alterations to the brain and behavior. GCs are implicated in emotional dysregulation and mood disorders. Chronic administration of corticosterone in rats increases depressive-like behavior in rats[443,444], and elevated levels of cortisol have been linked with depression in humans[445–447]. Chronic activation of the HPA axis and elevated GCs likely lead to altered emotion regulation through the binding of GRs in corticolimbic regions that are involved in emotion processing[16,22], leading to long-term changes in structure and function.

Inflammatory proteins are another potential biological signal linking hypercaloric diet (and chronic stress) with compromised brain structure and function. Peripheral and central measures of inflammation are increased by the consumption of an obesogenic diet[448,449], and chronic inflammation is a well-documented outcome of obesity[450,451]. Cytokines can cross the blood brain barrier, and cells within the brain parenchyma produce pro-inflammatory cytokines, both of which can contribute to neuroinflammation. Neuroinflammation can have deleterious effects on the brain. Stress activates microglia and microgliosis in the PFC, ACC, and INS has been associated with stress-related psychopathology[157,158]. Exogenous administration of inflammatory cytokines reduces DA release from the striatum[452,453], an effect that leads to altered activation of reward-related brain regions[454–458]. These findings suggest that elevations in proinflammatory cytokines affect neurobehavioral outcomes and may play a role mediating the effect of diet or chronic stress on the brain and behavior.

1.5 Overall Goal and Hypotheses

Based on the literature reviewed above, chronic activation of the stress response and prolonged exposure to elevated levels of GCs may interact with other factors—including timing and exposure to gonadal hormones during puberty, and dietary environment—to influence neurobehavioral and physiological outcomes during development. Brain alterations resulting from exposure to chronic stressors in development are thought to contribute to a higher risk for psychopathology in life[60,222] and warrant close examination. The overarching goals of this dissertation are to investigate the relationship between social subordination stress and neurobehavioral outcomes during development, and the modulation of this relationship by experiential factors, with an aim of understanding how stress becomes biologically embedded and increases the risk for psycho- and pathophysiology in adulthood.

The goals of chapter 2 were to (1) study the effects of social subordination stress on female macaque neurobehavioral development during the pubertal transition, (2) test whether experimentally-induced delay of pubertal timing and delayed exposure to gonadal hormones (e.g. E2, P4) modulate the impact of subordination stress on FC between corticolimbic regions important to emotion regulation, and (3) assess whether alterations to FC predicted behavioral and physiological outcomes. Because subordinate macaques manifest various stress-related phenotypes as adults[46,103,105,122–128], it is possible the pubertal delay they naturally experience contributes to these phenotypes, either as an additive factor or in interaction with chronic stress. Developmental increases in E2 may be important to the maturation of brain structure, function, and neurotransmitter systems important for mood and emotional regulation, and delayed

exposure could impair these maturational processes. On the other hand, a number of studies report an association between early puberty in females and emotional and behavioral problems during adolescence and early adulthood[314–317], suggesting that earlier pubertal timing may increase the risk for mood disorders, whereas on-time or late puberty may be protective. Some studies also report that late maturation is associated with improved performance in school[459] and abstinence from drugs[460], suggesting that late maturation may confer benefits for self-regulatory processes. Late maturation may promote better emotional regulation outcomes because the gonadal hormones may shape corticolimbic circuits when the PFC and its top-down projections are more developed, which may facilitate stronger top-down emotional regulation. Thus, it is possible that delayed puberty in individuals experiencing chronic stress may be protective. We hypothesized that the stress of subordinate social status would be associated with reduced FC between the AMYG and PFC, and impaired emotional regulation postpubertally. We hypothesized that experimentally-induced delayed pubertal timing would mitigate the negative effects of subordination stress in the subordinate monkeys; hence we expected an interaction between social status and pubertal timing. In other words, we expected more subordinate ranking monkeys to demonstrate poorer neurobehavioral outcomes, but no such linear relationship would be detected among females whose pubertal timing was delayed.

Prior work in our lab demonstrated effects of subordinate status on stress physiology and WM tracts in the brain in juvenile, prepubertal females during the second year of life[134]. Given that effects of subordinate status are detected in the second year of life, it is possible that subordination effects emerge even earlier in development. Due to the limited research on this topic, however, it is unclear when early life stress begins to become biologically embedded,

especially in primates, and how the effects unfold over time. For this reason, the next set of questions addressed in Chapter 3 of this dissertation shift focus to developmental effects of social subordination during infancy. By studying resting-state FC data collected longitudinally at 2 weeks and 6 months of life in female macaques, we sought to examine social status effects on corticolimbic FC in early development, broadening the scope to include resting-state FC between the PFC and the following limbic regions: AMYG, NACC, and INS. We reasoned that subordination stress effects on resting-state FC would not be evident at 2 weeks of age, when infants are buffered from social stress in the first few months of life, but would become apparent at 6 months of age. Subordinate infants may start to receive aggression by this point, albeit minimal[131], or perceive harassment directed to their mothers or other family members as a stressor. Additionally, effects of subordinate status may be transmitted via biological signals (GCs, cytokines, etc.) passed from mother to infant through lactation[461–466]. In addition to addressing questions about social subordination on early development, another goal of this project was to understand how subordination effects on neurobehavioral development are modified by the consumption of a HCD, obesogenic diet. A dietary intervention was included not only to examine how the consumption of a palatable diet interacts with subordination stress to shape brain development, but also to study how it influences pubertal timing in a continued effort to understand inter-relationships between stress, diet, pubertal timing, and neurobehavioral outcomes. Whereas in macaques, the chronic stress of social subordination has been associated with delayed pubertal timing, early life stress in humans has generally been associated with precocious pubertal timing[467–469]. Dietary environment may explain these discrepant findings, as girls exposed to chronic stressors have higher BMIs on average, and higher fat mass accelerates puberty in humans and NHPs[325–327]. To disentangle the contribution of stress and diet to pubertal

timing, as well as understand how these factors interact to impact emotional regulation and maturation of corticolimbic circuits, the project sought to understand how longitudinal development in rhesus macaques is impacted by these experiential and developmental factors. Due to the longitudinal nature of the project and ongoing data collection, however, Chapter 3 of this dissertation will focus only on data collected during the infants' first six months of life. We hypothesized that social subordination will be associated with decreased FC in emotion regulation circuits and increased calorie consumption of a HCD by 6 months of age, and that consumption of a HCD will further reduce FC in emotion regulation corticolimbic circuits and impair the development of FC in circuits involved in reward and saliency processing. We also hypothesized that subordinate status and consumption of a HCD would be associated with alterations in the HPA axis (higher basal cortisol and cortisol reactivity) and increased inflammatory markers by 6 months of age, and these alterations may play a role in linking subordination and dietary environment to changes in neurobehavioral development.

Chapter 2. Effect of Pubertal Delay and Social Status on Amygdala Functional Connectivity in Adolescent Rhesus Macaques: Associations with Behavior and Stress Physiology

2.1 Abstract

Puberty is a transitional stage marked by extensive brain remodeling and increased vulnerability to the effects of stress. Puberty-induced increases in gonadal hormones are involved in shaping the brain during adolescence, and may modulate the effects of stress on brain development during this critical period. In this study, we examined how psychosocial stress and the timing of the development rise in estradiol (E2) influence neurobehavioral development using socially-housed adolescent female rhesus monkeys, an ethologically valid model of chronic psychosocial stress. Female macaques were randomly assigned to either experience puberty spontaneously (n=34), or receive monthly injections of the gonadotropin-releasing hormone (GnRH) agonist Lupron from 14-36 months of age to delay puberty (n=36). Resting-state fMRI scans and observations of social and emotional behaviors were acquired postpubertally (43-46 months), after Lupron was discontinued and all subjects had begun puberty. At this postpubertal time point, more subordinate status was associated with decreased functional connectivity (FC) between dorsolateral prefrontal cortex (dlPFC) and amygdala (AMYG), which may reflect stress-induced impairments to this circuit and its regulatory functions. Lupron treatment during adolescence resulted in postpubertal increases in FC between orbitofrontal cortex (OFC) and AMYG, decreased anxiety-like behavior, increased frequency of submissive behavior, lower frequency of initiating proximity towards non-kin, and more inhibition during an acute stressor, indicating that pubertal delay with Lupron treatment may have positive effects on some neurobehavioral outcomes, but adverse consequences for others. Taken together, these data suggest that pubertal timing and chronic stress impact the maturation of AMYG FC and socioemotional behaviors during adolescence.

2.2 Introduction

Exposure to chronic stressors during early development is a major risk factor for psychopathology[21,222], which dramatically increases during adolescence after the onset of puberty[207,217,470,471], implicating the pubertal transition as a critical period of enhanced vulnerability. Puberty refers to the activation of the hypothalamic-pituitary-gonadal (HPG) axis and resulting gonadal maturation, whereas adolescence refers to the maturation of adult social and cognitive behaviors[205,472]. While gonadal and behavioral maturation are distinct processes, they are also linked via iterative interactions between the central nervous system and the HPG axis[473]. Adolescence is characterized by heightened developmental plasticity[207,474], which may contribute to vulnerability for the development of mental disease[475]. The increase in psychopathology is particularly pronounced for females[217], and evidence suggests that developmental changes in levels of E2 may play a role in the emergence of stress-related mood disorders during adolescence[207,476]. During adolescence, there is profound reorganization of corticolimbic brain circuits involved in emotional and stress regulation, including circuits connecting the AMYG and prefrontal cortex (PFC)[88,274,477–479], in particular the mPFC[275,276]. These circuits are known to be vulnerable to early life stress owing in part to their protracted development and dense expression of GC receptors[16,21,22,113,475]. Pubertal reorganization of these circuits may provide a window of opportunity for plasticity to experiences, but also a neurobiological mechanism of increased vulnerability to the effects of stress, which may help explain the emergence of stress-related psychopathologies during adolescence[257]. The neurobehavioral link between stress, adolescence and puberty, and the risk for psychopathology remains incompletely characterized, as it is not yet well understood how gonadal hormones

shape the brain during adolescence or modulate the effect of stress during this critical window of enhanced vulnerability.

Corticolimbic circuits, including those connecting PFC with AMYG, are important for assessing threats and regulating emotional and neuroendocrine responses[179,480–484], and are vulnerable to chronic stress[60,75,485,486], especially during childhood and adolescence[113,475]. Acute stressors activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to systemic release of GCs that work to maintain homeostasis and respond to the threat posed by the stressor[16]. Chronic stress involves repeated activation of the HPA axis, leading to impaired negative feedback of the system and excessive and prolonged GC release[19,26–28]. GC receptors are densely expressed in the PFC, AMYG, and other limbic regions (including the hippocampus)[16,22], and stress-induced structural and functional changes to corticolimbic regions are believed to be mediated by GC receptor binding[487]. Chronic stress (CS) is generally associated with decreases in the volume of the hippocampus, PFC, and AMYG, and with altered functional activity in the AMYG and PFC[10-17], indicating effects of stress on structure and function in corticolimbic regions. Weaker resting-state FC between the PFC and AMYG was reported for adults reporting chronic work-related stress[98], and similar alterations are reported in stress-related psychopathology[488,489], suggesting weaker communication between these regions and possible impairments to top-down emotional regulation. Moreover, structural connectivity (SC) and FC has been shown to be negatively associated with trait anxiety in healthy individuals[490]. Due to a lack of longitudinal studies, though, it is not well understood how stress affects brain development or interacts with the neurodevelopmental actions of rising gonadal hormonal levels during the pubertal transition.

During the pubertal transition, the hypothalamus-pituitary-gonadal (HPG) axis is reactivated after having been quiescent since the postnatal period, signaling greater release of gonadotropin releasing hormone from the hypothalamus. This hormone binds to receptors in the pituitary, stimulating the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which in turn promotes E2 production and release by the ovaries in females. During the pubertal transition, there is a gradual rise in production of E2 and release into systemic circulation (but no comparable rise in P4; levels of P4 only transiently increase to biologically significant concentrations following ovulation). E2 is a gonadal steroid that impacts mood regulation[190,192] and influences structural and neurochemical changes in brain systems responsible for emotional responses[190–192,194–196,491,492], including during the pubertal transition[260,261,473,478]. E2 has been shown to have anxiolytic and anti-depressive effects in animal models[230–242], and hormone replacement therapy with E2 tends to decrease depressive symptoms in postmenopausal women[242]. However, mixed findings have been reported in the literature, and E2's actions on mood are influenced by a number of other factors, including E2 dose[247,248], ER subtypes[244,246], environmental context[243], and stressor exposure[228,243,245,249]. A history of stressor exposure in rodents has been shown to attenuate E2's anxiolytic effects in the open field and elevated plus maze[245]. In rhesus macaques, E2 administration reduced anxiety-like behavior in females with high baseline anxiety levels, but socially subordinate females with the short promoter allele of the serotonin transporter did not demonstrate a similar anxiolytic response to the E2 treatment[228], suggesting that the chronic stress of social subordination attenuated E2's anxiolytic effects.

Growing evidence points to the role of E2 in influencing mood during adolescence and increasing vulnerability to stress-related psychopathology in females[216,217,493,494].

During adolescence, higher levels of E2 have been associated with depressed mood and shown to mediate the relationship between pubertal stage and depression[217]. However, it has also been proposed that rapid fluctuations in E2, or low levels of E2, may underlie dysregulated emotion and the rise of mood disorders in adolescence[195], and it is still not well understood how developmental changes in E2 levels relate to the emergence of psychopathology in adolescence. Furthermore, chronic stress early in life may interact with pubertal onset to exacerbate the rise in depressed mood during adolescence; stressful life events were associated with stronger depression scores in girls who had started puberty compared to prepubertal girls with similar life histories (controlling for age)[223]. Further research is needed to understand how chronic stress and pubertal increase in gonadal hormones interact to shape the brain and emotion regulation during the adolescent transition.

During puberty and adolescence, E2 and other gonadal hormones have been shown to have a second phase of organizational effects on the brain, influencing structural, functional, and neurochemical changes, including changes to corticolimbic circuits important to emotion regulation[190,191,195,260–264]. In rats, for example, the number of ventral mPFC neurons is similar in male and females early in adolescence, but cell death in females leads them to have significantly fewer neurons than males by postnatal day 90[271]. Frontal cortex white matter volume linearly increases during adolescence in rats, but more so in males than females[272]. Prepubertal gonadectomy prevents these sexual dimorphisms from emerging in females, and results in higher number of glial cells in the mPFC, suggesting that these changes in the mPFC and frontal cortex are driven by organizational effects of ovarian hormones[273]. While it has become increasingly clear that gonadal hormones have organizational effects during adolescence, it's not well

understood how these effects would be modified by exposure to chronic stressors. Research with adult animal models suggests that stress and E2 interact to promote dendritic remodeling of the mPFC in females; stress increases the dendritic length of BLA-projecting neurons in ovariectomized females, but only with E2 replacement, as no remodeling is seen without E2 replacement[184]. These results raise the possibility that rising levels of E2 during puberty could interact with stress to alter the maturation and development of the brain in females, but this question has yet to be explored.

Myelination of the human brain increases through adolescence[162], with boys demonstrating a steeper slope in global white matter volume compared to girls, and these changes have been linked with gonadal hormones[495–497]. At an early pubertal stage, a positive correlation between LH and overall white matter volume was reported in a sample of children[495], and in an older adolescent sample, pubertal stage predicted maturation of white matter structure integrity throughout the brain, as measured by diffusion tensor imaging (DTI)[496]. While testosterone is positively correlated with white matter development in boys, E2 levels are negatively correlated with white matter development in adolescent females[497]. It has been hypothesized that increases in myelination support the increased volume of white matter in both sexes, and that gonadal hormonal changes during puberty slow this process in females[272]. Maturation and strengthening of structural connections may support changes in FC, as white matter tracts form the basis for FC between distant brain regions[498].

Functional activity and connectivity have been shown to undergo developmental changes during adolescence, and growing evidence suggests that some of these changes may be influenced by

gonadal hormones[278,499–501]. In task-based functional MRI (fMRI) studies, adolescence is characterized by heightened activity in subcortical regions like the AMYG and NACC relative to childhood and adulthood[502–504], and immature prefrontal function relative to adulthood[503–505]. These findings have given rise to the "imbalance" model, which posits that a temporal asynchrony unfolds during adolescent development with enhanced bottom-up signaling from the limbic systems coming online before top-down signals from the later-developing PFC fully mature[506]. This models appears to be consistent with studies demonstrating that functional coupling during task and resting-state FC between AMYG and PFC changes during adolescence, potentially facilitating greater top-down regulation, but remains comparatively immature relative to an adult connectivity pattern[275,277]. FC between the AMYG and anterior cingulate cortex (ACC) was found to increase with age across middle childhood (5-11 years of age) during an emotional task, but was weaker relative to adult levels[275]. AMYG-medial PFC (mPFC) functional coupling during task was shown to reverse valence from positive to negative in the transition from childhood to puberty (between ages 10-13)[277], suggesting greater communication between these regions and possibly the emergence of a regulatory role of the mPFC during adolescence. A cross-sectional study of developmental changes in resting-state FC reported that FC between the AMYG and mPFC begins to change from zero to positive coupling around 11 years of age[276], providing further evidence that PFC top-down regulation of subcortical structures may come online during adolescence.

It is not well understood whether developmental changes in functional activity and FC of corticolimbic circuits are shaped by the changing hormonal milieu during puberty, but findings of corticolimbic FC changes across the menstrual cycle[278] and in response to exogenous gonadal

steroids, including E2,[279] demonstrate that gonadal hormones influence FC. In rats and humans, E2 has also been shown to modulate reactivity of the ventromedial PFC (vmPFC) and AMYG during fear extinction[499]; thus it is plausible that rising levels of E2 during puberty may be linked with development changes in functional activity and FC. The brain regions primarily reported to have altered FC and activity in response to changing E2 (or P4 following ovulation)—the AMYG, hippocampus, thalamus, basal ganglia, and PFC—are known for their high density of gonadal steroid receptors[507], providing a tractable means by which these hormones influence corticolimbic FC. Rodent work demonstrates that E2 modulates the presynaptic release of and postsynaptic responsiveness to many neurotransmitters, and intracellular recordings *in vitro* demonstrate reduced excitatory postsynaptic potentials of BLA neurons in bath application of E2 versus saline[508]. These effects of E2 at the cellular level represent a mechanism by which E2 may influence functional activity and FC. Taken together with findings that AMYG-PFC FC mature during adolescence—when puberty commences and E2 levels rise—it is possible that E2 plays a role in adolescent-related FC changes in females. Stress may interact with E2 during adolescence and influence FC between corticolimbic circuits, given other evidence that E2's effects on brain and behavior are modified by the experience of chronic stress[184,228,243,245,249].

Importantly, there is cross-talk between the HPA and HPG axes and interaction between these axes is relevant to the pubertal transition. E2 has been shown to potentiate GC secretion in response to an acute stressor and diminish GC negative feedback in ovariectomized rodents given E2 replacement[252,253]. In female adult macaques receiving Lupron drug treatment to suppress the HPG axis, E2 replacement resulted in decreased GC negative feedback to a dexamethasone suppression test, and increased GC response to a combined dexamethasone-

suppression-CRF stimulation test[48]. In this experiment, a significant and earlier escape from the dexamethasone suppression test, as well as a heightened GC response to CRF stimulation, was detected in socially subordinate females compared to dominant females receiving E2 replacement, suggesting that E2 actions on the HPA axis are modulated by the experience of chronic psychosocial stress. Conversely, activation of the HPA axis has been shown to modulate behavioral and physiological sensitivity to E2, suggesting complex interactions between stress and E2[509]. Chronic stress attenuates HPG activity via suppression of hypothalamic GNRH release, pituitary LH and FSH pulsatile release, and E2 synthesis in the ovary[311,312]. Subordination in adult female macaques is associated with impaired ovarian function, including reductions in E2 and P4, and subordinate status has been linked with delayed timing of menarche and first ovulation in adolescent macaques[108,313].

Although pubertal onset has been implicated in the emergence of mood disorders and psychopathology, it is not well understood how the timing of puberty contributes to the etiology of mood disorders, or how it interacts with a history of chronic stress to influence neurobehavioral development. Because subordinate macaques manifest various stress-related phenotypes as adults[46,103,105,122–128], it is possible the pubertal delay they naturally experience contributes to these phenotypes, either as an additive factor or in interaction with chronic stress. Developmental increases in E2 may be important to the maturation of brain structure, function, and neurotransmitter systems important for mood and emotional regulation, and delayed exposure to E2 could impair these maturational processes. On the other hand, a number of studies report an association between early puberty in females and emotional and behavioral problems during adolescence and early adulthood[314–317], suggesting that earlier pubertal timing

may increase the risk for mood disorders, whereas on-time or late puberty may be protective. Some studies also report that late maturation is associated with improved performance in school[459] and abstinence from drugs[460], suggesting that late maturation may confer benefits for self-regulatory processes. It's not clear whether these findings would apply to macaques, as some have proposed that earlier pubertal timing is a risk factor in girls because it places girls in situations for which they are not cognitively or emotionally "developmentally ready"[318], thereby increasing their exposure to stressors. However, others have put forth biological theories based on findings that the timing of puberty onset results in long lasting effects to neural circuits sensitive to hormones[261]. Sisk and colleagues have proposed that, with age, the brain gradually becomes less sensitive to the organizing effects of gonadal hormones, with early puberty having stronger effects on neural circuits and later puberty having weaker effects[320]. This is an interesting observation, since in light of the "imbalance" model discussed above[506], early maturers may experience the greatest impact of gonadal hormone effects on corticolimbic circuits when the cognitive control systems are the least developed. Accordingly, top-down and bottom-up communication between these regions may develop in such a way that ultimately leads to imbalanced communication and emotional dysregulation. In contrast, on time and late maturers would experience the impact of gonadal hormones on the limbic system when the PFC and its top-down projections were more developed, which may promote better emotional regulation outcomes. Thus, it is possible that delayed puberty in individuals experiencing chronic stress may be protective. In summary, pubertal onset, pubertal timing, and stress influence neurobehavioral development, but it is not clear how these factors interact with each other and impact circuits important for emotional and neuroendocrine regulation during the adolescent period.

The goal of the present study is to better understand the inter-related effects of continual adverse social experience and pubertal timing on neurobehavioral development during adolescence, which is difficult to study in humans. Social subordination in rhesus macaques provides a naturalistic model for studying the impacts of chronic psychosocial stress on neurobehavioral development[108]. Social subordination is a form of chronic psychosocial stress that results in various stress-related phenotypes, including hypercortisolemia[46], cardiovascular disease[122], compromised immune system[123,124], reproductive dysfunction[125–127], psychostimulant self-administration[128], emotional feeding[105], and alterations in emotion regulation[103]. Prior studies demonstrate that subordination impacts behavioral development[108], delays the onset of puberty[108] and affects the development of white matter tracts in the PFC pre-pubertally[134]. It remains an open question, though, whether subordination affects the maturation of corticolimbic FC during adolescence, particularly connectivity of AMYG-PFC circuits important for stress and emotional regulation, and whether stress-related impacts on the brain are modified by pubertal timing. To address these questions and as part of a longitudinal study using social subordination as a model of continual psychosocial stress through development, female macaques were randomly assigned to either progress through puberty spontaneously or receive monthly injections of the GnRH agonist Lupron to experimentally delay puberty beyond the age it would normally occur. The Lupron treatment was included as part of the experimental design to allow us to disentangle the effects of subordination status and pubertal timing, as these were previously found to be associated in the untreated group[108], with lower-ranking (subordinate) females showing delayed pubertal onset in comparison to dominant animals. We examined the effects of social rank and delayed exposure to the pubertal rise of E2 on socioemotional behavior and FC in corticolimbic

circuits that regulate emotional/stress reactivity in adolescent females macaques. We hypothesized that the stress of subordinate social status would be associated with reduced FC between the AMYG and PFC, and impaired emotional regulation at a postpubertal time point. We hypothesized that delayed pubertal timing would mitigate the negative effects of subordination stress in the subordinate monkeys; hence we expected an interaction between social status and pubertal timing. Among the untreated monkeys, we expected more subordinate ranking monkeys to demonstrate poorer neurobehavioral outcomes, but no such linear relationship would be detected among the females who had received Lupron to delay puberty.

2.3 Methods

2.3.1 Subjects

Subjects were 70 adolescent female rhesus macaques (*Macaca mulatta*) housed at the Yerkes National Primate Research Center Field Station in Lawrenceville, GA. The subjects lived in 4 large social groups made up of 2-3 adult males, 30-60 adult females, and their offspring. The social groups were housed in outdoor enclosures measuring three-quarters of an acre with access to climate-controlled indoor facilities. Subjects had *ad libitum* access to water and a standard low-fat, high-fiber diet (Purina Mills Int., Lab Diets, St. Louis, MO, USA) that was supplemented daily with fresh fruit and vegetables. As shown in Figure 2.1 (experimental design) subjects were studied at 43.43 ± 0.14 months of age (mean ± standard error of the mean-SEM-), after all experimental groups had reached puberty. All procedures were approved by the Emory University Institutional Animal Care and Use Committee in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals."

2.3.2 Determination of Social Rank

Each subject's relative dominance rank was determined from the outcome of dyadic agonistic interactions that occurred in formal group checks, as described previously[510], and during 30 minute focal observations collected when the subjects were 1.5 years old, 2.5 years old, and 3.5 years old. In dyadic interactions, subordination status is assigned to the individual who unequivocally emits submissive behavior in response to a more dominant animal approaching or aggressing[115]. A subject's relative rank was calculated as the ratio of her rank divided by the total number of animals in the social group, excluding animals younger than 12 months old. Accordingly, a subject with a rank of 40 out of 100 animals was assigned a relative rank of 0.40. Typically, the relative rank of an animal does not change, but unchecked group aggression resulted in one matriline being removed from the group a few months before the data collection at 3.5 years of age. For the subjects whose ranks were affected by the overthrow, we chose to use the relative ranks at 2.5 –rather than at 3.5- years of age because the rank at 2.5 years of age represents a greater extent of the subjects' accumulated life history in the social hierarchy.

2.3.3 Lupron Administration

Female subjects were randomly assigned to either a group that received depot Lupron treatment (n=34) and had delayed pubertal onset (38.07 ± 0.34 months), or were part of a control group (n=36) that received no exogenous treatment and reached puberty spontaneously (30.88 ± 0.64 months (Figure 2.1)). Menarche is the first menstrual cycle, typically followed by a period of adolescent sterility, during which time females have anovulatory cycles punctuated by menstruation, and is ended by the first ovulation[511]. Lupron depot is a GnRH agonist that down

regulated GnRH receptors in the pituitary suppressing developmental increases in gonadotropins and E2[512] and it was administered monthly (0.25 μg/kg/mo, IM) from prepuberty 16.36 ± 0.23 months to 32.56 ± 0.23 months of age, encompassing the interval from prepuberty through post-menarche typical for this species[108]. Once Lupron treatment was discontinued, subjects that had been previously treated with Lupron began to experience menarche and by the time of data collection for this study, all subjects had reached menarche (approximately 4-12 months delay from the control group). Control subjects reached menarche an average of 12.78 ± 0.71 months prior to scanning, and subjects who received Lupron reached menarche an average of 5.12 ± 0.29 months prior to neuroimaging data collection. Once menarche was observed, serum samples were collected twice weekly and assayed for P4 levels to determine first ovulation, as previously described[108]. With the exception of three subjects, all females experienced first ovulation by completion of the study.

2.3.4 Functional MRI Data Acquisition

Subjects were transported from the YNPRC Field Station on the day before the scan to the Imaging Core Lab at Yerkes Main Station. Resting-state-fMRI and T1 images (for co-registration)were acquired at 43.43 ± 0.14 months of age using a 3T Siemens Magentom Trim Trio MRI system (Siemens Med. Sol., Malvern, PA, USA), and an 8-channel phase array coil during a single session. Following initial induction with telazol (3.81 ± 0.05 mg/kg IM), isoflurane anesthesia inhalation was kept to the lowest possible to minimize potential impact on BOLD signal (1.03 ± 0.01%). This range of isoflurane is comparable to levels used in previous macaque studies reporting patterns of coherent BOLD activity and detecting networks similar to those found in awake, behaving monkeys in sensory, motor and cognitive systems[513–516]. To

minimize motion artifacts, the subjects' heads were immobilized in a custom head holder with ear bars and a mouthpiece, and all subjects were scanned supine in the same orientation. Four 15-minute resting-state fMRI scans were acquired using a T2*-weighted gradient-echo echo-plan imaging (EPI) sequence (400 volumes, TR/TE=2060/25ms, voxel size=1.5mm^3 isotropic). The high-resolution anatomical scans were acquired using a T1-weighted 3-dimensional (3D) magnetization-prepared rapid gradient-echo (3D-MPRAGE) parallel imaging sequence (128 coronal slices, TR/TE=3000/3.52ms, voxel size=0.5mm^3-isotropic) and a T2-weighted scan (50 coronal slices, TR/TE=7900/125ms, TE=125ms, voxel size=0.5x0.5x1mm^3). Subjects were returned to their social group the following day after fully recovering from anesthesia. Imaging data were not collected from one subject.

2.3.5 Functional MRI data pre-processing

All imaging data were pre-processed with an in-house pipeline built using Nipype[517] that incorporates tools from the FMRIB Software Library (FSL, Oxford, UK, RRID: SCR_002823[518,519]) and 4dfp library. The pipeline has been modified and adapted for analyzing imaging data from rhesus monkeys[520–524]. The pipeline performs the following steps: 1) slice-time correction, 2) one-step resampling of rigid body head motion correction, distortion correction using diffusion field maps, structural-functional co-registration, and non-linear registration of the T1-weighted structural image to the 112RM-SL atlas in F99 space[525,526], 4) BOLD signal normalized to a mode of 1000, 5) BOLD signal detrending, 6) regression of rigid body head motion parameters, whole-brain, ventricle, and white matter signal, and all first-order derivatives, and 7) low-pass filtering[520–522,524]. Global signal was regressed based on current literature underscoring the importance of removing non-neuronal, physiological noise (e.g.,

cardiac and respiration cycles) common across the entire brain[527,528]. Frames with displacement (FD) greater than 0.2 mm were also removed from analysis to minimize influence of motion artifacts[528,529].

2.3.6 Resting-state Functional Connectivity Analysis

Region of Interests (ROIs) for PFC were defined based on the Lewis and Van Essen[530] and Markov et al.[531] anatomical parcellations in F99 space. The left and right AMYG ROIs were manually drawn by experts using cytoarchitectonic maps in a UNC-Wisconsin adolescent atlas (RRID: SCR_002570) [532], and then propagated to the 112 atlas in F99 space with flirt and fnirt tools (FSL). Each ROI was then manually edited in the adult macaque F99 atlas following established anatomical landmarks[533,534] for neuroanatomical accuracy and to avoid ROI overlap and the inclusion of voxels outside the atlas brain or in white matter. For this study, the AMYG and lateral PFC ROIs were modified further to exclude any voxels where at least one subject had signal dropout, using the pre-processed EPI image registered to atlas space to determine dropout. The dropout threshold for each subject was calculated as the mean intensity of the subjects' EPI signal across a whole-brain mask minus two standard deviations. Using this study-specific set of ROIs, the average time course of the BOLD signal across the voxels within each ROI was extracted, and correlated with the time course of all other ROIs. Correlation coefficients (r-values) between ROIs were Fisher Z-transformed.

2.3.7 Quality Control Before Statistical Analysis

One subject was dropped from analysis upon visual inspection, due to a large lesion in the brain. A second subject was excluded due to an ill-conditioned design matrix in the nuisance

regression. All raw EPI images were visually inspected for Nyquist ghosting artifact and 7 subjects with intense artifact (criteria: voxels in the artifact had intensity values exceeding 200) were excluded from further analysis, (n=7); two subjects with a moderate artifact (intensity range from 150-200) were also excluded from further analyses because the ghosting artifact overlapped the brain; thirteen other subjects had a moderate intensity artifact outside (non-overlapping with) the brain and we ran statistical models with and without them to confirm there was no confounding effect on the results, and we are reporting both sets of findings. An additional quality control was performed, using the AMYG ROI seed-based whole-brain FCmaps for each subject., which were visually inspected for artifacts in the correlation patterns that were not biologically plausible (n=6 were excluded from further analysis).

2.3.8 Behavioral Data

Social Behavior Around the month of scanning, seven to eight 30-minute focal behavioral observations of each subject were collected from towers above the social groups using an established rhesus ethogram[535,536]. The ethogram includes aggressive (threat, display, attack chase), submissive (withdraw, fear grimace), affiliative (proximity, groom, wrestle), and anxiety-like behaviors (yawn, body shake, self-scratch). Observers used laptops with the WinObs software program[537] to record the behavior of the initiator and recipient in real time. Inter-observer reliability was greater than 92%, reflecting high agreement on frequencies and durations of behaviors across observers. For statistical analysis, the durations and frequencies of behaviors were averaged across the observation sessions. Key behaviors of interest—frequency of initiating proximity and grooming, submitting to others, and receiving aggression were

analyzed using hierarchical models described in the Statistical Analysis section below. One subject was excluded from the analysis due to missing data.

Human Intruder Task: Emotional Reactivity The Human Intruder task activates the HPA axis and elicits a range of defensive and aggressive responses[186]. The task has been used to gauge emotional and neuroendocrine by the PFC and AMYG[538,539], and to examine the effects of early life stress[188,540]. We have previously reported that subordinate, lupron-untreated (control) females from this study showed higher fear-related behaviors in the HI at prepuberty[134]. The HI task was administered prior to scanning, according to previously published procedures[108]. The HI paradigm consists of three consecutive 10-minute sessions: an alone condition, an intruder profile condition (experimenter enters the room and sits with his/her profile toward the monkey), and an intruder stare condition (experiment turns and makes eye contact with the subject). Sessions were videotaped and a previously published ethogram and methods were used to score the frequency and duration of behaviors[541]. Three subjects were excluded from analysis due to missing HI data..

2.3.9 Statistical Analysis

We first sought to confirm that prior treatment with Lupron (PTL) delayed the pubertal milestones of menarche and first ovulation using regression models that included PTL, relative rank at 2.5 years of age, and an interaction term for PTL x relative rank as regressors. Relative rank and the interaction term were included to test whether rank was associated with the timing of puberty and whether this relationship differed for the control and PTL group.

Although anesthetics levels (isoflurane, telazol) were kept at the minimum necessary for sedation and below levels reported in the literature to affect FC[513–516], we nevertheless tested whether isoflurane levels or telazol (corrected by body weight) had any effect on the AMYG-PFC FC, but found no significant correlations. Therefore, we did not include anesthesia as a covariate in the final statistical models testing effects of pubertal timing and relative rank on FC.

To test the hypothesis that social subordination stress and PTL affected AMYG-PFC FC, behavior, and stress physiology, we used a hierarchical regression statistical approach, in which successive linear regression models are built, each adding additional predictors. PTL, relative rank, and an interaction term between PTL and relative rank were entered as predictors in the first model (Model I). The subjects' age at menarche was entered as an additional predictor in the next regression model (Model II) to account for individual variability in menarche age among the PTL and control subjects. Model I and Model II were compared, and if the addition of menarche age did not significantly improve the explanatory power of Model II, we retained and interpreted Model I. If menarche age significantly increased the variance explained by Model II, we retained and interpreted Model II. In addition to testing the effects of relative rank, PTL and menarche age on outcomes of interest (AMYG-PFC FC), we also tested whether there were effects on brain global signal. To summarize, the models tested in a hierarchical approach include:

Model I: $FC = \beta_0 + \beta_1$ Relative rank $+ \beta_2$ PTL $+ \beta_3$ PTL x Relative rank

Model II: $FC = \beta_0 + \beta_1$ Relative rank $+ \beta_2$ PTL $+ \beta_3$ PTL x Relative rank $+ \beta_4$ Menarche age

For analysis of the HI behaviors, a Principal Component Analysis was performed using SPSS to discern behavioral phenotypes, as has been done previously with the HI and rhesus monkeys[542]. Varimax rotation method was chosen and behaviors with loading scores < 0.4 were excluded from components. Composite scores were calculated for each PCA component and the subjects' scores were entered in regression models as the dependent variable in the statistical models described above.

When menarche age had a significant effect in Model II, a secondary analysis was performed to test whether individual variability in menarche age predicted functional outcomes. The analysis was performed separately in the Control and PTL groups because puberty onset (defined here as menarche) likely reflects a different neuroendocrine process in Control females compared with PTL subjects. Whereas in Control subjects, menarche may represent cumulative exposure to gradually increasing levels of E2, PTL females likely experienced menarche as an abrupt elevation in E2 over a short period after the Lupron treatment was discontinued. The relationships between menarche age and functional outcomes may differ as a result of these disparate neuroendocrine experiences. To test how individual variability in puberty timing impacted functional outcomes, then, the Control and PTL groups were analyzed in separate regression models with menarche age and relative rank as predictors. If outliers were detected in any of the statistical models described above, models were run with and without outliers and differences in results were reported.

Lastly, we tested whether AMYG-PFC FC predicted behavior and GC negative feedback, constraining this analysis to those measures with statistically significant effects of relative rank,

PTL, or a relative rank x PTL interaction in the full model. If PTL predicted either outcome measure, we analyzed the PTL and Control groups separately to avoid spurious associations driven by group difference between the PTL and Control group. Models that were significant at $p \leq 0.5$ are reported below. Multiple comparisons corrections were not performed with this dataset given the relatively small sample size for detecting main effects and interactions, and to minimize the risk of making type II errors[543].

2.4 Results

2.4.1 Pubertal Timing

PTL was associated with age at menarche (b=0.758, t(65)=10.573, $p < 0.0005$), and first ovulation(b=0.671, t(65)=8.618, $p < 0.001$), confirming the efficacy of the Lupron treatment in delaying these pubertal milestones (Table 2.1, Figure 2.2A). Control and PTL subjects differed in their timing of menarche age (Control: 30.88 ± 0.64 months; PTL: 38.07 months ± 0.34 months), with PTL subjects going through menarche on average 7.19 months later than Controls. PTL subjects were also delayed in the timing of first ovulation relative to the Control subjects (Control: 34.82 ± 0.81 months; PTL: 41.61 ± 0.24 months). There was also a significant association between relative rank and age of menarche, (b=0.383, t(65)=3.902, $p < 0.001$) and first ovulation (b=0.536, t(65)=5.067, $p < 0.001$; Figure 2.2B), and age at menarche and first ovulation was also predicted by a significant relative rank x PTL interaction, with more subordinate monkeys having delayed menarche (b=-0.287, t(65)=-2.922, $p = 0.005$), and first ovulation (b=-0.356, t(65)=-3.364, $p = 0.001$), relative to more dominant monkeys in the Control, but not in the PTL group.

2.4.2. Behavioral Data

Social Behavior Regression analysis revealed effects of PTL and relative rank on subject behaviors in their social groups (Table 2.2). As would be expected, more subordinate status was associated with significantly higher rates of submissive behaviors toward non-kin (b=0.426, t(65)=3.188, p=0.002; Figure 2.3A). However, there was also a significant association between PTL and rates of submissive behaviors, with PTL females engaging in submissive behaviors more frequently than Controls (b=0.333, t(65)=3.351, p=0.001). Frequency of aggression received was not predicted by subordination status or PTL. Anxiety in the social compound was a composite score comprising the frequency of yawning, body shaking, and self-scratching, and was predicted by PTL, with PTL subjects displaying a lower frequency of anxiety-like behaviors than Controls, (b=-0.725, t(65)=-4.061, $p < 0.0005$; Figure 2.3B). Relative rank and PTL were negatively associated with the rate of initiating proximity with non-kin in the social group, so that more subordinate monkeys initiated proximity less frequently than dominant monkeys (b=-0.519, t(65)=-3.667, $p < 0.0005$), and PTL resulted in lower rates of initiating proximity than in untreated individuals (b=-0.31, t(65)=-2.943, $p = 0.005$; Figure 2.3C). No main or interaction effects of relative social rank or PTL were detected for any other behaviors.

HI Task: Emotional Reactivity

Three components were identified in the HI paradigm PCA (Table 2.3); the items that cluster together suggest that component 1 represents 'Reactivity', component 2 'Behavioral inhibition', and component 3 'Explore' (high exploration/low freezing). The first component was classified as reactive on account of behaviors from the stare condition that loaded positively—including avert gaze, anxiety-like behaviors (yawn, body shake, self-scratch), threat, appease, and

locomote—and accounted for 31.43 % of the variance. Whereas freezing in the alone and profile conditions loaded positively on the 'behavioral inhibition' component, and locomotion loaded negatively; altogether this component accounted for 22.07% of the variance. The third component explained 17.51% of the variance in the data and comprised exploration in the alone condition—which loaded positively—and freezing in the alone condition—which loaded negatively. A positive score on this component is consistent with high exploration and low freezing in the alone condition. Table 2.2 shows that the subjects' component 3 'Explore' scores were predicted by both PTL and relative rank, with PTL females showing lower 'Explore' scores than Controls, (b=-0.744, $t(65)$=-4.102, $p < 0.0005$; Figure 2.4), and more subordinate status being associated with lower 'Explore' scores than more dominant ranks (b=-0.372, $t(65)$=-2.242, $p = 0.029$). However, there was also a trend for PTL by relative rank interaction, with a negative relationship between subordination status and 'Explore' for the Control subjects and no relationship between subordination status and 'Explore' for PTL subjects.

2.4.3 Resting-state Functional Connectivity

Regression analysis of the FC data identified PFC circuits whose connectivity with the AMYG was predicted by subordination status or PTL. Connectivity between the Right AMYG and ipsilateral dlPFC (Brodmann Area 46 (BA46)) was negatively associated with subordination status, with the more subordinate monkeys showing weaker FC than dominant ones (b=-0.479, $t(65)$=-2.234, $p = 0.03$; Figures 2.5 and 2.6; Table 2.4. PTL resulted in stronger FC between the left AMYG and ipsilateral OFC (BA13) than in the Control group (b=0.348, $t(65)$=2.65, $p = 0.011$; Figure 2.7). Excluding subjects with mild artifact in the resting-state fMRI scans produces similar results, though with higher p-values. No other associations of relative rank or treatment

were detected in resting-state FC between the AMYG and PFC ROIs. There were also no effects of relative rank, PTL, or relative rank x PTL interaction on the global brain signal itself.

2.4.4 Associations between Resting-state Functional Connectivity and Behavior

To probe whether FC in AMYG-PFC circuits predicted behavior and/or responses to the dexamethasone suppression test, we analyzed the Control and PTL group separately. In the primary set of models, PTL significantly predicted either the dependent variable (behavior and cortisol), or independent variable (FC) tested, so caution was taken to analyze the groups separately to avoid spurious correlations driven by group differences between PTL and Control subjects. Results are reported in Table 2.5. In PTL subjects, FC between the Right AMYG and dlPFC was negatively associated with frequency of initiating submissive behavior (b=-0.516, t(65)=-3.501, p = 0.002). This linear relationship was not evident in the Control subjects. FC between the Left AMYG and mPFC (BA32) predicted rates of proximity initiated with non-kin in the PTL group (b=0.445, t(65)=2.556, p = 0.018), but not in the Control group. No associations were detected between FC in AMYG-PFC circuits and cortisol responses to dexamethasone. Correlations between all variables found to be significantly predicted by relative rank, PTL, or relative rank x PTL interaction are reported in Table 2.6.

2.4.5 Secondary Analysis: Menarche Age

To account and control for group effects on and individual variability in the timing of menarche, we included age of menarche in Model II described above. When menarche age was a significant covariate, we performed a secondary analysis, probing whether menarche age predicted FC, cortisol, or behavioral outcomes separately in the Control and PTL groups (See Table 2.7).

Menarche age was a significant covariate in the model of FC between Right AMYG and BA46, and the secondary analysis revealed a significant and positive association between menarche age and FC for the Control group (b=0.472, t(65)=-2.388, p = 0.026; Figure 2.8C). The relationship between menarche age and FC for the PTL group was similarly positive, but not statistically significant. Menarche age was a significant covariate in the full model for left AMYG-mPFC (BA32) FC. Although the full model was not significant, it became significant when the interaction term was dropped, underscoring the strength of the relationship between menarche age and the FC outcome. We therefore proceeded to a secondary analysis and found that menarche age was positively associated with left AMYG-BA32 FC in the Control group (b=0.48, t(65)=2.375, p = 0.026; Figure 2.8B). The relationship in the PTL group was in the same direction (positive) but not significant.

2.4.6 Influence of Outliers

The following measures included outliers: rate of submission, anxiety-like behavior, and initiating proximity in the social group. The models reported above remained significant when outliers were excluded from the analysis.

2.5 Discussion

The goal of the present study was to determine the effects of social status and experimentally-induced delayed puberty on female macaque neurobehavioral outcomes during adolescence. We hypothesized that social subordination stress would reduce AMYG-PFC FC and impair emotional regulation postpubertally, while pubertal delay would mitigate the adverse effects of subordination stress at a postpubertal developmental time point. Accordingly, we expected an

interaction between treatment and social status; among untreated females, we expected a linear relationship with more subordinate monkeys having poorer neural and emotional outcomes, while no such relationship would be evident among the PTL females. The hypotheses concerning social subordination were partially supported, in that social subordination adversely impacted AMYG-dlPFC FC and some socioemotional outcomes, but no significant interactions between PTL and relative rank were detected; the study was underpowered for detecting interactions and dropout in some of the ROIs may have prevented detection of meaningful FC differences. FC between the right AMYG and dlPFC (BA46) was weaker in more subordinate subjects, who also showed lower 'Explore' scores in the alone condition of the HI paradigm and initiated less proximity with non-kin in their social groups, suggesting impairments in emotion regulation circuits and behavior. While no interactions between PTL and relative rank were detected, a number of main effects of PTL were unexpectedly found. PTL resulted in stronger AMYG-OFC FC and lower anxiety-like behaviors in the social groups, suggesting that delayed puberty may strengthen corticolimbic communication critical for some aspects of emotion regulation. These beneficial outcomes may confer some protection against the negative impacts of chronic stress for the lower-ranking macaques who received Lupron treatment. However, prior Lupron treatment also resulted in increased frequency of submissive behavior, lower 'Explore' scores in the HI paradigm, and lower frequency of initiating proximity towards non-kin, indicating that pubertal delay with Lupron treatment may have adverse consequences for some socioemotional outcomes. For the PTL group only, stronger AMYG-dlPFC FC predicted lower rates of initiating submissive behavior, and stronger AMYG-mPFC FC predicted higher rates of initiating proximity with non-kin. These results are summarized in Figure 2.9. The effects of Lupron treatment may be a consequence of E2 having different organizational effects on particular

circuits or neurotransmitter systems due to delayed pubertal timing, or a consequence of the subjects who were treated with Lupron being studied in a relatively earlier developmental stage of adolescence.

The Lupron treatment was effective in delaying the onset of puberty, evidenced by the delayed age of menarche and first ovulation in the PTL females. The relationship between subordination status and pubertal onset detected in the control group (later menarche age and first ovulation in more subordinate animals, previously reported in this species[108,125,544]) was not present in the PTL group. This could be due to the fact that all PTL females had their first menarche and ovulation in a narrow time window after Lupron treatment was discontinued, compared to the broader and more variable range of spontaneous pubertal timing for the Control females. This finding suggests that the HPG axis may have been primed for puberty onset at a specific age for each animal and social rank under the potential control of a biological clock[545,546] or critical body weight[547,548], but suppressed until Lupron treatment was discontinued. If so, this may explain why the relationship between subordination and pubertal timing was abolished in the PTL group.

The relative ranks of the subjects were determined by the outcomes of dyadic interactions in the social groups. The positive association between more subordinate rank and frequency of initiating submissive-like behavior confirms the hallmark feature of the social subordination model[104,115,118]. The lack of association between subordination status and aggression received from more dominant animals may be explained by the fact that these monkeys live in large outdoor enclosures and are able to avoid harassment by maintaining physical distance from aggressors. Lower-ranking monkeys also initiated proximity with non-kin less frequently than

more dominant-ranking monkeys, a finding that is consistent with other reports of subordinate rhesus monkeys engaging in less affiliative behavior in their social groups[104]. More subordinate monkeys explored less and froze more in the alone condition of the HI, indicative of higher behavioral inhibition—a species-typical stress-related behavior that has been associated with subordinate status in previous studies[134,186].

Social status also had an effect on the connectivity of the corticolimbic circuits examined in this study, with weaker FC between right AMYG and dlPFC (BA46) in more subordinate monkeys. FC between the AMYG and PFC is critical for emotion regulation[362] and at rest this circuitry is hypoconnected in individuals who experienced chronic early life stress and suffer from anxiety disorders as adults[94,95], suggesting impaired top-down regulation of fear and anxiety signals from the AMYG. The dlPFC is believed to exert higher-level control over subcortical regions in the context of emotion regulation[549–551]. Compared to healthy controls, patients with depression—a mood disorder involving dysregulated emotions—demonstrate decreases in dlPFC blood flow and glucose metabolism, ineffective functioning, impaired activation, and reduced regulatory modulation of the AMYG[552–556]. FC between the dlPFC and AMYG during an emotion regulation task is reduced in patients with depression vs. healthy controls[556], and an effective connectivity study using magnetoencephalography—an imaging method with very high temporal resolution—reported reduced top-down endogenous effective connectivity from the dlPFC to the AMYG in patients with depression, reflecting impaired emotion regulation[557]. Furthermore, resting-state fMRI scans collected after an acute stress test with healthy participants revealed that higher HPA-axis reactivity to the test was associated with reduced AMYG-dlPFC FC[558], suggesting that robust FC in this circuit may be important for regulating emotional and/or stress

responses. The negative relationship between AMYG-dlPFC FC and subordination found in this study may be indicative of heightened emotional and stress reactivity in more subordinate ranking monkeys, consistent with their increased freezing and reduced exploration during the HI task (as captured by the lower 'Explore' score. Reduced AMYG-dlPFC FC could reflect impairments to the circuitry associated with the chronic stress of being socially subordinate[45,46,48,49,52,54,134]. In a study of chronic occupational stress in humans, stressed subjects showed weaker FC between the AMYG and dlPFC[98], consistent with our finding of reduced AMYG-dlPFC FC in more subordinate monkeys. A study of childhood stress found that physical and emotional neglect during childhood was associated with reductions in gray matter volume of dlPFC [559], providing further evidence of vulnerability of the dlPFC to the insults of chronic stress. GC receptors are expressed at high levels in human[560] and primate PFC[22], and rodent studies have demonstrated stress-induced retraction of apical dendritic branches and reduced spine density in pyramidal neurons in the mPFC[184,561], which, although different from the primate dlPFC[562,563], provides a potential biological mechanism explaining the impact of stress hormones on PFC connectivity by affecting dendritic and synaptic structure.

There were no significant interactions between social status and drug treatment, which may be attributable to the study being underpowered or difficulty detecting FC differences due to exclusion of voxels with dropout. Nevertheless, PTL was associated with numerous neurobehavioral effects, including lower rates of anxiety-like behavior in the social groups and stronger AMYG-OFC FC--suggesting strengthened emotional regulation--and increased frequency of submissive behavior, lower 'Explore' scores on the HI task, and less frequent initiation of proximity with non-kin--indicating greater timidity and reduced sociability in this

group of females. This pattern of results suggests that delayed exposure to the pubertal rise in E2 may have both positive and negative effects on limbic regulatory processes at a postpubertal time point. This set of results may be explained by alterations in the way E2 organizes circuits and neurotransmitter systems in the brain as a result of pubertal timing, or by relative differences in stage of adolescent development between the control and PTL group, as explained below. Adolescence is a time of immense emotional, social, and cognitive change[564], marked by increases in emotional and stress reactivity[207,309,565,566], anxiety[208], negative affect and depressed mood[209,210]. For most girls, these mood states are transient and resolve by early adulthood[567]; however some individuals—including those who experience chronic early life stress—are at increased risk for developing long-lasting psychopathology during this developmental period[207,217,470,471]. The developmental changes in E2 levels have been implicated in transient mood changes and the emergence of the mood disorders during puberty[207,476], although it's unclear if it is high levels of E2[217], rapid fluctuations in E2, low or declining E2 levels during the menstrual cycle that trigger disturbances in mood[195]. E2 impacts brain and neurochemical systems regulating stress and emotional responses to stimuli[190–192,194–196,491,492], although the particular effects of E2 on emotionality vary depending on environmental context[243] and history of stressor exposure[228,245], among other factors. Growing evidence demonstrates that E2 and other gonadal steroid hormones secreted in adolescence remodel and refine neural circuits[473,564,568], and may even influence the maturation of the HPA axis[258] and NT systems[569]; these organizational effects may underlie some of the transient and long-lasting changes to emotionality and mood that emerge during adolescence. Delayed onset of puberty with Lupron treatment may alter the organizational effects of E2 on neural systems or the HPA axis, potentially explaining some of the neurobehavioral outcomes we found. It is also possible that

some PTL effects reflect an immature developmental phenotype owing to the PTL subjects being studied at a relatively earlier adolescent stage than the untreated controls. These possibilities are not mutually exclusive and may help explain some of the main effects of PTL on neurobehavioral outcomes.

PTL resulted in higher FC between the OFC and left AMYG, indicating that delayed exposure to the rise in pubertal hormones alters FC in circuitry relevant to emotion regulation. The OFC has been shown to mediate extinction learning[570], and is believed to play a role in modulating fear responses through its connections with the AMYG[571]. Reduced coupling between the OFC and AMYG has been reported during an emotional processing task in anxiety-prone individuals[572], and patients with social anxiety have been shown to have weaker resting-state FC and white matter deficits in the AMYG-OFC circuit[488,573]. Lesion work in rhesus monkeys also supports a role for the OFC in mediating fear-related responses during adolescence[538]. The finding that PTL subjects had stronger FC than Controls suggests that delayed exposure to pubertal hormones may strengthen AMYG-OFC communication, with possible consequences for some aspects of top-down emotion regulation. For example, the reduced anxiety-like behaviors seen in the PTL animals suggests that delayed exposure to pubertal hormones had salutary effects on this dimension of emotional regulation, possibly via impacts to the AMYG-OFC circuit, though no associations between AMYG-OFC FC and anxiety-like behaviors were found in this study. Stronger AMYG-OFC FC among the PTL subjects and reduced anxiety-like behaviors, compared to the untreated controls, may reflect group differences in organizational effects of E2 as a result of differences in the timing of puberty of the PTL and control females.

Mounting evidence suggests that FC, SC, and functional activity in corticolimbic circuits are remodeled during adolescence[88,274,478,479], and the pubertal increase in gonadal hormones, as well as pubertal timing, play a role in shaping these circuits[473,564]. Interestingly, a cross-sectional study in humans reported a switch from negative to positive resting-state FC between the AMYG and mPFC in 10-13 years old children, which was interpreted as the emergence of PFC top-down regulation of the AMYG during adolescence[276]. Although this maturational pattern has not been reported for AMYG-OFC connectivity and no studies have experimentally demonstrated whether alterations in AMYG-PFC FC are linked with gonadal hormone fluctuations in puberty, resting-state FC is, indeed, sensitive to fluctuations in E2 levels in adults[278,500,501], supporting a putative role for E2 to shape FC changes during adolescence. In naturally cycling women, for example, women with higher E2 levels demonstrate generally increased resting-state FC between AMYG subnuclei and PFC compared to women with lower E2 levels[501]. Exogenous treatment with E2 in postmenopausal women increased FC between the AMYG and superior, ventrolateral, and medial prefrontal cortices[279]. What structural and cellular mechanisms may underlie effects of E2 on FC during puberty? A study by Asato et al. reported that WM maturation increased in parallel with progression through Tanner stages[496], suggesting that pubertal hormones may strengthen structural connections in the brain, thereby altering FC. SC changes, in turn, can be explained by changes at the cellular level[265,574], including E2 promotion of myelination[575–577] in part through an ERE that regulates expression of the myelin gene in oligodendrocytes[575,578] , and increased spine density on BLA-projecting neurons from the PFC[579]. However, white matter volumes increase more slowly in females than males during adolescence[272], and E2 levels are negatively correlated with white matter development in adolescent females[497], leading some to argue that rising E2 levels slow the development of white matter in adolescence[272]. In addition to effects via

changes to SC, E2 may also influence FC through its actions at the cellular level; E2 modulates the presynaptic release of and postsynaptic responsiveness to different neurotransmitters[280–282,574], and has been shown to reduce the excitatory postsynaptic potential of BLA neurons when applied *in vitro*[508]. Developmental changes in E2 levels at puberty, then, could alter FC in corticolimbic circuits through activational or organizational effects on neurotransmitter systems and excitation.

Importantly, the timing of exposure to increases in gonadal hormones may impact how pubertal changes in SC and FC unfold. In light of research indicating that the brain becomes less sensitive to the organizational action of gonadal hormones over time[564], early vs. late exposure may modulate the degree and even the developmental trajectories of connectivity changes induced by the pubertal transition (e.g. switches from positive to negative FC coupling between regions, or vice versa). It has been proposed that adolescent corticolimbic development is characterized by a temporal asynchrony with heightened bottom-up emotional processing emerging before top-down signals from the prefrontal circuits fully matures, and this imbalance may contribute to the increased incidence of mood disorders during this time[506]. Given findings that sensitivity to gonadal hormones decreases over time[320], relatively late maturers may experience the impact of gonadal hormones when the PFC and its top-down projections are more developed, which may promote better communication between the PFC and AMYG and facilitate some aspects of top-down emotional regulation. With respect to structural maturation during adolescence, rodent work has demonstrated that male rats show larger increases in frontal cortex white matter volume than females across development, and that prepubertal gonadectomy significantly increases white matter volume in females under the frontal cortex and number of glia cells in the mPFC[273].

Although speculative, it is possible that a delayed tempo of rising E2 levels, as seen in PTL females, permits an opportunity for myelination to progress significantly more than it otherwise would, analogous to the effect of prepubertal gonadectomy on white matter development in adolescent female rats. If so, stronger SC between the PFC and AMYG due to increased myelination may facilitate increased FC between the AMYG and PFC.

The secondary analysis involving menarche age revealed associations between individual variability in pubertal timing and FC in the AMYG-mPFC and AMYG-dlPFC circuits in the control, but not PTL group. Among control subjects, later menarche timing was associated with stronger FC in these circuits; the direction was similar for PTL subjects, but the associations were not significant, likely owing to the narrow variability in menarche timing for this group. The association between later pubertal timing and stronger FC in these circuits provides additional evidence that delayed exposure to puberty may strengthen FC in AMYG-PFC circuits, with possible benefits for some aspects of emotion regulation, as discussed above.

PTL was also associated with increased frequency of submissive behavior, less frequent initiation of proximity to non-kin, and lower 'Explore' scores on the HI task. These outcomes suggest an immature developmental phenotype that may be the result of differing organizational effects due to delayed pubertal timing on particular circuits or neurotransmitter systems, or a consequence of the PTL subjects being studied at an earlier development phase in puberty and adolescence. For most girls, adolescence is a time of transient shifts in negative affect and heightened emotional and stress reactivity[207,209,210], with curvilinear trajectories in psychopathology and depression that peak in mid puberty[580]; however, dysregulated emotional

moods typically resolve by adulthood[567]. Adolescence is also a period of social re-orientation, facilitated by changes in functional and structural development of brain networks and activation of motivational tendencies by gonadal hormones[581]. As such, when given the opportunity, humans and nonhuman primates typically spend less time with their families and more time with their peer groups during adolescent development[582,583]. The PTL females were likely studied at an earlier stage in adolescent development than the untreated controls, as they had experienced menarche on average 5.12 ± 0.29 months before data collection, compared to 12.78 ± 0.71 months elapsing between when the controls had their first menarche and data collection. Therefore, the PTL females may have had lower 'Explore' scores in the HI task (exploring less and freezing more), because they were studied at a relatively earlier adolescent stage of development closer to late-puberty (when fear reactivity is heightened[309]), whereas the control females would have reached a more adult-like stage of maturational development by comparison. Being less far along in adolescence than the untreated controls, the PTL females may have also had comparatively underdeveloped motivation to socialize, hence their lower tendency to initiate proximity with non-kin. As discussed above, the PTL subjects may have experienced different organizational effects of E2 compared to the untreated controls due to the late pubertal onset, which could account for some of these socioemotional outcomes. For example, E2 may play a role in the maturation of the oxytocin system, considering findings that E2-related increases in OTR binding have been detected in various studies[584,585], and at the onset of puberty, oxytocin receptor binding increases in the ventromedial hypothalamic nucleus of rats—which has a high density of ERs[586]. If E2 plays a role in organizing the oxytocin system, which itself strongly influences social motivation[587], delayed exposure to E2 with Lupron treatment may alter the organization of the oxytocin system and social behaviors.

There were several limitations of this study. This was a cross-sectional study, where we collected resting-state FC data at only one time-point, which was fixed based on chronological age (i.e. all data was collected within 6 months' age in all animals), and all animals had reached puberty. While controlling for chronological age, time from pubertal onset (menarche) varied, not only between PTL and Control subjects, but even within the Control group. As such, some of the effects of PTL or individual variability in pubertal timing may be attributable to differences in the pubertal or adolescent stage at time of data collection, rather than to lasting differences in the timing of pubertal onset. Future longitudinal studies collecting multiple data points during puberty could allow one to control for chronological age and pubertal age and parse their separable contributions; additionally, including hormone assays to chronicle the pubertal transition could help disentangle the specific effects of pubertal timing and pubertal stage on adolescence neurobehavioral development. The current study did not permit us to examine whether pubertal delay or social rank effects reported here persist into adulthood; future studies are needed to address this question. Another point worth mentioning is that we cannot determine whether neurobehavioral effects of subordinate status are related to the monkeys' postnatal experience in the social group, or to prenatal factors including maternal gestational stress or inherited genetic/epigenetic differences. Methodologically, this study was also limited by the resolution of the fMRI images. For example, the AMYG is divided into functionally distinct subnuclei[588–590], but our scanning protocol lacked the anatomical resolution to analyze connectivity patterns of these subnuclei. Recent work in humans found that resting-state FC networks of laterobasal and centromedial subnuclei of the AMYG were differentially effected by E2 levels[501]. Thus, it is likely that our findings represent an oversimplified view of underlying

neurobiological changes associated with the pubertal transition due to usage of a global AMYG ROI. Future studies are necessary to investigate the effects of subordination status and delayed exposure to gonadal hormones on connectivity of the AMYG subnuclei, as well as to examine other circuits that may be impacted by pubertal timing and social subordination stress. For example, circuits connecting the PFC with the hippocampus may be impacted by these experiential factors; the circuit plays a role in emotion regulation and is impaired by early life stress[94], and the hippocampus has a high expression of E2 receptors and its maturation has been linked with puberty[479,507]. Another limitation of the study concerns the use of anesthesia during the collection of the resting state scans. The isoflurane levels used in this study were comparable to those administered in previous publications reporting patterns of coherent BOLD fluctuations in the macaque brain that were similar to the awake state[513–516]. We also found no significant associations between anesthesia levels and FC in our subjects. Nonetheless, we cannot rule out the possibility for potential differences in FC between the anesthetized and awake states. Lastly, the resting-state FC analysis we performed does not permit detection of directionality in the FC signals; thus, we cannot determine whether the FC alterations reflect changes in top-down or bottom-up communication, only that they reflect changes in communication between the PFC and AMYG. This is a particularly important point to make for a developmental study, as the developing brain has been understudied and stronger FC may in fact reflect stronger bottom-up signals, given that the AMYG develops earlier than the PFC[159–161] and may drive heavier bottom-up signaling before top-down regulation matures. This possibility is supported by the observation of AMYG-originating inputs to the mPFC earlier than mPFC-originating inputs to the AMYG in rodents[591].

In summary, our findings of an association between social subordination and weaker AMYG-dlPFC FC during adolescence suggests that this circuit may be particularly vulnerable to social stress during the pubertal transition. We also documented neurobehavioral outcomes suggesting that some facets of emotion regulation were strengthened by pubertal delay with Lupron, including stronger AMYG-OFC FC and reduced anxiety-like behavior in the social group, and other outcomes suggesting impaired or immature socioemotional development, e.g. increased frequency of submissive behavior, lower 'Explore' scores in the HI paradigm, and lower frequency of initiating proximity towards non-kin. Delayed onset of puberty with Lupron treatment may alter the organizational effects of E2 on neural systems, potentially explaining some of the neurobehavioral outcomes we found. In addition, the PTL subjects were studied at a relatively immature developmental stage, compared to the control females, and the behavioral and neural differences we detected between the PTL females and the controls may have been diminished if the PTL females were studied farther along in their adolescent development. Whether stronger FC in the AMYG-OFC circuit in the PTL group, or stronger FC in AMYG-mPFC and AMYG-dlPFC in individuals who naturally entered puberty later, endures into adulthood and/or facilitates greater emotion regulation at a later developmental point is unclear, and will need to be addressed in future studies. These findings have broader relevance to the societal shift toward earlier pubertal timing and medicalized efforts to address this shift. The timing of puberty has steadily become more precocious in the last 150 years, with the age of menarche declining 1-4 months per decade and although this trend appeared to stabilize in the 1960s, there has been a resurgence of the trend in the last two decades[493]. In the 18th century, menarche occurred between 17 to 18 years of age[493]; currently, the average age of menarche in the United States is 12.5 years[592]. It is still an open question as to the causal forces behind this

trend, but findings that earlier pubertal timing may be a risk factor for psychopathology[314] raises concerns about the declining menarche age in this country. The medical community has responded by administering Lupron to treat girls showing signs of precocious puberty. The current study suggests that these treatments may, indeed, be protective for some neural and socioemotional outcomes, but potentially deleterious for others. Natural variability in pubertal timing and pharmacological suppression of puberty influenced social and emotional behaviors, as well as FC in corticolimbic circuitry important for emotion regulation, and further investigation is warranted giving the implications of these neurobehavioral changes for adolescent girls. It is unclear whether the changes we documented are lasting and whether they will mitigate or exacerbate emotion regulation in adulthood. Future work should be undertaken to elucidate the lasting impact of pubertal timing and pharmacological suppression of puberty with Lupron on the brain and behavior, as well as to better understand the underlying biological mechanisms of these effects, including possible E2 organizational effects on circuits and neurotransmitter systems.

Table 2.1. Regression Analysis of Pubertal Timing. Relative rank and PTL predicted both menarche age and first ovulation age, with PTL females and more subordinate ranking females experiencing delayed menarche and first ovulation, relative to untreated controls and dominant females, respectively. A relative rank x PTL interaction revealed a strong positive association between relative rank and pubertal timing for the control subjects, but not for the PTL subjects. * and **bold font** indicate a significant *p* value at < 0.05.

			TABLE 2.1				
			Regression Analysis of Pubertal Timing				
Menache Age			R^2	*F(df)*	*b*	*t*	*p*
	Overall Model		0.662	43.036 (3, 66)			
	Relative rank				0.383	3.902	**< 0.001***
	PTL				0.758	10.573	**< 0.001***
	Relative rank x PTL				-0.287	-2.922	**0.005***
First Ovulation Age							
	Overall Model		0.634	37.009 (3, 64)			
	Relative rank				0.468	4.527	**< 0.001***
	PTL				0.711	9.396	**< 0.001***
	Relative rank x PTL				-0.314	-3.038	**0.003***

Table 2.2 Social Behaviors: Hierarchical Regression Models. Model I examined effects of relative rank, PTL and relative rank x PTL on behavior. Model II examined the effects of those factors when menarche age was also included in the model. The yellow shading highlights which model was retained, depending on the significance of the change in the F-value. Results are shown for behaviors with significant effects in each of the models. * and **bold font** indicate a significant *p* value at < 0.05.

TABLE 2.2
Hierarchical Regression Analysis of Behaviors

	Model I					Model II					
Submissive Behavior toward Non-kin (Frequency)	R^2	*F(df)*	*b*	*t*	*p*	R^2	*F(df)*	*p - ΔF*	*b*	*t*	*p*
Overall Model	0.362	12.303(3,65)				0.362	9.093(4,64)	0.885			
Relative rank			0.426	3.188	**0.002***				0.435	2.913	0.005
PTL			0.333	3.351	**0.001***				0.352	2.158	0.035
Relative rank x PTL			0.071	0.527	0.6				0.064	0.448	0.656
Menarche age			-	-	-				-0.025	-0.145	0.885
Initiate Proximity with Non-kin (Frequency)											
Overall Model	0.285	8.623(3,65)				0.286	6.395(4,64)	0.784			
Relative rank			-0.519	-3.667	**< 0.001***				-0.5	-3.159	0.002
PTL			-0.31	-2.943	**0.005***				-0.272	-1.578	0.12
Relative rank x PTL			0.185	1.305	0.197				0.171	1.135	0.261
Menarche age			-	-	-				-0.05	-0.275	0.784
Anxiety-Like Behavior (Frequency)											
Overall Model	0.165	4.28(3,65)				0.235	4.915(4,64)	0.018*			
Relative rank			-0.145	-0.947	0.347				-0.317	-1.936	0.057
PTL			-0.384	-3.377	0.001				-0.725	-4.061	**< 0.001***
Relative rank x PTL			0.059	0.383	0.703				0.182	1.165	0.248
Menarche age			-	-	-				0.452	2.42	**0.018***
HI - Component 3 Explore Scores											
Overall Model	0.151	3.729(3,65)				0.238	4.836(4,64)	0.01*			
Relative rank			-0.177	-1.134	0.261				-0.372	-2.242	**0.029***
PTL			-0.363	-3.116	0.003				-0.744	-4.102	**< 0.001***
Relative rank x PTL			0.155	0.991	0.325				0.29	1.843	0.07
Menarche age			-	-	-				0.506	2.66	**0.01***

Table 2.3. Factor Loadings from HI PCA analysis. Three components were identified from the HI analysis. The first component was labeled 'Reactive' and explained 31.43% of the variance. The second component was labeled 'Behavioral Inhibition' and explained 22.07% of the variance. The third component was classified as 'Explore' (High Explore/Low Freeze) and explained 17.51% of the variance.

TABLE 2.3
PCA of Human Intruder

FACTOR LOADINGS	Component 1	Component 2	Component 3
Freeze – Alone (dur)		0.476	-0.821
Freeze – Profile (dur)		0.813	
Freeze – Stare (dur)	-0.885		
Avert gaze – Stare (freq)	0.832		
Anxiety – Stare (freq)	0.561		
Threat – Stare (freq)	0.868		
Appease – Stare (freq)	0.557		
Explore – Alone (dur)			0.927
Locomote – Alone (freq)		-0.852	
Locomote – Stare (freq)	0.46	-0.661	
VARIANCE Explained	0.3143	0.2207	0.1751
LABELS	Reactive	Behavioral Inhibition	Explore

Table 2.4. Hierarchical Regression of AMYG-PFC FC. Model I examined effects of relative rank, PTL, relative rank x PTL on AMYG-PFC FC. Model II examined the effects of those factors when menarche age was included in the hierarchical regression. Yellow shading indicates which model was retained, and results are shown for the circuits that were significantly predicted by one of the regressors. * and **bold font** indicate a significant *p* value at < 0.05.

TABLE 2.4

Hierarchical Regression Analysis of AMYG-PFC FC

	Model I					Model II					
Right AMYG-BA 46	R^2	*F(df)*	*b*	*t*	*p*	R^2	*F(df)*	*p - ΔF*	*b*	*t*	*p*
Overall Model	0.084	1.476 (3,48)				0.18	2.587 (4,47)	**0.023***			
Relative rank			-0.292	-1.402	0.167				-0.479	-2.234	**0.03***
PTL			0.004	0.031	0.975				-0.419	-1.868	0.068
Relative rank x PTL			0.001	0.005	0.996				0.146	0.706	0.484
Menarche age			-	-	-				0.553	2.346	**0.023***
Left AMYG-BA 32											
Overall Model	0.037	0.612 (3,48)				0.169	2.387 (4, 47)	**0.009***			
Relative rank			0.212	0.992	0.326				-0.008	-0.035	0.972
PTL			0.064	0.445	0.659				-0.433	-1.916	0.062
Relative rank x PTL			-0.059	-0.279	0.781				0.111	0.532	0.597
Menarche age			-	-	-				0.648	2.732	**0.009***
Left AMYG-BA 13											
Overall Model	0.189	3.718 (3, 48)				0.19	2.762	0.751			
Relative rank			-0.016	-0.081	0.935				0.009	0.044	0.965
PTL			0.348	2.65	**0.011***				0.406	1.817	0.076
Relative rank x PTL			-0.266	-1.368	0.178				-0.286	-1.388	0.172
Menarche age			-	-	-				-0.075	-0.319	0.751

Table 2.5. Regression Models testing Associations between Resting-state Functional Connectivity and Behavior. Regression models tested associations between FC and behavior for Control and PTL subjects, controlling for the effect of relative rank. * and **bold font** indicate a significant *p* value at < 0.05.

			TABLE 2.5				
			Associations between Resting-state FC and Behavior				
Submissive Behavior toward Non-kin (Frequency)				R^2	*F(df)*	*b*	*t*	*p*
	Control							
		Overall Model		0.227	3.372(2,23)			
		Right AMYG-BA46				0.249	1.3	0.206
		Relative rank				0.483	2.525	0.019
	PTL							
		Overall Model		0.563	14.145(2,22)			
		Right AMYG-BA46				-0.516	-3.501	**0.002***
		Relative rank				0.416	2.827	**0.01***
Initiate Proximity with Non-kin (Frequency)				R^2	*F(df)*	*b*	*t*	*p*
	Control							
		Overall Model		0.144	1.941 (2,23)			
		Left AMYG-BA32				-0.12	-0.609	0.549
		Relative rank				-0.338	-1.715	0.1
	PTL							
		Overall Model		0.347	5.854 (2,22)			
		Left AMYG-BA32				0.445	2.556	**0.018***
		Relative rank				-0.455	-2.616	**0.016***

Table 2.6. Correlation Matrix. Correlations between all variables found to be significantly predicted by relative rank, PTL, or relative rank x PTL interaction are reported below.

CORRELATION MATRIX													
	Relative rank	**Menarche age**	**First Ovulation Age**	**Anxiety-Like Behavior (Frequency)**	**Agonism received by non-kin (Frequency)**	**Submissive Behavior toward Non-kin (Frequency)**	**Initiate Proximity with Non-kin (Frequency)**	**Component 1 from HI PCA -- Reactive**	**Component 2 from HI PCA -- Inhibition**	**Component 3 from HI PCA -- Explore**	**Right AMY-BA 46 FC**	**Left AMY-BA 32 FC**	**Left AMY-BA 13 FC**
Relative rank	1	0.221	**.284***	-0.143	0.13	**.493****	**-.415****	0.178	0.018	-0.094	**-.312***	0.18	-0.16
Menarche age	0.221	1	**.868****	-0.186	0.145	**.359****	**-.364****	-0.142	-0.024	-0.141	0.065	**.324***	**.301***
First Ovulation age	**.284***	**.868****	1	-0.169	0.197	**.359****	**-.401****	-0.037	0.021	-0.014	-0.139	0.259	**.302***
Anxiety-Like Behavior (Frequency)	-0.143	-0.186	-0.169	1	0.004	-0.103	0.236	0.15	0.088	**.350****	0.093	0.029	0.158
Agonism received by non-kin (Frequency)	0.13	0.145	0.197	0.004	1	**.641****	-0.042	0.156	0.071	-0.147	**-.399****	-0.116	0.169
Submissive Behavior toward Non-kin (Frequency)	**.493****	**.359****	**.359****	-0.103	**.641****	1	-0.236	**.292***	-0.064	-0.211	**-.364****	0.044	0.021
Initiate Proximity with Non-kin (Frequency)	**-.415****	**-.364****	**-.401****	0.236	-0.042	-0.236	1	0.062	0.081	**.261***	0.167	-0.053	-0.181
Component 1 from HI PCA -- Reactive	0.178	-0.142	-0.037	0.15	0.156	**.292***	0.062	1	0.025	0.003	-0.162	0.002	-0.13
Component 2 from HI PCA -- Inhibition	0.018	-0.024	0.021	0.088	0.071	-0.064	0.081	0.025	1	-0.002	0.177	0.167	0.12
Component 3 from HI PCA -- Explore	-0.094	-0.141	-0.014	**.350****	-0.147	-0.211	**.261***	0.003	-0.002	1	-0.105	-0.115	-0.01
Right AMY-BA 46 FC	**-.312***	0.065	-0.139	0.093	**-.399****	**-.364****	0.167	-0.162	0.177	-0.105	1	0.259	0.018
Left AMY-BA 32 FC	0.18	**.324***	0.259	0.029	-0.116	0.044	-0.053	0.002	0.167	-0.115	0.259	1	-0.13
Left AMY-BA 13 FC	-0.16	**.301***	**.302***	0.158	0.169	0.021	-0.181	-0.13	0.12	-0.01	0.018	-0.13	1

Table 2.7. Secondary Regression Analysis with Menarche Age. When menarche age was significant in Model II with all subjects, we proceeded to a secondary analysis to test whether menarche age predicted FC, behavior, or cortisol outcomes. Control and PTL subjects were analyzed separately and relative rank was included to control for its effect. * and **bold font** indicate a significant *p* value at < 0.05.

TABLE 2.7

Secondary Regression Analysis with Menarche Age

			R^2	*F(df)*	*b*	*t*	*p*
Right AMYG-BA 46							
	Control						
		Overall Model	0.263	4.110 (2,23)			
		Menarche age			0.472	2.388	**0.026***
		Relative rank			-0.484	-2.45	**0.022***
	PTL						
		Overall Model	0.111	3.000 (2,23)			
		Menarche age			0.16	0.814	0.424
		Relative rank			-0.286	-1.454	0.159
Left AMYG-BA 32							
	Control						
		Overall Model	0.23	3.431 (2,23)			
		Menarche age			0.48	2.375	**0.026***
		Relative rank			-0.001	-0.005	0.996
	PTL						
		Overall Model	0.099	1.266 (2,23)			
		Menarche age			0.284	1.436	0.165
		Relative rank			0.148	0.749	0.461

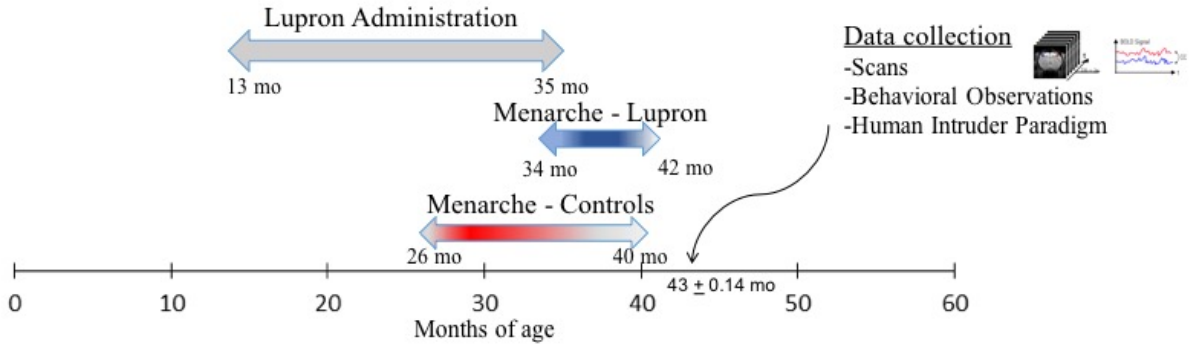


Figure 2.1. Experimental Timeline. Lupron was administered from prepuberty (16.36 ± 0.23 months of age) to a time point that that is typical of post-menarche for this species[108] (32.56 months ± 0.23 months of age). The range of Lupron administration was a minimum of 13 months of age through a maximum of 35 months of age. Control subjects had onset of menarche at 30.88 ± 0.64 months. Once Lupron was discontinued, PTL subjects began experiencing menarche around 38.07 months ± 0.34 months. MRI Scans, behavioral observations, and Human Intruder data was collected based on the individual's age; all subjects had reached menarche by the time of data collection (43 ± 0.14 months).

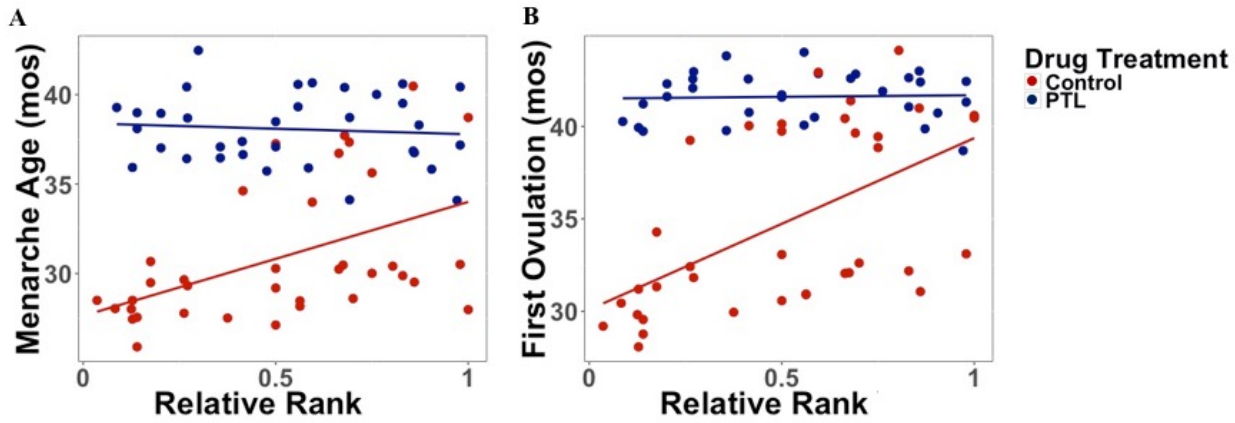


Figure 2.2. Effect of PTL and Relative rank on Pubertal Timing. A) Menarche age was significantly delayed in PTL subjects in comparison to Controls. Relative rank was positively associated with menarche age for the Control, but not PTL group (R^2=0.662, F(3,66)=43.036, p < 0.0005; PTL: b=0.758, t(65)=10.573, p < 0.0005; Relative rank – Control group: b=0.383, t(65)=3.902, p < 0.0005). **B)** PTL subjects experienced first ovulation at a significantly later age than Controls. A positive linear relationship was detected between relative rank and age at first ovulation for the Control group only. (R^2=0.619, F(3,63)=34.133, p < 0.001; PTL: b=0.671, t(65)=8.618, p < 0.001; Relative rank: b=0.536, t(65)=5.067, p < 0.001; Interaction: b=-0.356, t(65)=-3.364, p = 0.001).

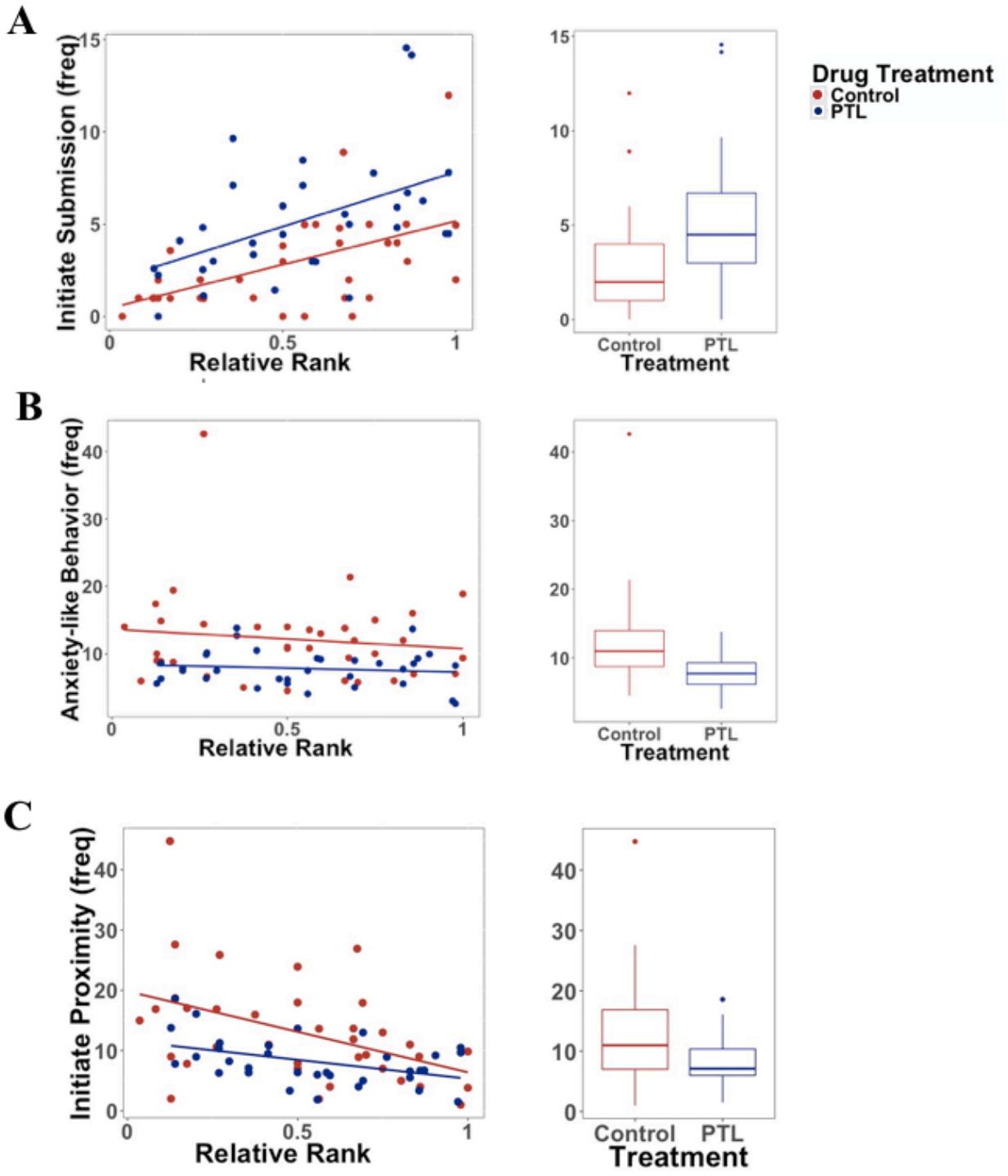


Figure 2.3. Associations between Relative rank, PTL, and Social behaviors. **A)** Rate of submissive behaviors toward non-kin was predicted by relative rank and PTL, with PTL subjects displaying higher rates of submission than Controls, and Subordinate animals more submissive

behaviors than dominants (Model I: R^2=0.362, F(3,65)=12.303, p < 0.0005; Relative rank: b=0.426, t(65)=3.188, p=0.002; PTL: b=0.333, t(65)=3.351, p=0.001). **B)** PTL subjects displayed lower anxiety-like behavior in their social groups (Model II: R^2=0.235, F(4.64)=4.915, p = 0.002; PTL: b=-0.725, t(65)=-4.061, p < 0.0005). **C)** Rates of proximity initiated with non-kin was negatively predicted by lower social rank and PTL (Model I: R^2=0.285, F(3,65)=8.623, p < 0.0005; Relative rank: b=-0.519, t(65)=-3.667, p < 0.0005; PTL: b=-0.31, t(65)=-2.943, p=0.005).

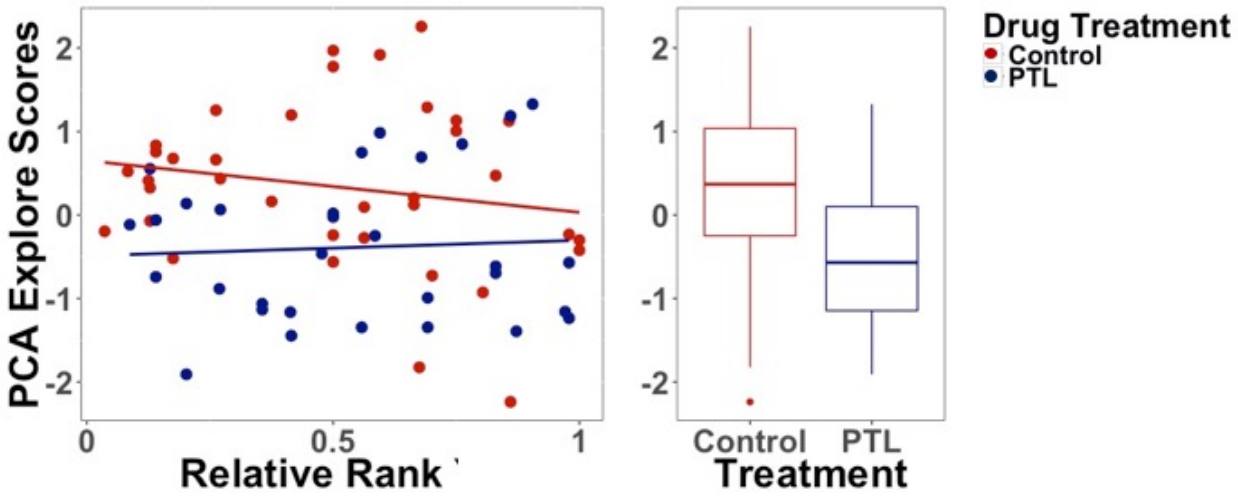


Figure 2.4. Explore Scores on HI paradigm predicted by PTL. PTL subjects had lower 'Explore' scores than Controls, and more subordinate monkeys had lower 'Explore' scores than more dominant animals. However, there was also a trend for a PTL by relative rank interaction. (Model II: R^2=0.238, F(4,62)=4.836, p = 0.016; PTL: b=-0.744, t(65)=-4.102, $p < 0.0005$; relative rank: b=-0.372, t(65)=-2.242, p = 0.029; PTL x relative rank Interaction: b=0.506, t(65)=2.66, p = 0.01).

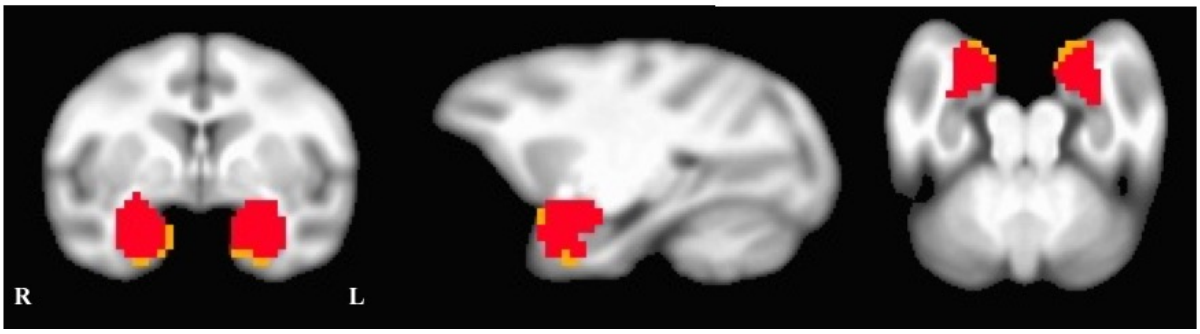


Figure 2.5. Right and Left AMYG ROIs used in the AMYG-PFC Resting-state FC Analysis. The left and right AMYG ROIs were manually drawn by experts using cytoarchitectonic maps in a UNC-Wisconsin adolescent atlas[532], and then propagated to the 112 atlas in F99 space[593]. Each ROI was then manually edited in the adult macaque F99 atlas following established anatomical landmarks[533,534] for neuroanatomical accuracy and to avoid the inclusion of voxels outside the atlas brain or in white matter. Voxels were excluded if they had signal dropout in at least one subject (shaded orange). The red voxels represent those retained in the ROI used for the Resting-state FC analysis.

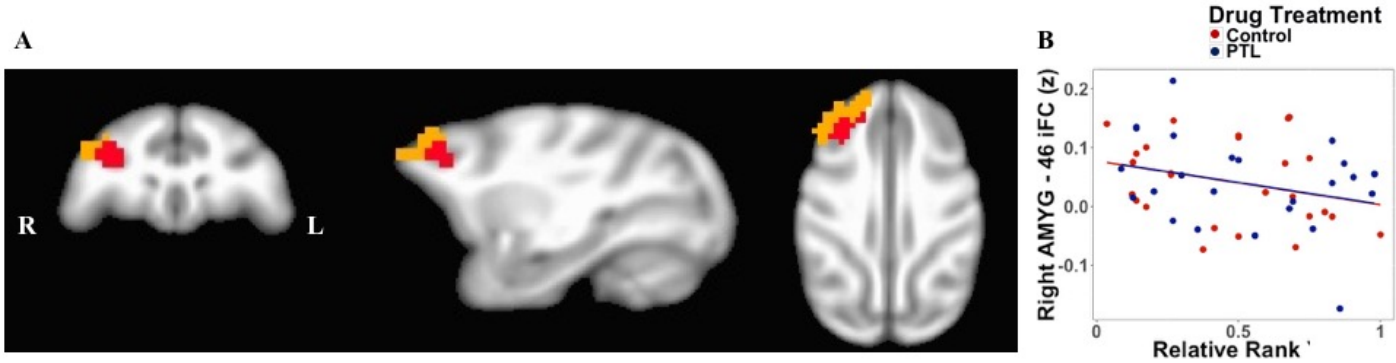


Figure 2.6. Right dlPFC FC with AMYG. A) Markov ROI of Right BA46 used in ROI-to-ROI analysis to asses FC with AMYG. Red voxels represent those remaining in ROI after (orange) voxels excluded due to signal dropout. **B)** Resting-state FC of Right AMYG with BA46 was negatively associated with relative rank, such that more subordinate subjects had lower FC (Model II: R^2=0.18, F(4,47)=2.587, p = 0.049; Relative rank: b=-0.479, t(65)=-2.234, p = 0.03).

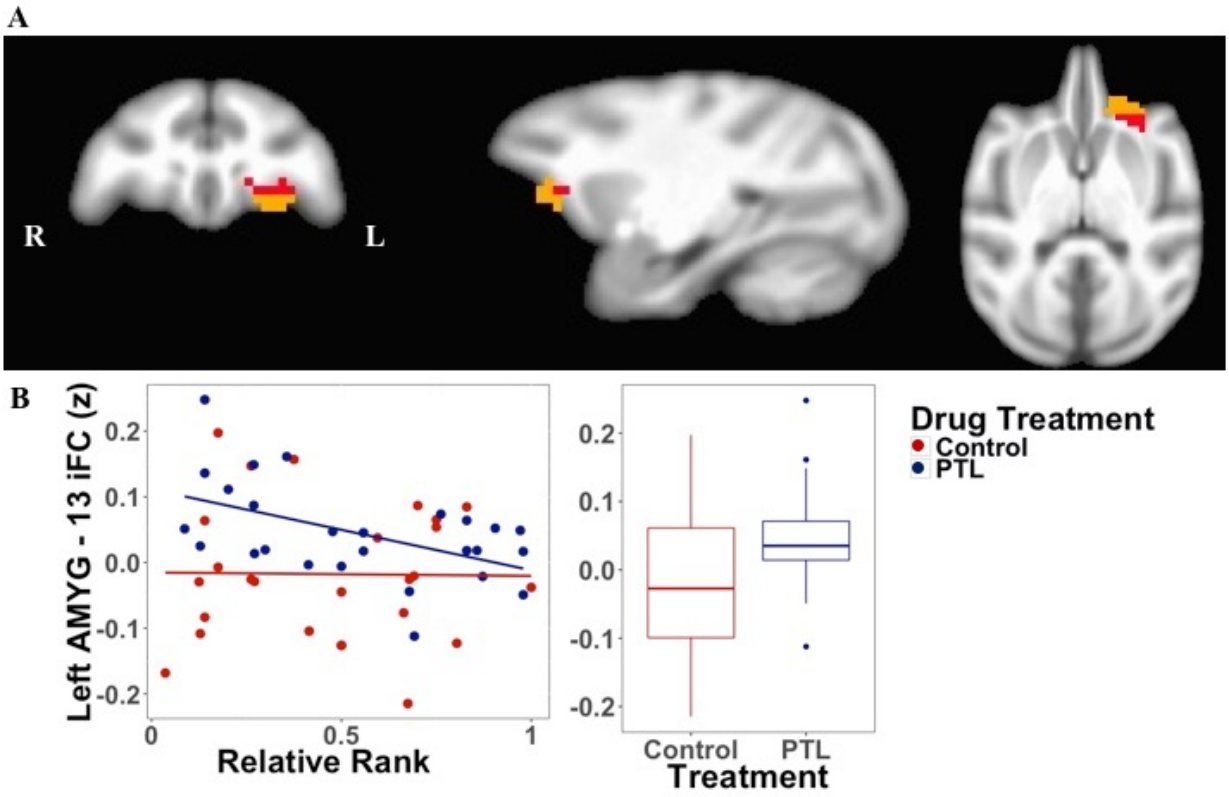


Figure 2.7. FC between Left AMYG and OFC. A) Markov ROI of Left BA13 used in ROI-to-ROI analysis to assess FC between OFC and Left AMYG. Red voxels represent those included in the FC analysis of Left AMY-BA13 ROI; the orange voxels were part of the original BA13 ROI and were excluded due to signal dropout. **B)** Resting-state FC of Left AMYG with BA13 was positive and higher in PTL subjects relative to Controls (Model I: R^2=0.189, F(3,48)=3.718, p = 0.017; Treatment: b=0.348, t(65)=2.65, p = 0.011).

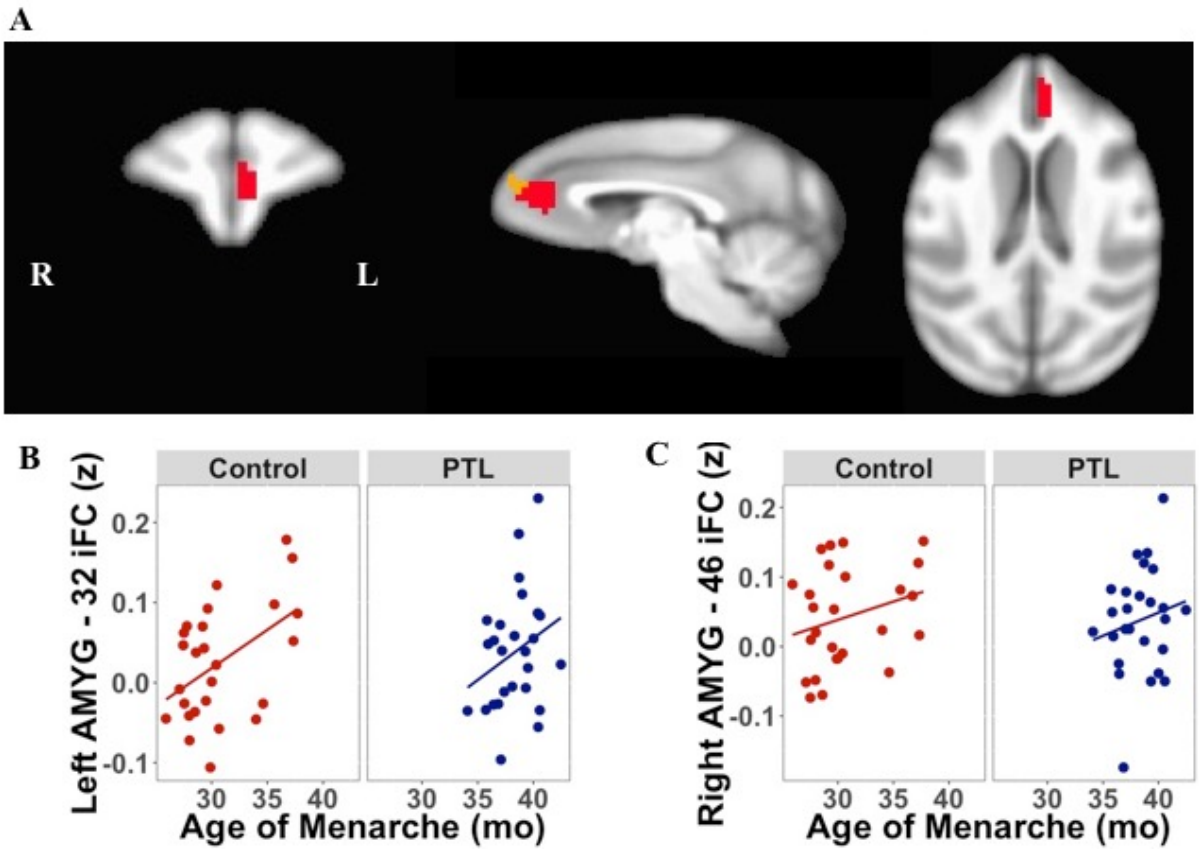
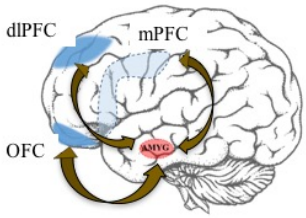


Figure 2.8. Secondary Analysis of the Effects of Menarche Age. **A)** mPFC ROI of Left BA32 defined in Markov Atlas. Red voxels represent those with sufficient signal that were included in the ROI used for the ROI-to-ROI Resting-state FC. **B)** Menarche age had a positive association with FC between Left BA32 and AMYG, but this relationship was only significant for the Control subjects (R^2 = 0.23, F (2,23)= 3.431, p=0.05; Menarche Age: b=0.48, t(24)=2.375, p=0.026). **C)** Menarche age positively predicted FC between the Right AMYG and BA46 for Control subjects (R^2 = 0.263, F(2,23)=, p=0.03; Menarche Age: b=0.472, t(24)=2.388, p=0.026). The relationship was similar for PTL subjects, but it did not reach significance.

Summary of Findings

Subordinate Status	Prior Lupron Treatment	Menarche Age
↓ AMYG-dlPFC FC	Delayed Onset of Menarche and First Ovulation	Controls only:
↓ Rate Initiating Proximity with Non-kin	⊘ Abolish Rank ~ Pubertal Timing Association	↑ AMYG-dlPFC FC
↑ Rate Submissive Behaviors	↑ AMYG-OFC FC	↑ AMYG-mPFC FC
↓ 'Explore' Scores on HI task	↓ Rate Anxiety Behaviors in Social Group	
	↓ Rate Initiating Proximity with Non-kin	
	↓ 'Explore' Scores on HI task	



Associations between FC and Behavior
PTL only:
AMYG-dlPFC FC ~ Rate Submissive Behavior (negative)
AMYG-mPFC FC ~ Rate Initiating Proximity with Non-kin (positive)

Figure 2.9. Summary of Findings. The following summarizes the main effects of subordinate status, PTL, and menarche age on neurobehavioral outcomes. No significant interactions between subordinate status and PTL were detected. Two significant associations between FC and social behaviors were detected for the PTL females, but not for the controls (bottom right box).

CHAPTER 3. Social Subordination Stress and an Obesogenic Diet Affect Neurobehavioral Development and Physiology in Infant Rhesus Macaques

3.1 Abstract

Chronic social stress has adverse effects on brain structure and function, including corticolimbic circuits, and these neural alterations are linked with the emergence of stress-related psychopathology and mood disorders. It is not well understood how these alterations emerge during development and interact with other risk factors, including the consumption of obesogenic diets. Research suggests that consumption of an obesogenic diet compromises brain structure and function, particularly in many of the same circuits adversely impacted by chronic stress. Chronic stress promotes emotional eating and increases the drive to eat palatable food, and it is not well understood how these experiential factors—chronic stress and diet--interact and impact neurobehavioral development. Here we examined the potential for a synergistic impact of social stress and consumption a highly caloric, obesogenic, diet on the development of the nonhuman primate (NHP) infant brain circuits that control stress/emotional responses and reward processes using a translational macaque model of social subordination stress. Infants assume their mother's rank at birth and their early neurobehavioral development may be shaped by their subordinate status, either through their social experiences or biological signals transmitted from their mother. Forty-three socially-housed infant-mother pairs (n=21 dominant-ranking, n=20 subordinate-ranking) were randomly assigned to either a chow-only, low-calorie diet (LCD) condition, or to a Choice condition with access to both a LCD and high-calorie diet (HCD) from birth, and all feeding behavior was continuously quantified. Resting-state functional MRI scans were acquired in all infants at 2 weeks and 6 months of age (comparable to 2 and 24 months in humans) to examine effects of social rank and diet on the development of prefrontal cortex (PFC, with a focus on medial PFC –mPFC-, orbitofrontal cortex –OFC-, anterior cingulate cortex–ACC-, and dorsolateral PFC –dlPFC- subregions) functional connectivity (FC) with the

amygdala (AMYG), nucleus accumbens (NACC), and insula (INS), brain regions involved in reward processing, emotion regulation, and interoception. We also measured baseline levels of the stress hormone cortisol and inflammatory markers longitudinally, and behavioral and cortisol reactivity to an acute stressor at 6 months of age. Infants in the Choice dietary condition ate significantly more kilocalories (kcals) than infants on the LCD-only diet, but we did not find evidence that differences in social rank promoted more feeding in the first 6 months of life. We did find several effects of diet condition and social status on resting-state FC in corticolimbic circuits involved in salience detection, reward processing, interoception, and emotion regulation. Consumption of the Choice diet by mothers predicted weaker AMYG-OFC FC in their infants at 2 weeks, compared to the infants of mothers consuming the LCD-only diet. Subordinate status predicted stronger AMYG-mPFC and INS-ACC FC at 6 months, and diet and social status had complex interaction effects on INS-PFC and INS-ACC FC at 6 months. The Choice diet increased baseline cortisol and cortisol reactivity at 6 months, depending on the social status of the animal, providing evidence that diet condition affects the hypothalamic-pituitary-adrenal (HPA) axis early in development. In the LCD-only diet group, levels of the pro-inflammatory marker CRP were higher in subordinates than dominants at 6 months, indicating that the biological embedding of social status emerges as early as 6 months of life. Cumulative consumption of HCD, but not LCD, kcals predicted inflammation (CRP levels) at 6 months of life, suggesting that the association between HCD diet and inflammation depends on both the source and number of calories consumed. We found no association between caloric intake and resting-state FC, behavior, or cortisol levels, however, suggesting that these diet effects may have been driven by differences in the levels of saturated fat and sugar between the HCD and LCD-only diets, rather than simply differences in total calories between the diets. Because the

infants did not significantly differ in their body weight, the observed diet effects add to growing evidence that diet-induced alterations to neurobehavioral outcomes can emerge before significant body fat accumulates.

3.2 Introduction

Early life stress influences neurobehavioral development and produces long-term alterations in stress physiology, placing individuals at increased risk for psychopathology and adverse health outcomes throughout life[594–596]. More recently, chronic stress has also been found to be a cumulative risk factor for obesity in children[341,597], especially girls[342–344]. Understanding the etiology of childhood obesity is critical given that childhood obesity is an urgent public health concern and economic burden[334,339,598–602]. Obese children are more likely to be obese as adults[330], and to develop more severe obesity-related diseases[331–333] at earlier ages[334,335]. In the U.S., childhood obesity has more than doubled in children and quadrupled in adolescence in the past 30 years, underscoring the urgency to better understand the biological origins of this disease. Several gaps in the literature stand out. It is not well understood how consumption of an obesogenic diet impacts neurobehavioral development and physiology before frank obesity emerges. It is even less clear how chronic early life stress and consumption of an obesogenic diet interact to alter neurobehavioral development during critical periods in childhood, leading to obesity. The potential exists for both environmental insults to impact the development of emotional/stress regulatory and reward circuits in the brain, increasing susceptibility to non-homeostatic or "emotional" eating and the development of an addictive-like phenotype[350,399,603].

A dietary environment that includes calorically dense and highly palatable food has been shown to result in increased caloric intake and subsequent obesity[604]. Studies of diet-induced obesity in adult animals finds that some individuals are resistant to diet-induced obesity[605], and stressor exposure appears to be one factor that explains individual susceptibility to excessive caloric

intake[603,606]. Repeated stressor exposure, indeed, can result in increased calorie consumption and preference for a calorically dense diet in animal models[607–610]. Similar findings have been reported for women[603,606], with female adults increasing consumption of calorically dense food in response to stressors. Growing evidence demonstrates a bias toward stress-eating in women[611] and gender differences in obesity risk factors[612]. A recent study found that emotional and uncontrolled eating strongly differentiated women at low and high-risk for obesity; for men, eating competence (a measure of eating attitudes and meal planning) was a more important factor in discriminating the low and high-risk groups [612]. Among children, emotional eating has also been linked with reported stress[345], with higher rates of obesity among children in families reporting significant stressors in their lives, including poverty, domestic violence, drug abuse and childhood maltreatment[346–349], a trend that is particularly pronounced for girls[342–344]. Evolutionarily, consuming more calories may be advantageous when confronted with an acute stressor, but this strategy can become maladaptive if sustained chronically and adverse health outcomes ensue.

While there is increasing evidence that both obesogenic diets and chronic stress alter brain structure and function, it is not well understood how these environmental insults affect the brain during development and whether they interact to compromise neurobehavioral development and physiological outcomes. Exposure to chronic stressors alters brain structure and function[487,613–616], with pronounced effects on corticolimbic circuits important for emotional and stress regulation, reward processes, and interoception (sensing and evaluating the physiological condition of the entire body[617]) that involve the PFC, ACC, INS, AMYG, and ventral striatal structures such as the NACC. The AMYG is instrumental in the generation of

behavioral/emotional, autonomic and stress neuroendocrine responses to threats through its connections with the hypothalamus and brainstem[618,619], and is also involved in stimulus-reward learning[358]. Importantly, its connections with the PFC serve a critical role in emotion regulation[362], with the PFC believed to exert top-down regulatory influence on the AMYG[352,620]. Stress has been shown to alter activity in the PFC and AMYG and to weaken AMYG-PFC FC[94,95], with negative consequences for emotion regulation, anxiety, and mood disorders[95,488,621]. Individuals with weaker functional coupling between the AMYG and PFC exhibit higher levels of trait anxiety[622], a finding that has been replicated in clinical populations with anxiety disorders[488,490].

Chronic exposure to stressors and elevated glucocorticoid (GC) levels during development have also been associated with alterations to the corticolimbic reward system[623], implicating the NACC and its connections with the PFC. The NACC integrates information related to stress and reward processes[624,625]: ventral tegmental area (VTA) dopaminergic (DA) projections are part of the mesolimbic pathway and release DA onto the NACC in response to rewards[626] and acute stressors[627]. Chronic stress results in sustained DA release in the NACC and a compensatory down-regulation of D2 receptors (D2R)[67,68]. Because D2Rs in the striatum mediate neurotransmission in PFC-striatal pathways and modulate PFC activity, D2R down-regulation has consequences for PFC-striatal pathways and has been associated with decreased activity of the PFC[428]. The PFC, in turn, exerts an inhibitory influence on subcortical DA transmission[429], and stress-induced alterations to the PFC may impair top-down inhibitory process. FC studies have noted aberrant frontal-striatal connectivity alterations in anxiety disorders[96,97], providing evidence that activity in and connectivity between the PFC and NACC may be compromised in

stress-related neuropsychiatric disorders, potentially leading to impairments in reward and motivational processes and increasing the risk of developing a drug addiction[623]. Interestingly, similar impairments in these PFC-NACC circuits have been recently proposed to mediate overeating in obesity, which is currently viewed as a form of food addiction[399].

In coordination with the PFC and subcortical regions, the INS is involved in interoception, salience and reward processing[371], and is also affected by chronic stress[66]. The posterior INS receives interoceptive information about the physiological condition of the body, including information about pain, temperature, visceral sensations, thirst, and hunger[628]. Interoceptive information is integrated with affective information conveyed to the INS through reciprocal connections with the AMYG and NACC. In addition to its role in interoception, the INS is also considered a member of the 'salience network', which is believed to mark salient information for additional processing and facilitate bottom-up access to attentional and working memory resources through its connections with the PFC executive network[371,629]. The volume of the INS is smaller in children who suffered maltreatment[66], indicating that this region is sensitive to chronic early life stress and trauma. Chronic work-related stress in humans has been associated with stronger intra-INS and INS-AMYG FC in stressed compared to healthy controls[98], while a task-based fMRI study found decreased functional coupling between PFC and INS in chronically stressed individuals[183]. It has been proposed that regulation of anxious responsiveness may require robust INS-PFC connectivity[99,100]; inefficient prefrontal regulation of INS responsiveness could result in over-estimation of threat and an exaggerated response to a perceived threat.

One way in which stress may lead to excessive feeding or a preference for an obesogenic diet is through stress-induced alterations in the corticolimbic circuits described above. Stress-induced alterations to reward systems increase addiction vulnerability[623], a finding that may be applicable to excessive emotional non-homeostatic feeding. According to models that frame obesogenic eating as an addiction[355], overeating reflects an imbalance between reward-saliency and motivation-drive circuits, which include the OFC, AMYG, INS, and ACC, and inhibitory-control pathways connecting the PFC with limbic region, with dysregulated DA neurotransmission playing a role in promoting this imbalanced state, as is the case with other addictions[431]. Stress-induced emotional eating is especially problematic, given findings that an obesogenic diet and weight gain impact many of the same circuits compromised by chronic stress[435,630]. Excessive feeding and resulting obesity are associated with reduced activity in the PFC, which may reflect deficits in top-down control over feeding[630]. Sustained consumption of an obesogenic diet has also been found to impair DA neurotransmission[356,379,631–633], which may exacerbate changes in PFC-NACC circuitry[634] and AMYG-PFC circuitry by altering glutamatergic innervation of the PFC via PFC-striatal pathways[355]. Similar to acute stressors, calorically dense foods elicit DA release in the NACC of animal models[635], and sustained consumption ultimately results in D2R down-regulation[379,627]. This is consistent with findings that obese people have reduced availability of D2R in the striatum, which is associated with lower levels of activity in the PFC and ACC[403]. In summary, both chronic stress and consumption of an obesogenic diet impair neurotransmission in the striatum and activity in the PFC, but further research is needed to assess whether these alterations contribute to an imbalance between reward-saliency and top-down inhibitory pathways control, and to test if the combined insults of chronic stress and hypercaloric intake have a synergistic impact on these corticolimbic regions.

The AMYG and INS—regions impacted by chronic stress—also appear to be involved in and/or altered by the consumption of a HCD and ensuing obesity[406,430,636,637]. Both regions contribute to reward processing and interoception—two processes that are highly relevant to feeding behaviors. Food intake is regulated by complementary drives; a homeostatic pathway responds to interoceptive signaling and increases motivation to eat when energy stores are depleted, and a hedonic reward-based regulation pathway which can supersede the homeostatic pathway and increase the drive to consume palatable foods[350]. Repeated consumption of a palatable HCD may result in alterations to either or both pathways. The consumption of a HCD has been associated with compromised development of the AMYG in a rat model, with the offspring of dams fed a high-fat diet exhibiting atrophy of pyramidal dendrites in the basolateral AMYG and weaker fear conditioning[386]. Thus, obesogenic diet-induced and/or stress-induced alterations to the AMYG may contribute to the etiology of obesity, given findings of altered AMYG activity in obese compared to lean individuals[405,406,636]. Obese children show a greater neural response to taste of sucrose and water in the bilateral AMYG and INS compared to lean children[406], and similar findings have been reported in adults[405,636], suggesting that food tastes trigger heightened responses in key reward and interoceptive areas. The INS may be particularly important to feeding behavior given its role in interoception, gustation (it includes the primary gustatory cortex), reward/addiction, and emotion[360]. Dysregulated interoception has been posited to contribute to addiction generally, and obesity specifically[355]. For example, gastric distention in lean, but not obese, subjects results in activation of the AMYG and deactivation of the INS, a finding the authors interpreted as blunted interoceptive awareness of satiety information in obese individuals regarding the fullness of the stomach[430].

Resting-state fMRI measures temporal correlations in the blood oxygen level dependent (BOLD) signal activity between two brain regions, and resting-state FC studies comparing obese and lean humans detect altered communication between the PFC, AMYG, NACC, and INS, suggesting these circuits are dysregulated in obesity[422,423,638]. High BMI was correlated with stronger NACC-ACC and NACC-ventromedial PFC (vmPFC) connectivity in women[422], and stronger connectivity between the NACC and mPFC was reported in adults with excess body weight[423], suggesting overactive reward circuitry. An effective connectivity study found relatively decreased modulation of the OFC and the NACC by the AMYG in obese adults compared to lean adults, possibly indicating impaired flexibility in updating the reward value of food, and increased modulation of the NACC by the OFC, which might contribute to a heightened drive to eat in response to a food cue[424]. Opposite patterns of AMYG-INS connectivity were reported for obese and lean individuals during a fast, with obese adults showing stronger connectivity between these regions and lean subjects showing weaker connectivity[425], suggesting obesity-related alterations in interoceptive processing. Altogether these connectivity results demonstrate that obesity is associated with region-specific alterations to circuits involved in emotion, reward processing and interoception that may reflect heightened reward-saliency and incentive processing and/or impaired top-down control.

While the bulk of the research on stress and diet have focused on adult outcomes, research has started to increasingly examine how the experiential factors of diet and early life stress influence the development of the CNS, HPA axis, and behavior. In rodent pups, prolonged maternal separation reduces CRH binding sites in the pituitary, and leads to increased density of CRH

binding sites in the PFC, AMYG, and hypothalamus post-infancy[639]. Studies in monkeys have shown that unpredictable separations from mother, or spontaneous maternal abusive behavior, increases CRH concentrations in the cerebrospinal fluid and alters the diurnal activity of the HPA axis[21,640]. In rhesus macaques, peer-rearing is an established model of early life adversity and has been shown to increase the volume of region-specific areas of the PFC, including the dorsomedial PFC and the dorsal ACC in juveniles[87]. Post-institutionalized children have increased AMYG volume and reactivity compared to healthy controls[80,81], and decreased structural integrity of tracts connecting the PFC with AMYG and striatum[82,83]. More recently, low socioeconomic status was associated with decreased brain surface area, and increased hippocampal and AMYG volumes[85,86]. Limited resting-state FC studies indicate that early life stress in children may be associated with alterations to the control network and default mode network. Early life stress was associated with negative correlations between the left lateral frontal cortex and regions in the temporal cortex and hippocampus[101]. Higher interparental conflict—a common form of early life stress—was correlated with stronger FC between the posterior cingulate cortex (PCC) and mPFC, and between the PCC and AMYG[102]. These findings differ from those typically reported in adults who experienced chronic stress, and could be due to brain circuitry reorganization the occurs during adolescence[163,641], the nature and severity of the stressor[177], and heterogeneity of the populations studied.

Maternal consumption of a high-fat diet has been associated with a number of neuroadaptations in offspring, including alterations to the central nervous system, including decreased dendritic arborization in the AMYG[386] (as discussed above), increased neurogenesis of orexigenic cells in the hypothalamus[387], and differential expression of genes related to serotonergic and DA

neurotransmission[388,389], inflammation[390], and neuropeptides that regulate food intake[387,391]. The association between maternal consumption of a high-fat diet in the perinatal period and higher expression of orexigenic peptides in the lateral hypothalamus[387]—suggestive of increased neurogenesis of orexigenic neurons—may have implications for homeostatic regulation of food intake in offspring. Accumulating evidence also indicates the maternal consumption of a high-fat diet alters reward circuitry in offspring, possibly mediating an increased drive for non-homeostatic eating. For example, early life consumption of a high-fat diet in the 3rd postnatal week of life in rodent models altered DA-related gene expression in the NACC that was suggestive of reduced DA transduction, and increased a preference for fat as an adult[397]. In macaques, maternal obesity and high-fat diet consumption decreased the abundance of DA fiber projections to the PFC and reduced D2R and D1R protein levels in the PFC of their offspring; offspring of obese dams also demonstrated a preference for food high in fat and sugar compared to offspring from lean mothers fed a control diet[389]. The bulk of the developmental studies that have been carried out in rodent models have focused on maternal consumption, with very little research examining how infant consumption of a HCD affects early development. There is a gap in the literature concerning how consumption of a HCD by mothers and infants influences development, including systems-level effects on corticolimbic circuits, and how HCD consumption interacts with early life stress to shape the brain, HPA axis, and behavior.

The biological mechanisms by which chronic stress and obesogenic diets alter brain structure and function remains poorly understood, particularly during development, but evidence points to GCs and inflammatory cytokines as potentially important in this regard. Sustained intake of an obesogenic diet has been reliably shown to increase GC secretion in response to

stressors[105,441,442], and obesity is characterized by hypercortisolemia[441]. GCs affect structural connectivity (SC) and FC in the brain by remodeling dendritic length and branching, synapses and myelin through a number of transcriptional and non-transcriptional mechanisms[642]. As such, elevated GCs may play a crucial role mediating the adverse consequences of both chronic stress and consumption of a HCD. Proinflammatory cytokines may also prove to be important biological signals mediating the effects of chronic stress and obesity on neurobehavioral development. Chronic inflammation is a well-documented consequence of obesity[450,451]; importantly, consumption of an obesogenic diet has been shown to increase central and peripheral measures of inflammation before frank obesity is even evident[448,449]. Chronic stress has also been shown to promote a pro-inflammatory state[643,644]. The possibility that pro-inflammatory markers and GCs could mediate effects of stress and diet on neurobehavioral development is supported by recent findings. Consumption of a high-fat diet during pre-weaning development in rats was linked with increased expression of both corticosterone receptors in the AMYG in adulthood, and pro-inflammatory and anti-inflammatory genes in the AMYG and hippocampus that are known to be regulated by GRs[395]. Importantly, these alterations, in turn, predicted increased anxiety behavior, decreased basal corticosterone levels, and a prolonged stress response following an acute stress challenge. Inflammation has been directly linked with alterations in functional activity and FC in adults, supporting the possibility that inflammation may alter the development of corticolimbic circuits, though this is an underexplored area of research. Exogenous administration of inflammatory cytokines alters activation of reward-related brain regions[454–458], an effect that appears to be mediated by cytokine-induced reductions in DA release in the ventral striatum[452,453]. More recently, studies found increased c-reactive protein (CRP; another inflammatory marker) levels predicted decreased FC between dorsal striatum and

vmPFC[645], and levels of IL-6 were associated with altered connectivity within the default mode network[646], suggesting that pro-inflammatory markers may influence the organization of brain networks. In summary, stress-induced or diet-induced changes in the development of the brain and behavior may be mediated, in part, by elevated GCs and pro-inflammatory markers.

In this study, we sought to investigate the potential for a synergistic impact of a social stressor and consumption of an obesogenic diet on infant brain development longitudinally. Infancy is a development period marked by significant and rapid changes in the brain, creating windows of vulnerability during which time adverse experiences can be readily encoded[475,647]. Cells begin proliferating, migrating and making synaptic connections prenatally, producing an overabundance of neuron dendrites, axons, and synapses at birth that are selectively pruned during development[648]. Cortical synaptic density and neurotransmitter receptor density reaches a peak between postnatal month 2 and 4 in rhesus macaques, and then declines to adult levels by 3 years of age in most areas, though declines continue in the PFC for another twenty years[649–651]. In monkeys, peak synapse density in limbic regions occurs at postnatal month 2[652], coinciding with the onset of limbic functions including memory and social fear, and achieves functional maturity at 4 months[653]. A comparable process unfolds in human development, but the process occurs over a longer time and a region-specific manner, with the PFC not reaching peak synaptic density until 3.5 years[654]. During infancy, gray matter volume increases, likely driven by 'exuberant synaptogenesis'[164,165], and white matter volume increases as cortical myelination[166] progresses. These developmental changes represent an opportunity for the postnatal environment to make long lasting impacts to the brain and behavior.

Disentangling the contributions of stress, diet, and feeding behavior on neurobehavioral development, as well as the underlying biological mechanisms (e.g. increased GCs, proinflammatory signals, both) in children is difficult, considering the challenges associated with prospective, longitudinal studies that standardize and monitor accurate food intake under rigorous experimental control. Social subordination in female rhesus macaques is a useful and well-validated translational animal model of chronic psychosocial stress[104,107]. Social subordination is a naturalistic stressor that produces a number of distinct stress-related phenotypes[104]. Infants acquire the relative ranks of their mothers rank at birth[115], and their early neurobehavioral development may be shaped by their subordinate status, either through their social experiences or biological signals transmitted from their mother. Through observational learning, the infant observes its mother's rank-related social interactions early in development and learn its own position in the social hierarchy through imitation or identification with the mother[655]. Subordinate infants may start to receive aggression by this point, albeit minimal[131], or perceive harassment directed to their mothers or other family members as stressful. Social status effects in infancy may also be communicated through immunological and hormonal constituents in breastmilk, such as immunoglobulins, cytokines, GCs, and other hormones like leptin[656–658]. Studying rhesus macaques in infancy affords an opportunity to better understand how social status influences neurobehavioral development via biological signals and/or vicarious learning about the social environment. Importantly, subordinate macaques have also been shown to be susceptible to stress-induced overeating of an obesogenic diet[105,610]. Adult subordinate females have been found to eat approximately twice as many calories when a diet of fat and sugar is available[610], and juvenile females have been shown to be susceptible to diet-induced increased fat mass[326]. Using the social subordination model in rhesus macaques, we sought to investigate

how social subordination interacts with dietary environment during infancy to affect the development of stress neuroendocrine systems (HPA axis), proinflammatory markers, emotional reactivity, feeding behavior, as well as FC in corticolimbic circuits important to interoception, reward processing, and stress/emotional regulation. Additionally, we examined whether stress-induced or diet-induced neurobiological alterations were associated with elevations in GC or pro-inflammatory cytokines. We hypothesized that social subordination will begin to promote hypercaloric consumption and decrease FC in circuits subserving emotion regulation and impulse control by 6 months of age, and consumption of an obesogenic diet will further reduce FC in these corticolimbic circuits and impair the development of FC in circuits involved in reward-saliency processing. We also hypothesized that subordination status and consumption of an obesogenic diet, particularly calories derived from a HCD, would be associated with alterations to the HPA axis (higher basal cortisol and cortisol reactivity) and increased inflammatory markers by 6 months of age, and these alterations may be linked with changes in neurobehavioral development.

3.3 Methods

3.3.1 Subjects

Subjects were 44 infant female rhesus macaques (*Macaca mulatta*) born at the Yerkes National Primate Research Center Field Station in Lawrenceville, GA. The infants were reared by dams in large social groups compromised of approximately 2-3 males, 30-60 adult females and their offspring. The social groups lived in outdoor enclosures comprising three-quarters of an acre with attached climate-controlled indoor quarters. Data was collected longitudinally during the infants' first 6 months of life (see Figure 3.1 for summary). All procedures were approved by the

Emory University Institutional Animal Care and Use Committee, in accordance with the Animal Welfare Act and the U.S. Department of Health and Human Services "Guide for Care and Use of Laboratory Animals." Three subjects were excluded from all analyses because they were chronically unthrifty early in life and released early from the study.

3.3.2 Dam Selection and Prenatal Conditions

Macaque groups are organized by a dominance hierarchy, with lower social ranking animals unequivocally emitting a submissive behavior in response to an approach or aggression from a more dominant individual[115]. The social status of the individuals in the social groups was determined from formal group checks, in which the outcomes of dyadic agonistic interactions were recorded. Dams were selected for the study from families at the extremes of the social hierarchy within their groups, with dominant dams recruited from families ranked in the top third of the group and subordinate dams recruited from families in the bottom third. Exclusionary criteria included primiparous females and/or females with histories of infant physical abuse or neglect. Because prenatal diet has been shown to have programming effects on neurobehavioral development[388,397,432], all pregnant females were maintained on a LCD-only diet through gestation to control for prenatal dietary environment. Dam-infant pairs were eligible for the study if the above criteria were met and the infant's birth weight > 450g, to avoid effects of prematurity/low birth weight on brain development[659].

3.3.3 Infant Assignment and Cross Fostering

To disentangle the impact of prenatal maternal stress and postnatal stress, we used a cross fostering design. Using pseudo-random assignment, approximately half of the newborns were

cross fostered to dams of the same or different social status as their biological mother within 48 hours of birth using well-established procedures[660,661]. The remaining subjects were reared by their biological mother. Because rhesus offspring assume the social status of their mothers[115], cross fostered infants assumed the social status of their foster mothers, and infants who remained with their biological mothers inherited their social status. Twenty-one dominant-ranking mother-infant pairs, and twenty subordinate-ranking pairs were followed after birth. Ranks were assessed on a monthly basis to monitor changes in the hierarchy of the group. Relative social status of the infant was calculated as the ratio of its postnatal dam's social status divided by the total number of animals > 3 years of age in the group, excluding adult males.

3.3.4 Diet Intervention

Table 3.1 summarizes the distribution of subjects across the experimental cells of the study design. The Choice condition closely models the human dietary environment and has been shown to sustain stress-induced emotional feeding in animal models [54,353,662,663] and promotes obesity more effectively than a HCD diet alone[663]. The LCD was LabDiet Monkey Diet 503A, a pelleted version of the standard LabDiet Monkey Diet 5038 (Purina Mills International, St. Louis, MO). The diet contained 3.46 kcals/gram and the calories are distributed as 14% fat, 18% protein, and 68% carbohydrates. The HCD pellets (D14051502B, Research Diets, Inc.) contained 4.25 kcal/gram and the caloric composition was 30% fat, 20% protein, and 50% carbohydrate. The HCD pellets included substantially more sugar carbohydrates (29.84% of total calories) compared to the LCD pellets (6.11% of total calories). The LCD and HCD pellets were comparable in the amounts of vitamins and minerals they provided.

Subjects accessed the feeders with radio-frequency identification (RFID) chips subcutaneously embedded in their wrists; dams had RFID chips at the outset of the experiment, and infants received RFID chips at 6 weeks of age, before these animals transition to solid food[664]. The automated feeders were outfitted with radio-frequency antenna that scanned the subjects' RFID chips, dispensing a single pellet at a time and quantifying each subject's caloric intake. This set-up has been previously validated to allow monkeys to consume food *ad libitum,* and allowing systematic control over access to one of the two experimental diets and careful recording of caloric intake[54,610]. Calorie intake was monitored continually for the first 6 months of life to determine how social status and dietary environment predicts caloric intake. One infant-dam pair was assigned to the LCD-only condition, but the dam was able to access the HCD feeder in the infant's first two weeks of life. We excluded this infant from analyses that included the 2 week time point.

3.3.5. Emotional Behavior

The Human Intruder (HI) task is a standardized task that elicits behavioral reactivity[186] and HPA axis activation[538], and responses to the task have been shown to be under the control of AMYG and PFC circuits[538,539]. Behavioral responses to the task emerge during infancy and include anxiety-like, fear-like, aggressive, and exploratory behaviors[186,187,665,666]. Prior work by our group demonstrated that early life stress in rhesus macaques increased behavioral reactivity on the task. To assess effects of social status and diet on behavioral and stress responses, the task was administered at 6 months of age only, immediately following collection of a baseline blood sample to examine the HPA axis stress response during the HI task (see below). The task comprised three consecutive ten-minute conditions: (alone) the animal is alone in a testing cage,

(profile) an unfamiliar human intruder enters the room and sits with its profile to the subject, (stare) the intruder turns and stares at the subject making direct eye contact. The tests were recorded and scored using an established ethogram[134].

3.3.6 Physiology

Stress Physiology: Basal Cortisol Levels and Stress Reactivity

Dam-infant pairs were habituated to being removed from their group for awake venipuncture using previously described methods[667]. Baseline blood samples were collected from the infants in the early AM (at sunrise) to control for circadian changes in cortisol secretion at ages postnatal day (PND) 2-4, 2 weeks, and 6 months and following previously published methods[44,668,669]. Blood samples were also collected immediately following the 30-minute HI task, and again 4 hours after completion of the HI task (recovery), to test whether social status and dietary environment influences stress-induced cortisol reactivity in response to an acute stressor and GC negative feedback. Efforts were made to obtain all blood samples within 10 minutes from initial group disturbance to avoid confounding effects of arousal[670]. Blood samples were collected in pre-chilled tubes containing EDTA, immediately placed on ice, centrifuged and plasma stored at -80°C until assayed.

All plasma cortisol assays were performed by the YNPRC Biomarker Core Laboratory. After thawing at 4°C, 20µl of plasma were placed into a 96-well block with 100 µl of 50ng/ml internal standard (d4-cortisol) in acetonitrile, spun and extracted. Extractions solution was analyzed by ultra-performance liquid chromatography electrospray ionization (UPLC-ESI) tandem mass spectrometry. Cortisol and d4-Cortisol were eluted by Acquity UPLC@ BEH C18, 1.7µm,

50X2.1mm column (Waters, Milford, MA). Cortisol and d4-Cortisol were identified at m/z pairs of 363.1/121.1 and 367.3/125.2 by AB Sciex TripleQuad 6500. Cortisol concentrations for each sample were calculated using linear regression analysis of a standard curve. The quantification range for the assay was 0.1 -100 µg/dl cortisol. For each run, calibration standards were prepared at concentrations of 0, 0.1, 0.5, 1, 5, 10, 20, 50, 75, 100 µg/dl. Three fortified quality control (QC) samples were also analyzed in duplicate in each run. The intra- and inter-assay precision [percentage coefficient of variation (CV%)] are 1.43% and 6.71%.

Markers of Peripheral Inflammation

Peripheral inflammatory markers were assayed from the baseline plasma samples collected at sunrise at 2 weeks and 6 months of age. Concentrations of C-reactive protein (CRP), the pro-inflammatory cytokine interleukin 6 (IL-6), and the chemokine monocyte chemoattractant protein-1 (MCP1) were measured using Meso Scale Discovery electrochemiluminescent (ECL) immunoassay V-PLEX human panels on the MSD SECTOR Imager 2400-A (Meso Scale Diagnostics, LLC, Rockville MD). The pro-inflammatory cytokine TNFα was measured similarly using a NHP V-plex panel. Samples were diluted by a factor of 1000 for CRP, 4 for MCP-1, 2 for IL-6, and 1 for TNFα, and run in duplicate. The assay includes running the samples in duplicate and converting the ECL signal to pg/mL values based on standard curves of calibrator proteins. All samples included in the analysis had CV values <10%.

Hair Cortisol Accumulation

Accumulation of cortisol in hair can be a useful marker of chronic stress[671,672]. To assay exposure to cortisol levels prenatally and from birth through the first 6 months of postnatal life, hair was

shaved from the back of the neck at birth and 6 months, respectively. Hair cortisol measures were analyzed as previously described[672,673]. Briefly, each sample was weighed, washed twice in isopropanol to remove external contamination, ground to a fine powder, and then extracted with methanol overnight. The methanol was evaporated, the residue was redissolved in the assay buffer, and then cortisol was measured using the Salimetrics (Carlsbad, CA) enzyme immunoassay kit (cat. # 1-3002) according to the manufacturer's directions. Intra- and inter-assay coefficients of variation were <10%.

Body Weight measurements

Body weight (BW) was measured at 2 weeks and 6 months of age.

3.3.7 Resting-state fMRI

Neuroimaging Scanning

Neuroimaging data were collected from the infants longitudinally at 2 weeks and 6 months of age. Infants were transported with their mother from their social groups to the YNPRC Imaging Center on the day before or day of the scan. All scans were collected in a single session using a 3T Siemens Magnetom Trio Tim system scanner (Siemens Med. Sol., Malvern, PA, USA) with an 8-channel phase array coil and under anesthesia. Following initial telazol induction (2 weeks: 3.82 ± 0.08 mg/kg BW, 6 months: 2.25 ± 0.04 mg/kg BW, i.m.), infants were intubated to administer isoflurane during the scanning session. Low isoflurane levels were administered (0.8 – 1.0%) to maintain a sedated and relatively immobile state while minimizing effects to BOLD signal. These isoflurane levels are below those used in previous macaque studies demonstrating patterns of coherent BOLD signal comparable to those found in awake monkeys[513–516]. Animals

were scanned in the supine position and their heads were immobilized by a custom-made head holder with ear bars and a mouthpiece. Eight high-resolution T1-weighted structural MRI scans were acquired for registration purposes using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel image sequence with TR/TE=2600/3.38msec, FoV=128mm, voxel size=0.5mm^3 isotropic, GRAPPA, R=2). Two 15 min resting-state fMRI (T2*-weighted) scans were collected following the T1-weighted scans to allow approximately 40 minutes to elapse from initial telazol induction for stabilization of physiological variables. These scans were collected using a T2*-weighted gradient-echo echoplanar imaging (EPI) sequence with 400 volumes, TR/TE=2160/25msec, voxel size=1.5mm^3 isotropic). A short reverse-phase encoding scan was acquired to enable unwarping distortions in the EPI scans using previously validated methods[674].

During the scanning procedures, physiological measurements were monitored using an oximeter, ECG, rectal thermistor and blood pressure monitor. An intravenous catheter maintained hydration with a dextrose/NaCl solution (0.45%), and an MRI-compatible heating pad was used to stabilize subjects' body temperature. At the end of the scanning session and upon anesthesia recovery infants joined their mothers in shared housing, and the pair was returned to their social group the following day. Three infants could not be scanned at 2 weeks either because the infant or mother was sick.

Functional MRI data pre-processing

All data was preprocessed using an in-house Nipype-based pipeline[517] with modifications to previously published resting-state FC preprocessing methods[520–523] and customized alterations

specific to processing macaque brains[524]. Files were converted to nifti format and the first 3 volumes of each resting-state fMRI scan were discarded, as these may have been collected before scanner equilibrium was reached. The preprocessing pipeline included unwarping of the EPI scans using a reverse phase-encoding distortion correction method[674], slice-timing correction, and one-step resampling of rigid body head motion-correction, linear registration of the EPI to the T1 scan, and affine followed by non-linear registration of the T1-MRI image to a 6 month age-specific T1-weighted rhesus infant MRI atlas. The 6 month T1-weighted atlas was developed by our group in collaboration with Martin Styner (UNC)[593] and based on scans acquired longitudinally from 40 infant rhesus monkeys from the YNPRC colony, balanced by sex, and early social experience and status/rank. This 6 month infant atlas was registered to the 112RM-SL atlas in F99 space[525,526] for use in the nipype pipeline.

Additional processing steps included normalization of the signal to a whole brain mode of 1000, detrending of the BOLD signal after the EPI functional series were concatenated, nuisance regression of the rigid body head motion parameters, whole-brain, ventricle, and white matter signal, and all their first-order derivatives, and low-pass filtering with second-order Butterworth filter. Frames with displacement greater than 0.2mm were censored from the FC analysis[675]. Global signal was regressed based on current literature highlighting the importance of removing non-neuronal, physiological noise (e.g., cardiac and respiration cycles) common across the entire brain[527,528].

Resting-state FC Analysis

Corticolimbic FC between Regions of interest (ROIs) in the PFC [dlPFC: Brodmann area 46 -BA46-, BA9), mPFC (BA32), ACC (BA24, BA25), and OFC (BA11 and BA13)] and the AMYG and NACC was analyzed. Given its role in processing salient stimuli, natural reinforcers, and interoceptive information[371], we also analyzed FC between the INS and PFC regions with which it has established structural connections in the macaque (BA46, BA24, BA11, BA13)[373,676]. ROIs were defined based on Lewis and Van Essen[530] and Markov[677] anatomical parcellations mapped onto the cortical surface of the UNC-Emory rhesus infant atlases in F99 space. The left and right AMYG ROIs were hand-drawn by experts using cytoarchitectonic maps derived from a UNC-Wisconsin adolescent atlas[532] (RRID: SCR_002570), and propagated to the infant atlas using deformable registration via ANTS. Using established anatomical landmarks[533,534], the ROIs were manually edited over the infant atlases to improve neuroanatomical accuracy and avoid ROI overlap. As described in the previous chapter, this set of ROIs was modified further to remove any voxels where at least one subject had signal dropout, with dropout being defined as the mean intensity of the subject's EPI signal minus two standard deviations. Using this modified set of ROIs, the average BOLD timecourse was extracted for each ROI, and correlated with the average timecourse from the other ROIs. Lastly, Pearson correlation coefficients were Fisher z-transformed.

Quality Control of Scanning data

Upon visual inspection, one subject was noted for having a possible lesion on the right side of the brain at 6 months of age that resulted in signal loss in the resting-state fMRI scans in the following ROIs: BA9, BA46, BA11, BA32. Due to this abnormality, we excluded this subject from any FC analysis in circuits involving these four ROIs (right-side only). As described in the

previous chapter, when mild to moderate Nyquist ghosting was observed in the raw EPI scans, we pursued a second quality-control test by creating AMYG seed-based whole-brain, voxel-wise FC maps to determine whether Nyquist ghosting affected BOLD signal in the brain by altering coherent networks. After these AMYG whole-brain voxelwise seed maps were examined we noted that 8 subjects at 2 weeks of age and 8 subjects at 6 months of age had a moderate artifact pattern. Analysis was performed with and without these cases; we reported how the models were changed by exclusion of the moderate artifact cases.

3.3.8 Statistical Analysis

Overall Model and Rationale

Our aim was to test the effect of the infants' social status and experimental diet on various outcomes, including cumulative kcals consumed, resting-state FC in corticolimbic circuits, cortisol in plasma and hair, pro-inflammatory markers, and emotional behavior. Due to limitations of our relatively small final sample size, we do not have sufficient power to analyze and interpret effects of biological mother's rank/status (including genetic heritability factors and potential prenatal maternal stress in the subordinate animals) and cross-fostering (CF). Thus, our general approach was to first test whether Biological mom's social status and CF had detectable effects on functional outcomes in preliminary models, and control for these factors in the main models, when necessary. In preliminary ANCOVA models or rmANCOVA models, biological mom's social status was entered as a between-subjects factor, controlling for CF as a covariate. In the main ANOVA or rmANOVA models, we tested effects of infant social status (i.e. foster mom's social status: dominant (DOM) vs. subordinate (SUB)) and postnatal diet (LCD-only vs. Choice) on the outcome measures, treating these variables as between-subjects factors. If

biological mom's social status or CF were significant predictors in the preliminary model, one (or both) were entered as covariates in the main models. In the case of rmANOVA models, biological mom or CF was also included as a covariate in the main model if either factor interacted with the repeated measure (age or time).

Analysis of Feeding Data and BW

We first sought to determine whether there were effects of social status or diet on the number of kcals consumed by the dams during the infants' first 2 weeks of life, and by the infants during their first 6 months of life. Because the infants are exclusively breastfeeding through their 2 week scans, the dams' kcals consumption of either diet during this period served as a proxy for the infants' caloric intake during this time. The cumulative number of total kcals, LCD kcals, and HCD kcals consumed failed Kolmogorov-smirnov tests of normality, and the feeding data was transformed with a square-root transformation of the total kcals, and a square-root + 1 transform of the LCD-kcals and HCD-kcals. Because of the variability in timing of infant weaning, we first tested whether kcals consumed by infants during the first 6 months (total, HCD, and LCD) was dependent on the beginning of weaning (defined at first age of consumption of solid food from the automated feeders), by correlating Kcals with 'feeding duration', measured as the number of days between infants first recorded feeding and their 6 month scan. If the correlation was significant, feeding duration was included as a covariate in the ANOVA models described above. A t-test compared consumption of the HCD kcals by DOMs and SUBs on the Choice diet, and a paired t-test examined whether Choice subjects consumed significantly different number of kcals of LCD vs. HCD chow. Similar ANOVA models to those described above were used to analyze effects of social status and diet on BW at 2 weeks and 6 months.

.

Analysis of Resting-State FC Data in Corticolimbic Brain Circuits

We next tested whether social status and diet impacted brain activity outcomes in the first 6 months of life. Although we kept the levels of anesthetics (telazol, isoflurane) to the lowest minimum, and left a 40 min a lag time between telazol administration and the resting-state fMRI scans for clearance, we also tested potentially confounding effects of isoflurane and telazol on FC by performing correlations between their respective doses per animal and FC at 2 week and 6 months. The preliminary and main ANOVA models described above ("Overall Model and Rationale" section) were run separately for FC at 2 weeks and 6 months, with isoflurane and telazol dose(s) added as a covariate(s) when significantly correlated with FC.

Analysis of Stress Physiology

To assess effects of social status and diet on baseline HPA axis function early in life, we analyzed the effect of these factors on baseline plasma cortisol levels in the blood samples collected at sunrise (early AM) at PND2-4, 2 weeks, and 6 months using rmANOVA models (described above), treating Age as a repeated, within-subject, measure. To measure stress-induced HPA axis reactivity and recovery to baseline at 6 months of age, plasma cortisol levels were measured immediately before (baseline/sunrise: pre-HI), and after administration of the HI task (post-HI), and 4 hours post-HI. These measures were analyzed with rmANOVA models, with time point (baseline/sunrise, post-HI, 4-hours post-HI) as the repeated, within-subject, measure. Three subjects were excluded from the cortisol reactivity analysis at 6 months, and 8 subjects were excluded from the baseline cortisol analysis, because their samples were collected >10 minutes from disturbance.

Analysis of Hair Cortisol and Inflammatory Markers

Due to non-normality of the distribution, hair cortisol and inflammatory markers were log-transformed (with a constant added when zeros were present). The transformed hair cortisol levels at PND 2-4 and 6 months, and inflammatory markers at 2 weeks and 6 months were entered into rmANOVA models, with age as a repeated measure.

Analysis of Emotional Behavior during the HI task

The following HI composite scores were calculated by adding rate or duration of ethologically similar behaviors: freeze (duration of freeze and crouch-freeze), anxiety-like (rates of self-scratch, yawn, self-grooming, and tooth grinding), aggressive (rates of threat, threat bark, crooked tail, lunge, and display), and explore (duration of tactile explore and visual explore). In addition to these composite scores, visual inspection of the intruder (duration), visual avoidance of the intruder (duration), locomotion (duration), and coo (rate) were analyzed. Non-normally distributed behaviors or composites that failed the Kolmogorov-smirnov test were transformed with a natural log, and a constant was added when zeros were present. Phases of the HI task were excluded from analysis if the behavior/composite was zero-inflated in the phase, with a zero-inflation threshold set at more than 40% of subjects having zeros. If two or more phases were entered into the statistical model, rmANOVA models were performed with phase as the within-subject repeated measure; otherwise ANOVA models were run.

Multiple Regression Models: Do Kcals Consumed and Social Status Predict Functional Outcomes?

Following the statistical models detailed above, we tested whether the number of kcals consumed and relative rank predicted functional outcomes, constraining this analysis to outcomes with statistically significant effects of social status, diet, or a social status x diet interaction. The regression model tested included the following terms:

$$\text{Outcome} = \text{Relative rank} + \text{LCD kcals} + \text{HCD kcals}$$

The dams' kcal data was entered into models predicting infant outcomes at 2 weeks, and the infants' kcal data was entered into models predicting functional outcomes at 6 months. Modeling the quantitative number of kcals permits us to probe whether effects of diet were driven by quantitative differences in kcals consumed or qualitative aspects of the different diets. The relative rank of the infant's postnatal dam was included in the model to test whether there was a 'dose effect' of social experience (i.e. whether being more or less extreme on the continuum of social status predicted functional outcomes). If the LCD or HCD kcals was a significant regressor in the model, an interaction term between relative rank and the kcals term was successively entered in the model and the models were compared to test whether the addition of the interaction term significantly increased the variance explained by the model.

Associations between outcomes (FC, cortisol, proinflammatory markers, emotional behavior)

Lastly, we tested whether there were significant associations between outcomes with statistically significant effects of social status, diet, or a social status x diet interaction . Bivariate correlations were performed to test associations between FC, stress and inflammatory physiology, and emotional behavior.

At 6 months of age, the models were analyzed with and without the infant whose mother consumed HCD despite assignment to the LCD-only group. By this time point, the infant had access to the LCD-only diet (through breast-milk and her own consumption) for the majority of her development (5.5 months). We reported instances when significant models were changed by her exclusion. When outliers were present (defined as being above or below 3 SDs), analyses were performed with and without outliers, and differences in the models are reported.

3.4 Results

3.4.1 Predictors of Feeding

Feeding data is summarized in Table 3.2. DOM dams consumed significantly more cumulative total kcals during their infants' first 2 weeks of life compared to SUB dams ($F(3,31)=5.165$, $p=0.030$, $\eta^2=.143$; Figure 3.2B). Dams in the LCD-only condition consumed significantly more cumulative LCD kcals than dams in the Choice condition ($F(3,31)=28.844$, $p< 0.001$, $\eta^2=.482$). For the Choice dams, there were no significant differences between the number of cumulative HCD kcals consumed by the DOMs and SUBs. In addition, Choice dams did not differ significantly in their consumption of LCD kcals and HCD kcals.

For infant calorie consumption in the first 6 months of life, there was a main effect of diet on cumulative total kcals, with Choice infants eating more cumulative total kcals through the age of the 6 month MRI scan than LCD-only infants ($F(3,34)=6.338$, $p=0.017$, $\eta^2=.157$; Figure 3.2A). No main effects of social status or interactions between social status and diet condition were detected on cumulative total kcals consumed. The number of days the infants spent feeding in their first 6 months of life was a significant covariate in the model of cumulative LCD kcals

($F(4,33)=10.215$, $p=0.003$, $\eta^2=.236$), but there were no effects of social status or diet on cumulative LCD kcals consumed. For the Choice infants, there was no significant difference in cumulative HCD kcals consumed by the DOM and SUB infants. Furthermore, the number of cumulative HCD kcals and LCD kcals consumed by the Choice infants did not significantly differ. There were no effects of social rank or diet condition on BW at 2weeks or 6 months.

3.4.2 Resting-state FC

At 2 weeks of age, infants consuming the LCD-only diet through maternal lactation showed significantly stronger, more positive FC between right AMYG and OFC (BA13) than infants in the Choice condition, whose moms were consuming the HCD and LCD chow ($F(4,31)=6.947$, $p=0.013$, $\eta^2=.183$; Figure 3.3). No other main effects of diet were found in ROI-to-ROI FC at 2 weeks or 6 months of age.

At 6 months of age, SUB infants had stronger positive FC between left AMYG and mPFC (BA32) ($F(3,37)=4.130$, $p=0.049$, $\eta^2=.100$; Figure 3.4 A,B). There was also a main effect of social status on FC between left INS and ACC (BA24), ($F(3,37)=4.820$, $p=0.034$, $\eta^2=.115$; Figure 3.4 C,D), with SUB infants having significantly stronger, more positive FC than DOM infants. No main effects of social status were found in other FC models at 2 weeks and 6 months.

A social status x diet interaction was also detected at 6 months of age for FC between the right INS and dlPFC (BA46) ($F(3,36)=7.386$, $p=0.01$, $\eta^2=.170$; Figure 3.5 A,B). The post-hoc tests revealed that SUBs on LCD-only diet had significantly stronger FC compared to both SUBs on the Choice diet ($p=0.01$, $\eta^2=.172$), and to DOMs on the LCD-only diet ($p=0.018$, $\eta^2=.145$). There

was also an interaction effect of social status x diet on FC between the right INS and OFC at 6 months (BA13) ($F(3,37)=4.964$, $p=0.032$, $\eta^2=.118$, Figure 3.5 C,D). Post-hoc tests revealed that DOMs on Choice diet had significantly higher FC than DOMs on LCD-only diet ($p=0.02$, $\eta^2=.137$), and SUBs on the Choice diet ($p=0.015$, $\eta^2=.149$). FC between the right INS and ACC (BA24) also showed a social status x diet interaction effect at 6 months ($F(3,37)=5.653$, $p=0.023$, $\eta^2=.133$; Figure 3.5 E,F). Although the post-hoc tests did not yield significant contrasts, there was a trend for DOMs on the Choice diet to have significantly higher FC than DOMs on the LCD-only diet ($p=0.051$, $\eta^2=.099$). There were no other significant interaction effects observed in other 2 week and 6 month models. Imaging results are summarized in Table 3.3.

The significant results reported for the ROI-to-ROI models were not changed when the analysis excluded the LCD-only infant whose foster mom was able to access the HCD feeders during the infants' first two weeks of life. When the models were re-run excluding the subjects with artifacts in the AMYG seed maps, the significance of the results was unchanged with the exception of the model of FC between left INS and BA24 (where the directionality of the results remained the same, and there was a statistical trend for a main effect of social status ($p=0.076$), suggesting that the difference was most likely due to loss of statistical power to detect an effect given the smaller sample size).

3.4.3 Stress Physiology and Peripheral Inflammation

When baseline cortisol levels sampled at sunrise were analyzed longitudinally across PND2-4, 2 weeks of age, and 6 months of age, no effects of infant social status or diet on baseline cortisol were detected. At 6 months of age, plasma cortisol samples were also collected immediately

following the HI task to gauge stress reactivity of the HPA axis, and 4 hours later to measure recovery of the HPA axis to baseline. There was one cortisol outlier that seemed to drive a CF effect as a covariate in the preliminary model (since it was not significant when the outlier was excluded); therefore, we did not include CF in the main model. A rmANOVA model of the 6 months plasma cortisol levels at sunrise (baseline), post-HI, and 4 hours post-HI excluding the cortisol outlier detected a main effect of sample time point collection ($F(2,62)=220.697$, $p < 0.001$, $\eta^2=.877$), and a social status x diet x time point interaction ($F(2,62)=4.292$, $p=0.018$, $\eta^2=.122$; Figure 3.6). Post-hoc analyses revealed that at sunrise, DOMs in the Choice diet group had significantly higher cortisol than DOMs in the LCD-only group ($p=0.028$, $\eta^2=.147$), and SUBS had significantly higher cortisol levels than DOMs in the LCD-only group ($p=0.047$, $\eta^2=0.121$). Immediately following the HI task, SUBs on the Choice diet had significantly higher cortisol levels compared to SUBS on the LCD-only diet ($p=0.015$, $\eta^2=.178$). The results were similar when the outlier was included in the analysis, with the following exceptions: a main effect of diet was detected ($F(1,32)=4.133$, $p=0.05$, $\eta^2=0.114$), and there was no significant difference in baseline cortisol between SUBs and DOMs on the LCD-only diet. Additionally, with the outlier included, a significant effect was detected wherein SUBs on the Choice diet had significantly higher cortisol levels than DOMs on the Choice diet immediately following the HI task ($p=0.027$, $\eta^2=0.144$). There was no effects of social status or diet on cortisol accumulation in hair at birth or 6 months of age.

When two outliers were excluded from the CRP model at 2 weeks and 6 months of age, there was a main effect of social status ($F(1,34)=4.524$, $p=0.041$, $\eta^2=0.117$), and a social status x diet interaction ($F(1,34)=4.270$, $p=0.046$, $\eta^2=0.112$; Figure 3.7). The post-hoc tests revealed SUBs in

the LCD-only group had significantly higher CRP levels than DOMs in the LCD-only group ($p=0.005$, $\eta^2=0.205$). This model was not significant when outliers were included. None of the other inflammatory cytokines showed effects of social status, diet, or social status x diet interactions.

3.4.4 Emotional Behavior

In the Stare phase of the HI task, a main effect of social status emerged, with DOMs exhibiting higher rates of anxiety-like behavior than SUBs ($F(3,36)=6.1$, $p=0.018$, $\eta^2=0.145$; Figure 3.8A), regardless of diet condition. There was a HI phase x diet interaction for the duration of time spent visually inspecting the intruder ($F(1,34)=4.279$, $p=0.046$; Figure 3.8B). Post-hoc tests showed that the LCD-only infants spent significantly more time visually inspecting the intruder in the Profile phase than the Stare phase ($p=0.015$, $\eta^2=0.161$), whereas there was no difference for the Choice infants. Because biological mom's social status had a significant effect on this behavior, it was included as a covariate in the model, where a phase x biological mom social status interaction was detected as well ($F(1,34)=5.695$, $p=0.023$, $\eta^2=.143$). No other significant main or interactions effects were found.

3.4.5 Regression Models of Feeding behavior

Because CRP levels at 6 months were predicted by cumulative HCD kcals in the basic regression model, an interaction term between relative rank and the HCD kcals was entered. CRP levels were better predicted by the regression model that included the infant's relative rank, cumulative LCD kcals, cumulative HCD kcals, and an interaction between HCD kcals and relative rank ($R^2=0.359$, $p=0.007$; HCD kcals: $b=1.149$, $t(34)=3.861$, $p < 0.001$, Interaction: $b=-.900$, $t(34)=-$

2.902, p=0.007; Figure 3.9). There was a positive relationship between the cumulative HCD kcals consumed and CRP levels at 6 months for the DOM infants, but no linear relationship for the SUB infants. No other outcomes were predicted by the regression models.

3.4.6 Associations between Outcomes with Significant Social Status or Diet effects

At 2 weeks of age, no significant association was found between right AMYG-BA13 FC and CRP levels—the two outcomes with significant diet effects. At 6 months of age, we tested associations between FC in the circuits with significant effects of diet, social status, or diet x social status, and baseline cortisol, cortisol reactivity, CRP levels, rate of anxiety-like behavior in the HI task, and duration of time spent visually inspecting the intruder in the HI task, but found no significant associations. We also tested whether baseline cortisol levels and cortisol reactivity at 6 months predicted CRP levels at 6 months, but again found no significant associations.

3.5 Discussion

The results of the present study demonstrate that social subordination stress and dietary environment exert influences on neurobehavioral development and stress physiology in infant rhesus macaques, as early as 2 weeks and 6 months of age (equivalent to 2-24 months in humans). We hypothesized that social subordination would be associated with decreased FC in circuits involved in emotion regulation and promote greater caloric consumption by 6 months of age. In addition, we hypothesized that consumption of a HCD would further reduce FC in these emotion regulation circuits and impair the development of FC in circuits involved in reward-saliency processing. We posited that subordinate status and consumption of an obesogenic diet, notably a HCD, would be associated with higher basal cortisol and cortisol reactivity and

increased inflammatory markers by 6 months of age. Our findings generally supported the latter hypotheses regarding diet and status effects on physiology. However, we did not find evidence of status effects on feeding behavior, and our hypotheses about effects of social status and diet on corticolimbic circuits during infancy were not generally supported, as subordination was associated with heightened, rather than reduced, FC in emotion regulation circuits. SUB infants did not show evidence of stress-induced eating. Instead, Choice infants ate more kcals than infants on the LCD-only diet, confirming that a highly caloric dietary environment increases the number of kcals consumed, independently of social rank, and affected various outcome measures. Diet and social status impacted resting-state FC in corticolimbic circuits involved in reward processing, interoception, salience detection, and emotion regulation. The Choice diet consumed by the mother decreased AMYG-OFC FC in the infants at 2 weeks, as compared to infants raised by mothers consuming the LCD-only diet, and subordination effects on corticolimbic circuits emerged at 6 months. Subordinate infants had stronger AMYG-mPFC and INS-ACC FC than DOM infants, and there were complex interaction effects between diet and social status on INS-PFC and INS-ACC FC at 6 months. Diet condition also interacted with social status to predict baseline cortisol, cortisol reactivity, and inflammation at 6 months. Inflammation was predicted by cumulative consumption of HCD, but not LCD, kcals at 6 months, and by an interaction between HCD kcal consumption and social status; DOM, but not SUB, infants who ate more HCD kcals had higher CRP levels. No other associations between caloric intake and FC, behavior, or cortisol levels were detected, suggesting that the other diet effects reported here may be explained more by qualitative, rather than quantitative, differences between the HCD and LCD diets. Moreover, there were no differences between the infant groups in BW at 2 weeks or 6 months, supporting other findings of diet effects on neurobehavioral

development and physiology, independent of an obesogenic phenotype[388,395,448,449]. Altogether, these data indicate that subordinate status and consumption of a HCD may adversely impact physiology during infancy, and influence corticolimbic circuits involved in emotion regulation and reward processing in complex ways, as summarized in Figure 3.10. Effects of social status in infancy may be due to the infants' social experiences, exposure to biological signals through their mother's milk, or the maternal care they received, though further work is needed to explore these possibilities.

There was a main effect of social status on the dams' feeding behavior during their infant's first two weeks of life. DOM dams consumed more total kcals in the first two weeks postpartum than SUB dams. There was no difference in total kcals consumed by dams on the Choice and LCD-only diets; Choice dams ate significantly less LCD kcals than LCD-only dams, but this decrement in calories was offset by the HCD kcals they consumed. The dams' consumption pattern differed from previous reports of adult female feeding behavior, where SUBs have been reported to consume more total kcals and showed preference for the HCD diet in the Choice condition, when compared with DOMs[610]. This difference may be partly attributable to life stage: during the postpartum period dams are under a strong hormonal flux that may support different feeding preferences and behaviors. In addition, these previous reports studied unrelated small groups of females in a different housing configuration, a context that may exacerbate non-homeostatic feeding.

In the first 6 months of life, infants in the Choice group consumed more total kcals than infants in the LCD-only condition, confirming the efficacy of the diet-intervention in increasing caloric

intake during infancy. However, we did not find an effect of social status on consumption at this age point. In previous studies with adult female macaques, subordinate adult females consumed significantly more calories than dominant females when a diet of fat and sugar is available[105,610], which has been interpreted as evidence of stress-induced emotional eating. Subordinate infants may face minimal stressors in the first 6 months of life, as they typically receive little aggression[131], and therefore may not be susceptible to stress-induced eating by this developmental stage. Alternatively, subordinate infants may be exposed to stressors, possibly by observing their mother being harassed, but their stress response may be somewhat mitigated by maternal buffering, at least in the first few months of life[678]. However, the cortisol and stress physiology results suggest that the physiology of SUB infants is impacted by their low ranking social status by 6 months, although some of the subordination effects on development may be cumulative and gradual. Accordingly, the neurobehavioral adaptations that lead to stress-induced eating may not emerge until later in development.

At 2 weeks of age, consumption of the Choice diet by mothers resulted in their infants having weaker right AMYG-OFC FC compared to infants whose mothers consumed the LCD-only diet. The AMYG and OFC have been implicated in feeding behavior and are both impacted by consumption of a HCD, obesogenic diet, and/or obesity[358,386,419,424,665,679–681]. The OFC processes the reward value of taste, with neurons in this region responding to both the sight and delivery of food cues, and to the taste, texture, and macronutrient fat in food[359,682]. Taste and flavor reward neurons in the OFC modulate their firing rate in response to the quality of the good and the amount delivered[359,368,369], and decrease their firing rates as satiety is reached[683], indicating that they are tuned to the reward value of food[682]. The OFC supports conditioned-cue elicited

feeding[679], and lesions of the OFC have been linked to overeating[665]. In humans, activation in the OFC has also been found to reflect the subjective pleasantness of taste[366,684]. Neurons in the primate AMYG also respond to taste and oral texture, as well as to the sight and smell of food[685–687]. Lesions of the AMYG in rodents induces hyperphagia, adding to accumulating evidence that the AMYG modulates motivation for non-homeostatic eating[358,680,681]. The OFC and AMYG project to the hypothalamus[359], a central player in the orexigenic-satiety network, providing a tractable pathway by which these regions may modulate feeding behaviors. Diet-induced alterations to both of these regions have also been noted in the literature. Adolescent obesity has been associated with decreased OFC volume[419], and enhanced activation to visual food cues in the OFC[688]. Prenatal consumption of a high-fat diet by rat dams induced dendritic atrophy in the basolateral AMYG of offspring[386], and perinatal high-fat diet increased expression of corticosterone receptors and altered the expression of pro-inflammatory and anti-inflammatory genes in the AMYG of rodents[395]. In humans, the AMYG shows a hyper-active response to food cues in obese children[406], and deficient AMYG modulation of the OFC in a task-based fMRI study of food cues in obese vs. lean adults[424], which the authors interpreted as a reflection of impaired flexibility in updating the reward value of food. OFC dysfunction impairs the ability to update the incentive motivational value of a reinforcer as a function of context, for example in response to satiety, and can result in compulsive consumption[689]. Therefore, reduced AMYG-OFC FC in the Choice infants may reflect weakened AMYG modulation of the OFC and impairments in reward value updating, and increased motivation for hypercaloric intake regardless of satiety. At the same time, prior research also supports a role for the OFC in mediating anxious temperament and fear-related responses in adolescent monkeys[538], and reduced functional coupling between the OFC and AMYG has been reported in anxiety-prone

people and people with social anxiety disorder[488,572]. Although speculative, the finding of reduced AMYG-OFC FC in the infants exposed to the HCD through breast milk may reflect the emergence of functional changes in brain circuits that could be linked to impaired emotion regulation early in development.

Effects of social status on corticolimbic FC emerged at 6 months. Infants of SUB dams demonstrated stronger, positive FC between the left AMYG-mPFC (BA32) and left INS-ACC (BA24) compared to DOM infants. Strong FC/coupling between the AMYG and vmPFC is believed to support emotion regulation, with the vmPFC providing top-down signaling to actively suppress AMYG over-activity[362,549,690]. Studies of humans diagnosed with anxiety disorders consistently find increased AMYG reactivity during the processing of emotional stimuli[691–693], and weaker coupling between the mPFC and AMYG has been linked with increased anxiety levels during the presentation of fearful faces[549], and at rest in healthy individuals[490]. Reduced AMYG-mPFC resting-state FC has been observed after chronic stress[98], and in anxiety-related neuropsychiatric disorders[362,694]. Contrary to our expectations, SUB infants at 6 months had stronger positive FC than DOMs. One possibility is that enhanced FC in this circuit reflects a neuroadaptation early in life to prepare SUB infants for their social environment. Although they are receiving minimal aggression themselves at this age, they are likely learning about their place in the social hierarchy by observing their mother[655], and aggression directed toward their mother or other family members may be a stressor for the infant. Studies of patients with PTSD report stronger FC between the mPFC and AMYG compared to trauma-exposed controls, a finding the authors interpret as reflecting increased sensitivity to salient events and biased attentional processing[695]. It may be the case that increased AMYG-

mPFC FC in the SUB infants facilitates vigilance and increased monitoring of the environment for salient stimuli, including potential threats.

Another possibility is that SUB infants may be exposed to elevated levels of cytokines or cortisol through their mothers' breast milk, as compared to DOM infants, which may contribute to social status effects on FC in the brain. GCs are transferred from plasma to milk[696,697] (there is no evidence for mammary synthesis of GCs), and the levels in the mother's plasma and milk are highly correlated[698]. Accordingly, HPA axis activation in the mother has implications for GC levels in the milk. In fact, exposure to stressors increases GC levels in the milk of rats[699], and studies with rodent models demonstrate that GCs ingested through milk cross the infant's epithelial barrier and are present in the neonatal plasma and brain[700]. Furthermore, animals exposed to increased milk GCs during infancy display altered HPA regulation and behavioral responses to stress[461–464]. Higher GC levels in milk are linked with higher confidence in 3-4 month old male rhesus monkeys[466], while infant fear behavior was shown to be positively correlated with mother's plasma cortisol concentrations in humans, but only in infants who were breast-fed, suggesting lactational programming effects on temperament[465]. Thus, it is possible that SUB mothers, being exposed to continual stressors, had elevated cortisol levels, which were transmitted to their infants through lactation, altering neurobehavioral development, in turn. If social subordination promoted a proinflammatory state in the SUB mothers, these mothers may also transmit elevated cytokine levels through lactation. During development cytokines influence neurogenesis, differentiation, migration, neural plasticity, and synapse formation[701–704]. Cytokines have been shown to alter neurotransmitter levels[452,453], influence synaptic transmission[705,706] and FC[645,646], and elevated levels have been shown to activate microgliosis in

the PFC, ACC, and INS and play a role in stress-related disorders[157,158]. Because the DOM mothers consumed significantly more calories than SUB moms, social status effects may also arise from differences in the nutritional and bioactive signals in their milk. Lastly, status effects on neurobehavioral development may also reflect rank-related differences in maternal care during infancy, as social rank has been shown to determine patterns of maternal care in NHPs (e.g. dominant ranking vervet monkeys are more rejecting toward daughters)[707], and maternal care influences neurobehavioral development[536]. There are very few studies investigating the impact of early life stress on resting-state FC, and further longitudinal research is needed to better understand how the impacts of subordinate status on corticolimbic FC unfold developmentally and what the underlying causal mechanisms are.

Like the AMYG, the INS is also implicated in processing emotional and salient information, and is hyper-reactive to salient stimuli in anxiety-related disorders[708–710]. It has been proposed that regulation of anxious responsiveness depends on robust FC between the INS and PFC, and weak coupling is believed to provide insufficient regulation of INS reactivity[99,100]. Indeed, resting-state FC and task-based fMRI studies find that INS-PFC coupling tends to be compromised in individuals who are anxiety-prone or have been diagnosed with anxiety-related disorders[100,572,711]. In one task-based study, patients with generalized social anxiety disorder demonstrated stronger reactivity in the anterior INS (aINS) and weaker aINS-dorsal ACC (dACC) coupling when processing fearful faces, whereas healthy controls failed to show significant activity in the aINS, but had robust aINS-dACC coupling[100]. Trait anxiety levels in adolescence were also found to inversely correlate with AI-dACC FC at rest[712]. Based on this adult and adolescence literature, we hypothesized that subordinate social status would predict

weaker INS-PFC FC, but we found, instead, that SUBs had stronger FC between left INS-ACC than DOMs. This circuit has been proposed to be important in the salience network, with the INS playing a key role in detecting salient stimuli, and the ACC involved in processing signal ambiguity during learning (e.g. during fear learning and extinction), error monitoring, and recruiting the executive network to focus attention and engage higher-order cognitive processes on external stimuli[371]. Increased FC in this circuit may prepare SUB infants to quickly and effectively respond to stressors in their environment. It may be possible that by 6 months of age, SUB infants mount a stress response when their mother or other family members are the targets of threats or aggression. If so, their experience may be representative of early stages of stress and state anxiety. A resting-state FC study in adults of state anxiety found that pre-scan anxiety levels predicted stronger connectivity of the dACC with other nodes in the salience network (including the INS)[713]. The social status-related alterations to INS-ACC FC we observed may represent changes seen early in the etiology of chronic stress, and thus may be more similar in nature to state anxiety. Alternatively, SUB infants may be exposed to altered levels of hormonal and immunological signals in their moms' breast milk, e.g. cortisol and/or proinflammatory cytokines, compared to DOM infants, and these signals may shape corticolimbic circuits in the brain, as explained above.

A number of interesting diet by social status interactions were observed in FC between the INS and PFC/ACC at 6 months of age. In the circuitry connecting the right INS and dlPFC (BA46), SUBs on the LCD-only diet had stronger FC compared to DOM infants, but assignment to the Choice diet abolished this difference by significantly reducing INS-dlPFC FC in the SUB group. The circuitry connecting the INS, ACC, and dlPFC is considered part of the 'salience network',

which has been theorized to integrate external sensory and internal physiological signals, mark salient events for additional processing, and facilitate access to attention, working memory, and other cognitive resources by disengaging the default mode network and engaging the executive network, of which the dlPFC is also a key member[371]. The INS receives multimodal input and is believed to play a prominent role in bottom-up detection of salient stimuli, while the ACC is well-positioned to modulate responses in the sensory, motor, and association cortices through its connections with supplementary motor cortex and other motor areas. The dlPFC is at the interface of both the salience network and the central executive network (CEN); in this pivotal position, it is thought to facilitate access to attentional resources and provide goal-directed cognitive control over behavior[370]. An analysis of network connectivity using granger causality demonstrated that activity in the right anterior INS temporally precedes activity in the other nodes of the CEN and DMN and plays a causal role engaging the CEN and deactivating the DMN[714]. While few studies of stress/anxiety have reported alterations in dlPFC FC with the other nodes of the salience network, one study reported pre-scan state anxiety to be associated with stronger intrinsic FC between the dlPFC and the other nodes of the salience network. Increased FC between the right INS and dlPFC in SUB, LCD-only diet infants may reflect the early stages of stress and/or serve as a neurobiological adaptation that facilitates quick and efficient responses to salient social stimuli in the environment, an adaptation that may benefit them in their subordinate status, especially as development progresses. There was no social status difference in FC among the Choice infants, however, and the Choice diet reduced INS-dlPFC FC in SUB infants, a finding that could be related to neuroimaging evidence supporting a role for the dlPFC in the cognitive control of eating behavior[715]. In healthy women, but not men, activity in the right dlPFC increased in response to hedonic food, and higher activity predicted

less subsequent energy-intake in an *ad libitum* environment[715]. In obese females, grey matter volume and metabolic activity of the dlPFC was negatively associated with measures of obesity (BMI and central leptin-levels), suggesting this region may play a role in the etiology of obesity and/or is impacted by the consumption of an obesogenic diet. Our findings provide novel evidence that consumption of a HCD, as a part of a dietary choice condition, in the first 6 months of life decreases right INS-dlPFC FC in SUB infants, which may influence the infants' detection of and responsiveness to salient events in their environment, as well as control of overeating. An effect of diet on vigilance was detected in the HI task, with LCD-only infants inspecting the intruder significantly more in the Profile phase than the Stare phase, and Choice infants showing no difference between the phases. This behavioral finding supports the notion that consumption of a HCD through the Choice diet condition may influence attention to salient and threatening stimuli in the environment.

A social status by diet interaction effect was also detected in resting-state FC in the circuitry connecting the right INS and OFC (BA13) at 6 months. In the LCD-only condition, SUBs and DOMs had positive, but relatively weak resting-state FC between these regions; in the Choice group, however, DOMs showed strong positive FC that was significantly higher compared to SUBs and to DOMs in the LCD-only group. This particular circuit may be relevant to the reward-based regulation of food intake that underlies motivation to consume palatable foods. The INS includes the primary taste cortex, with neurons that respond to taste and are tuned to sweet, salt, bitter, sour, and umami, as well as viscosity, fat texture, temperature, and capsaicin[682]. A secondary cortical taste area is located in the primate OFC and it receives direct inputs from the primary taste cortex in the INS[716–718]. In addition to responding to different

tastes, neurons in the OFC also encode the reward value of tastes, as discussed previously. Thus, the circuitry connecting OFC and INS may play a role in the motivational salience of obtaining food, and of reinforcers more generally. Indeed, the INS has been implicated in craving and urges for drugs of abuse, and in the addiction literature, INS activity is increased by smoking and cocaine-related cues, is related to the intensity of a craving, and predicts likelihood of smoking relapse[719–721]. Damage to the INS, as opposed to other brain regions studied, resulted in easy and immediate cessation of smoking[722]. The INS is also implicated in cravings for food[723], and in support of the model of obesity as a food addiction, obese individuals show increased neural activation in the INS to cues of high-calorie foods[724], including in children[406], and when tasting a liquid meal[405]. The OFC also features prominently in studies of both addiction and obesity[724–726], and is believed to code the value of rewarding stimuli and their motivational significance. In a task-based fMRI study of smokers, stronger functional coupling of the OFC and INS was observed during the presentation of smoking cues vs. food cues, providing evidence that stronger connectivity in these regions may be associated with heightened incentive salience processing[727]. Altogether, this evidence supports our interpretation that consumption of the HCD diet through the Choice condition during the first 6 months of life could result in alterations to the INS-OFC circuitry, perhaps reflecting diet-induced changes that alter the hedonic drive to consume a more energy-dense, and tasty, diet. This effect was apparent only in the DOM subjects, who ate more of the HCD kcals than subordinate infants; FC remained low in the SUBs on the Choice diet. In other words, social subordination counteracted the diet-induced increases to FC, resulting in low connectivity for SUB infants. As proposed earlier, harassment toward their mother by higher-ranking adults may have been a stressor for the SUB infants, or the SUB infants may have been exposed to different levels of nutrition and biological signals in their mothers' breast milk, which

may have influenced the development of the INS-PFC circuit. Early life stress is associated with smaller INS volumes in children[66], and with increased cumulative adversity in adulthood[74], suggesting the region is sensitive to stress. While stress and anxiety-related alterations to INS FC have been reported in adults[100,712], it is unclear how stress impacts INS-PFC circuits early in development. Here we document that social status and diet interact to alter INS FC in complex ways.

At 6 months of age, social status by diet interactions also began to emerge on baseline cortisol and cortisol stress reactivity. DOMs infants in the LCD-only condition had the lowest baseline cortisol levels at sunrise; their levels were significantly lower than DOMs in the Choice condition and SUBs in the LCD-only condition. These results suggest that consumption of the HCD diet on the Choice diet (or consumption of more total kcals), and social subordination, were both experiential factors that elevated cortisol levels during infancy. The diet effect on cortisol levels is consistent with reports in the literature of obese individuals experiencing hypercortisolemia[441], and was likely detected in the dominant-ranking infants because they were eating more HCD kcals than the subordinate-ranking infants, though this difference was not statistically significant. The finding that subordination was associated with elevated baseline cortisol in 6-month olds is similar to that reported in an earlier study of subordination in 1 year-old macaque females[134], and suggests that subordination effects on cortisol levels may emerge as early as 6 months of age, possibly due to the infants' social experiences, exposure to biological signals through their mother's milk, or the maternal care they received. Interestingly, the Choice diet resulted in the highest stress-induced cortisol elevations to the acute stressor of the HI task, but uniquely for the SUB infants. Hence, the combined social stress and obesogenic diet

environment may have had a 'double hit' on cortisol reactivity at 6 months. Sustained intake of an obesogenic diet increases GC secretion in response to stressors in adult maques[105,441,442], and the current results demonstrate that diet-induced alterations to HPA axis reactivity emerge in infancy as well. No social status or diet effects were reported on baseline cortisol at birth, or 2 weeks. Furthermore, no social status or diet effects were detectable in hair cortisol at birth, or at 6 months. Altogether, these findings suggest that the effects of diet and social status on the HPA axis begin emerging around 6 months of age, otherwise they would likely have been detected in the hair cortisol measurement at 6 months.

Analysis of pro-inflammatory markers at 2 weeks and 6 months of age revealed a diet by social status interaction on CRP levels, with SUB infants having higher CRP levels than DOM infants at both ages, but only for those infants on the LCD-only diet. This finding is consistent with a rich literature documenting an association between chronic stress and persistent inflammation[643,644]. Infants are exposed to inflammatory markers and GCs through consumption of their mother's breast milk[697], which influences the infants' immune system[728]. The social status effects evident at 2 weeks could be the result of transmission of inflammatory markers from SUB dams to their infants, if the dams' levels of cytokines or GCs are elevated due to their subordinate status. Descriptively speaking, infants in the Choice condition had CRP levels that were midway between the SUB and DOM infants in the LCD-only group, with levels increasing from 2 weeks to 6 months and closely approximating the high levels seen in the SUB-LCD-only infants at 6 months, suggesting that the Choice diet elevates CRP levels, though not significantly. In the regression analyses of kcals consumed, the number of cumulative HCD kcals consumed by the infants in the first 6 months of life predicted CRP levels at 6 months, and there was a

significant interaction between HCD kcals and social status. The DOM infants show a strong linear relationship between cumulative HCD kcals consumed and CRP levels, and SUB infants show no relationship. These findings imply that diet effects on CRP levels slowly emerged in infancy, becoming evident by 6 months of age, and the effect was driven in part by the cumulative number of HCD kcals consumed, not simply consumption of the HCD diet. Obesogenic diets increase peripheral and central measures of inflammation prior to the onset of obesity[448,449], and our results suggest that diet-induced inflammation is detectable as early as 6 months (roughly equivalent to 2 years of age in human infants).

There were few effects of social status or diet on emotional behaviors in the HI task. As discussed above, the effect of diet on the duration of time spent visually inspecting the intruder may reflect diet-induced alterations to the circuitry involved in processing salient threats. A social status effect was also observed for the composite of anxiety-like behavior in the Stare condition. Dominant-ranking subjects exhibited higher rates of anxiety-like behaviors, a counter-intuitive finding that may be explained by subordinate-ranking infants' habituation to threats via observing their low-ranking mothers' receipt of intimidation. This effect of social status may also be explained by differences in maternal care provided by DOM and SUB mothers, or by differences in the nutritional and bioactive components of milk consumed by DOM versus SUB infants. The DOM dams were eating significantly more total kcals in their infants' first two weeks of life, potentially influencing both the nutritional and bioactive signals in their milk, and the SUB dams may have elevated GCs or pro-inflammatory cytokines as a result of chronic social subordination stress[643,644]. Social status differences in milk, in turn, may shape the way the infants' HPA axis and limbic regions develop and may explain the SUBS'

reduced anxiety-like behaviors during an acute stressor.

We tested whether number of kcals consumed by the dams in the first two weeks of their infants' lives, and by the infants in the first 6 months of life, predicted any of the outcomes with statistically significant effects of social status, diet, or social status x diet interaction. Other than the outcome of 6 month CRP levels, no other outcomes were associated with number of kcals consumed of either the LCD or HCD diet. This lends support to the interpretation that—aside from the CRP outcome--observed diet effects or diet by social status interactions may have been attributable to qualitative differences in the LCD and HCD diets, rather than to differences in the number of kcals consumed *per se*. The HCD diet was higher in saturated fats and sugar than the LCD diet and these macronutrient differences may influence neurobehavioral and physiological development, perhaps more so than the total caloric intake. Consumption of the HCD by dams in the Choice group may alter the macronutrient and non-nutritional components of their breast milk, for example by increasing levels of cortisol or pro-inflammatory cytokines, with possible implications for their infants' development. The differences between the LCD-only and Choice diet may also influence the quality of maternal care provided by the dams, which, in turn, may shape neurobehavioral development and account for some of the diet effects we detected. Although research on this topic is very limited, consumption of a high-fat diet increases nursing and grooming in rodent models[729,730], and higher grooming rates have beneficial effects on offspring behavior[731,732]. We also examined whether there were associations between the outcome measures to understand if GCs or pro-inflammatory markers mediated any of the observed effects, and to probe whether alterations to corticolimbic FC predicted behavioral changes. No significant associations were detected. Given the relatively small sample size in

each group, we may have been underpowered to detect such associations. It is also possible that changes in plasma cortisol and peripheral CRP levels were beginning to emerge at 6 months and were not 'online' long enough to make a discernable impact on neurobehavioral development. Future analyses with additional age points will help elucidate whether the inflammatory and cortisol changes detect in the first 6 months of life are lasting and partially mediate effects of diet and social status on the brain and behavior. Many of the FC circuits impacted by social status and diet are involved in salience detection, reward processing and interoception and, as such, may not be directly relevant to the types of behaviors evoked in the HI task—behaviors that tap emotion regulation, including anxiety-like and fear behaviors. Studies in humans and animal models find evidence of diet-induced alterations to reward-seeking and impulsive behaviors[733,734], and the circuitry altered by diet and social status in this study may relate more closely to these behaviors, which were not studied. Future work is warranted to examine whether social status and diet-induced changes to FC in infancy predicts alterations in reward-seeking behaviors and saliency detection.

It is worth noting several limitations. Some of the ROIs used in the analyses represent conglomerations of sub-nuclei or distinct sub-regions that may have unique resting-state FC patterns in the brain. The AMYG subnuclei are known to have unique connectivity patterns in the brain[501,735], and using a ROI that includes all subnuclei together may obfuscate true ROI-to-ROI FC. For example, at a given point in development, if one subnuclei has a positive FC pattern with a ROI, and another subnuclei has a negative FC pattern with a ROI, the distinct patterns may be muddled when merged together in one, unified ROI. As with the AMYG, the anterior and posterior sub-regions of the INS have been shown to have discrete connectivity patterns[736],

and we may be obscuring these nuanced patterns by using a unified ROI of the INS. Future research with subdivided ROIs could help elucidate patterns of connectivity that may have been obscured with the use of a larger ROI. Interpretation of the FC findings is also limited because we are not able to determine directionality with the resting state FC analysis we performed. As such, we cannot determine whether status or diet effects on FC between the PFC and subcortical regions reflect changes in top-down or bottom-up communication, only that they reflect altered communication between regions. The developing brain has been understudied and stronger FC with the PFC may in fact reflect stronger bottom-up signals. For example, the AMYG matures earlier than the PFC[159–161] and may drive heavier bottom-up signaling; this possibility is supported by the observation of AMYG-originating inputs to the mPFC developing earlier than mPFC-originating inputs to the AMYG in rodents[591]. Anesthesia was administered during the acquisition of the resting-state fMRI scans, presenting another limitation given the potential for differences in FC between the anesthetized and awake states. However, the isoflurane levels used in this study were lower than those administered in previous publications reporting resting-state network dynamics in the macaque brain that were similar to the awake state[513–516]. We also tested whther FC values were significantly associated with anesthesia levels and controlled for them in our models when necessary. Another limitation concerns nutritional differences between the diets. The HCD diet contained a higher ratio of ω-6 to ω-3fatty acids compared to the LCD diet. Deficiencies in ω-3 during prenatal and postnatal development result in changes to neurotransmitter metabolism[737,738], and synaptogenesis during infancy depend on the large-scale incorporation of DHA into synaptic membranes[739]. Diets deficient in DHA may have a formative and lasting impact on brain development, and recent resting-state FC work with macaque adults found that monkeys fed low levels of ω-3 throughout their lives demonstrated impairment of

distributed cortical networks[740]. Accordingly, we cannot discount the possibility that diet-related differences we observed may be attributable to differences in the availability of ω-3 in the two diet conditions. Due to small sample size, we also did not have the power to probe the effects of the prenatal environment on the outcomes of interest. Future research is warranted to disentangle the effects of prenatal and postnatal stress and diet on neurobehavioral and physiological outcomes.

In summary, the present study revealed several effects of social status and diet on resting-state FC in corticolimbic circuits, stress physiology, inflammation, and behavior during infancy. Infants from SUB mothers had higher baseline cortisol and higher inflammation in the LCD-only dietary group, and subordinate status was associated with stronger AMYG-mPFC and INS-ACC resting-state FC regardless of diet group. Remarkably, these effects suggest that subordinate status effects, whether experienced by the infant or derived through biological signals from her mother, start to impact development in the first six months of life, either due to biological signals from the mothers' breast milk, the infants learning about their status through observing their mom and/or mounting a stress response when their mom receives threats and aggression, or differences in maternal care. Consumption of the Choice diet increased cortisol reactivity to an acute stressor in SUB infants, decreased AMYG-OFC FC at 2 weeks, and altered salience detection and reward processing pathways involving INS-PFC FC in complex ways depending on the social status of the infants. The observed effects of diet on stress physiology, inflammation, and resting-state FC in stress-sensitive corticolimbic circuits during infancy are troubling, especially if they increase vulnerability to stress or lead to uncontrolled eating as development progresses. Future work is warranted to investigate whether the effects of social

status and diet detected in the first 6 months of life continue to progress and predict feeding and socioemotional behaviors further in development.

Table 3.1. Experimental Groups. SUB and DOM dams were followed during pregnancy, and at birth, approximately half the newborns remained with their biological mom and half were cross fostered to dams of the same or different social status as their biological mom within 48 hours using well-established protocols[660]. The infant-dam pairs were pseudo-randomly assigned to the LCD-only diet or the Choice diet with access to both the LCD and HCD food pellets.

	Foster Mom				Biological Mom	
Dam's Prenatal Rank	SUB	DOM	DOM	SUB	DOM	SUB
Infant's Postnatal Rank	DOM	SUB	DOM	SUB		
LCD-only	4	1	2	3	5	6
Choice (LCD + HCD)	4	2	2	3	4	5

Table 3.2. Summary of Feeding Behavior. This table summarizes kcal consumption of the LCD and HCD by the infants through the first 6 months of life, and by the dams in their infants' first two weeks of life.

	INFANT FEEDING			
	Choice		LCD-only	
Mean ± SE:	**DOM**	**SUB**	**DOM**	**SUB**
Accumulated LCD kcals	5382 ± 982	3725 ± 527	7879 ± 1186	5803 ± 839
Accumulated HCD kcals	8058 ± 1532	6712 ± 2521	376 ± 313	35 ± 17
Accumulated Total Kcals	13440 ± 2075	10438 ± 2644	8255 ± 1281	5838 ± 845
	DAM FEEDING			
	Choice		LCD-only	
Mean ± SE:	**DOM**	**SUB**	**DOM**	**SUB**
Accumulated LCD kcals	5370 ± 676	4764 ± 412	12595 ± 2037	9046 ± 1163
Accumulated HCD kcals	5833 ± 1447	3415 ± 896	25 ± 15	12 ± 8
Accumulated Total Kcals	11254 ± 1466	8179 ± 881	12620 ± 2038	9058 ± 1158

Table 3.3. Summary of Significant Effects of Social Status and Diet on Resting-state FC in Corticolimbic Circuitry: ANOVA Analysis. * and **bold font** indicate a significant *p* value at < 0.05.

ANOVA Analysis of Resting-state FC in Corticolimbic Circuitry												
Effects of Diet												
	2wks Right AMYG - BA13 iFC (z)											
	F	p	η	Main Effect Direction								
Infant Social Status	0.647	0.427	0.02									
Diet	6.947	**0.013***	0.183	LCD > Choice								
Infant Social Status x Diet	0.586	0.45	0.019									
Telazol	0	0.989	0									
Effects of Social Status												
	6mo Left INS - BA24 iFC (z)				**6mo Left AMY - BA32 iFC (z)**							
	F	p	η	Main Effect Direction	F	p	η	Main Effect Direction				
Infant Social Status	4.82	**0.034***	0.115	SUB > DOM	4.13	**0.049***	0.1	SUB > DOM				
Diet	0.763	0.388	0.02		0.243	0.625	0.007					
Infant Social Status x Diet	0.021	0.887	0.001		1.101	0.301	0.029					
2wk FC												
Telazol												
Effects of Diet x Social Status												
	6mo Right INS - BA46 iFC (z)				**6mo Right INS - BA24 iFC (z)**				**6mo Right INS - BA13 iFC (z)**			
	F	p	η	Post-Hoc Tests	F	p	η	Post-Hoc Tests	F	p	η	Post-Hoc Tests
Infant Social Status	0.471	0.497	0.013		0.172	0.681	0.005		2.001	0.166	0.051	
Diet	1.304	0.261	0.035		0.198	0.659	0.005		1.347	0.253	0.035	
Infant Social Status x Diet	7.386	**0.01***	0.17	SUB-LCD-only > SUB-Choice DOM-LCD-only < SUB-LCD-	5.653	**0.023***	0.133	DOM-Choice > DOM-LCD-only	4.964	**0.032***	0.118	SUB-Choice < DOM-Choice DOM-Choice > DOM-LCD-
2wk FC												
Telazol												

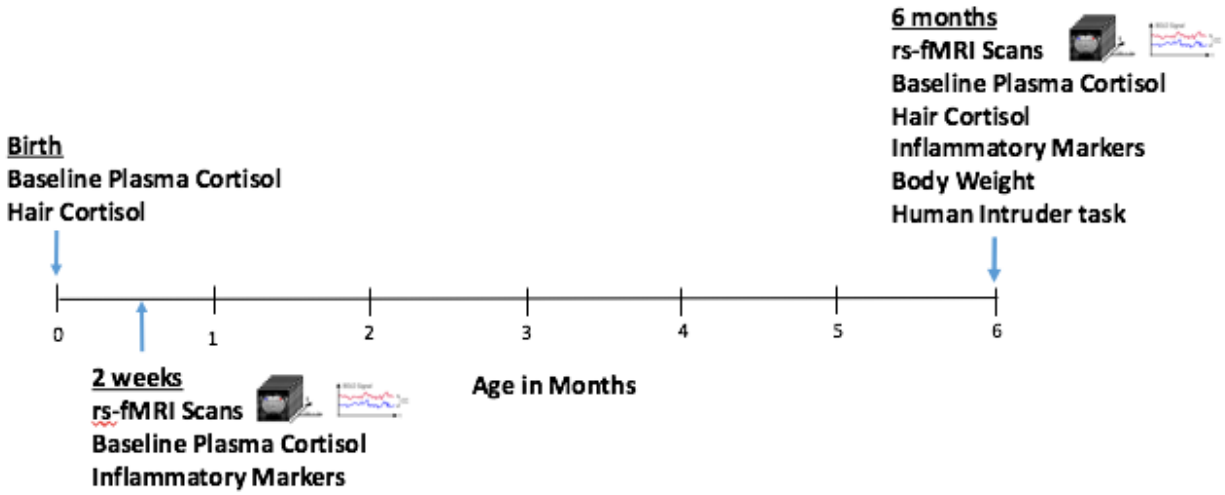


Figure 3.1 Experimental Timeline. At birth, hair was shaved from the back of the neck and blood was collected in the early AM (sunrise); both were assayed for cortisol levels. At 2 weeks of age, blood was collected at sunrise and assayed for baseline cortisol and pro-inflammatory cytokines. The infants were also brought to the MRI center for the collection of resting-state fMRI scans. At 6 months of age, we collected blood at sunrise to assay for baseline cortisol and pro-inflammatory cytokines. The back of the infants' neck was shaved to assay the hair for cortisol accumulation during the first 6 months of life. The HI task was administered and blood was collected immediately following the test and 4-hours after the test. BWs were recorded and resting-state fMRI scans were collected at this age point. Feeding was measured from birth through 6 months.

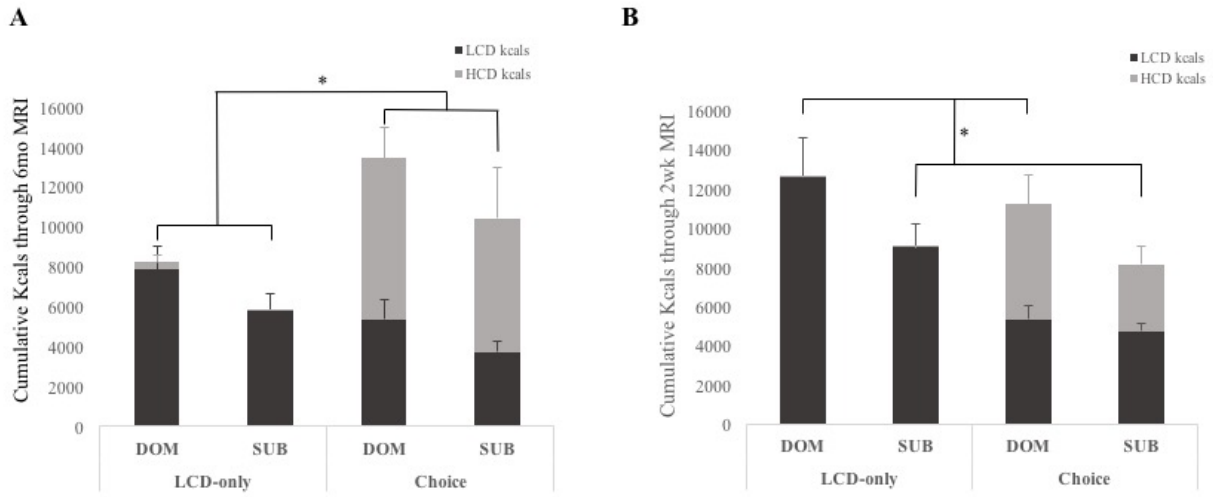


Figure 3.2. Cumulative Consumption of LCD and HCD kcals by Dams and Infants.

A) Infant Feeding: Choice infants consumed more total cumulative kcals through the 6 month MRI scan than infants on the LCD-only diet ($F(3,34)=6.338$, $p=0.017$, $\eta^2=.157$). The Choice infants did not show a significant preference for the HCD kcals, and DOM and SUB infants on the Choice diet did not differ in the number of HCD kcals they consumed. B) Dam Feeding: The dams showed a different consumption pattern in the first 2 weeks of their infants' lives. DOM dams consumed significantly more total kcals than SUB dams ($F(3,31)=5.165$, $p=0.030$, $\eta^2=.143$). Dams on the LCD diet consumed significantly more of the LCD diet than dams on the Choice diet ($F(3,31)=28.844$, $p< 0.001$, $\eta^2=.482$). The dams on the Choice diet did not significantly differ in the amount of HCD and LCD diet they consumed, and there was no effect of social status on kcals consumed of the HCD pellets within the Choice group. * indicates a significant *p* value at < 0.05.

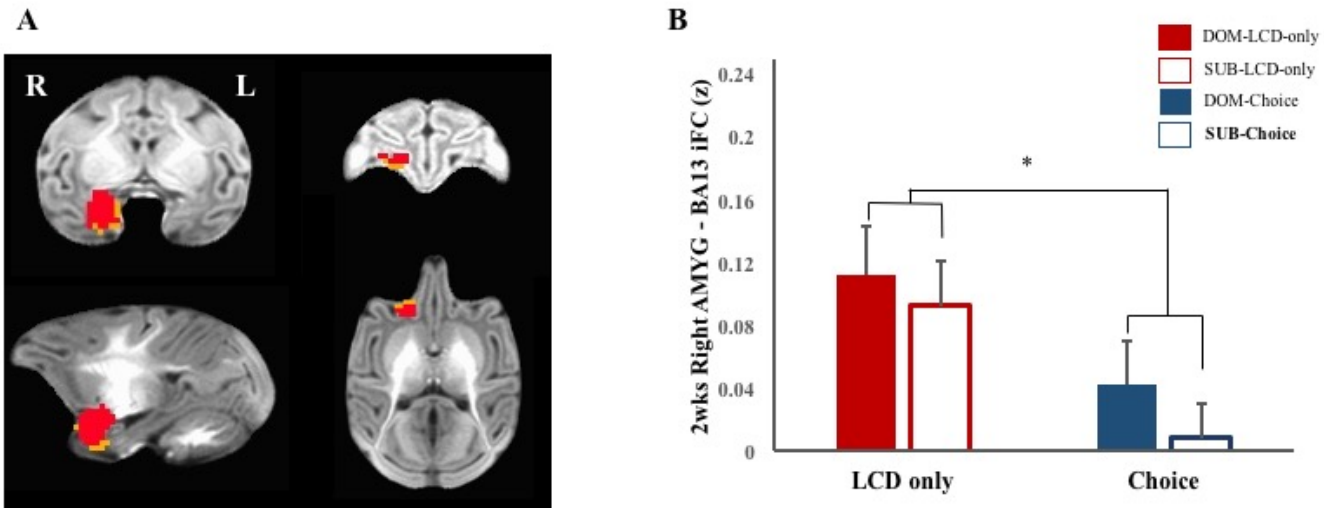


Figure 3.3. Main Effect of Diet on Right AMYG-BA13 FC at 2 weeks of age. A) We analyzed FC between the Right AMYG[532] (Left panel) and the OFC (Markov-defined BA13[677] in right panel). The ROIs were eroded to exclude voxels with low signal intensity (dropout). The orange voxels represent voxels that were excluded due to dropout. B) At 2 weeks of age, LCD-only infants had significantly more positive FC between right AMYG and OFC (BA13) compared to infants on the Choice diet ($F(4,31)=6.947$, $p=0.013$, $\eta^2=.183$). * indicates a significant *p* value at < 0.05.

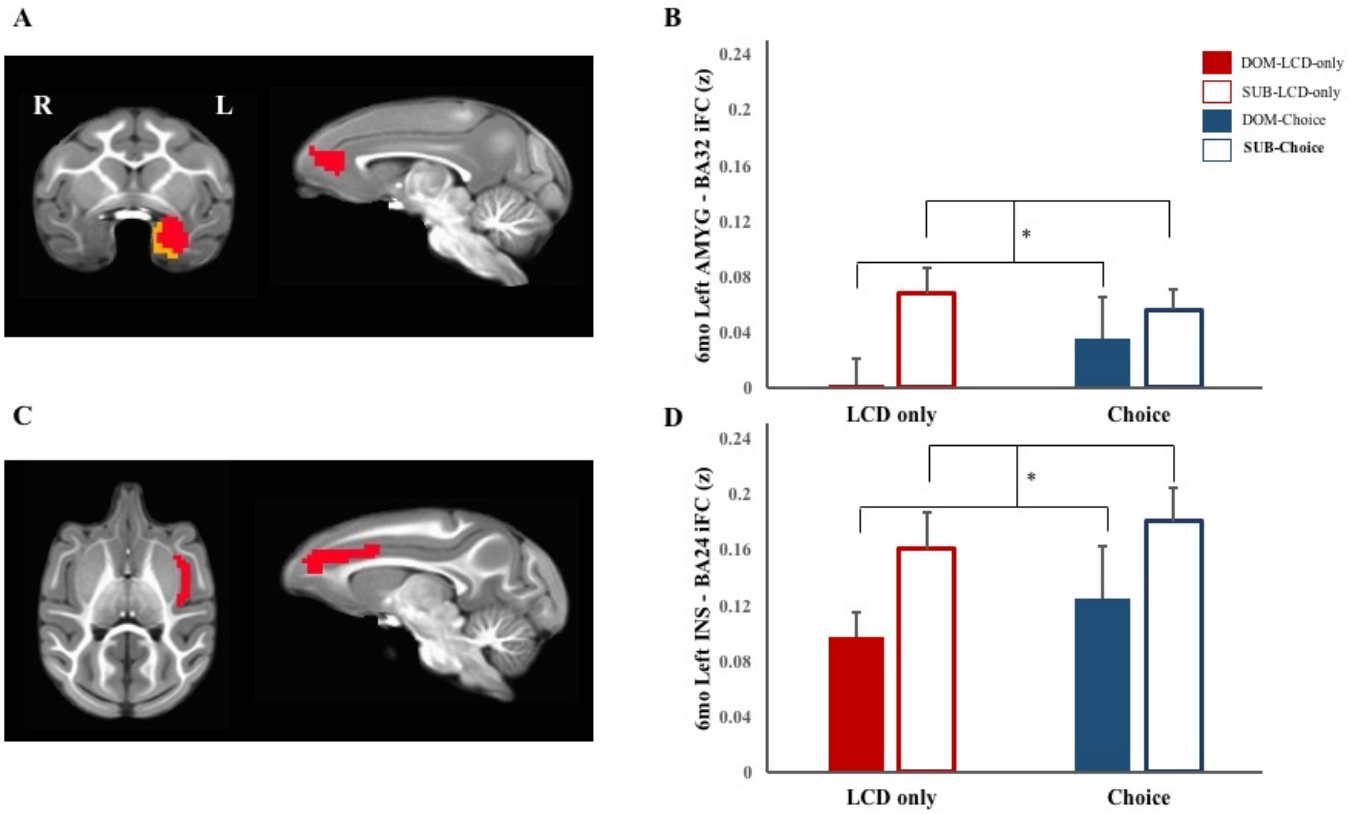


Figure 3.4. Main Effects of Social Status on FC at 6 months of age. A) Signal was extracted from a previously defined AMYG ROI[532] that was eroded to exclude voxels with low signal intensity (signal dropout; shaded orange), and the BA32 Markov ROI[677]. B) The SUB infants had stronger positive FC between left AMYG and mPFC (BA32) ($F(3,37)=4.130$, $p=0.049$, $\eta^2=.100$). C) The left INS ROI was defined by the Lewis and Van Essen anatomical parcellations[530], and the BA24 ROI was defined by the Markov parcellations[677]. D) There was a main effect of social status on FC between left INS and ACC (BA24) ($F(3,37)=4.820$, $p=0.034$, $\eta^2=.115$), with SUB infants having significantly stronger, more positive, FC than DOM infants. * indicates a significant *p* value at < 0.05.

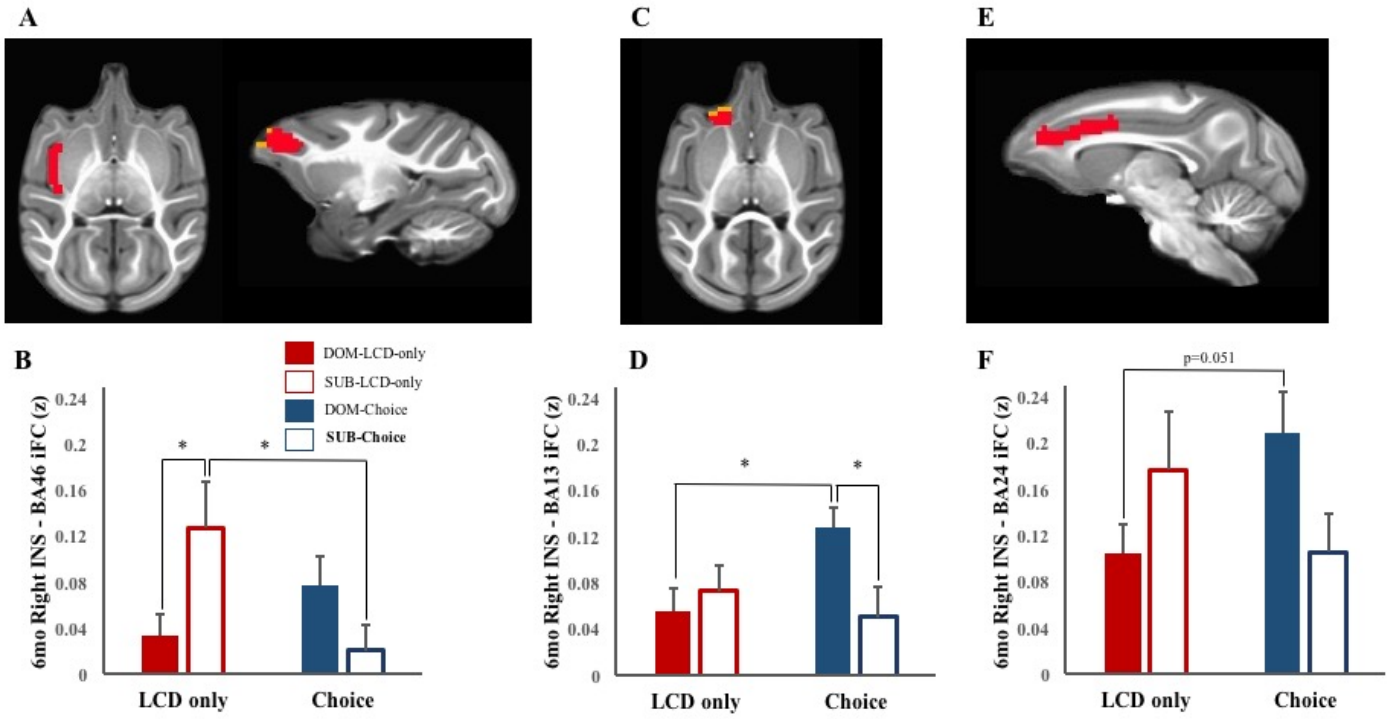


Figure 3.5. Social Status x Diet Interaction Effects on FC at 6 months of age. A) Location of the right INS ROI (based on LVE macaque brain parcellations[55]) and the BA46 Markov ROI[677] used to analyze FC in this circuitry at 6 months of age. The BA46 ROI was eroded to exclude voxels where signal intensity was low (signal dropout; shaded orange). B) There was a social status x diet interaction on right INS-BA46 FC ($F(3,36)=7.386$, $p=0.01$, $\eta^2=.170$), with SUBs on LCD-only diet showing significantly stronger FC compared to both SUBs on the Choice diet ($p=0.01$, $\eta^2=.172$), and to DOMs on the LCD-only diet ($p=0.018$, $\eta^2=.145$). C) FC between the right INS shown in panel A and a BA13 Markov ROI was also analyzed. D) A social status x diet interaction effect ($F(3,37)=4.964$, $p=0.032$, $\eta^2=.118$) was detected, with DOMs on Choice diet showing significantly higher FC vs. DOMs on LCD-only diet ($p=0.02$, $\eta^2=.137$), and SUBs on Choice ($p=0.015$, $\eta^2=.149$). E) FC between the right INS (shown in panel A) and right ACC (BA24) was analyzed. F) A social status x diet significant interaction was also detected for the

Right INS-BA24 FC ($F(3,37)=5.653$, $p=0.023$, $\eta^2=.133$); although the post-hoc tests were not significant, there was a trend for DOMs on the Choice diet to have significantly higher FC than DOMs on the LCD-only diet ($p=0.051$, $\eta^2=.099$). * indicates a significant *p* value at < 0.05.

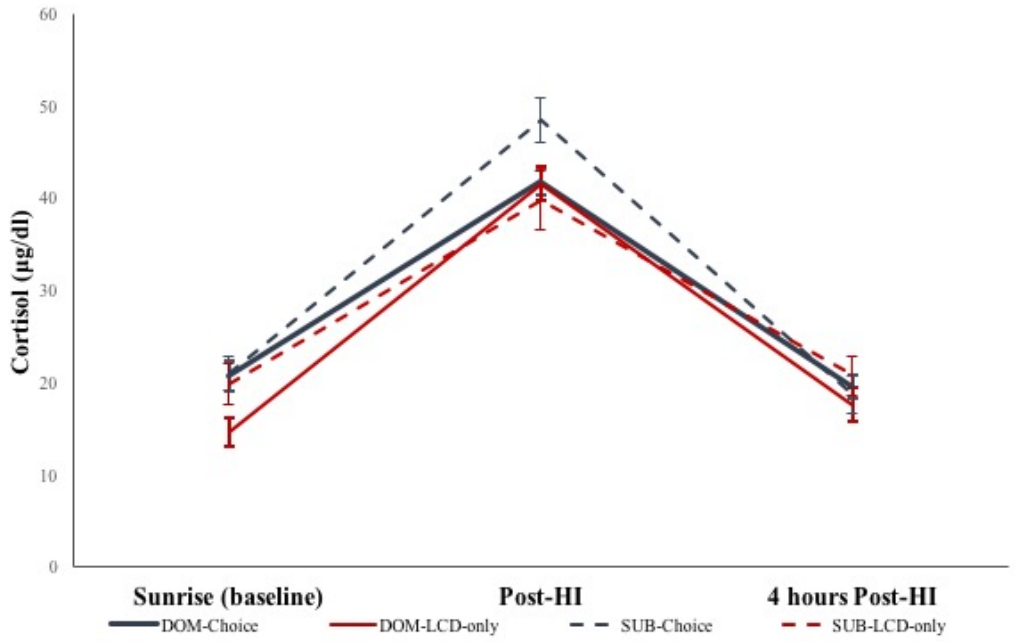


Figure 3.6. Effects of Social Status and Diet on Baseline Cortisol and Reactivity to the HI task. With a cortisol outlier excluded, rmANOVA of the 6 month plasma cortisol levels at sunrise, post-HI, and 4 hours post-HI detected a main effect of time point ($F(2,64)=220.697$, $p < 0.001$, $\eta^2=0.877$), and a social status x diet x time point interaction ($F(2,64)=4.292$, $p=0.018$, $\eta^2=0.122$). Post-hoc tests revealed that at sunrise, DOMs in the Choice diet group had significantly higher cortisol than DOMs in the LCD-only group ($p=0.028$, $\eta^2=0.147$), and SUBS had higher cortisol than DOMS in the LCD-only group ($p=0.047$, $\eta^2=0.121$). SUBs on the Choice diet mounted a significantly higher cortisol stress response than SUBS on the LCD-only diet ($p=0.015$, $\eta^2=0.178$).

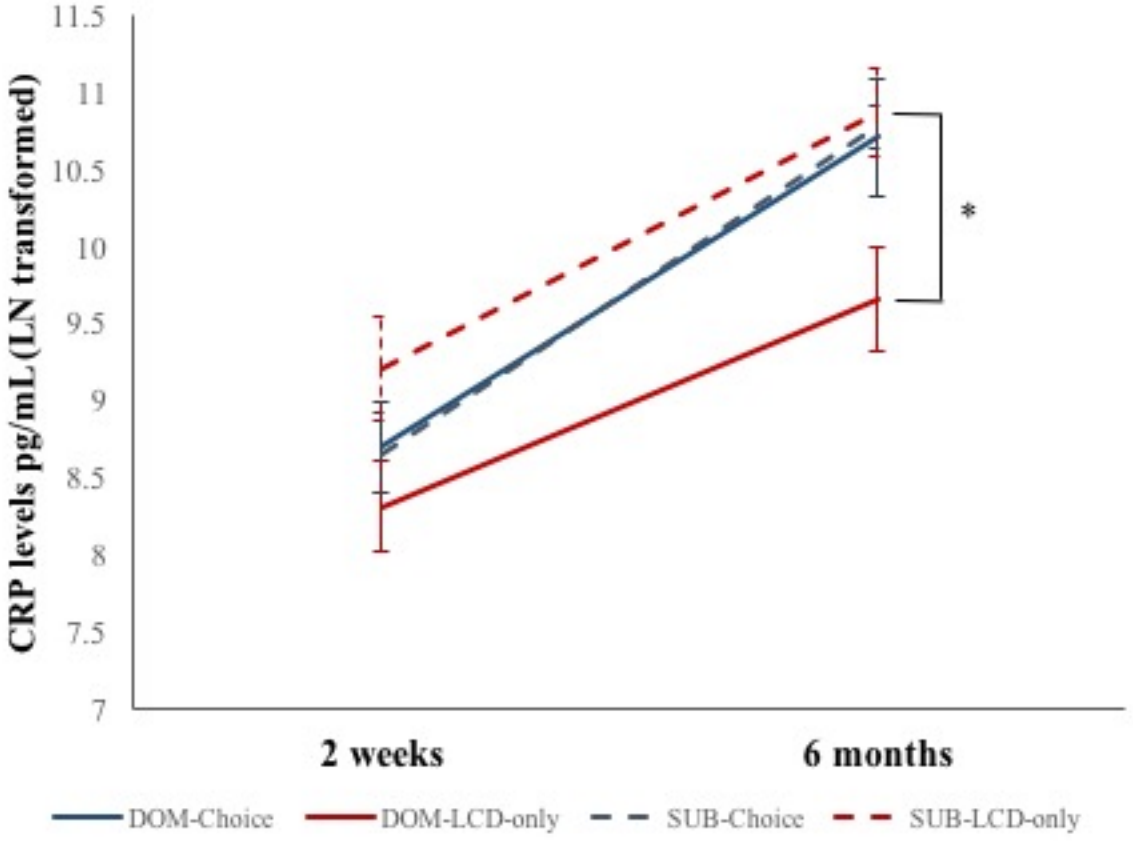


Figure 3.7 CRP levels at 2 weeks and 6 months are impacted by Social Status and Diet. When outliers were excluded from the model, there was a main effect of social status on CRP levels at 2 weeks and 6 months ($F(1,34)=4.524$, $p=0.041$, $\eta^2=0.117$) that was qualified by a social status x diet interaction ($F(1,34)=4.270$, $p=0.046$, $\eta^2=0.112$). SUBs in the LCD-only group had significantly higher CRP levels than DOMs in the LCD-only group ($p=0.005$, $\eta^2=0.205$).* indicates a significant *p* value at < 0.05.

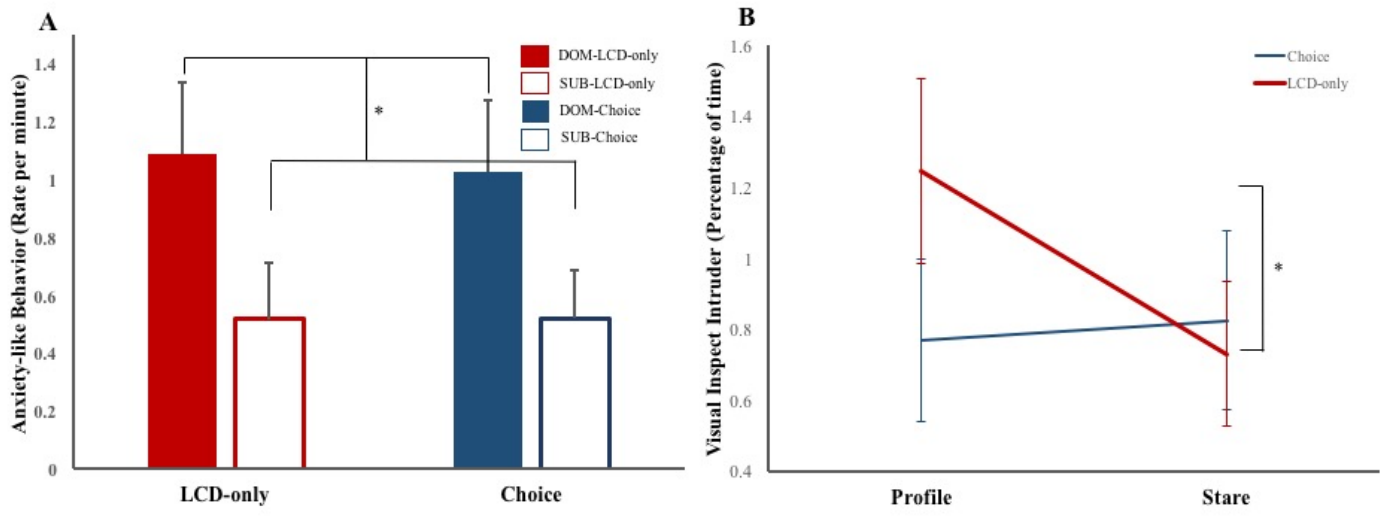


Figure 3.8 Behaviors in the HI task show effects of social status and diet condition. A) DOMs exhibited higher rates of anxiety-like behavior than SUBs in the Stare phase of the HI task ($F(3,36)=6.1$, $p=0.018$, $\eta^2=0.145$), regardless of diet condition. B) There was a HI phase x diet interaction for the duration of time spent visually inspecting the intruder ($F(1,34)=4.279$, $p=0.046$), with LCD-only infants spending significantly more time visually inspecting the intruder in the Profile phase than the Stare phase ($p=0.015$, $\eta^2=0.161$), whereas there was no difference across the two conditions for the Choice infants. .* indicates a significant *p* value at < 0.05.

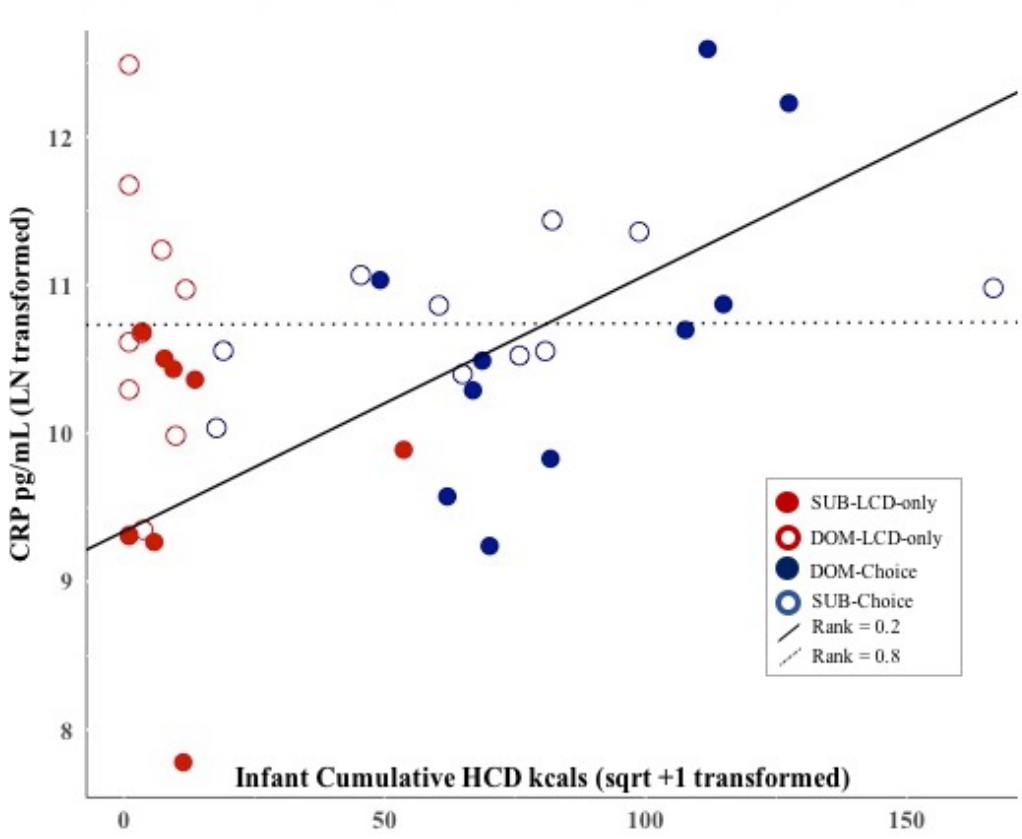


Figure 3.9 Cumulative HCD kcals consumed by infants in first 6 months of life interact with Relative rank to Predict Inflammation. The CRP levels at 6 months were predicted by a regression model that included the relative rank of the infant's mom, the LCD kcals, the HCD kcals, and an interaction between HCD kcals and relative social status (R^2=0.359, p=0.007; HCD kcals: *b* =1.149, t(34)=3.861, p < 0.001, Interaction: *b* =-.900, t(34)=-2.902, p=0.007). There is a strong positive relationship between HCD kcals consumed by the DOM infants and CRP levels at 6 months, but no relationship for the SUB infants. The solid line indicates the trend line for infants with relative rank = 0.2 (DOM), and the dashed line indicates the trend line for infants with relative rank = 0.8 (SUB).

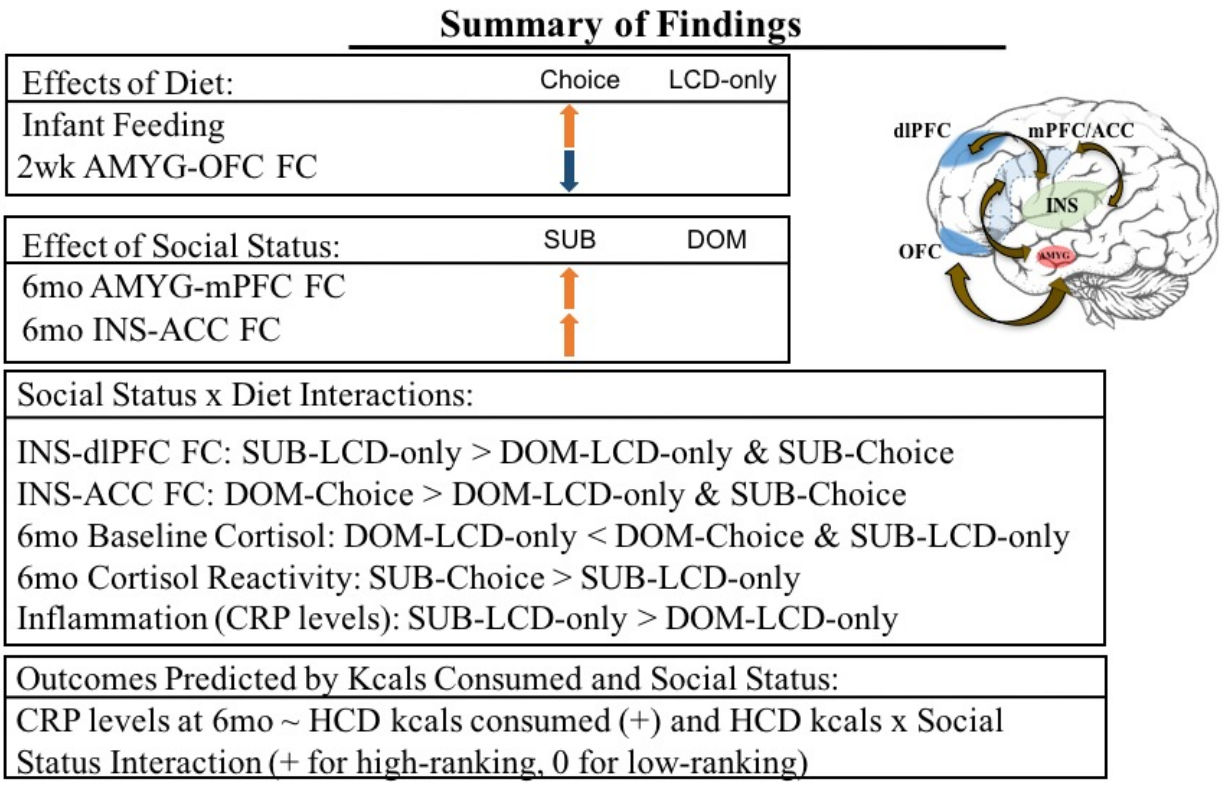


Figure 3.10 Summary of Findings. The figure summarizes the main effects of diet, social status, and diet x social status interactions, in addition to the findings from the regression models with Kcals consumed and social status as predictors.

Chapter 4. General Discussion

4.1 Summary of Results

4.1.1 Summary of Chapter 2

The goal of chapter 2 was to examine the interaction effects of chronic stress and pubertal timing on neurobehavioral development during adolescence, which is difficult to study in humans. Prior studies have shown that subordination impacts behavioral development[108], delays the onset of puberty[108] and affects the development of white matter tracts in the prefrontal cortex (PFC) in pre-pubertal juveniles[134]. It remains an open question, though, whether subordination affects the maturation of corticolimbic functional connectivity (FC) during adolescence, particularly connectivity between the amygdala (AMYG) and PFC important for stress and emotional regulation, and whether stress-related impacts on the brain are modified by pubertal timing. To address these questions and as part of a longitudinal study using social subordination as a model of psychosocial stress, female macaques were randomly assigned to either experience puberty spontaneously or receive monthly injections of the gonadotropin-releasing hormone (GnRH) agonist Lupron to experimentally delay puberty. The Lupron treatment was included as part of the experimental design to allow us to disentangle the effects of subordination status and pubertal timing, as these were found to be associated in the untreated group[108], with lower-ranking (subordinate) females showing delayed pubertal onset in comparison to dominant animals. We examined the effects of social rank and delayed exposure to the pubertal rise of estradiol (E2) on socioemotional behavior and resting-state functional connectivity (FC) in corticolimbic circuits that regulate emotional/stress reactivity in adolescent female macaques.

We hypothesized that social subordination stress would predict reduced AMYG-PFC FC and impaired emotional regulation postpubertally, while pubertal delay would mitigate the adverse

effects of subordination stress at a postpubertal developmental time point. Accordingly, we expected an interaction between treatment and social status; among untreated females, we expected a linear relationship with more subordinate monkeys having poorer neural and emotional outcomes, while no such relationship would be evident among the females previously treated with Lupron (PTL). The hypotheses concerning social subordination were partially supported, in that social subordination adversely impacted AMYG-dorsolateral prefrontal cortex (dlPFC) FC and some socioemotional outcomes, but no significant interactions between PTL and relative rank were detected; the study was underpowered for detecting interactions and dropout in some of the ROIs may have prevented detection of meaningful FC differences. FC between the right AMYG and dlPFC (BA46) was weaker in more subordinate subjects, who also showed lower 'Explore' scores in the alone condition of the HI paradigm and initiated less proximity with non-kin in their social groups, suggesting impairments in emotion regulation circuits and behavior. While no interactions between PTL and relative rank were detected, a number of main effects of PTL were unexpectedly found. PTL resulted in stronger AMYG-orbitofrontal cortex (OFC) FC and lower anxiety-like behaviors in the social groups, suggesting that delayed puberty may strengthen corticolimbic communication critical for some aspects of emotion regulation. These beneficial outcomes may confer some protection against the negative impacts of chronic stress for the lower-ranking macaques who received Lupron treatment. However, prior Lupron treatment also resulted in increased frequency of submissive behavior, lower 'Explore' scores in the HI paradigm, and lower frequency of initiating proximity towards non-kin, indicating that pubertal delay with Lupron treatment may have adverse consequences for some socioemotional outcomes. For the PTL group only, stronger AMYG-dlPFC FC predicted lower rates of initiating submissive behavior, and stronger AMYG-medial PFC (mPFC) FC predicted higher rates of initiating proximity with non-

kin. The effects of Lupron treatment may be a consequence of E2 having different organizational effects on particular circuits or neurotransmitter systems due to delayed pubertal timing, or a consequence of the subjects who were treated with Lupron being studied in a relatively earlier developmental stage of adolescence.

4.1.2 Summary of Chapter 3

The aim of chapter 3 was to investigate the potential for a synergistic impact of social subordination and an obesogenic diet on infant brain development longitudinally, using a translational macaque model of social subordination stress. Disentangling the contributions of stress, diet, and feeding behavior on neurobehavioral development, as well as the underlying biological mechanisms (e.g. increased glucocorticoids (GCs), proinflammatory signals, both) in children is difficult, considering the challenges associated with prospective, longitudinal studies that standardize and monitor accurate food intake under rigorous experimental control. Social subordination in female rhesus macaques is a useful and well-validated translational animal model of chronic psychosocial stress[104,107]. Social subordination is a naturalistic stressor that produces a number of distinct stress-related phenotypes[104]. Infants acquire the relative ranks of their mothers rank at birth[115], and their early neurobehavioral development may be shaped by their subordinate status, either through their social experiences or biological signals transmitted from their mother. Through observational learning, the infant observes its mother's rank-related social interactions early in development and learn its own position in the social hierarchy through imitation or identification with the mother[655]. Subordinate infants may start to receive minimal aggression by this point, albeit minimal[131], or perceive harassment directed to their mothers or other family members as stressful. Social status effects in infancy may also be communicated

through immunological and hormonal constituents in breastmilk, such as immunoglobulins, cytokines, GCs, and other hormones like leptin[656–658]. Studying rhesus macaques in infancy affords an opportunity to better understand how social status influences neurobehavioral development via biological signals and/or vicarious learning about the social environment. Importantly, subordinate macaques have also been shown to be susceptible to stress-induced overeating of an obesogenic diet[105,610]. Adult subordinates have been found to eat approximately twice as many calories when a diet of fat and sugar is available[610], and juvenile females have been shown to be susceptible to diet-induced increased fat mass[326]. Using the social subordination model in rhesus macaques, we sought to investigate how social subordination interacts with dietary environment during infancy to affect the development of stress neuroendocrine systems (Hypothalamic-pituitary-adrenal (HPA) axis), proinflammatory markers, emotional reactivity, feeding behavior, as well as FC in corticolimbic circuits important to interoception, reward processing, and stress/emotional regulation. Additionally, we examined whether stress-induced or diet-induced neurobiological alterations were associated with elevations in GC or pro-inflammatory cytokines. We hypothesized that social subordination will begin to promote hypercaloric consumption and decrease FC in circuits subserving emotion regulation and impulse control by 6 months of age, and consumption of a high-calorie diet (HCD) will further reduce FC in these corticolimbic circuits and impair the development of FC in circuits involved in reward-saliency processing. We also hypothesized that subordination status and consumption of a HCD would predict alterations in the HPA axis (higher basal cortisol and cortisol reactivity) and increased inflammatory markers by 6 months of age, and these alterations may be associated with changes in neurobehavioral development.

The results of this study demonstrated that social subordination stress and dietary environment exert influences on neurobehavioral development and stress physiology in infant rhesus macaques, as early as 2 weeks and 6 months of age (equivalent to 2-24 months in humans). Our findings generally support the hypotheses regarding diet and status effects on physiology. However, we did not find evidence of status effects on feeding behavior, and our hypotheses about effects of social status and diet on corticolimbic circuits during infancy were not generally supported, as subordination was associated with heightened, rather than reduced, FC in emotion regulation circuits. SUB infants did not show evidence of stress-induced eating. Instead, Choice infants ate more kcals than infants on the low-calorie-diet only (LCD-only), confirming that a highly caloric dietary environment increases the number of kcals consumed, independently of social rank, and affected various outcome measures. Diet and social status impacted resting-state FC in corticolimbic circuits involved in reward processing, interoception, salience detection, and emotion regulation. The Choice diet consumed by the mother decreased AMYG-OFC FC in the infants at 2 weeks, as compared to infants raised by mothers consuming the LCD-only diet, and subordination effects on corticolimbic circuits emerged at 6 months. Subordinate infants had stronger AMYG-mPFC and Insula-Anterior Cingulate Cortex (INS-ACC) FC than DOM infants, and there were complex interaction effects between diet and social status on INS-PFC and INS-ACC FC at 6 months. Diet condition also interacted with social status to predict baseline cortisol, cortisol reactivity, and inflammation at 6 months. Inflammation was predicted by cumulative consumption of HCD, but not LCD, kcals at 6 months, and by an interaction between HCD kcal consumption and social status; DOM, but not SUB, infants who ate more HCD kcals had higher CRP levels. No other associations between caloric intake and FC, behavior, or cortisol levels were detected, suggesting that the other diet effects reported here may be explained more by

qualitative, rather than quantitative, differences between the HCD and LCD diets. Moreover, there were no differences between the infants groups in body weight at 2 weeks or 6 months, supporting other findings of diet effects on neurobehavioral development and physiology, independent of an obesogenic phenotype[388,395,448,449]. Altogether, these data indicate that subordinate status and consumption of a HCD may adversely impact physiology during infancy, and influence corticolimbic circuits involved in emotion regulation and reward processing in complex ways, as summarized in Figure 3.10. Effects social status in infancy may be due to the infants' social experiences, exposure to biological signals through their mother's milk, or the maternal care they received, though further work is needed to explore these possibilities.

4.2 Integration of Findings

The overarching goals of this dissertation were to investigate the neurobehavioral impact of social subordination stress during development and examine how experiential factors modify chronic stress-related changes to corticolimbic circuits, behavior, and stress physiology. Social subordination in rhesus macaques provides a naturalistic model for studying the impacts of chronic psychosocial stress on neurobehavioral development[108]. Social subordination in rhesus and other macaques is a useful translational model for studying the effects of chronic social stress[46,52,103–109]. Subordinate status has been associated with hypercortisolemia[46], cardiovascular disease[122], compromised immune system[123,124], reproductive dysfunction[125–127], psychostimulant self-administration[128], emotional feeding[105], and alterations in emotion regulation[103]. Using the social subordination model of chronic stress, we found effects of social status on corticolimbic FC and emotional behaviors at 6 months and 3.5-years of age. These findings suggest that biological embedding of subordinate status emerges as early as 6 months of age in female rhesus

macaques, consistent with earlier work demonstrating effects of subordination on white matter tracts in prepubertal female macaques—alterations which themselves were associated with increased emotionality in subordinates[134]. While subordinate status was associated with alterations to corticolimbic FC during both infancy and adolescence, the particular circuits affected, and the direction of change (higher or lower), differed at the two developmental time points. The findings reported here suggest the possibility that subordination affects corticolimbic FC differently in early life vs. during the pubertal transition. For example, FC between the AMYG-mPFC was stronger in 6 month old subordinate infants compared to dominant infants, but no effects of subordination were detected in AMYG-mPFC FC in adolescent macaques. Instead, subordinate adolescent females had weaker FC between the AMYG and dlPFC compared to dominant females. The fact that subordination had different effects in infancy versus adolescence may be due to developmental variation in social experience, maternal biological signals, and the region-specific maturation of neural circuits. For example, subordinate infants at 6 months of age receive little direct aggression from other group members at this time[131] and may benefit from social buffering from their mother[678], whereas subordinate adolescents receive harassment from other group members[134] and may receive inadequate social buffering from their peers, given the finding that they are less likely to initiate proximity with non-kin. Infants are breast feeding early in life, and the biological signals transmitted through milk, including cortisol and cytokines[697], may affect neurobehavioral outcomes differently, as compared to the biological mechanisms by which subordination stress may impact the brain and behavior during adolescence, e.g. endogenous changes in cortisol and pro-inflammatory cytokines. The HPA axis and PFC circuits mature from infancy to adolescence[159–161,565], so differences in neural outcomes between these developmental time points may also be due to

differences in HPA axis function (and glucocorticoid levels), and the vulnerability of particular circuits to allostatic load, depending on when they are undergoing rapid change and may be more vulnerable to the effects of stress. Alternatively, it is possible that the detection of subordination effects on AMYG-dlPFC FC in adolescence but not infancy suggests that alterations to AMYG-dlPFC FC begin to emerge only after years of chronic stress, independently of the developmental time point.

It is also noteworthy that subordinate infants exhibited *higher* AMYG-mPFC and INS-ACC FC at 6 months of life, while subordinate adolescents demonstrated *lower* AMYG-dlPFC, compared to dominant females. Whereas the finding of decreased AMYG-dlPFC FC in adolescent subordinates is consistent with research demonstrating associations between chronic occupation stress and reduced AMYG-dlPFC FC[98], the finding of increased AMYG-mPFC and INS-ACC FC in subordinate infants is an unexpected finding and generally inconsistent with other research findings of early life stress effects on resting-state FC[100,362,572,694,711]. However, these differences may be due to the chronicity of the stressor. Chronic stress has been shown to reduce AMYG-mPFC resting-state FC in humans[98], and AMYG-mPFC and INS-PFC/ACC FC is reduced in individuals who are anxiety-prone or diagnosed with anxiety-related disorders[100,362,572,694,711], with impairments in these circuits linked to insufficient regulation of AMYG and INS reactivity[99,100,549]. However, this literature is in adults and it is possible that the inconsistencies are due to developmental differences in the circuit. It is also possible that increased FC in these circuits at 6 months reflect a neuroadaptation that enables subordinate infants to effectively cope with their unpredictable social environment. Subordinate infants may be exposed to stressors, possibly by observing their mother being targeted and harassed. Social status effects at this age

may also be influenced by biological signals transmitted to the infants from their mothers through lactation, including cortisol and cytokines[697]. The dominant-ranking dams ate significantly more calories on both the LCD-only and Choice diet during their infants' first two weeks of life, and higher calorie consumption may influence the nutritional and non-nutritional composition of their milk, which may have influenced unexpected social status effects on infant development, through lactational programming. There are very few studies investigating the impact of early life stress on the development of resting-state FC, and ongoing collection of longitudinal research from the stress and diet study, and analysis of social behaviors and the nutritional and biological signals in the mothers' milk, will allow a better understanding of how these impacts unfold across development and what underlying biological mechanisms may be driving the effects.

The findings presented in this dissertation demonstrate that, in addition to social status, dietary environment and pubertal timing are important experiential factor that influence neurobehavioral and physiological development. We detected interactions between social status and diet on corticolimbic FC, inflammation, and stress physiology during infancy, suggesting that dietary condition modifies the impact of social subordination stress on neurobehavioral and stress physiology outcomes during infancy. Dominant infants on the Choice diet had significantly lower baseline cortisol levels than subordinate infants on the same diet and compared to dominant infants on the LCD-only. In other words, there was a social status difference between the infants on the LCD-only diet (dominants have lower baseline cortisol than subordinates), but the Choice diet abolished this social status difference. A similar pattern was true of inflammation as well. Among the infants on the LCD-only diet, CRP levels were significantly higher in the

subordinate infants compared to dominant infants, but there was no social status difference for the Choice infants. By 6 months, CRP levels in the Choice infants had increased to the levels seen in subordinates in the LCD-only condition, suggesting that the absence of a social status effect in Choice infants was because the Choice diet increased CRP levels for both subordinate and dominant infants to similar (and high) levels. These findings are novel and indicate that consumption of a HCD (through the Choice dietary environment) has adverse effects on development early in life by elevating levels of cortisol and CRP in a non-human primate model. While the results do not suggest a synergistic interaction between diet and social status, they demonstrate that dietary environment is an important experiential factor that may influence development and interact with early life stress to shape the way physiological systems develop.

Interactions between diet and subordination were also evident in corticolimbic FC at 6 months of age. Among the LCD-only infants, INS-dlPFC FC was stronger in subordinates compared to dominants—a neuroadaptation that may facilitate recruitment of the salience network to cope with the challenges of being subordinate. Consumption of the HCD (by assignment to the Choice condition) may interfere with the development of this neuroadaptation, as subordinates on the Choice diet had significantly reduced FC compared to subordinates on the LCD-only diet. Infants on the Choice diet demonstrated lower vigilance of the human intruder during the profile phase of the human intruder task, supporting the possibility that a hypercaloric diet negatively impacts the salience network. Among dominant infants, those on the Choice diet had significantly higher INS-OFC FC compared to those on the LCD-only diet—perhaps a reflection of diet-induced alterations to reward processing pathways that increases motivation to consume food. The stress of social subordination may have had counteracting effects on FC in this circuit,

given that subordinates on the Choice diet had significantly lower FC than the dominant infants, and the INS is sensitive to the insults of early life stress[66]. It is unclear if the social status effects are the result of the infants' social experience (stress response to the observation of their mother and family members receiving harassment), differences in maternal care received by subordinate versus dominant infants, biological signals transmitted through the mother's milk; further work is needed to explore these possibilities. It is clear, however, that diet is an important experiential factor in early development that interacts with social status to influence FC in cortical circuits important for emotion regulation, reward processing, and salience detection.

We did not detect interactions between social status and pubertal timing on neurobehavioral outcomes in adolescence, but we were statistically underpowered to do so. Therefore, it is unclear how pubertal timing modifies the effect of social subordination on brain, behavior, and physiology. Nonetheless, we did find several main effects of prior treatment with Lupron on emotion regulation circuitry and socioemotional behaviors, providing evidence that the timing of the rise in pubertal hormones—including E2—may impact the maturation of socioemotional behaviors and the corticolimbic regions that regulate them. Some facets of emotion regulation were strengthened by pubertal delay with Lupron, including stronger AMYG-OFC FC and reduced anxiety-like behavior in the social group, and other outcomes suggested impaired or immature socioemotional development, e.g. increased frequency of submissive behavior, lower 'Explore' scores in the HI paradigm, and lower frequency of initiating proximity towards non-kin. Delayed onset of puberty with Lupron treatment may alter the organizational effects of E2 on neural systems, potentially explaining some of the neurobehavioral outcomes we found. The subjects treated with Lupron during the pubertal transition were studied at a relatively immature

developmental stage, compared to the control females, and some of the behavioral and neural differences we detected as a result of the Lupron treatment may be an artifact of those females being studied earlier in adolescent development. Delayed timing of menarche in the untreated females was also associated with higher FC in AMYG-PFC circuits, suggesting a general association between later timing of puberty and increased corticolimbic FC, which may have consequences for emotion regulation during adolescence and into adulthood, but future work is needed to explore this question.

4.3 Conclusions and Future Directions

Overall, this dissertation provides evidence that (1) social subordination impacts the strength of FC in corticolimbic circuits involved in emotion and stress regulation, though whether subordination results in higher or lower FC depends on the circuit and the developmental time point, (2) delayed pubertal timing is generally associated with stronger AMYG-PFC FC, and both positive and negative socioemotional outcomes (3) consumption of an obesogenic diet early in development increases levels of stress hormones and inflammation, and impacts the development of salience and reward circuits involved in addictive phenotypes, at the same time that it modifies the consequences of social subordination on corticolimbic FC, inflammation, and stress physiology. Future studies are warranted to probe several questions that remain unanswered. Given that infants and adolescent female macaques demonstrated different patterns of AMYG-PFC FC, how does the impact of chronic social subordination stress on the brain and behavior developmentally progress? Do Lupron-induced alterations to corticolimbic FC and socioemotional outcomes persist into adulthood? How would advancing, instead of delaying, pubertal timing with a pharmacological intervention impact neurobehavioral development? How

do prenatal effects, including gestational stress and consumption of a HCD, affect neurobehavioral development of offspring and interact with postnatal experiences of social stress? Are there additional brain circuits impacted by social subordination, pubertal timing, or consumption of an obesogenic diet outside of the corticolimbic circuits investigated in this dissertation? How would social subordination, pubertal tempo, and diet condition differentially impact development in male rhesus macaques? Further research investigating these questions would help elucidate the neurobiological mechanisms by which stress 'gets under the skin', and interacts with experiential factors like dietary environment and pubertal timing, to alter neurobehavioral and physiological development.

Chapter 5. REFERENCES

1. Ganzel BL, Morris PA, Wethington E. Allostasis and the human brain: Integrating models of stress from the social and life sciences. *Psychol Rev*. 2010;117(1):134-174. doi:10.1037/a0017773.
2. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. *Physiol Rev*. 2007;87(3):873-904. doi:10.1152/physrev.00041.2006.
3. McEwen BS, Gianaros PJ. Stress- and allostasis-induced brain plasticity. *Annu Rev Med*. 2011;62(1):431-445. doi:10.1146/annurev-med-052209-100430.
4. McEwen BS. Brain on stress: How the social environment gets under the skin. *Proc Natl Acad Sci*. 2012;109(4):17180-17185. doi:10.1073/pnas.1121254109.
5. Chrousos GP. Stress and disorders of the stress system. *Nat Rev Endocrinol*. 2009;5(7):374-381. doi:10.1038/nrendo.2009.106.
6. Chrousos GP, Gold PW. The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA*. 1992;267(9):1244-1252. http://www.ncbi.nlm.nih.gov/pubmed/1538563. Accessed October 23, 2017.
7. Selye H. STRESS AND DISEASE. *Laryngoscope*. 1955;65(7):500???514. doi:10.1288/00005537-195507000-00002.
8. Juster R-P, McEwen BS, Lupien SJ. Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neurosci Biobehav Rev*. 2010;35(1):2-16. doi:10.1016/j.neubiorev.2009.10.002.
9. Belar CD, Salomon M, Vella A, Blumenthal J, Bourdeau TL, Millard R. Our Health at Risk Stress in America ™ : RELEASED JANUARY 11, 2012 @BULLET Mind/Body Health: For a Healthy Mind and Body, Talk to a Psychologist. https://www.apa.org/news/press/releases/stress/2011/final-2011.pdf. Accessed October 23,

2017.

10. Brunner E. Stress and the biology of inequality. *BMJ*. 1997;314(7092):1472-1476. http://www.ncbi.nlm.nih.gov/pubmed/9167568. Accessed October 23, 2017.
11. Baum A, Posluszny DM. Health psychology: mapping biobehavioral contributions to health and illness. *Annu Rev Psychol*. 1999;50(1):137-163. doi:10.1146/annurev.psych.50.1.137.
12. Dallman MF, Pecoraro N, Akana SF, et al. Chronic stress and obesity: a new view of "comfort food". *Proc Natl Acad Sci U S A*. 2003;100(20):11696-11701. doi:10.1073/pnas.1934666100.
13. Segerstrom SC, Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. *Psychol Bull*. 2004;130(4):601-630. doi:10.1037/0033-2909.130.4.601.
14. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci*. 2009;10(6):434-445. doi:10.1038/nrn2639.
15. Kaplow JB, Widom CS. Age of onset of child maltreatment predicts long-term mental health outcomes. *J Abnorm Psychol*. 2007;116(1):176-187. doi:10.1037/0021-843X.116.1.176.
16. Ulrich-Lai Y, Herman J. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci*. 2009. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4240627/. Accessed August 20, 2017.
17. Kvetnansky R, Sabban EL, Palkovits M. Catecholaminergic systems in stress: structural and molecular genetic approaches. *Physiol Rev*. 2009;89(2):535-606.

doi:10.1152/physrev.00042.2006.

18. Johnson EO, Kamilaris TC, Chrousos GP, Gold PW. Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis. *Neurosci Biobehav Rev*. 1992;16(2):115-130. http://www.ncbi.nlm.nih.gov/pubmed/1630726. Accessed October 23, 2017.
19. Myers B, McKlveen JM, Herman JP. Neural Regulation of the Stress Response: The Many Faces of Feedback. *Cell Mol Neurobiol*. 2012;32(5):683-694. doi:10.1007/s10571-012-9801-y.
20. Charmandari E, Tsigos C, Chrousos G. ENDOCRINOLOGY OF THE STRESS RESPONSE. *Annu Rev Physiol*. 2005;67(1):259-284. doi:10.1146/annurev.physiol.67.040403.120816.
21. Sanchez MM. The impact of early adverse care on HPA axis development: Nonhuman primate models. *Horm Behav*. 2006;50:623-631. doi:10.1016/j.yhbeh.2006.06.012.
22. Sánchez M, Young L, Plotsky P. Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation. *J*. 2000. http://www.jneurosci.org/content/20/12/4657.short. Accessed August 13, 2017.
23. Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends Neurosci*. 1997;20(2):78-84. http://www.ncbi.nlm.nih.gov/pubmed/9023876. Accessed October 22, 2017.
24. Droste SK, de Groote L, Atkinson HC, Lightman SL, Reul JMHM, Linthorst ACE. Corticosterone levels in the brain show a distinct ultradian rhythm but a delayed response to forced swim stress. *Endocrinology*. 2008;149(7):3244-3253. doi:10.1210/en.2008-0103.
25. de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain Corticosteroid Receptor Balance in

Health and Disease. *Endocr Rev*. 1998;19(3):269-301. doi:10.1210/edrv.19.3.0331.

26. Makino S, Hashimoto K, Gold PW. Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress. *Pharmacol Biochem Behav*. 2002;73(1):147-158. http://www.ncbi.nlm.nih.gov/pubmed/12076734. Accessed October 23, 2017.
27. Makino S, Smith MA, Gold PW. Increased expression of corticotropin-releasing hormone and vasopressin messenger ribonucleic acid (mRNA) in the hypothalamic paraventricular nucleus during repeated stress: association with reduction in glucocorticoid receptor mRNA levels. *Endocrinology*. 1995;136(8):3299-3309. doi:10.1210/endo.136.8.7628364.
28. Raadsheer FC, Hoogendijk WJ, Stam FC, Tilders FJ, Swaab DF. Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients. *Neuroendocrinology*. 1994;60(4):436-444. http://www.ncbi.nlm.nih.gov/pubmed/7824085. Accessed October 23, 2017.
29. Kolber BJ, Roberts MS, Howell MP, Wozniak DF, Sands MS, Muglia LJ. Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning. *Proc Natl Acad Sci U S A*. 2008;105(33):12004-12009. doi:10.1073/pnas.0803216105.
30. Shepard JD, Barron KW, Myers DA. Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior. *Brain Res*. 2000;861(2):288-295. http://www.ncbi.nlm.nih.gov/pubmed/10760490. Accessed October 23, 2017.
31. Meloni EG, Reedy CL, Cohen BM, Carlezon WA. Activation of Raphe Efferents to the Medial Prefrontal Cortex by Corticotropin-Releasing Factor: Correlation with Anxiety-

Like Behavior. *Biol Psychiatry*. 2008;63(9):832-839. doi:10.1016/j.biopsych.2007.10.016.

32. Mora F, Segovia G, Del Arco A, de Blas M, Garrido P. Stress, neurotransmitters, corticosterone and body-brain integration. *Brain Res*. 2012;1476:71-85. doi:10.1016/j.brainres.2011.12.049.

33. Mcewen BS, Gianaros PJ. Central role of the brain in stress and adaptation : Links to socioeconomic status , health , and disease. 2010;1186:190-222. doi:10.1111/j.1749-6632.2009.05331.x.

34. Herman JP, Figueiredo H, Mueller NK, et al. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol*. 2003;24(3):151-180. http://www.ncbi.nlm.nih.gov/pubmed/14596810. Accessed October 22, 2017.

35. Goldstein LE, Rasmusson AM, Bunney BS, Roth RH. Role of the amygdala in the coordination of behavioral, neuroendocrine, and prefrontal cortical monoamine responses to psychological stress in the rat. *J Neurosci*. 1996;16(15):4787-4798. http://www.ncbi.nlm.nih.gov/pubmed/8764665. Accessed October 26, 2017.

36. Jankord R, Herman JP. Limbic Regulation of Hypothalamo-Pituitary-Adrenocortical Function during Acute and Chronic Stress. *Ann N Y Acad Sci*. 2008;1148(1):64-73. doi:10.1196/annals.1410.012.

37. López JF, Akil H, Watson SJ. Neural circuits mediating stress. *Biol Psychiatry*. 1999;46(11):1461-1471. http://www.ncbi.nlm.nih.gov/pubmed/10599476. Accessed October 26, 2017.

38. Dedovic K, Duchesne A, Andrews J, Engert V, Pruessner JC. The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *Neuroimage*.

2009;47(3):864-871. doi:10.1016/j.neuroimage.2009.05.074.

39. Ghashghaei HT, Barbas H. Pathways for emotion: Interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey. *Neuroscience*. 2002;115(4):1261-1279. doi:10.1016/S0306-4522(02)00446-3.

40. Ongür D, An X, Price JL. Prefrontal cortical projections to the hypothalamus in macaque monkeys. *J Comp Neurol*. 1998;401(4):480-505. http://www.ncbi.nlm.nih.gov/pubmed/9826274. Accessed October 26, 2017.

41. Barbas H. Flow of information for emotions through temporal and orbitofrontal pathways. *J Anat*. 2007;211(2):237-249. doi:10.1111/j.1469-7580.2007.00777.x.

42. Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci*. 1993;13(9):3839-3847. http://www.ncbi.nlm.nih.gov/pubmed/8396170. Accessed October 26, 2017.

43. Cerqueira JJ, Almeida OFX, Sousa N. The stressed prefrontal cortex. Left? Right! *Brain Behav Immun*. 2008;22(5):630-638. doi:10.1016/j.bbi.2008.01.005.

44. Sánchez MM, Noble PM, Lyon CK, et al. Alterations in diurnal cortisol rhythm and acoustic startle response in nonhuman primates with adverse rearing. *Biol Psychiatry*. 2005;57(4):373-381. doi:10.1016/j.biopsych.2004.11.032.

45. Shively C a, Laber-Laird K, Anton RF. Behavior and physiology of social stress and depression in female cynomolgus monkeys. *Biol Psychiatry*. 1997;41(8):871-882. doi:10.1016/S0006-3223(96)00185-0.

46. Shively CA. Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys. *Biol Psychiatry*. 1998;44(9):882-891. doi:10.1016/S0006-

3223(97)00437-X.

47. Jarrell H, Hoffman J, Kaplan J, Berga S. Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys. *Physiol*. 2008. http://www.sciencedirect.com/science/article/pii/S0031938407004830. Accessed August 21, 2017.

48. Wilson ME, Legendre A, Pazol K, Fisher J, Chikazawa K. Gonadal steroid modulation of the limbic-hypothalamic- pituitary-adrenal (LHPA) axis is influenced by social status in female rhesus monkeys. *Endocrine*. 2005;26(2):89-97. doi:10.1385/ENDO:26:2:089.

49. Paiardini M, Hoffman J, Cervasi B, et al. T-cell phenotypic and functional changes associated with social subordination and gene polymorphisms in the serotonin reuptake transporter in female rhesus monkeys. *Brain Behav Immun*. 2009;23(2):286-293. doi:10.1016/j.bbi.2008.10.006.

50. Rosenblum LA, Forger C, Noland S, Trost RC, Coplan JD. Response of adolescent bonnet macaques to an acute fear stimulus as a function of early rearing conditions. *Dev Psychobiol*. 2001;39(1):40-45. http://www.ncbi.nlm.nih.gov/pubmed/11507708. Accessed October 26, 2017.

51. Lopez-duran NL, Kovacs M, George CJ. Hypothalamic — pituitary — adrenal axis dysregulation in depressed children and adolescents : A meta-analysis. 2009:1272-1283. doi:10.1016/j.psyneuen.2009.03.016.

52. Michopoulos V, Reding KM, Wilson ME, Toufexis D. Social subordination impairs hypothalamic-pituitary-adrenal function in female rhesus monkeys. *Horm Behav*. 2012;62(4):389-399. doi:10.1016/j.yhbeh.2012.07.014.

53. Nader M a., Nader SH, Czoty PW, et al. Social dominance in female monkeys: Dopamine receptor function and cocaine reinforcement. *Biol Psychiatry*. 2012;72(5):414-421. doi:10.1016/j.biopsych.2012.03.002.

54. Wilson ME, Fisher J, Fischer A, Lee V, Harris RB, Bartness TJ. Quantifying food intake in socially housed monkeys: social status effects on caloric consumption. *Physiol Behav*. 2008;94(4):586-594. doi:10.1016/j.physbeh.2008.03.019.

55. Vyas A, Bernal S, Chattarji S. Effects of chronic stress on dendritic arborization in the central and extended amygdala. *Brain Res*. 2003;965(1-2):290-294. http://www.ncbi.nlm.nih.gov/pubmed/12591150. Accessed October 26, 2017.

56. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci*. 2002;22(15):6810-6818. doi:20026655.

57. Weniger G, Lange C, disorders EI-J of affective, 2006 undefined. Abnormal size of the amygdala predicts impaired emotional memory in major depressive disorder. *Elsevier*. http://www.sciencedirect.com/science/article/pii/S0165032706001893. Accessed January 13, 2018.

58. Frodl T, Meisenzahl E, Zetzsche T, … RB-B, 2002 undefined. Enlargement of the amygdala in patients with a first episode of major depression. *Elsevier*. http://www.sciencedirect.com/science/article/pii/S0006322301013592. Accessed January 13, 2018.

59. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct*. 2008;213(1-2):93-118. doi:10.1007/s00429-008-0189-x.

60. Sánchez MM, Ladd CO, Plotsky PM. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev Psychopathol*. 2001;13:419-449. doi:10.1017/S0954579401003029.

61. Heim C, Nemeroff CB. The impact of early adverse experiences on brain systems involved in the pathophysiology of anxiety and affective disorders. *Biol Psychiatry*. 1999;46(11):1509-1522. http://www.ncbi.nlm.nih.gov/pubmed/10599479. Accessed October 26, 2017.

62. Rauch SL, Shin LM, Wright CI. Neuroimaging studies of amygdala function in anxiety disorders. *Ann N Y Acad Sci*. 2003;985:389-410. http://www.ncbi.nlm.nih.gov/pubmed/12724173. Accessed October 26, 2017.

63. Shekhar A, Truitt W, Rainnie D, Sajdyk T. Role of stress, corticotrophin releasing factor (CRF) and amygdala plasticity in chronic anxiety. *Stress*. 2005;8(4):209-219. doi:10.1080/10253890500504557.

64. Reynolds SM, Zahm DS. Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala. *J Neurosci*. 2005;25(50):11757-11767. doi:10.1523/JNEUROSCI.3432-05.2005.

65. Liberzon I, King AP, Britton JC, Phan KL, Abelson JL, Taylor SF. Paralimbic and medial prefrontal cortical involvement in neuroendocrine responses to traumatic stimuli. *Am J Psychiatry*. 2007;164(8):1250-1258. doi:10.1176/appi.ajp.2007.06081367.

66. Dannlowski U, Stuhrmann A, Beutelmann V, et al. Limbic scars: long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging. *Biol Psychiatry*. 2012;71(4):286-293. doi:10.1016/j.biopsych.2011.10.021.

67. Shively CA, Grant KA, Ehrenkaufer RL, Mach RH, Nader MA. Social stress, depression,

and brain dopamine in female cynomolgus monkeys. *Ann N Y Acad Sci*. 1997;807:574-577. http://www.ncbi.nlm.nih.gov/pubmed/9071402. Accessed October 16, 2017.

68. Izzo E, Sanna PP, Koob GF. Impairment of dopaminergic system function after chronic treatment with corticotropin-releasing factor. *Pharmacol Biochem Behav*. 2005;81(4):701-708. doi:10.1016/j.pbb.2005.04.017.

69. Hains AB, Vu MAT, Maciejewski PK, van Dyck CH, Gottron M, Arnsten AFT. Inhibition of protein kinase C signaling protects prefrontal cortex dendritic spines and cognition from the effects of chronic stress. *Proc Natl Acad Sci U S A*. 2009;106(42):17957-17962. doi:10.1073/pnas.0908563106.

70. Liston C, Miller MM, Goldwater DS, et al. Stress-induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci*. 2006;26(30):7870-7874. doi:10.1523/JNEUROSCI.1184-06.2006.

71. Arnsten AFT. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci*. 2009;10(juNe):410-422. doi:10.1038/nrn2648.

72. Kempton MJ, Salvador Z, Munafò MR, et al. Structural Neuroimaging Studies in Major Depressive Disorder. *Arch Gen Psychiatry*. 2011;68(7):675. doi:10.1001/archgenpsychiatry.2011.60.

73. Southwick SM, Bremner JD, Rasmusson A, Morgan CA, Arnsten A, Charney DS. Role of norepinephrine in the pathophysiology and treatment of posttraumatic stress disorder. *Biol Psychiatry*. 1999;46(9):1192-1204. http://www.ncbi.nlm.nih.gov/pubmed/10560025. Accessed January 13, 2018.

74. Ansell EB, Rando K, Tuit K, Guarnaccia J, Sinha R. Cumulative Adversity and Smaller Gray Matter Volume in Medial Prefrontal, Anterior Cingulate, and Insula Regions. *Biol*

Psychiatry. 2012;72(1):57-64. doi:10.1016/j.biopsych.2011.11.022.

75. Bremner JD. Long-term effects of childhood abuse on brain and neurobiology. 2003;12:271-292. doi:10.1016/S1056-4993(02)00098-6.

76. Bremner JD, Randall P, Vermetten E, et al. Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse--a preliminary report. *Biol Psychiatry*. 1997;41(1):23-32. http://www.ncbi.nlm.nih.gov/pubmed/8988792. Accessed January 13, 2018.

77. Choi J, Jeong B, Rohan ML, Polcari AM, Teicher MH. Preliminary evidence for white matter tract abnormalities in young adults exposed to parental verbal abuse. *Biol Psychiatry*. 2009;65(3):227-234. doi:10.1016/j.biopsych.2008.06.022.

78. Kertes DA, Gunnar MR, Madsen NJ, Long JD. Early deprivation and home basal cortisol levels: A study of internationally adopted children. *Dev Psychopathol*. 2008;20(2):473-491. doi:10.1017/S0954579408000230.

79. Gunnar MR, Morison SJ, Chisholm K, Schuder M. Salivary cortisol levels in children adopted from romanian orphanages. *Dev Psychopathol*. 2001;13(3):611-628. http://www.ncbi.nlm.nih.gov/pubmed/11523851. Accessed January 15, 2018.

80. Tottenham N, Sheridan MA. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Front Hum Neurosci*. 2009;3:68. doi:10.3389/neuro.09.068.2009.

81. Tottenham N. Human amygdala development in the absence of species-expected caregiving. *Dev Psychobiol*. 2012;54(6):598-611. doi:10.1002/dev.20531.

82. Behen ME, Muzik O, Saporta ASD, et al. Abnormal fronto-striatal connectivity in children with histories of early deprivation: A diffusion tensor imaging study. *Brain*

Imaging Behav. 2009;3(3):292-297. doi:10.1007/s11682-009-9071-6.

83. Eluvathingal TJ, Chugani HT, Behen ME, et al. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. *Pediatrics*. 2006;117(6):2093-2100. doi:10.1542/peds.2005-1727.

84. Lupien SJ, King S, Meaney MJ, McEwen BS. Child's stress hormone levels correlate with mother's socioeconomic status and depressive state. *Biol Psychiatry*. 2000;48(10):976-980. http://www.ncbi.nlm.nih.gov/pubmed/11082471. Accessed October 26, 2017.

85. Noble KG, Houston SM, Kan E, Sowell ER. Neural correlates of socioeconomic status in the developing human brain. *Dev Sci*. 2012;15(4):516-527. doi:10.1111/j.1467-7687.2012.01147.x.

86. Noble KG, Houston SM, Brito NH, et al. Family income, parental education and brain structure in children and adolescents. *Nat Neurosci*. 2015;18(5):773-778. doi:10.1038/nn.3983.

87. Spinelli S, Chefer S, Suomi SJ, Higley JD, Barr CS, Stein E. Early-life stress induces long-term morphologic changes in primate brain. *Arch Gen Psychiatry*. 2009;66(6):658-665. doi:10.1001/archgenpsychiatry.2009.52.

88. Peper JS, van den Heuvel MP, Mandl RCW, Pol HEH, van Honk J. Sex steroids and connectivity in the human brain: A review of neuroimaging studies. *Psychoneuroendocrinology*. 2011;36:1101-1113. doi:10.1016/j.psyneuen.2011.05.004.

89. Honey CJ, Kotter R, Breakspear M, Sporns O. Network structure of cerebral cortex shapes functional connectivity on multiple time scales. *Pnas*. 2007;104(24):10240-10245. doi:10.1073/pnas.0701519104.

90. Logothetis NK, Wandell B a. Interpreting the BOLD signal. *Annu Rev Physiol*.

2004;66:735-769. doi:10.1146/annurev.physiol.66.082602.092845.

91. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci*. 2009;10(3):186-198. doi:10.1038/nrn2575.

92. Biswal BB, Yetkin FZ, Haughton VM, et al. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995;34(4):537-541. doi:10.1002/mrm.1910340409.

93. Sporns O. Contributions and challenges for network models in cognitive neuroscience. *Nat Neurosci*. 2014;17(5):652-660. doi:10.1038/nn.3690.

94. Herringa RJ, Birn RM, Ruttle PL, et al. Childhood maltreatment is associated with altered fear circuitry and increased internalizing symptoms by late adolescence. *Proc Natl Acad Sci U S A*. 2013;110(47):19119-19124. doi:10.1073/pnas.1310766110.

95. Burghy C a, Stodola DE, Ruttle PL, et al. Developmental pathways to amygdala-prefrontal function and internalizing symptoms in adolescence. *Nat Neurosci*. 2012;15(12):1736-1741. doi:10.1038/nn.3257.

96. Harrison BJ, Soriano-Mas C, Pujol J, et al. Altered corticostriatal functional connectivity in obsessive-compulsive disorder. *Arch Gen Psychiatry*. 2009;66(11):1189-1200. doi:10.1001/archgenpsychiatry.2009.152.

97. Giménez M, Pujol J, Ortiz H, et al. Altered brain functional connectivity in relation to perception of scrutiny in social anxiety disorder. *Psychiatry Res*. 2012;202(3):214-223. doi:10.1016/j.pscychresns.2011.10.008.

98. Golkar A, Johansson E, Kasahara M, Osika W, Perski A, Savic I. The influence of work-related chronic stress on the regulation of emotion and on functional connectivity in the brain. *PLoS One*. 2014;9(9). doi:10.1371/journal.pone.0104550.

99. Paulus MP, Stein MB. Interoception in anxiety and depression. *Brain Struct Funct*. 2010;214(5-6):451-463. doi:10.1007/s00429-010-0258-9.

100. Klumpp H, Angstadt M, Phan KL. Insula reactivity and connectivity to anterior cingulate cortex when processing threat in generalized social anxiety disorder. *Biol Psychol*. 2012;89(1):273-276. doi:10.1016/j.biopsycho.2011.10.010.

101. Demir-Lira ÖE, Voss JL, O'Neil JT, Briggs-Gowan MJ, Wakschlag LS, Booth JR. Early-life stress exposure associated with altered prefrontal resting-state fMRI connectivity in young children. *Dev Cogn Neurosci*. 2016;19:107-114. doi:10.1016/j.dcn.2016.02.003.

102. Graham AM, Pfeifer JH, Fisher PA, Carpenter S, Fair DA. Early life stress is associated with default system integrity and emotionality during infancy. *J Child Psychol Psychiatry*. 2015;56(11):1212-1222. doi:10.1111/jcpp.12409.

103. Shively CA, Register TC, Friedman DP, Morgan TM, Thompson J, Lanier T. Social stress-associated depression in adult female cynomolgus monkeys (Macaca fascicularis). *Biol Psychol*. 2005;69(1 SPEC. ISS.):67-84. doi:10.1016/j.biopsycho.2004.11.006.

104. Michopoulos V, Higgins M, Toufexis D, Wilson ME. Social subordination produces distinct stress-related phenotypes in female rhesus monkeys. 2012. doi:10.1016/j.psyneuen.2011.12.004.

105. Michopoulos V, Toufexis D, Wilson ME. Social stress interacts with diet history to promote emotional feeding in females. *Psychoneuroendocrinology*. 2012;37(9):1479-1490. doi:10.1016/j.psyneuen.2012.02.002.

106. Reding K, Michopoulos V, Wallen K, Sanchez M, Wilson ME, Toufexis D. Social status modifies estradiol activation of sociosexual behavior in female rhesus monkeys. *Horm Behav*. 2012;62(5):612-620. doi:10.1016/j.yhbeh.2012.09.010.

107. Shively C a., Willard SL. Behavioral and neurobiological characteristics of social stress versus depression in nonhuman primates. *Exp Neurol*. 2012;233(1):87-94. doi:10.1016/j.expneurol.2011.09.026.

108. Wilson ME, Bounar S, Godfrey J, Michopoulos V, Higgins M, Sanchez M. Social and emotional predictors of the tempo of puberty in female rhesus monkeys. *Psychoneuroendocrinology*. 2013;38(1):67-83. doi:10.1016/j.psyneuen.2012.04.021.

109. Sapolsky RM. The Influence of Social Hierarchy on Primate Health. *Science (80-)*. 2005;308(5722):648-652. doi:10.1126/science.1106477.

110. Petrides M, Tomaiuolo F, Yeterian EH, Pandya DN. The prefrontal cortex: Comparative architectonic organization in the human and the macaque monkey brains. *Cortex*. 2012;48(1):46-57. doi:10.1016/j.cortex.2011.07.002.

111. Byrne RW, Whiten A. *Machiavellian Intelligence : Social Expertise and the Evolution of Intellect in Monkeys, Apes, and Humans*. Clarendon Press; 1988.

112. Augustine JR. Circuitry and functional aspects of the insular lobe in primates including humans. *Brain Res Rev*. 1996;22(3):229-244. doi:10.1016/S0165-0173(96)00011-2.

113. Knickmeyer RC, Styner M, Short SJ, et al. Maturational Trajectories of Cortical Brain Development through the Pubertal Transition : Unique Species and Sex Differences in the Monkey Revealed through Structural Magnetic Resonance Imaging. 2010;(May):1053-1063. doi:10.1093/cercor/bhp166.

114. Hertzman C. The biological embedding of early experience and its effects on health in adulthood. *Ann N Y Acad Sci*. 1999;896:85-95. http://www.ncbi.nlm.nih.gov/pubmed/10681890. Accessed October 23, 2017.

115. Bernstein IS. Dominance, aggression and reproduction in primate societies. *J Theor Biol*.

1976;60:459-472. doi:10.1016/0022-5193(76)90072-2.

116. Bernstein IS, Gordon TP. Behavioral research in breeding colonies of Old World monkeys. *Lab Anim Sci*. 1977;27(4):532-540. http://www.ncbi.nlm.nih.gov/pubmed/409882. Accessed October 23, 2017.

117. Marmot MG, Sapolsky R. Of Baboons and Men: Social Circumstances, Biology, and the Social Gradient in Health. September 2014. https://www.ncbi.nlm.nih.gov/books/NBK242456/?report=reader. Accessed October 23, 2017.

118. Bernstein IS, Gordon TP, Rose RM. Aggression and social controls in rhesus monkey (Macaca mulatta) groups revealed in group formation studies. *Folia Primatol (Basel)*. 1974;21(2):81-107. http://www.ncbi.nlm.nih.gov/pubmed/4471987. Accessed October 23, 2017.

119. Bernstein IS, Gordon TP. The function of aggression in primate societies. *Am Sci*. 62(3):304-311. http://www.ncbi.nlm.nih.gov/pubmed/4857115. Accessed October 23, 2017.

120. Silk JB. Practice Random Acts of Aggression and Senseless Acts of Intimidation: The Logic of Status Contests in Social Groups. *Evol Anthropol*. 2002;11(6):221-225. doi:10.1002/evan.10038.

121. Abbott DH, Keverne EB, Bercovitch FB, et al. Are subordinates always stressed? A comparative analysis of rank differences in cortisol levels among primates. *Horm Behav*. 2003;43(1):67-82. http://www.ncbi.nlm.nih.gov/pubmed/12614636. Accessed October 23, 2017.

122. Kaplan JR, Adams MR, Clarkson TB, Manuck SB, Shively CA, Williams JK.

Psychosocial factors, sex differences, and atherosclerosis: lessons from animal models. *Psychosom Med*. 58(6):598-611. http://www.ncbi.nlm.nih.gov/pubmed/8948008. Accessed October 23, 2017.

123. Gust DA, Gordon TP, Wilson ME, Ahmed-Ansari A, Brodie AR, McClure HM. Formation of a new social group of unfamiliar female rhesus monkeys affects the immune and pituitary adrenocortical systems. *Brain Behav Immun*. 1991;5(3):296-307. http://www.ncbi.nlm.nih.gov/pubmed/1954404. Accessed October 23, 2017.

124. Snyder-Mackler N, Sanz J, Kohn JN, et al. Social status alters immune regulation and response to infection in macaques. *Science (80-)*. 2016;354(6315):1041-1045. doi:10.1126/science.aah3580.

125. Zehr JL, Van Meter PE, Wallen K. Factors regulating the timing of puberty onset in female rhesus monkeys (Macaca mulatta): role of prenatal androgens, social rank, and adolescent body weight. *Biol Reprod*. 2005;72(5):1087-1094. doi:10.1095/biolreprod.104.027755.

126. Kaplan JR, Manuck SB. Ovarian dysfunction, stress, and disease: a primate continuum. *ILAR J*. 2004;45(2):89-115. http://www.ncbi.nlm.nih.gov/pubmed/15111730. Accessed October 23, 2017.

127. Wilson ME, Kinkead B. Gene-Environment Interactions, Not Neonatal Growth Hormone Deficiency, Time Puberty in Female Rhesus Monkeys1. *Biol Reprod*. 2008;78(4):736-743. doi:10.1095/biolreprod.107.065953.

128. Morgan D, Grant K a, Gage HD, et al. Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration. *Nat Neurosci*. 2002;5:169-174. doi:10.1038/nn798.

129. Bernstein I. *Primate Behaviour : Developments in Field and Laboratory Research. Vol. 1*.; 1970. https://books.google.com/books?hl=en&lr=&id=tSrgBAAAQBAJ&oi=fnd&pg=PA71&dq=Primate+status+hierarchies+berstein+1970&ots=KedHGEee2-&sig=MY53HEXU3V50jxSktTidhQSErXQ#v=onepage&q&f=false. Accessed October 23, 2017.

130. Berman CM. Mother-infant relationships among free-ranging rhesus monkeys on Cayo Santiago: A comparison with captive pairs. *Anim Behav*. 1980;28(3):860-873. doi:10.1016/S0003-3472(80)80146-1.

131. Spencer-Booth Y. The behaviour of group companions towards rhesus monkey infants. *Anim Behav*. 1968;16(4):541-557. http://www.ncbi.nlm.nih.gov/pubmed/4974615. Accessed October 23, 2017.

132. Hinde RA, Spencer-Booth Y. Effects of brief separation from mother on rhesus monkeys. *Science*. 1971;173(3992):111-118. http://www.ncbi.nlm.nih.gov/pubmed/4996549. Accessed October 23, 2017.

133. Bernstein IS, Ehardt CL. Age-sex differences in the expression of agonistic behavior in rhesus monkey (Macaca mulatta) groups. *J Comp Psychol*. 1985;99(2):115-132. http://www.ncbi.nlm.nih.gov/pubmed/4040001. Accessed October 23, 2017.

134. Howell BR, Godfrey J, Gutman D a, et al. Social Subordination Stress and Serotonin Transporter Polymorphisms: Associations With Brain White Matter Tract Integrity and Behavior in Juvenile Female Macaques. *Cereb Cortex*. 2013;(December):1-16. doi:10.1093/cercor/bht187.

135. Brooke SM, de Haas-Johnson AM, Kaplan JR, Manuck SB, Sapolsky RM.

Dexamethasone resistance among nonhuman primates associated with a selective decrease of glucocorticoid receptors in the hippocampus and a history of social instability. *Neuroendocrinology*. 1994;60(2):134-140. http://www.ncbi.nlm.nih.gov/pubmed/7969770. Accessed October 23, 2017.

136. Kohn JN, Snyder-Mackler N, Barreiro LB, Johnson ZP, Tung J, Wilson ME. Dominance rank causally affects personality and glucocorticoid regulation in female rhesus macaques HHS Public Access. *Psychoneuroendocrinology*. 2016;74:179-188. doi:10.1016/j.psyneuen.2016.09.005.

137. Sapolsky RM, Meaney MJ, McEwen BS. The development of the glucocorticoid receptor system in the rat limbic brain. III. Negative-feedback regulation. *Brain Res*. 1985;350(1-2):169-173. http://www.ncbi.nlm.nih.gov/pubmed/3986611. Accessed October 23, 2017.

138. Haroon E, Raison CL, Miller AH. Psychoneuroimmunology Meets Neuropsychopharmacology: Translational Implications of the Impact of Inflammation on Behavior. *Neuropsychopharmacology*. 2012;37(1):137-162. doi:10.1038/npp.2011.205.

139. Johnson JD, Campisi J, Sharkey CM, et al. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. *Neuroscience*. 2005;135(4):1295-1307. doi:10.1016/j.neuroscience.2005.06.090.

140. Bierhaus A, Wolf J, Andrassy M, et al. A mechanism converting psychosocial stress into mononuclear cell activation. *Proc Natl Acad Sci U S A*. 2003;100(4):1920-1925. doi:10.1073/pnas.0438019100.

141. Rhen T, Cidlowski JA. Antiinflammatory Action of Glucocorticoids — New Mechanisms for Old Drugs. *N Engl J Med*. 2005;353(16):1711-1723. doi:10.1056/NEJMra050541.

142. Hodge S, Hodge G, Flower R, Han P. Methyl-prednisolone up-regulates monocyte

interleukin-10 production in stimulated whole blood. *Scand J Immunol*. 1999;49(5):548-553. http://www.ncbi.nlm.nih.gov/pubmed/10320649. Accessed January 13, 2018.

143. Carpenter LL, Gawuga CE, Tyrka AR, Lee JK, Anderson GM, Price LH. Association between plasma IL-6 response to acute stress and early-life adversity in healthy adults. *Neuropsychopharmacology*. 2010;35(13):2617-2623. doi:10.1038/npp.2010.159.

144. Pace TWW, Mletzko TC, Alagbe O, et al. Increased stress-induced inflammatory responses in male patients with major depression and increased early life stress. *Am J Psychiatry*. 2006;163(9):1630-1633. doi:10.1176/ajp.2006.163.9.1630.

145. Silverman MN, Sternberg EM. Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction. *Ann N Y Acad Sci*. 2012;1261(1):55-63. doi:10.1111/j.1749-6632.2012.06633.x.

146. Reader BF, Jarrett BL, McKim DB, Wohleb ES, Godbout JP, Sheridan JF. Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety. *Neuroscience*. 2015;289:429-442. doi:10.1016/j.neuroscience.2015.01.001.

147. Pace TWW, Hu F, Miller AH. Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression. *Brain Behav Immun*. 2007;21(1):9-19. doi:10.1016/j.bbi.2006.08.009.

148. Barnes PJ. Corticosteroid effects on cell signalling. *Eur Respir J*. 2006;27(2):413-426. doi:10.1183/09031936.06.00125404.

149. De Bosscher K, Vanden Berghe W, Haegeman G. Cross-talk between nuclear receptors and nuclear factor κB. *Oncogene*. 2006;25(51):6868-6886. doi:10.1038/sj.onc.1209935.

150. Tung J, Barreiro LB, Johnson ZP, et al. Social environment is associated with gene regulatory variation in the rhesus macaque immune system. *Proc Natl Acad Sci*.

2012;109:6490-6495. doi:10.1073/pnas.1202734109.

151. Pollitt RA, Kaufman JS, Rose KM, Diez-Roux A V, Zeng D, Heiss G. Cumulative life course and adult socioeconomic status and markers of inflammation in adulthood. *J Epidemiol Community Heal*. 2008;62(6):484-491. doi:10.1136/jech.2006.054106.

152. Loucks EB, Pilote L, Lynch JW, et al. Life course socioeconomic position is associated with inflammatory markers: the Framingham Offspring Study. *Soc Sci Med*. 2010;71(1):187-195. doi:10.1016/j.socscimed.2010.03.012.

153. Barres BA, Schmid R, Sendnter M, Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development*. 1993;118(1):283-295. http://www.ncbi.nlm.nih.gov/pubmed/8375338. Accessed January 16, 2018.

154. Miller AH, Haroon E, Raison CL, Felger JC. Cytokine targets in the brain: impact on neurotransmitters and neurocircuits. *Depress Anxiety*. 2013;30(4):297-306. doi:10.1002/da.22084.

155. Palin K, Bluthé R-M, Verrier D, Tridon V, Dantzer R, Lestage J. Interleukin-1beta mediates the memory impairment associated with a delayed type hypersensitivity response to bacillus Calmette-Guérin in the rat hippocampus. *Brain Behav Immun*. 2004;18(3):223-230. doi:10.1016/j.bbi.2003.09.002.

156. Frank MG, Baratta M V, Sprunger DB, Watkins LR, Maier SF. Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses. *Brain Behav Immun*. 2007;21(1):47-59. doi:10.1016/j.bbi.2006.03.005.

157. Steiner J, Bielau H, Brisch R, et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. *J Psychiatr Res*. 2008;42(2):151-157. doi:10.1016/j.jpsychires.2006.10.013.

158. Setiawan E, Wilson AA, Mizrahi R, et al. Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes. *JAMA psychiatry*. 2015;72(3):268-275. doi:10.1001/jamapsychiatry.2014.2427.

159. GIEDD JN. Structural Magnetic Resonance Imaging of the Adolescent Brain. *Ann N Y Acad Sci*. 2004;1021(1):77-85. doi:10.1196/annals.1308.009.

160. Gogtay N, Giedd JN, Lusk L, et al. Dynamic mapping of human cortical development during childhood through early adulthood. *Proc Natl Acad Sci*. 2004;101(21):8174-8179. doi:10.1073/pnas.0402680101.

161. Shaw P, Kabani NJ, Lerch JP, et al. Neurodevelopmental Trajectories of the Human Cerebral Cortex. *J Neurosci*. 2008;28(14):3586-3594. doi:10.1523/JNEUROSCI.5309-07.2008.

162. Giedd JN, Blumenthal J, Jeffries NO, et al. Brain development during childhood and adolescence: a longitudinal MRI study. *Nat Neurosci*. 1999;2(10):861-863. doi:10.1038/13158.

163. Giedd JN, Rapoport JL. Structural MRI of pediatric brain development: what have we learned and where are we going? *Neuron*. 2010;67(5):728-734. doi:10.1016/j.neuron.2010.08.040.

164. LaMantia AS, Rakic P. Axon overproduction and elimination in the anterior commissure of the developing rhesus monkey. *J Comp Neurol*. 1994;340(3):328-336. doi:10.1002/cne.903400304.

165. Huttenlocher PR, de Courten C, Garey LJ, Van der Loos H. Synaptogenesis in human visual cortex--evidence for synapse elimination during normal development. *Neurosci Lett*. 1982;33(3):247-252. http://www.ncbi.nlm.nih.gov/pubmed/7162689. Accessed

October 24, 2017.

166. Gibson KR. Myelination and behavioral development: A comparative perspective on questions of neoteny, altriciality and intelligence. In: *Brain Maturation and Cognitive Development: Comparative and Cross-Cultural Perspectives.* Foundations of human behavior. Hawthorne, NY, US: Aldine de Gruyter; 1991:29-63.

167. Kim J, Cicchetti D. Longitudinal pathways linking child maltreatment, emotion regulation, peer relations, and psychopathology. *J Child Psychol Psychiatry*. 2009;51(6):706-716. doi:10.1111/j.1469-7610.2009.02202.x.

168. Humphrey T. The development of the human amygdala during early embryonic life,. *J Comp Neurol*. 1968;132(1):135-165. doi:10.1002/cne.901320108.

169. Kordower JH, Piecinski P, Rakic P. Neurogenesis of the amygdaloid nuclear complex in the rhesus monkey. *Brain Res Dev Brain Res*. 1992;68(1):9-15. http://www.ncbi.nlm.nih.gov/pubmed/1521327. Accessed October 24, 2017.

170. Kling A. Ontogenetic and phylogenetic studies on the amygdaloid nuclei. *Psychosom Med*. 28(2):155-161. http://www.ncbi.nlm.nih.gov/pubmed/4956464. Accessed October 24, 2017.

171. Chareyron LJ, Lavenex PB, Amaral DG, Lavenex P. Postnatal development of the amygdala: A stereological study in macaque monkeys. *J Comp Neurol*. 2012;520(9):1965-1984. doi:10.1002/cne.23023.

172. Payne C, Machado CJ, Bliwise NG, Bachevalier J. Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study. *Hippocampus*. 2010;20(8):922-935. doi:10.1002/hipo.20688.

173. Machado CJ, Bachevalier J. Non-human primate models of childhood psychopathology:

the promise and the limitations. *J Child Psychol Psychiatry*. 2003;44(1):64-87. http://www.ncbi.nlm.nih.gov/pubmed/12553413. Accessed October 24, 2017.

174. Noonan MP, Sallet J, Mars RB, et al. A Neural Circuit Covarying with Social Hierarchy in Macaques. *PLoS Biol*. 2014;12(9):e1001940. doi:10.1371/journal.pbio.1001940.

175. Tottenham N, Hare TA, Quinn BT, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci*. 2010;13(1):46-61. doi:10.1111/j.1467-7687.2009.00852.x.

176. Tottenham N. A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing. *Front Hum Neurosci*. 2009;3(January):1-18. doi:10.3389/neuro.09.068.2009.

177. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull*. 2007;133(1):25-45. doi:10.1037/0033-2909.133.1.25.

178. Grant K a, Shively C a, Nader M a, et al. Effect of social status on striatal dopamine D2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography. *Synapse*. 1998;29(1):80-83. doi:10.1002/(SICI)1098-2396(199805)29:1<80::AID-SYN7>3.0.CO;2-7.

179. Quirk GJ, Beer JS. Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. *Curr Opin Neurobiol*. 2006;16(6):723-727. doi:10.1016/j.conb.2006.07.004.

180. Amodio DM, Frith CD. Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci*. 2006;7(4):268-277. doi:10.1038/nrn1884.

181. Sallet J, Mars RB, Noonan MP, et al. Social network size affects neural circuits in

macaques. *Science*. 2011;334(6056):697-700. doi:10.1126/science.1210027.

182. Fuchs E, Flügge G. Chronic social stress: Effects on limbic brain structures. *Physiol Behav*. 2003;79:417-427. doi:10.1016/S0031-9384(03)00161-6.

183. Liston C, McEwen BS, Casey BJ. Psychosocial stress reversibly disrupts prefrontal processing and attentional control. *Proc Natl Acad Sci*. 2009;106(3):912-917. doi:10.1073/pnas.0807041106.

184. Shansky RM, Morrison JH. Stress-induced dendritic remodeling in the medial prefrontal cortex: effects of circuit, hormones and rest. *Brain Res*. 2009;1293:108-113. doi:10.1016/j.brainres.2009.03.062.

185. Le Bihan D, Mangin JF, Poupon C, et al. Diffusion tensor imaging: concepts and applications. *J Magn Reson Imaging*. 2001;13(4):534-546. http://www.ncbi.nlm.nih.gov/pubmed/11276097.

186. Kalin NH, Shelton SE. Defensive behaviors in infant rhesus monkeys: Environmental cues and neurochemical regulation. *Science*. 1989;243(4899):1718-1721. doi:10.1126/science.2564702.

187. Meunier M, Bachevalier J, Murray EA, M??lkov?? L, Mishkin M. Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci*. 1999;11(12):4403-4418. doi:10.1046/j.1460-9568.1999.00854.x.

188. Howell BR, Grand AP, Mccormack KM, et al. Early adverse experience increases emotional reactivity in juvenile rhesus macaques: Relation to amygdala volume. *Dev Psychobiol*. 2014;56(8):1735-1746. doi:10.1002/dev.21237.

189. Embree M, Michopoulos V, Votaw JR, et al. The relation of developmental changes in brain serotonin transporter (5HTT) and 5HT1A receptor binding to emotional behavior in

female rhesus monkeys: Effects of social status and 5HTT genotype. *Neuroscience*. 2013;228:83-100. doi:10.1016/j.neuroscience.2012.10.016.

190. Pfaff DW, Vasudevan N, Kia HK, et al. Estrogens, brain and behavior: studies in fundamental neurobiology and observations related to women's health. *J Steroid Biochem Mol Biol*. 2000;74(5):365-373. doi:10.1016/S0960-0760(00)00114-X.

191. Bethea CL, Mirkes SJ, Su A, Michelson D. Effects of oral estrogen, raloxifene and arzoxifene on gene expression in serotonin neurons of macaques. *Psychoneuroendocrinology*. 2002;27(4):431-445. doi:10.1016/S0306-4530(01)00054-3.

192. Stoppe G, Dören M. Critical appraisal of effects of estrogen replacement therapy on symptoms of depressed mood. *Arch Womens Ment Health*. 2002;5(2):39-47. doi:10.1007/s00737-002-0139-1.

193. Dreher J-C, Schmidt PJ, Kohn P, Furman D, Rubinow D, Berman KF. Menstrual cycle phase modulates reward-related neural function in women. *Proc Natl Acad Sci U S A*. 2007;104(7):2465-2470. doi:10.1073/pnas.0605569104.

194. Bethea C, Reddy A. Effect of ovarian hormones on survival genes in laser captured serotonin neurons from macaques. *J Neurochem*. 2008. http://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2008.05213.x/full. Accessed August 8, 2017.

195. Martel M, Klump K, Nigg J, Breedlove S. Potential hormonal mechanisms of attention-deficit/hyperactivity disorder and major depressive disorder: a new perspective. *Horm*. 2009. http://www.sciencedirect.com/science/article/pii/S0018506X09000294. Accessed August 8, 2017.

196. Zadran S, Qin Q, Bi X, Zadran H. 17-β-estradiol increases neuronal excitability through

MAP kinase-induced calpain activation. *Proc*. 2009. http://www.pnas.org/content/106/51/21936.short. Accessed August 8, 2017.

197. Galvin C, Ninan I. Regulation of the mouse medial prefrontal cortical synapses by endogenous estradiol. *Neuropsychopharmacology*. 2014;39(9):2086-2094. doi:10.1038/npp.2014.56.

198. Hines M. Gender Development and the Human Brain. *Annu Rev Neurosci*. 2011;34(1):69-88. doi:10.1146/annurev-neuro-061010-113654.

199. McCarthy MM. Estradiol and the developing brain. *Physiol Rev*. 2008;88(1):91-124. doi:10.1152/physrev.00010.2007.

200. Apter D, Bützow TL, Laughlin GA, Yen SS. Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins. *J Clin Endocrinol Metab*. 1993;76(4):940-949. doi:10.1210/jcem.76.4.8473410.

201. Pohl CR, deRidder CM, Plant TM. Gonadal and nongonadal mechanisms contribute to the prepubertal hiatus in gonadotropin secretion in the female rhesus monkey (Macaca mulatta). *J Clin Endocrinol Metab*. 1995;80(7):2094-2101. doi:10.1210/jcem.80.7.7608261.

202. Rapisarda JJ, Bergman KS, Steiner RA, Foster DL. Response to estradiol inhibition of tonic luteinizing hormone secretion decreases during the final stage of puberty in the rhesus monkey. *Endocrinology*. 1983;112(4):1172-1179. doi:10.1210/endo-112-4-1172.

203. Wilson ME, Fisher J, Chikazawa K. Estradiol negative feedback regulates nocturnal luteinizing hormone and follicle-stimulating hormone secretion in prepubertal female rhesus monkeys. *J Clin Endocrinol Metab*. 2004;89(8):3973-3978. doi:10.1210/jc.2004-

0148.

204. Wilson ME, Schwartz SM, Walker ML, Gordon TP. Oestradiol and somatomedin-C influence body weight patterns in premenarchial rhesus monkeys housed outdoors. *J Endocrinol*. 1984;102(3):311-317. http://www.ncbi.nlm.nih.gov/pubmed/6384413. Accessed October 24, 2017.

205. Sisk CL, Foster DL. The neural basis of puberty and adolescence. 2004;7(10):1040-1047. doi:10.1038/nn1326.

206. Forbes EE, Williamson DE, Ryan ND, Dahl RE. Positive and negative affect in depression: influence of sex and puberty. *Ann N Y Acad Sci*. 2004;1021(1):341-347. doi:10.1196/annals.1308.042.

207. Dahl RE, Gunnar MR. Heightened stress responsivity and emotional reactivity during pubertal maturation: Implications for psychopathology. *Dev Psychopathol*. 2009;21(1):87-97. doi:10.1017/S0954579409000066.

208. Buchanan CM, Eccles JS, Becker JB. Are adolescents the victims of raging hormones: evidence for activational effects of hormones on moods and behavior at adolescence. *Psychol Bull*. 1992;111(1):62-107. http://www.ncbi.nlm.nih.gov/pubmed/1539089. Accessed December 12, 2017.

209. Petersen AC, Compas BE, Brooks-Gunn J, Stemmler M, Ey S, Grant KE. Depression in adolescence. *Am Psychol*. 1993;48(2):155-168. http://www.ncbi.nlm.nih.gov/pubmed/8442570. Accessed October 28, 2017.

210. Rutter M, Graham P, Chadwick OFD, Yule W. ADOLESCENT TURMOIL: FACT OR FICTION? *J Child Psychol Psychiatry*. 1976;17(1):35-56. doi:10.1111/j.1469-7610.1976.tb00372.x.

211. Nolen-Hoeksema S, Girgus JS. The emergence of gender differences in depression during adolescence. *Psychol Bull*. 1994;115(3):424-443. doi:10.1037/0033-2909.115.3.424.

212. Resnick MD, Bearman PS, Blum RW, et al. Protecting Adolescents From Harm. *JAMA*. 1997;278(10):823. doi:10.1001/jama.1997.03550100049038.

213. Arnett JJ. Adolescent storm and stress, reconsidered. *Am Psychol*. 1999;54(5):317-326. doi:10.1037/0003-066X.54.5.317.

214. Weissman MM, Bland RC, Canino GJ, et al. Cross-National Epidemiology of Major Depression and Bipolar Disorder. https://msrc.fsu.edu/system/files/Cross National Epidemiology of Major Depression and Bipolar Disorder.pdf. Accessed December 12, 2017.

215. Bebbington PE, Dunn G, Jenkins R, et al. The influence of age and sex on the prevalence of depressive conditions: report from the National Survey of Psychiatric Morbidity . *Psychol Med*. 1998;28(1):9-19. http://www.ncbi.nlm.nih.gov/pubmed/9483679. Accessed December 12, 2017.

216. Angold A, Costello EJ, Worthman CM. Puberty and depression: the roles of age, pubertal status and pubertal timing. *Psychol Med*. 1998;28(1):51-61. http://www.ncbi.nlm.nih.gov/pubmed/9483683. Accessed October 26, 2017.

217. Angold A, Costello EJ, Erkanli A, Worthman CM. Pubertal changes in hormone levels and depression in girls. *Psychol Med*. 1999;29(5):1043-1053. doi:10.1017/S0033291799008946.

218. Nelson R. *Intro to Behavioral Endocrinology*. Sinauer Associates; 2015.

219. Bloch M, Schmidt PJ, Danaceau M, Murphy J, Nieman L, Rubinow DR. Effects of Gonadal Steroids in Women With a History of Postpartum Depression. *Am J Psychiatry*.

2000;157(6):924-930. doi:10.1176/appi.ajp.157.6.924.

220. Freeman EW, Sammel MD, Lin H, Nelson DB. Associations of Hormones and Menopausal Status With Depressed Mood in Women With No History of Depression. *Arch Gen Psychiatry*. 2006;63(4):375. doi:10.1001/archpsyc.63.4.375.

221. Paikoff RL, Brooks-Gunn J, Warren MP. Effects of girls' hormonal status on depressive and aggressive symptoms over the course of one year. *J Youth Adolesc*. 1991;20(2):191-215. doi:10.1007/BF01537608.

222. Gunnar M, Quevedo K. The Neurobiology of Stress and Development. *Annu Rev Psychol*. 2007;58(1):145-173. doi:10.1146/annurev.psych.58.110405.085605.

223. Silberg J, Pickles A, Rutter M, et al. The influence of genetic factors and life stress on depression among adolescent girls. *Arch Gen Psychiatry*. 1999;56(3):225-232. http://www.ncbi.nlm.nih.gov/pubmed/10078499. Accessed December 12, 2017.

224. Vaillancourt C, Cyr M, Rochford J, Boksa P, Di Paolo T. Effects of ovariectomy and estradiol on acoustic startle responses in rats. *Pharmacol Biochem Behav*. 2002;74(1):103-109. http://www.ncbi.nlm.nih.gov/pubmed/12376157. Accessed October 26, 2017.

225. Swerdlow NR, Hartman PL, Auerbach PP. Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders. *Biol Psychiatry*. 1997;41(4):452-460. doi:10.1016/S0006-3223(96)00065-0.

226. Zehr JL, Maestripieri D, Wallen K. Estradiol Increases Female Sexual Initiation Independent of Male Responsiveness in Rhesus Monkeys. *Horm Behav*. 1998;33(2):95-103. doi:10.1006/hbeh.1998.1440.

227. Beach FA. Sexual attractivity, proceptivity, and receptivity in female mammals. *Horm Behav*. 1976;7(1):105-138. doi:10.1016/0018-506X(76)90008-8.

228. Michopoulos V, Checchi M, Sharpe D, Wilson ME. Estradiol effects on behavior and serum oxytocin are modified by social status and polymorphisms in the serotonin transporter gene in female rhesus monkeys. *Horm Behav*. 2011;59(4):528-535. doi:10.1016/j.yhbeh.2011.02.002.

229. WALLEN K, TANNENBAUM PL. Hormonal Modulation of Sexual Behavior and Affiliation in Rhesus Monkeys. *Ann N Y Acad Sci*. 1997;807(1 Integrative N):185-202. doi:10.1111/j.1749-6632.1997.tb51920.x.

230. Ogawa S, Eng V, Taylor J, Lubahn DB, Korach KS, Pfaff DW. Roles of estrogen receptor-alpha gene expression in reproduction-related behaviors in female mice. *Endocrinology*. 1998;139(12):5070-5081. doi:10.1210/endo.139.12.6357.

231. Díaz-Véliz G, Alarcón T, Espinoza C, Dussaubat N, Mora S. Ketanserin and anxiety levels: influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats. *Pharmacol Biochem Behav*. 1997;58(3):637-642. http://www.ncbi.nlm.nih.gov/pubmed/9329052. Accessed December 10, 2017.

232. Pandaranandaka J, Poonyachoti S, Kalandakanond-Thongsong S. Differential effects of exogenous and endogenous estrogen on anxiety as measured by elevated T-maze in relation to the serotonergic system. *Behav Brain Res*. 2009;198(1):142-148. doi:10.1016/j.bbr.2008.10.043.

233. Diaz-Veliz G, Soto V, Dussaubat N, Mora S. Influence of the estrous cycle, ovariectomy and estradiol replacement upon the acquisition of conditioned avoidance responses in rats. *Physiol Behav*. 1989;46(3):397-401. http://www.ncbi.nlm.nih.gov/pubmed/2623060. Accessed December 10, 2017.

234. Mora S, Dussaubat N, Díaz-Véliz G. Effects of the estrous cycle and ovarian hormones on

behavioral indices of anxiety in female rats. *Psychoneuroendocrinology*. 1996;21(7):609-620. http://www.ncbi.nlm.nih.gov/pubmed/9044444. Accessed December 10, 2017.

235. Olivera-Lopez JI, Molina-Hernández M, Tellez-Alcántara NP, Jaramillo MT. Estradiol and neuropeptide Y (intra-lateral septal) reduce anxiety-like behavior in two animal models of anxiety. *Peptides*. 2008;29(8):1396-1403. doi:10.1016/j.peptides.2008.04.002.

236. Galeeva A, Tuohimaa P. Analysis of mouse plus-maze behavior modulated by ovarian steroids. *Behav Brain Res*. 2001;119(1):41-47. http://www.ncbi.nlm.nih.gov/pubmed/11164524. Accessed December 10, 2017.

237. Bernardi M, Vergoni A V, Sandrini M, Tagliavini S, Bertolini A. Influence of ovariectomy, estradiol and progesterone on the behavior of mice in an experimental model of depression. *Physiol Behav*. 1989;45(5):1067-1068. http://www.ncbi.nlm.nih.gov/pubmed/2780868. Accessed December 10, 2017.

238. Koss WA, Einat H, Schloesser RJ, Manji HK, Rubinow DR. Estrogen effects on the forced swim test differ in two outbred rat strains. *Physiol Behav*. 2012;106(2):81-86. doi:10.1016/j.physbeh.2012.01.004.

239. Okada M, Hayashi N, Kometani M, Nakao K, Inukai T. Influences of ovariectomy and continuous replacement of 17beta-estradiol on the tail skin temperature and behavior in the forced swimming test in rats. *Jpn J Pharmacol*. 1997;73(1):93-96. doi:10.1254/jjp.73.93.

240. Dalla C, Antoniou K, Papadopoulou-Daifoti Z, Balthazart J, Bakker J. Oestrogen-deficient female aromatase knockout (ArKO) mice exhibit depressive-like symptomatology. *Eur J Neurosci*. 2004;20(1):217-228. doi:10.1111/j.1460-9568.2004.03443.x.

241. Schmidt PJ, Rubinow DR. Sex Hormones and Mood in the Perimenopause. *Ann N Y Acad*

Sci. 2009;1179(1):70-85. doi:10.1111/j.1749-6632.2009.04982.x.

242. Zweifel JE, O'Brien WH. A meta-analysis of the effect of hormone replacement therapy upon depressed mood. *Psychoneuroendocrinology*. 1997;22(3):189-212. http://www.ncbi.nlm.nih.gov/pubmed/9203229. Accessed December 11, 2017.

243. Morgan M., Schulkin J, Pfaff D. Estrogens and non-reproductive behaviors related to activity and fear. *Neurosci Biobehav Rev*. 2004;28(1):55-63. doi:10.1016/J.NEUBIOREV.2003.11.017.

244. Lund TD, Rovis T, Chung WCJ, Handa RJ. Novel Actions of Estrogen Receptor-β on Anxiety-Related Behaviors. *Endocrinology*. 2005;146(2):797-807. doi:10.1210/en.2004-1158.

245. Walf AA, Frye CA. Antianxiety and Antidepressive Behavior Produced by Physiological Estradiol Regimen may be Modulated by Hypothalamic–Pituitary–Adrenal Axis Activity. *Neuropsychopharmacology*. 2005;30(7):1288-1301. doi:10.1038/sj.npp.1300708.

246. Walf AA, Frye CA. ERb-Selective Estrogen Receptor Modulators Produce Antianxiety Behavior when Administered Systemically to Ovariectomized Rats. doi:10.1038/sj.npp.1300713.

247. Tomihara K, Soga T, Nomura M, et al. Effect of ER-β gene disruption on estrogenic regulation of anxiety in female mice. *Physiol Behav*. 2009;96(2):300-306. doi:10.1016/j.physbeh.2008.10.014.

248. Morgan MA, Pfaff DW. Effects of Estrogen on Activity and Fear-Related Behaviors in Mice. *Horm Behav*. 2001;40(4):472-482. doi:10.1006/HBEH.2001.1716.

249. Olesen KM, Ismail N, Merchasin ED, Blaustein JD. Long-term alteration of anxiolytic effects of ovarian hormones in female mice by a peripubertal immune challenge. *Horm*

Behav. 2011;60(4):318-326. doi:10.1016/j.yhbeh.2011.06.005.

250. Ismail N, Kumlin AM, Blaustein JD. A pubertal immune challenge alters the antidepressant-like effects of chronic estradiol treatment in inbred and outbred adult female mice. *Neuroscience*. 2013;249:43-52. doi:10.1016/j.neuroscience.2012.09.047.

251. Toffol E, Heikinheimo O, Partonen T. Associations between psychological well-being, mental health, and hormone therapy in perimenopausal and postmenopausal women. *Menopause*. 2013;20(6):667-676. doi:10.1097/gme.0b013e318278eec1.

252. Burgess LH, Handa RJ. Chronic estrogen-induced alterations in adrenocorticotropin and corticosterone secretion, and glucocorticoid receptor-mediated functions in female rats. *Endocrinology*. 1992;131(3):1261-1269. doi:10.1210/endo.131.3.1324155.

253. Carey MP, Deterd CH, de Koning J, Helmerhorst F, de Kloet ER. The influence of ovarian steroids on hypothalamic-pituitary-adrenal regulation in the female rat. *J Endocrinol*. 1995;144(2):311-321. http://www.ncbi.nlm.nih.gov/pubmed/7706984. Accessed December 13, 2017.

254. Giussani DA, Farber DM, Jenkins SL, et al. Opposing effects of androgen and estrogen on pituitary-adrenal function in nonpregnant primates. *Biol Reprod*. 2000;62(5):1445-1451. http://www.ncbi.nlm.nih.gov/pubmed/10775199. Accessed August 8, 2017.

255. Roy BN, Reid RL, Vugt DAVAN, R DPBN, Gynecology RLR. The Effects of Estrogen and Progesterone on Corticotropin-Releasing Hormone and Arginine Vasopressin Messenger Ribonucleic Acid Levels in the Paraventricular Nucleus and Supraoptic Nucleus of the Rhesus Monkey. 1999;140(5).

256. Weiser MJ, Handa RJ. Estrogen impairs glucocorticoid dependent negative feedback on the hypothalamic–pituitary–adrenal axis via estrogen receptor alpha within the

hypothalamus. *Neuroscience*. 2009;159(2):883-895. doi:10.1016/j.neuroscience.2008.12.058.

257. Holder, Mary K., Blaustein J. Puberty and Adolescence as a Time of Vulnerability to Stressors that Alter Neurobehavioral Processes. 2012;76(October 2009):211-220. doi:10.1007/s11103-011-9767-z.Plastid.

258. Romeo RD. Pubertal maturation and programming of hypothalamic-pituitary-adrenal reactivity. *Front Neuroendocrinol*. 2010;31(2):232-240. doi:10.1016/j.yfrne.2010.02.004.

259. Wilson ME, Kinkead B. Gene-Environment Interactions, Not Neonatal Growth Hormone Deficiency, Time Puberty in Female Rhesus Monkeys. 2009;78(4):736-743. doi:10.1095/biolreprod.107.065953.Gene-Environment.

260. Ahmed E, Zehr J, Schulz K, Lorenz B. Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions. *Nature*. 2008. https://www.nature.com/articles/nn.2178. Accessed August 8, 2017.

261. Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. *Horm Behav*. 2009;55(5):597-604. doi:10.1016/j.yhbeh.2009.03.010.

262. Stoppe G, Dören M. Critical appraisal of effects of estrogen replacement therapy on symptoms of depressed mood. *Arch Womens Ment Health*. 2002;5(2):39-47. doi:10.1007/s00737-002-0139-1.

263. Bethea CL, Reddy AP. Effect of ovarian hormones on survival genes in laser captured serotonin neurons from macaques. *J Neurochem*. 2008;105(4):1129-1143. doi:10.1111/j.1471-4159.2008.05213.x.

264. Dreher J, Schmidt P, Kohn P. Menstrual cycle phase modulates reward-related neural

function in women. *Proc*. 2007. http://www.pnas.org/content/104/7/2465.short. Accessed August 8, 2017.

265. Cooke B, Woolley C. Gonadal hormone modulation of dendrites in the mammalian CNS. *Dev Neurobiol*. 2005. http://onlinelibrary.wiley.com/doi/10.1002/neu.20143/full. Accessed August 8, 2017.

266. Li C, Brake W, Romeo R. Estrogen alters hippocampal dendritic spine shape and enhances synaptic protein immunoreactivity and spatial memory in female mice. *Proc*. 2004. http://www.pnas.org/content/101/7/2185.short. Accessed August 8, 2017.

267. Sá S, Lukoyanova E, Madeira M. Effects of estrogens and progesterone on the synaptic organization of the hypothalamic ventromedial nucleus. *Neuroscience*. 2009. http://www.sciencedirect.com/science/article/pii/S030645220900712X. Accessed August 8, 2017.

268. Garcia-Segura L, Melcangi R. Steroids and glial cell function. *Glia*. 2006. http://onlinelibrary.wiley.com/doi/10.1002/glia.20404/full. Accessed August 8, 2017.

269. Koolschijn MP, Peper JS, Crone EA. The Influence of Sex Steroids on Structural Brain Maturation in Adolescence. 2014;9(1). doi:10.1371/journal.pone.0083929.

270. Brouwer RM, Hugo MMGK, Fsh ÁHÁ. Longitudinal Development of Hormone Levels and Grey Matter Density in 9 and 12-Year-Old Twins. *Behav Genet*. 2015:313-323. doi:10.1007/s10519-015-9708-8.

271. Markham JA, Morris JR, Juraska JM. Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. *Neuroscience*. 2007;144(3):961-968. doi:10.1016/J.NEUROSCIENCE.2006.10.015.

272. Willing J, Juraska JM. The timing of neuronal loss across adolescence in the medial

prefrontal cortex of male and female rats. *Neuroscience*. 2015;301:268-275. doi:10.1016/J.NEUROSCIENCE.2015.05.073.

273. Koss WA, Lloyd MM, Sadowski RN, Wise LM, Juraska JM. Gonadectomy before puberty increases the number of neurons and glia in the medial prefrontal cortex of female, but not male, rats. *Dev Psychobiol*. 2015;57(3):305-312. doi:10.1002/dev.21290.
274. Peper J, Brouwer R, Schnack H. Sex steroids and brain structure in pubertal boys and girls. 2009. http://www.sciencedirect.com/science/article/pii/S0306453008002539. Accessed August 8, 2017.
275. Perlman S, Pelphrey K. Developing connections for affective regulation: age-related changes in emotional brain connectivity. *J Exp Child Psychol*. 2011. http://www.sciencedirect.com/science/article/pii/S0022096510001657. Accessed August 8, 2017.
276. Gabard-Durnam LJ, Flannery J, Goff B, et al. The development of human amygdala functional connectivity at rest from 4 to 23 years: a cross-sectional study. *Neuroimage*. 2014;95:193-207. doi:10.1016/j.neuroimage.2014.03.038.
277. Gee DG, Humphreys KL, Flannery J, et al. A Developmental Shift from Positive to Negative Connectivity in Human Amygdala – Prefrontal Circuitry. 2013;33(10):4584-4593. doi:10.1523/JNEUROSCI.3446-12.2013.
278. Weis S, Hausmann M, Stoffers B, Vohn R. Estradiol modulates functional brain organization during the menstrual cycle: an analysis of interhemispheric inhibition. *J*. 2008. http://www.jneurosci.org/content/28/50/13401.short. Accessed August 8, 2017.
279. Ottowitz W, Derro D, Dougherty D. Analysis of Amygdalar-Cortical Network Covariance During Pre-versus Post-menopausal Estrogen Levels: Potential Relevance to Resting State

Networks,. *Neuro Endocrinol*. 2008. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3947844/. Accessed August 8, 2017.

280. Yankova M, Hart SA, Woolley CS. Estrogen increases synaptic connectivity between single presynaptic inputs and multiple postsynaptic CA1 pyramidal cells: a serial electron-microscopic study. *Proc Natl Acad Sci U S A*. 2001;98(6):3525-3530. doi:10.1073/pnas.051624598.

281. Maejima T, Masseck OA, Mark MD, Herlitze S. Modulation of firing and synaptic transmission of serotonergic neurons by intrinsic G protein-coupled receptors and ion channels. *Front Integr Neurosci*. 2013;7:40. doi:10.3389/fnint.2013.00040.

282. Yokomaku D, Numakawa T, Numakawa Y, et al. Estrogen Enhances Depolarization-Induced Glutamate Release through Activation of Phosphatidylinositol 3-Kinase and Mitogen-Activated Protein Kinase in Cultured Hippocampal Neurons. *Mol Endocrinol*. 2003;17(5):831-844. doi:10.1210/me.2002-0314.

283. Morissette M, Biron D, Di Paolo T. Effect of estradiol and progesterone on rat striatal dopamine uptake sites. *Brain Res Bull*. 1990;25(3):419-422. doi:10.1016/0361-9230(90)90231-N.

284. Lévesque D, Di Paolo T. Dopamine receptor reappearance after irreversible receptor blockade: effect of chronic estradiol treatment of ovariectomized rats. *Mol Pharmacol*. 1991;39(5):659-665. http://www.ncbi.nlm.nih.gov/pubmed/1674586. Accessed December 14, 2017.

285. Morissette M, Di Paolo T. Sex and estrous cycle variations of rat striatal dopamine uptake sites. *Neuroendocrinology*. 1993;58(1):16-22. http://www.ncbi.nlm.nih.gov/pubmed/8264850. Accessed December 14, 2017.

286. Morissette M, Di Paolo T. Effect of chronic estradiol and progesterone treatments of ovariectomized rats on brain dopamine uptake sites. *J Neurochem*. 1993;60(5):1876-1883. http://www.ncbi.nlm.nih.gov/pubmed/8473903. Accessed December 14, 2017.

287. Becker JB, Hu M. Sex differences in drug abuse. *Front Neuroendocrinol*. 2008;29(1):36-47. doi:10.1016/j.yfrne.2007.07.003.

288. Morissette M, Le Saux M, D'Astous M, et al. Contribution of estrogen receptors alpha and beta to the effects of estradiol in the brain. *J Steroid Biochem Mol Biol*. 2008;108(3-5):327-338. doi:10.1016/j.jsbmb.2007.09.011.

289. Leranth C, Roth RH, Elsworth JD, Naftolin F, Horvath TL, Redmond DE. Estrogen is essential for maintaining nigrostriatal dopamine neurons in primates: implications for Parkinson's disease and memory. *J Neurosci*. 2000;20(23):8604-8609. http://www.ncbi.nlm.nih.gov/pubmed/11102464. Accessed December 14, 2017.

290. Kritzer MF, Kohama SG. Ovarian hormones influence the morphology, distribution, and density of tyrosine hydroxylase immunoreactive axons in the dorsolateral prefrontal cortex of adult rhesus monkeys. *J Comp Neurol*. 1998;395(1):1-17. http://www.ncbi.nlm.nih.gov/pubmed/9590542. Accessed December 14, 2017.

291. Bethea CL, Gundlah C, Mirkes SJ. Ovarian steroid action in the serotonin neural system of macaques. *Novartis Found Symp*. 2000;230:112-30-3. http://www.ncbi.nlm.nih.gov/pubmed/10965505. Accessed December 14, 2017.

292. Martinowich K, Lu B. Interaction between BDNF and Serotonin: Role in Mood Disorders. *Neuropsychopharmacology*. 2008;33(1):73-83. doi:10.1038/sj.npp.1301571.

293. Di Paolo T, Lévesque D, Daigle M. A physiological dose of progesterone affects rat striatum biogenic amine metabolism. *Eur J Pharmacol*. 1986;125(1):11-16.

http://www.ncbi.nlm.nih.gov/pubmed/2426126. Accessed January 16, 2018.

294. Fabre-Nys C, Blache D, Hinton MR, Goode JA, Kendrick KM. Microdialysis measurement of neurochemical changes in the mediobasal hypothalamus of ovariectomized ewes during oestrus. *Brain Res*. 1994;649(1-2):282-296. http://www.ncbi.nlm.nih.gov/pubmed/7953644. Accessed January 16, 2018.

295. Renner KJ, Krey LC, Luine VN. Effect of progesterone on monoamine turnover in the brain of the estrogen-primed rat. *Brain Res Bull*. 1987;19(2):195-202. http://www.ncbi.nlm.nih.gov/pubmed/3664280. Accessed January 16, 2018.

296. Pecins-Thompson M, Brown NA, Kohama SG, Bethea CL. Ovarian steroid regulation of tryptophan hydroxylase mRNA expression in rhesus macaques. *J Neurosci*. 1996;16(21):7021-7029. http://www.ncbi.nlm.nih.gov/pubmed/8824338. Accessed December 14, 2017.

297. Beyer C, Feder HH. Sex Steroids and Afferent Input: Their Roles in Brain Sexual Differentiation. *Annu Rev Physiol*. 1987;49(1):349-364. doi:10.1146/annurev.ph.49.030187.002025.

298. Shively CA, Bethea CL. Cognition, mood disorders, and sex hormones. *ILAR J*. 2004;45(2):189-199. http://www.ncbi.nlm.nih.gov/pubmed/15111738. Accessed December 15, 2017.

299. Li K, Xu E. The role and the mechanism of gamma-aminobutyric acid during central nervous system development. *Neurosci Bull*. 2008;24(3):195-200. http://www.ncbi.nlm.nih.gov/pubmed/18500393. Accessed December 16, 2017.

300. Goldman-Rakic PS, Brown RM. Postnatal development of monoamine content and synthesis in the cerebral cortex of rhesus monkeys. *Brain Res*. 1982;256(3):339-349.

http://www.ncbi.nlm.nih.gov/pubmed/7104766. Accessed December 15, 2017.

301. Rosenberg DR, Lewis DA. Postnatal maturation of the dopaminergic innervation of monkey prefrontal and motor cortices: A tyrosine hydroxylase immunohistochemical analysis. *J Comp Neurol*. 1995;358(3):383-400. doi:10.1002/cne.903580306.

302. Rosenberg DR, Lewis DA. Changes in the dopaminergic innervation of monkey prefrontal cortex during late postnatal development: A tyrosine hydroxylase immunohistochemical study. *Biol Psychiatry*. 1994;36(4):272-277. doi:10.1016/0006-3223(94)90610-6.

303. Erickson SL, Akil M, Levey AI, Lewis DA. Postnatal development of tyrosine hydroxylase- and dopamine transporter-immunoreactive axons in monkey rostral entorhinal cortex. *Cereb Cortex*. 8(5):415-427. http://www.ncbi.nlm.nih.gov/pubmed/9722085. Accessed December 15, 2017.

304. Arain M, Haque M, Johal L, et al. Maturation of the adolescent brain. *Neuropsychiatr Dis Treat*. 2013;9:449-461. doi:10.2147/NDT.S39776.

305. Crews F, He J, Hodge C. Adolescent cortical development: A critical period of vulnerability for addiction. *Pharmacol Biochem Behav*. 2007;86(2):189-199. doi:10.1016/j.pbb.2006.12.001.

306. Depue RA, Spoont MR. Conceptualizing a serotonin trait. A behavioral dimension of constraint. *Ann N Y Acad Sci*. 1986;487:47-62. http://www.ncbi.nlm.nih.gov/pubmed/2436540. Accessed December 16, 2017.

307. Morilak DA, Ciaranello RD. Ontogeny of 5-hydroxytryptamine2 receptor immunoreactivity in the developing rat brain. *Neuroscience*. 1993;55(3):869-880. doi:10.1016/0306-4522(93)90447-N.

308. Haycock JW, Becker L, Ang L, Furukawa Y, Hornykiewicz O, Kish SJ. Marked disparity

between age-related changes in dopamine and other presynaptic dopaminergic markers in human striatum. *J Neurochem*. 2003;87(3):574-585. http://www.ncbi.nlm.nih.gov/pubmed/14535941. Accessed December 16, 2017.

309. Quevedo K, Benning S, Gunnar M. The onset of puberty: Effects on the psychophysiology of defensive and appetitive motivation. *Dev*. 2009. https://www.cambridge.org/core/journals/development-and-psychopathology/article/onset-of-puberty-effects-on-the-psychophysiology-of-defensive-and-appetitive-motivation/9AAB57C4F2E364AEC179481A1EB77A03. Accessed August 18, 2017.

310. Balada F, Torrubia R, Arqué JM. Gonadal hormone correlates of sensation seeking and anxiety in healthy human females. *Neuropsychobiology*. 1993;27(2):91-96. doi:10.1159/000118960.

311. Rivier C, Rivest S. Effect of stress on the activity of the hypothalamic-pituitary-gonadal axis: peripheral and central mechanisms. *Biol Reprod*. 1991;45(4):523-532. http://www.ncbi.nlm.nih.gov/pubmed/1661182. Accessed January 10, 2018.

312. Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr Rev*. 2000;21(1):55-89. doi:10.1210/edrv.21.1.0389.

313. Kaplan JR, Chen H, Appt SE, et al. Impairment of ovarian function and associated health-related abnormalities are attributable to low social status in premenopausal monkeys and not mitigated by a high-isoflavone soy diet. *Hum Reprod*. 2010;25(12):3083-3094. doi:10.1093/humrep/deq288.

314. Graber JA. Pubertal timing and the development of psychopathology in adolescence and

beyond. *Horm Behav*. 2013;64(2):262-269. doi:10.1016/j.yhbeh.2013.04.003.

315. Ge X, Conger RD, Elder GH. Pubertal transition, stressful life events, and the emergence of gender differences in adolescent depressive symptoms. *Dev Psychol*. 2001;37(3):404-417. http://www.ncbi.nlm.nih.gov/pubmed/11370915. Accessed October 24, 2017.
316. Kaltiala-Heino R, Marttunen M, Rantanen P, Rimpelä M. Early puberty is associated with mental health problems in middle adolescence. *Soc Sci Med*. 2003;57(6):1055-1064. http://www.ncbi.nlm.nih.gov/pubmed/12878105. Accessed October 24, 2017.
317. Angold A, Costello EJ. Puberty and Depression. *Child Adolesc Psychiatr Clin N Am*. 2006;15(4):919-937. doi:10.1016/j.chc.2006.05.013.
318. Rierdan J, Koff E. Developmental variables in relation to depressive symptoms in adolescent girls. *Dev Psychopathol*. 1993;5(3):485. doi:10.1017/S0954579400004533.
319. Piekarski DJ, Johnson CM, Boivin JR, et al. Does puberty mark a transition in sensitive periods for plasticity in the associative neocortex ? *Brain Res*. 2016. doi:10.1016/j.brainres.2016.08.042.
320. Schulz KM, Sisk CL. Neuroscience and Biobehavioral Reviews The organizing actions of adolescent gonadal steroid hormones on brain and behavioral development. *Neurosci Biobehav Rev*. 2016;70:148-158. doi:10.1016/j.neubiorev.2016.07.036.
321. Baker KD, Loughman A, Spencer SJ, Reichelt AC. The impact of obesity and hypercaloric diet consumption on anxiety and emotional behavior across the lifespan. *Neurosci Biobehav Rev*. 2017;83(June):173-182. doi:10.1016/j.neubiorev.2017.10.014.
322. Puder JJ, Munsch S. Psychological correlates of childhood obesity. *Int J Obes*. 2010;34(S2):S37-S43. doi:10.1038/ijo.2010.238.
323. Onyike CU, Crum RM, Lee HB, Lyketsos CG, Eaton WW. Is obesity associated with

major depression? Results from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. 2003;158(12):1139-1147. http://www.ncbi.nlm.nih.gov/pubmed/14652298. Accessed October 25, 2017.

324. Rofey DL, Kolko RP, Iosif A-M, et al. A Longitudinal Study of Childhood Depression and Anxiety in Relation to Weight Gain. *Child Psychiatry Hum Dev*. 2009;40(4):517-526. doi:10.1007/s10578-009-0141-1.

325. Wagner I V., Sabin MA, Pfäffle RW, et al. Effects of obesity on human sexual development. *Nat Rev Endocrinol*. 2012;8(4):246-254. doi:10.1038/nrendo.2011.241.

326. Terasawa E, Kurian JR, Keen KL, Shiel NA, Colman RJ, Capuano S V. Body weight impact on puberty: effects of high-calorie diet on puberty onset in female rhesus monkeys. *Endocrinology*. 2012;153(4):1696-1705. doi:10.1210/en.2011-1970.

327. Schwartz SM, Wilson ME, Walker ML, Collins DC. Dietary influences on growth and sexual maturation in premenarchial rhesus monkeys. *Horm Behav*. 1988;22(2):231-251. http://www.ncbi.nlm.nih.gov/pubmed/3294160. Accessed December 17, 2017.

328. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384(9945):766-781. doi:10.1016/S0140-6736(14)60460-8.

329. Center for Health Statistics N. Health, United States, 2011. 2011. https://www.cdc.gov/nchs/data/hus/hus11.pdf. Accessed October 24, 2017.

330. Whitaker RC, Wright JA, Pepe MS, Seidel KD, Dietz WH. Predicting obesity in young adulthood from childhood and parental obesity. *N Engl J Med*. 1997;337(13):869-873. doi:10.1056/NEJM199709253371301.

331. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 2012;380(9859):2224-2260. doi:10.1016/S0140-6736(12)61766-8.

332. Dietz WH. Health consequences of obesity in youth: childhood predictors of adult disease. *Pediatrics*. 1998;101(3 Pt 2):518-525. http://www.ncbi.nlm.nih.gov/pubmed/12224658. Accessed October 15, 2017.

333. Freedman DS, Mei Z, Srinivasan SR, Berenson GS, Dietz WH. Cardiovascular risk factors and excess adiposity among overweight children and adolescents: the Bogalusa Heart Study. *J Pediatr*. 2007;150(1):12-17.e2. doi:10.1016/j.jpeds.2006.08.042.

334. Freedman DS, Khan LK, Dietz WH, Srinivasan SR, Berenson GS. Relationship of childhood obesity to coronary heart disease risk factors in adulthood: the Bogalusa Heart Study. *Pediatrics*. 2001;108(3):712-718. http://www.ncbi.nlm.nih.gov/pubmed/11533341. Accessed October 15, 2017.

335. Dabelea D, Harrod CS. Role of developmental overnutrition in pediatric obesity and type 2 diabetes. *Nutr Rev*. 2013;71 Suppl 1:S62-7. doi:10.1111/nure.12061.

336. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev*. 2006;27(7):750-761. doi:10.1210/er.2006-0032.

337. Must A, McKeown NM. *The Disease Burden Associated with Overweight and Obesity*.; 2000. http://www.ncbi.nlm.nih.gov/pubmed/25905320. Accessed October 24, 2017.

338. Visscher TL, Seidell JC. The Public Health Impact of Obesity. *Annu Rev Public Health*. 2001;22(1):355-375. doi:10.1146/annurev.publhealth.22.1.355.

339. Withrow D, Alter DA. The economic burden of obesity worldwide: a systematic review of

the direct costs of obesity. *Obes Rev*. 2011;12(2):131-141. doi:10.1111/j.1467-789X.2009.00712.x.

340. Tsai AG, Williamson DF, Glick HA. Direct medical cost of overweight and obesity in the USA: a quantitative systematic review. *Obes Rev*. 2011;12(1):50-61. doi:10.1111/j.1467-789X.2009.00708.x.

341. Evans GW, Fuller-Rowell TE, Doan SN. Childhood cumulative risk and obesity: the mediating role of self-regulatory ability. *Pediatrics*. 2012;129(1):e68-73. doi:10.1542/peds.2010-3647.

342. Suglia SF, Duarte CS, Chambers EC, Boynton-Jarrett R. Cumulative Social Risk and Obesity in Early Childhood. *Pediatrics*. 2012;129(5):e1173-e1179. doi:10.1542/peds.2011-2456.

343. Noll JG, Zeller MH, Trickett PK, Putnam FW. Obesity Risk for Female Victims of Childhood Sexual Abuse: A Prospective Study. *Pediatrics*. 2007;120(1):e61-e67. doi:10.1542/peds.2006-3058.

344. Schneiderman JU, Mennen FE, Negriff S, Trickett PK. Overweight and obesity among maltreated young adolescents. *Child Abuse Negl*. 2012;36(4):370-378. doi:10.1016/j.chiabu.2012.03.001.

345. Mazur J, Dzielska A, Małkowska-Szkutnik A. Psychological determinants of selected eating behaviours in adolescents. *Med Wieku Rozwoj*. 2011;15(3):240-249. http://www.ncbi.nlm.nih.gov/pubmed/22006479. Accessed October 15, 2017.

346. Koch F-S, Sepa A, Ludvigsson J. Psychological Stress and Obesity. *J Pediatr*. 2008;153(6):839-844.e3. doi:10.1016/j.jpeds.2008.06.016.

347. Moens E, Braet C, Bosmans G, Rosseel Y. Unfavourable family characteristics and their

associations with childhood obesity: a cross-sectional study. *Eur Eat Disord Rev*. 2009;17(4):315-323. doi:10.1002/erv.940.

348. Gundersen C, Mahatmya D, Garasky S, Lohman B. Linking psychosocial stressors and childhood obesity. *Obes Rev*. 2011;12(5):e54-63. doi:10.1111/j.1467-789X.2010.00813.x.

349. Anderson SE, Gooze RA, Lemeshow S, Whitaker RC. Quality of early maternal-child relationship and risk of adolescent obesity. *Pediatrics*. 2012;129(1):132-140. doi:10.1542/peds.2011-0972.

350. Lutter M, Nestler EJ. Homeostatic and Hedonic Signals Interact in the Regulation of Food Intake. *J Nutr*. 2009;139(3):629-632. doi:10.3945/jn.108.097618.

351. Andermann ML, Lowell BB. Toward a Wiring Diagram Understanding of Appetite Control. *Neuron*. 2017;95(4):757-778. doi:10.1016/j.neuron.2017.06.014.

352. Ghashghaei HT, Hilgetag CC, Barbas H. Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage*. 2007;34(3):905-923. doi:10.1016/j.neuroimage.2006.09.046.

353. Warne JP. Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. *Mol Cell Endocrinol*. 2009;300(1-2):137-146. doi:10.1016/j.mce.2008.09.036.

354. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.

355. Volkow ND, Wang GJ, Fowler JS, Tomasi D, Baler R. Food and Drug Reward: Overlapping Circuits in Human Obesity and Addiction. *Brain Imaging Behav Neurosci*. 2012;(November 2011):289-320. doi:10.1007/7854.

356. Tomasi D, Volkow ND. Striatocortical pathway dysfunction in addiction and obesity:

differences and similarities. *Crit Rev Biochem Mol Biol*. 2013;48(1):1-19. doi:10.3109/10409238.2012.735642.

357. Wise RA. Role of brain dopamine in food reward and reinforcement. *Philos Trans R Soc B Biol Sci*. 2006;361(1471):1149-1158. doi:10.1098/rstb.2006.1854.

358. Baxter MG, Murray EA. The amygdala and reward. *Nat Rev Neurosci*. 2002;3(7):563-573. doi:10.1038/nrn875.

359. Rolls ET. Emotion and decision-making explained: a précis. *Cortex*. 2014;59:185-193. doi:10.1016/j.cortex.2014.01.020.

360. Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB. A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. *Brain Struct Funct*. 2010;214(5-6):519-534. doi:10.1007/s00429-010-0255-z.

361. Ma N, Liu Y, Li N, et al. Addiction related alteration in resting-state brain connectivity. *Neuroimage*. 2010;49(1):738-744. doi:10.1016/j.neuroimage.2009.08.037.

362. Kim MJ, Loucks RA, Palmer AL, et al. The structural and functional connectivity of the amygdala : From normal emotion to pathological anxiety. *Behav Brain Res*. 2011;223(2):403-410. doi:10.1016/j.bbr.2011.04.025.

363. Bush G, Luu P, Posner M. Cognitive and emotional influences in anterior cingulate cortex. *Trends Cogn Sci*. 2000;4(6):215-222. doi:10.1016/S1364-6613(00)01483-2.

364. Carter CS, Braver TS, Barch DM, Botvinick MM, Noll D, Cohen JD. Anterior cingulate cortex, error detection, and the online monitoring of performance. *Science*. 1998;280(5364):747-749. doi:10.1126/SCIENCE.280.5364.747.

365. Grabenhorst F, Rolls ET. Value, pleasure and choice in the ventral prefrontal cortex. *Trends Cogn Sci*. 2011;15(2):56-67. doi:10.1016/j.tics.2010.12.004.

366. Grabenhorst F, Rolls ET, Bilderbeck A. How cognition modulates affective responses to taste and flavor: top-down influences on the orbitofrontal and pregenual cingulate cortices. *Cereb Cortex*. 2008;18(7):1549-1559. doi:10.1093/cercor/bhm185.

367. Frank MJ, Claus ED. Anatomy of a decision: striato-orbitofrontal interactions in reinforcement learning, decision making, and reversal. *Psychol Rev*. 2006;113(2):300-326. doi:10.1037/0033-295X.113.2.300.

368. Padoa-Schioppa C, Assad JA. Neurons in the orbitofrontal cortex encode economic value. *Nature*. 2006;441(7090):223-226. doi:10.1038/nature04676.

369. Padoa-Schioppa C. Neurobiology of Economic Choice: A Good-Based Model. *Annu Rev Neurosci*. 2011;34(1):333-359. doi:10.1146/annurev-neuro-061010-113648.

370. Jimura K, Locke HS, Braver TS. Prefrontal cortex mediation of cognitive enhancement in rewarding motivational contexts. *Proc Natl Acad Sci U S A*. 2010;107(19):8871-8876. doi:10.1073/pnas.1002007107.

371. Menon V, Uddin L. Saliency, switching, attention and control: a network model of insula function. *Brain*. 2010;214:655-667. doi:10.1007/s00429-010-0262-0.Saliency.

372. Mesulam M, Mufson EJ. Insula of the Old World Monkey . I : Architectonics in the Insulo-orbito- temporal Component of the. 1982;22:1-22.

373. Mufson EJ, Mesulam M. Insula of the Old World Monkey . 11 : Afferent Cortical Input and Comments on the Claustrum. 1982;37:23-37.

374. Russo SJ, Bolanos CA, Theobald DE, et al. IRS2-Akt pathway in midbrain dopamine neurons regulates behavioral and cellular responses to opiates. *Nat Neurosci*. 2007;10(1):93-99. doi:10.1038/nn1812.

375. Nestler EJ. Molecular basis of long-term plasticity underlying addiction. *Nat Rev*

Neurosci. 2001;2(2):119-128. doi:10.1038/35053570.

376. Nestler EJ. Is there a common molecular pathway for addiction? *Nat Neurosci*. 2005;8(11):1445-1449. doi:10.1038/nn1578.

377. Teegarden SL, Bale TL. Decreases in Dietary Preference Produce Increased Emotionality and Risk for Dietary Relapse. *Biol Psychiatry*. 2007;61(9):1021-1029. doi:10.1016/j.biopsych.2006.09.032.

378. Olausson P, Jentsch JD, Tronson N, Neve RL, Nestler EJ, Taylor JR. DeltaFosB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. *J Neurosci*. 2006;26(36):9196-9204. doi:10.1523/JNEUROSCI.1124-06.2006.

379. Johnson PM, Kenny PJ. Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats. *Nat Neurosci*. 2010;13(5):635-641. doi:10.1038/nn.2519.

380. Alsiö J, Olszewski PK, Norbäck AH, et al. Dopamine D1 receptor gene expression decreases in the nucleus accumbens upon long-term exposure to palatable food and differs depending on diet-induced obesity phenotype in rats. *Neuroscience*. 2010;171(3):779-787. doi:10.1016/j.neuroscience.2010.09.046.

381. Geiger BM, Haburcak M, Avena NM, Moyer MC, Hoebel BG, Pothos EN. Deficits of mesolimbic dopamine neurotransmission in rat dietary obesity. *Neuroscience*. 2009;159(4):1193-1199. doi:10.1016/j.neuroscience.2009.02.007.

382. Barbano MF, Cador M. Opioids for hedonic experience and dopamine to get ready for it. *Psychopharmacology (Berl)*. 2007;191(3):497-506. doi:10.1007/s00213-006-0521-1.

383. Le Merrer J, Becker JAJ, Befort K, Kieffer BL. Reward Processing by the Opioid System in the Brain. *Physiol Rev*. 2009;89(4):1379-1412. doi:10.1152/physrev.00005.2009.

384. Zhang M, Gosnell BA, Kelley AE. Intake of high-fat food is selectively enhanced by mu

opioid receptor stimulation within the nucleus accumbens. *J Pharmacol Exp Ther*. 1998;285(2):908-914. http://www.ncbi.nlm.nih.gov/pubmed/9580643. Accessed October 25, 2017.

385. Vucetic Z, Kimmel J, Reyes TM. Chronic high-fat diet drives postnatal epigenetic regulation of μ-opioid receptor in the brain. *Neuropsychopharmacology*. 2011;36(6):1199-1206. doi:10.1038/npp.2011.4.

386. Janthakhin Y, Rincel M, Costa A, Darnaudéry M. Psychoneuroendocrinology Maternal high-fat diet leads to hippocampal and amygdala dendritic remodeling in adult male o ff spring. *Psychoneuroendocrinology*. 2017;83(April):49-57. doi:10.1016/j.psyneuen.2017.05.003.

387. Chang G-Q, Gaysinskaya V, Karatayev O, Leibowitz SF. Maternal High-Fat Diet and Fetal Programming: Increased Proliferation of Hypothalamic Peptide-Producing Neurons That Increase Risk for Overeating and Obesity. *J Neurosci*. 2008;28(46):12107-12119. doi:10.1523/JNEUROSCI.2642-08.2008.

388. Sullivan EL, Grayson B, Takahashi D, et al. Chronic consumption of a high-fat diet during pregnancy causes perturbations in the serotonergic system and increased anxiety-like behavior in nonhuman primate offspring. *J Neurosci*. 2010;30(10):3826-3830. doi:10.1523/JNEUROSCI.5560-09.2010.

389. Rivera HM, Kievit P, Kirigiti MA, et al. Maternal High-Fat Diet and Obesity Impact Palatable Food Intake and Dopamine Signaling in Nonhuman Primate Offspring. 2016;23(11):2157-2164. doi:10.1002/oby.21306.Maternal.

390. Bilbo SD, Tsang V. Enduring consequences of maternal obesity for brain inflammation and behavior of offspring. *FASEB J*. 2010;24(6):2104-2115. doi:10.1096/fj.09-144014.

391. Gupta A, Srinivasan M, Thamadilok S, Patel MS. Hypothalamic alterations in fetuses of high fat diet-fed obese female rats. *J Endocrinol*. 2009;200(3):293-300. doi:10.1677/JOE-08-0429.

392. Bayol SA, Farrington SJ, Stickland NC. A maternal “junk food” diet in pregnancy and lactation promotes an exacerbated taste for “junk food” and a greater propensity for obesity in rat offspring. *Br J Nutr*. 2007;98(4):843-851. doi:10.1017/S0007114507812037.

393. Ong ZY, Muhlhausler BS. Maternal "junk-food" feeding of rat dams alters food choices and development of the mesolimbic reward pathway in the offspring. *FASEB J*. 2011;25(7):2167-2179. doi:10.1096/fj.10-178392.

394. Wright TM, Fone KCF, Langley-Evans SC, Voigt J-PW. Exposure to maternal consumption of cafeteria diet during the lactation period programmes feeding behaviour in the rat. *Int J Dev Neurosci*. 2011;29(8):785-793. doi:10.1016/j.ijdevneu.2011.09.007.

395. Sasaki A, Vega WCDE, Pan P, Mcgowan PO. PERINATAL HIGH FAT DIET ALTERS GLUCOCORTICOID SIGNALING AND ANXIETY BEHAVIOR IN ADULTHOOD. *Neuroscience*. 2013;240:1-12. doi:10.1016/j.neuroscience.2013.02.044.

396. Peleg-Raibstein D, Luca E, Wolfrum C. Maternal high-fat diet in mice programs emotional behavior in adulthood. *Behav Brain Res*. 2012;233(2):398-404. doi:10.1016/j.bbr.2012.05.027.

397. Teegarden SL, Scott AN, Bale TL. Early life exposure to a high fat diet promotes long-term changes in dietary preferences and central reward signaling. *Neuroscience*. 2009;162(4):924-932. doi:10.1016/j.neuroscience.2009.05.029.

398. Wang GJ, Volkow ND, Logan J, et al. Brain dopamine and obesity. *Lancet (London,*

England). 2001;357(9253):354-357. http://www.ncbi.nlm.nih.gov/pubmed/11210998. Accessed October 25, 2017.

399. Volkow ND, Wang G-J, Fowler JS, Telang F. Overlapping neuronal circuits in addiction and obesity: evidence of systems pathology. *Philos Trans R Soc B Biol Sci*. 2008;363(1507):3191-3200. doi:10.1098/rstb.2008.0107.

400. de Weijer BA, van de Giessen E, van Amelsvoort TA, et al. Lower striatal dopamine D2/3 receptor availability in obese compared with non-obese subjects. *EJNMMI Res*. 2011;1(1):37. doi:10.1186/2191-219X-1-37.

401. Karlsson HK, Tuominen L, Tuulari JJ, et al. Obesity Is Associated with Decreased -Opioid But Unaltered Dopamine D2 Receptor Availability in the Brain. *J Neurosci*. 2015;35(9):3959-3965. doi:10.1523/JNEUROSCI.4744-14.2015.

402. Haltia LT, Rinne JO, Merisaari H, et al. Effects of intravenous glucose on dopaminergic function in the human brain in vivo. *Synapse*. 2007;61(9):748-756. doi:10.1002/syn.20418.

403. Volkow ND, Wang G-J, Telang F, et al. Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors. *Neuroimage*. 2008;42(4):1537-1543. doi:10.1016/j.neuroimage.2008.06.002.

404. Stoeckel LE, Weller RE, Cook EW, Twieg DB, Knowlton RC, Cox JE. Widespread reward-system activation in obese women in response to pictures of high-calorie foods. *Neuroimage*. 2008;41(2):636-647. doi:10.1016/j.neuroimage.2008.02.031.

405. DelParigi A, Chen K, Salbe AD, Reiman EM, Tataranni PA. Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast. *Neuroimage*. 2005;24(2):436-443.

doi:10.1016/j.neuroimage.2004.08.035.

406. Boutelle KN, Wierenga CE, Bischoff-Grethe A, et al. Increased brain response to appetitive tastes in the insula and amygdala in obese compared with healthy weight children when sated. *Int J Obes*. 2015;39(4):620-628. doi:10.1038/ijo.2014.206.

407. Stice E, Spoor S, Bohon C, Small DM. Relation between obesity and blunted striatal response to food is moderated by TaqIA A1 allele. *Science*. 2008;322(5900):449-452. doi:10.1126/science.1161550.

408. Stice E, Spoor S, Bohon C, Veldhuizen MG, Small DM. Relation of reward from food intake and anticipated food intake to obesity: A functional magnetic resonance imaging study. *J Abnorm Psychol*. 2008;117(4):924-935. doi:10.1037/a0013600.

409. Ward MA, Carlsson CM, Trivedi MA, Sager MA, Johnson SC. The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study. *BMC Neurol*. 2005;5:23. doi:10.1186/1471-2377-5-23.

410. Gunstad J, Paul RH, Cohen RA, et al. Relationship Between Body Mass Index and Brain Volume in Healthy Adults. *Int J Neurosci*. 2008;118(11):1582-1593. doi:10.1080/00207450701392282.

411. Debette S, Beiser A, Hoffmann U, et al. Visceral fat is associated with lower brain volume in healthy middle-aged adults. *Ann Neurol*. 2010;68(2):136-144. doi:10.1002/ana.22062.

412. Shott ME, Cornier M-A, Mittal VA, et al. Orbitofrontal cortex volume and brain reward response in obesity. *Int J Obes*. 2015;39(2):214-221. doi:10.1038/ijo.2014.121.

413. Veit R, Kullmann S, Heni M, et al. Reduced cortical thickness associated with visceral fat and BMI. *NeuroImage Clin*. 2014;6:307-311. doi:10.1016/j.nicl.2014.09.013.

414. Pannacciulli N, Del Parigi A, Chen K, Le DSNT, Reiman EM, Tataranni PA. Brain

abnormalities in human obesity: A voxel-based morphometric study. *Neuroimage*. 2006;31(4):1419-1425. doi:10.1016/j.neuroimage.2006.01.047.

415. Walther K, Birdsill AC, Glisky EL, Ryan L. Structural brain differences and cognitive functioning related to body mass index in older females. *Hum Brain Mapp*. 2010;31(7):1052-1064. doi:10.1002/hbm.20916.

416. Brooks SJ, Benedict C, Burgos J, et al. Late-life obesity is associated with smaller global and regional gray matter volumes: a voxel-based morphometric study. *Int J Obes (Lond)*. 2013;37(2):230-236. doi:10.1038/ijo.2012.13.

417. Figley CR, Asem JSA, Levenbaum EL, Courtney SM. Effects of Body Mass Index and Body Fat Percent on Default Mode, Executive Control, and Salience Network Structure and Function. *Front Neurosci*. 2016;10:234. doi:10.3389/fnins.2016.00234.

418. Marqués-Iturria I, Pueyo R, Garolera M, et al. Frontal cortical thinning and subcortical volume reductions in early adulthood obesity. *Psychiatry Res*. 2013;214(2):109-115. doi:10.1016/j.pscychresns.2013.06.004.

419. Maayan L, Hoogendoorn C, Sweat V, Convit A. Disinhibited eating in obese adolescents is associated with orbitofrontal volume reductions and executive dysfunction. *Obesity (Silver Spring)*. 2011;19(7):1382-1387. doi:10.1038/oby.2011.15.

420. Sharkey RJ, Karama S, Dagher A. Overweight is not associated with cortical thickness alterations in children. *Front Neurosci*. 2015;9(FEB):1-7. doi:10.3389/fnins.2015.00024.

421. Benson BL, Leburn IS, Rasmussen DW. The Impact of Drug Enforcement on Crime: An Investigation of the Opportunity Cost of Police Resources. *J Drug Issues*. 2001;31(4):989-1006. doi:10.1177/002204260103100410.

422. Coveleskie K, Gupta A, Kilpatrick LA, et al. Altered functional connectivity within the

central reward network in overweight and obese women. *Nutr Diabetes*. 2015;5(1):e148. doi:10.1038/nutd.2014.45.

423. Contreras-Rodríguez O, Martín-Pérez C, Vilar-López R, Verdejo-Garcia A. Ventral and Dorsal Striatum Networks in Obesity: Link to Food Craving and Weight Gain. *Biol Psychiatry*. 2017;81(9):789-796. doi:10.1016/j.biopsych.2015.11.020.

424. Stoeckel LE, Kim J, Weller RE, Cox JE, Cook EW, Horwitz B. Effective connectivity of a reward network in obese women. *Brain Res Bull*. 2009;79(6):388-395. doi:10.1016/j.brainresbull.2009.05.016.

425. Lips MA, Wijngaarden MA, van der Grond J, et al. Resting-state functional connectivity of brain regions involved in cognitive control, motivation, and reward is enhanced in obese females. *Am J Clin Nutr*. 2014;100(2):524-531. doi:10.3945/ajcn.113.080671.

426. Black WR, Lepping RJ, Bruce AS, et al. Tonic hyper-connectivity of reward neurocircuitry in obese children. *Obesity*. 2014;22(7):1590-1593. doi:10.1002/oby.20741.

427. Chodkowski BAA, Cowan RL, Niswender KD. Imbalance in resting state functional connectivity is associated with eating behaviors and adiposity in children. *Heliyon*. 2016;2(1). doi:10.1016/j.heliyon.2015.e00058.

428. Volkow ND, Wang G-J, Telang F, et al. Profound decreases in dopamine release in striatum in detoxified alcoholics: possible orbitofrontal involvement. *J Neurosci*. 2007;27(46):12700-12706. doi:10.1523/JNEUROSCI.3371-07.2007.

429. Doherty MD, Gratton A. Medial prefrontal cortical D1 receptor modulation of the meso-accumbens dopamine response to stress: an electrochemical study in freely-behaving rats. *Brain Res*. 1996;715(1-2):86-97. doi:10.1016/0006-8993(95)01557-4.

430. Tomasi D, Wang G-J, Wang R, et al. Association of body mass and brain activation

during gastric distention: implications for obesity. Gaetani S, ed. *PLoS One*. 2009;4(8):e6847. doi:10.1371/journal.pone.0006847.

431. Volkow ND, Wang GJ, Baler RD. Reward, dopamine and the control of food intake: Implications for obesity. *Trends Cogn Sci*. 2011;15(1):37-46. doi:10.1016/j.tics.2010.11.001.

432. Reyes TM. High-fat diet alters the dopamine and opioid systems: effects across development. *Int J Obes Suppl*. 2012;2(Suppl 2):S25-8. doi:10.1038/ijosup.2012.18.

433. Berthoud H-R, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. *AJP Regul Integr Comp Physiol*. 2011;300(6):R1266-R1277. doi:10.1152/ajpregu.00028.2011.

434. Michopoulos V, Diaz MP, Wilson ME. Social change and access to a palatable diet produces differences in reward neurochemistry and appetite in female monkeys. *Physiol Behav*. 2016;162:102-111. doi:10.1016/j.physbeh.2016.04.023.

435. Carnell S, Gibson C, Benson L, Ochner CN, Geliebter A. Neuroimaging and obesity: Current knowledge and future directions. *Obes Rev*. 2012;13(1):43-56. doi:10.1111/j.1467-789X.2011.00927.x.

436. Capuron L, Lasselin J, Castanon N. Role of Adiposity-Driven Inflammation in Depressive Morbidity. *Neuropsychopharmacology*. 2017;42(1):115-128. doi:10.1038/npp.2016.123.

437. Galvez JF, Bauer IE, Sanches M, et al. Shared clinical associations between obesity and impulsivity in rapid cycling bipolar disorder: a systematic review. *J Affect Disord*. 2014;168:306-313. doi:10.1016/j.jad.2014.05.054.

438. Zender R, Olshansky E. Women's Mental Health: Depression and Anxiety. *Nurs Clin North Am*. 2009;44(3):355-364. doi:10.1016/j.cnur.2009.06.002.

439. Hillman JB, Dorn LD, Bin Huang. Association of Anxiety and Depressive Symptoms and

Adiposity Among Adolescent Females, Using Dual Energy X-ray Absorptiometry. *Clin Pediatr (Phila)*. 2010;49(7):671-677. doi:10.1177/0009922810363155.

440. Rofey DL, Kolko RP, Iosif A-M, et al. A longitudinal study of childhood depression and anxiety in relation to weight gain. *Child Psychiatry Hum Dev*. 2009;40(4):517-526. doi:10.1007/s10578-009-0141-1.

441. Pasquali R, Ambrosi B, Armanini D, et al. Cortisol and ACTH response to oral dexamethasone in obesity and effects of sex, body fat distribution, and dexamethasone concentrations: a dose-response study. *J Clin Endocrinol Metab*. 2002;87(1):166-175. doi:10.1210/jcem.87.1.8158.

442. Legendre A, Harris RBS. Exaggerated response to mild stress in rats fed high-fat diet. *AJP Regul Integr Comp Physiol*. 2006;291(5):R1288-1294. doi:10.1152/ajpregu.00234.2006.

443. Kalynchuk LE, Gregus A, Boudreau D, Perrot-Sinal TS. Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats. *Behav Neurosci*. 2004;118(6):1365-1377. doi:10.1037/0735-7044.118.6.1365.

444. Gregus A, Wintink AJ, Davis AC, Kalynchuk LE. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav Brain Res*. 2005;156(1):105-114. doi:10.1016/j.bbr.2004.05.013.

445. Stetler C, Miller GE. Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research. *Psychosom Med*. 2011;73(2):114-126. doi:10.1097/PSY.0b013e31820ad12b.

446. Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. *Am J*

Psychiatry. 2003;160(9):1554-1565. doi:10.1176/appi.ajp.160.9.1554.

447. Parker KJ, Schatzberg AF, Lyons DM. Neuroendocrine aspects of hypercortisolism in major depression. *Horm Behav*. 2003;43(1):60-66. http://www.ncbi.nlm.nih.gov/pubmed/12614635. Accessed October 25, 2017.

448. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J*. 2014;13(1):61. doi:10.1186/1475-2891-13-61.

449. Vasconcelos AR, Cabral-Costa JV, Mazucanti CH, Scavone C, Kawamoto EM. The Role of Steroid Hormones in the Modulation of Neuroinflammation by Dietary Interventions. *Front Endocrinol (Lausanne)*. 2016;7:9. doi:10.3389/fendo.2016.00009.

450. Gregor MF, Hotamisligil GS. Inflammatory mechanisms in obesity. *Annu Rev Immunol*. 2011;29(1):415-445. doi:10.1146/annurev-immunol-031210-101322.

451. Miller AA, Spencer SJ. Obesity and neuroinflammation: A pathway to cognitive impairment. *Brain Behav Immun*. 2014;42:10-21. doi:10.1016/j.bbi.2014.04.001.

452. Felger JC, Hernandez CR, Miller AH. Levodopa reverses cytokine-induced reductions in striatal dopamine release. *Int J Neuropsychopharmacol*. 2015;18(4). doi:10.1093/ijnp/pyu084.

453. Felger JC, Mun J, Kimmel HL, et al. Chronic Interferon-α Decreases Dopamine 2 Receptor Binding and Striatal Dopamine Release in Association with Anhedonia-Like Behavior in Nonhuman Primates. *Neuropsychopharmacology*. 2013;38(11):2179-2187. doi:10.1038/npp.2013.115.

454. Eisenberger NI, Berkman ET, Inagaki TK, Rameson LT, Mashal NM, Irwin MR. Inflammation-induced anhedonia: endotoxin reduces ventral striatum responses to reward. *Biol Psychiatry*. 2010;68(8):748-754. doi:10.1016/j.biopsych.2010.06.010.

455. Brydon L, Harrison NA, Walker C, Steptoe A, Critchley HD. Peripheral Inflammation is Associated with Altered Substantia Nigra Activity and Psychomotor Slowing in Humans. *Biol Psychiatry*. 2008;63(11):1022-1029. doi:10.1016/j.biopsych.2007.12.007.

456. Harrison NA, Brydon L, Walker C, Gray MA, Steptoe A, Critchley HD. Inflammation causes mood changes through alterations in subgenual cingulate activity and mesolimbic connectivity. *Biol Psychiatry*. 2009;66(5):407-414. doi:10.1016/j.biopsych.2009.03.015.

457. Harrison NA, Cercignani M, Voon V, Critchley HD. Effects of Inflammation on Hippocampus and Substantia Nigra Responses to Novelty in Healthy Human Participants. *Neuropsychopharmacology*. 2015;40(4):831-838. doi:10.1038/npp.2014.222.

458. Capuron L, Pagnoni G, Drake DF, et al. Dopaminergic mechanisms of reduced basal ganglia responses to hedonic reward during interferon alfa administration. *Arch Gen Psychiatry*. 2012;69(10):1044-1053. doi:10.1001/archgenpsychiatry.2011.2094.

459. Dubas JS, Graber JA, Petersen AC. The Effects of Pubertal Development on Achievement during Adolescence. *Am J Educ*. 99:444-460. doi:10.2307/1085555.

460. Stattin H, Magnusson D. *Pubertal Maturation in Female Development*. L. Erlbaum Associates; 1990. https://books.google.com/books/about/Pubertal_maturation_in_female_developmen.html?id=_bAoAAAAYAAJ. Accessed January 16, 2018.

461. Catalani A, Marinelli M, Scaccianoce S, et al. Progeny of mothers drinking corticosterone during lactation has lower stress-induced corticosterone secretion and better cognitive performance. *Brain Res*. 1993;624(1-2):209-215. http://www.ncbi.nlm.nih.gov/pubmed/8252393. Accessed January 15, 2018.

462. Catalani A, Casolini P, Scaccianoce S, Patacchioli FR, Spinozzi P, Angelucci L. Maternal

corticosterone during lactation permanently affects brain corticosteroid receptors, stress response and behaviour in rat progeny. *Neuroscience*. 2000;100(2):319-325. http://www.ncbi.nlm.nih.gov/pubmed/11008169. Accessed January 15, 2018.

463. Catalani A, Casolini P, Cigliana G, et al. Maternal corticosterone influences behavior, stress response and corticosteroid receptors in the female rat. *Pharmacol Biochem Behav*. 2002;73(1):105-114. http://www.ncbi.nlm.nih.gov/pubmed/12076729. Accessed January 15, 2018.

464. Casolini P, Cigliana G, Alemà GS, Ruggieri V, Angelucci L, Catalani A. Effect of increased maternal corticosterone during lactation on hippocampal corticosteroid receptors, stress response and learning in offspring in the early stages of life. *Neuroscience*. 1997;79(4):1005-1012. http://www.ncbi.nlm.nih.gov/pubmed/9219963. Accessed January 15, 2018.

465. Glynn LM, Davis EP, Schetter CD, Chicz-DeMet A, Hobel CJ, Sandman CA. Postnatal maternal cortisol levels predict temperament in healthy breastfed infants. *Early Hum Dev*. 2007;83(10):675-681. doi:10.1016/j.earlhumdev.2007.01.003.

466. Sullivan RM, Gratton A. Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: Side matters. *Psychoneuroendocrinology*. 2002;27(1-2):99-114. doi:10.1016/S0306-4530(01)00038-5.

467. Ellis BJ, Shirtcliff EA, Boyce WT, Deardorff J, Essex MJ. Quality of early family relationships and the timing and tempo of puberty: Effects depend on biological sensitivity to context. *Dev Psychopathol*. 2011;23(1):85-99. doi:10.1017/S0954579410000660.

468. Ellis BJ, Essex MJ. Family Environments, Adrenarche, and Sexual Maturation: A Longitudinal Test of a Life History Model. *Child Dev*. 2007;78(6):1799-1817.

doi:10.1111/j.1467-8624.2007.01092.x.

469. Kim K, Smith PK. Childhood stress, behavioural symptoms and mother–daughter pubertal development. *J Adolesc*. 1998;21(3):231-240. doi:10.1006/JADO.1998.0149.

470. Thapar A, Stephan C, Pine D, Thapar A. Depression in adolescence. *Lancet*. 2012;379(9820):1056-1067. doi:10.1016/S0140-6736(11)60871-4.Depression.

471. Maughan B, Collishaw S, Stringaris A. Depression in childhood and adolescence. *J Can Acad Child Adolesc Psychiatry*. 2013;22(1):35-40. http://www.ncbi.nlm.nih.gov/pubmed/23390431. Accessed October 26, 2017.

472. Varlinskaya EI, Vetter-O'Hagen CS, Spear LP. Puberty and gonadal hormones: Role in adolescent-typical behavioral alterations. *Horm Behav*. 2013;64(2):343-349. doi:10.1016/j.yhbeh.2012.11.012.

473. Sisk CL, Zehr JL. Pubertal hormones organize the adolescent brain and behavior. *Front Neuroendocrinol*. 2005;26(3-4):163-174. doi:10.1016/j.yfrne.2005.10.003.

474. Patton GC, Viner R. Pubertal transitions in health. *Lancet*. 2007;369(9567):1130-1139. doi:10.1016/S0140-6736(07)60366-3.

475. Andersen SL. Trajectories of brain development: Point of vulnerability or window of opportunity? *Neurosci Biobehav Rev*. 2003;27(1-2):3-18. doi:10.1016/S0149-7634(03)00005-8.

476. Spear LP. Heightened stress responsivity and emotional reactivity during pubertal maturation: Implications for psychopathology. *Dev Psychopathol*. 2009;21(1):87-97. doi:10.1017/S0954579409000066.

477. Casey B, Duhoux S, Cohen M. Adolescence: what do transmission, transition, and translation have to do with it? *Neuron*. 2010.

http://www.sciencedirect.com/science/article/pii/S0896627310006768. Accessed August 8, 2017.

478. Romeo R. Puberty: a period of both organizational and activational effects of steroid hormones on neurobehavioural development. *J Neuroendocrinol*. 2003. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2826.2003.01106.x/full. Accessed August 8, 2017.

479. Goddings AL, Mills KL, Clasen LS, Giedd JN, Viner RM, Blakemore SJ. The influence of puberty on subcortical brain development. *Neuroimage*. 2014;88:242-251. doi:10.1016/j.neuroimage.2013.09.073.

480. Banks S, Eddy K, Angstadt M. Amygdala–frontal connectivity during emotion regulation. *Soc Cogn*. 2007. https://academic.oup.com/scan/article-abstract/2/4/303/1676121. Accessed August 9, 2017.

481. Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci*. 2011. http://www.sciencedirect.com/science/article/pii/S1364661310002524. Accessed August 9, 2017.

482. Wager T, Davidson M, Hughes B, Lindquist M. Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron*. 2008. http://www.sciencedirect.com/science/article/pii/S0896627308007538. Accessed August 9, 2017.

483. Sotres-Bayon F, Quirk GJ. Prefrontal control of fear: more than just extinction. *Curr Opin Neurobiol*. 2010;20(2):231-235. doi:10.1016/j.conb.2010.02.005.

484. Quirk GJ, Gehlert DR. Inhibition of the amygdala: key to pathological states? *Ann N Y*

Acad Sci. 2003;985:263-272. http://www.ncbi.nlm.nih.gov/pubmed/12724164. Accessed December 17, 2017.

485. Teicher MH, Anderson CM, Ohashi K, Polcari A. Childhood maltreatment: Altered network centrality of cingulate, precuneus, temporal pole and insula. *Biol Psychiatry*. 2014;76:297-305. doi:10.1016/j.biopsych.2013.09.016.

486. Dannlowski U, Stuhrmann A, Beutelmann V, et al. Limbic Scars : Long-Term Consequences of Childhood Magnetic Resonance Imaging. 2011. doi:10.1016/j.biopsych.2011.10.021.

487. Mcewen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure : Hippocampus , Amygdala , and Prefrontal Cortex. 2015;41(1):3-23. doi:10.1038/npp.2015.171.

488. Hahn A, Stein P, Windischberger C, et al. Reduced resting-state functional connectivity between amygdala and orbitofrontal cortex in social anxiety disorder. *Neuroimage*. 2011;56(3):881-889. doi:10.1016/j.neuroimage.2011.02.064.

489. Stevens J, Jovanovic T, Fani N, Ely T. Disrupted amygdala-prefrontal functional connectivity in civilian women with posttraumatic stress disorder. *J Psychiatr*. 2013. http://www.sciencedirect.com/science/article/pii/S0022395613001829. Accessed August 12, 2017.

490. Kim MJ, Gee DG, Loucks RA, Davis FC, Whalen PJ. Anxiety Dissociates dorsal and ventral medial prefrontal cortex functional connectivity with the amygdala at rest. *Cereb Cortex*. 2011;21(7):1667-1673. doi:10.1093/cercor/bhq237.

491. Dreher J-C. Sensitivity of the brain to loss aversion during risky gambles. *Trends Cogn Sci*. 2007;11(7):270-272. doi:10.1016/j.tics.2007.05.006.

492. Galvin C, Ninan I. Regulation of the mouse medial prefrontal cortical synapses by

endogenous estradiol. *Neuropsychopharmacology*. 2014. https://www.nature.com/articles/npp201456. Accessed August 8, 2017.

493. Lee Y, Styne D. Influences on the onset and tempo of puberty in human beings and implications for adolescent psychological development. *Horm Behav*. 2013;64(2):250-261. doi:10.1016/j.yhbeh.2013.03.014.

494. Martel MM, Klump K, Nigg JT, Breedlove SM, Sisk CL. Potential hormonal mechanisms of attention-deficit/hyperactivity disorder and major depressive disorder: a new perspective. *Horm Behav*. 2009;55(4):465-479. doi:10.1016/j.yhbeh.2009.02.004.

495. Peper JS, Brouwer RM, Schnack HG, et al. Cerebral white matter in early puberty is associated with luteinizing hormone concentrations. *Psychoneuroendocrinology*. 2008;33(7):909-915. doi:10.1016/j.psyneuen.2008.03.017.

496. Asato MR, Terwilliger R, Woo J, Luna B. White matter development in adolescence: A DTI study. *Cereb Cortex*. 2010;20(9):2122-2131. doi:10.1093/cercor/bhp282.

497. Herting MM, Maxwell EC, Irvine C, Nagel BJ. The impact of sex, puberty, and hormones on white matter microstructure in adolescents. *Cereb Cortex*. 2012;22(9):1979-1992. doi:10.1093/cercor/bhr246.

498. Huang H, Ding M. Linking Functional Connectivity and Structural Connectivity Quantitatively: A Comparison of Methods. *Brain Connect*. 2016;6(2):99-108. doi:10.1089/brain.2015.0382.

499. Zeidan MA, Igoe SA, Linnman C, et al. Estradiol Modulates Medial Prefrontal Cortex and Amygdala Activity During Fear Extinction in Women and Female Rats. *Biol Psychiatry*. 2011;70(10):920-927. doi:10.1016/j.biopsych.2011.05.016.

500. Ottowitz W, Siedlecki K, Lindquist M. Evaluation of prefrontal–hippocampal effective

connectivity following 24 hours of estrogen infusion: an FDG-PET study. 2008. http://www.sciencedirect.com/science/article/pii/S0306453008002540. Accessed August 8, 2017.

501. Engman J, Linnman C, Van Dijk KRA, Milad MR. Amygdala subnuclei resting-state functional connectivity sex and estrogen differences. *Psychoneuroendocrinology*. 2016;63:34-42. doi:10.1016/j.psyneuen.2015.09.012.

502. Ernst M, Nelson EE, Jazbec S, et al. Amygdala and nucleus accumbens in responses to receipt and omission of gains in adults and adolescents. *Neuroimage*. 2005;25(4):1279-1291. doi:10.1016/j.neuroimage.2004.12.038.

503. Galvan A, Hare TA, Parra CE, et al. Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *J Neurosci*. 2006;26(25):6885-6892. doi:10.1523/JNEUROSCI.1062-06.2006.

504. Monk CS, McClure EB, Nelson EE, et al. Adolescent immaturity in attention-related brain engagement to emotional facial expressions. *Neuroimage*. 2003;20(1):420-428. http://www.ncbi.nlm.nih.gov/pubmed/14527602. Accessed August 8, 2017.

505. Eshel N, Nelson EE, Blair RJ, Pine DS, Ernst M. Neural substrates of choice selection in adults and adolescents: development of the ventrolateral prefrontal and anterior cingulate cortices. *Neuropsychologia*. 2007;45(6):1270-1279. doi:10.1016/j.neuropsychologia.2006.10.004.

506. Hare TA, Tottenham N, Galvan A, Voss HU, Glover GH, Casey BJ. Biological Substrates of Emotional Reactivity and Regulation in Adolescence During an Emotional Go-Nogo Task. 2008;(19):927-934. doi:10.1016/j.biopsych.2008.03.015.

507. Simerly R, Swanson L, Chang C. Distribution of androgen and estrogen receptor mRNA-

containing cells in the rat brain: an in situ hybridization study. *J*. 1990. http://onlinelibrary.wiley.com/doi/10.1002/cne.902940107/full. Accessed August 13, 2017.

508. Womble MD, Andrew JA, Crook JJ. 17β-Estradiol reduces excitatory postsynaptic potential (EPSP) amplitude in rat basolateral amygdala neurons. *Neurosci Lett*. 2002;331(2):83-86. doi:https://doi.org/10.1016/S0304-3940(02)00871-6.

509. Michopoulos V, Berga SL, Kaplan JR, Wilson ME. Social Subordination and Polymorphisms in the Gene Encoding the Serotonin Transporter Enhance Estradiol Inhibition of Luteinizing Hormone Secretion in Female Rhesus Monkeys1. *Biol Reprod*. 2009;81(6):1154-1163. doi:10.1095/biolreprod.109.079038.

510. Michopoulos V, Embree M, Reding K, et al. CRH receptor antagonism reverses the effect of social subordination upon central GABAA receptor binding in estradiol-treated ovariectomized female rhesus monkeys. *Neuroscience*. 2013;250:300-308. doi:10.1016/j.neuroscience.2013.07.002.

511. Campbell CJ, Campbell, J. C. Adolescent Sterility. In: *The International Encyclopedia of Primatology*. Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2017:1-1. doi:10.1002/9781119179313.wbprim0479.

512. Golub M, Styne D, Wheeler M. Growth retardation in premenarchial female rhesus monkeys during chronic administration of a GnRH agonist (leuprolide acetate). *J Med*. 1997. http://onlinelibrary.wiley.com/doi/10.1111/j.1600-0684.1997.tb00219.x/full. Accessed August 20, 2017.

513. Vincent JL, Patel GH, Fox MD, et al. Intrinsic functional architecture in the anaesthetized monkey brain. *Nature*. 2007;447(7140):83-86. doi:10.1038/nature05758.

514. Hutchison R, Womelsdorf T, Gati J. Resting-state networks show dynamic functional connectivity in awake humans and anesthetized macaques. *Hum brain*. 2013. http://onlinelibrary.wiley.com/doi/10.1002/hbm.22058/full. Accessed August 20, 2017.

515. Tang C, Ramani R. fMRI and Anesthesia. *Int Anesthesiol Clin*. 2016. http://journals.lww.com/anesthesiaclinics/Citation/2016/05410/fMRI_and_Anesthesia.9.aspx. Accessed August 20, 2017.

516. Li C, Patel S, Auerbach E, Zhang X. Dose-dependent effect of isoflurane on regional cerebral blood flow in anesthetized macaque monkeys. *Neurosci Lett*. 2013. http://www.sciencedirect.com/science/article/pii/S0304394013001171. Accessed August 20, 2017.

517. Gorgolewski K, Burns C, Madison C. Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python. *Front*. 2011. https://www.ncbi.nlm.nih.gov/pmc/articles/pmc3159964/. Accessed August 20, 2017.

518. Smith S, Jenkinson M, Woolrich M, Beckmann C. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage*. 2004. http://www.sciencedirect.com/science/article/pii/S1053811904003933. Accessed August 20, 2017.

519. Woolrich M, Jbabdi S, Patenaude B, Chappell M. Bayesian analysis of neuroimaging data in FSL. *Neuroimage*. 2009. http://www.sciencedirect.com/science/article/pii/S1053811908012044. Accessed August 20, 2017.

520. Fair D, Dosenbach N, Church J. Development of distinct control networks through segregation and integration. *Proc*. 2007. http://www.pnas.org/content/104/33/13507.short.

Accessed August 20, 2017.

521. Fair D, Cohen A, Power J. Functional brain networks develop from a “local to distributed” organization. *PLoS Comput*. 2009. http://journals.plos.org/ploscompbiol/article?id=10.1371/journal.pcbi.1000381. Accessed August 20, 2017.

522. Fair D, Nigg J, Iyer S, Bathula D. Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data. *Front Syst*. 2012. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3563110/. Accessed August 20, 2017.

523. Iyer S, Shafran I, Grayson D, Gates K, Nigg J. Inferring functional connectivity in MRI using Bayesian network structure learning with a modified PC algorithm. *Neuroimage*. 2013. http://www.sciencedirect.com/science/article/pii/S1053811913001997. Accessed August 20, 2017.

524. Miranda-Dominguez O, Mills B. Bridging the gap between the human and macaque connectome: a quantitative comparison of global interspecies structure-function relationships and network. *J*. 2014. http://www.jneurosci.org/content/34/16/5552.short. Accessed August 20, 2017.

525. McLaren D, Kosmatka K, Kastman E, Bendlin B. Rhesus macaque brain morphometry: a methodological comparison of voxel-wise approaches. *Methods*. 2010. http://www.sciencedirect.com/science/article/pii/S1046202309002448. Accessed August 20, 2017.

526. McLaren D, Kosmatka K, Oakes T, Kroenke C. A population-average MRI-based atlas collection of the rhesus macaque. *Neuroimage*. 2009.

http://www.sciencedirect.com/science/article/pii/S1053811908011762. Accessed August 20, 2017.

527. Fox MD, Zhang D, Snyder AZ, Raichle ME. The Global Signal and Observed Anticorrelated Resting State Brain Networks. *J Neurophysiol*. 2009;101(6):3270-3283. doi:10.1152/jn.90777.2008.

528. Power J, Mitra A, Laumann T, Snyder A. Methods to detect, characterize, and remove motion artifact in resting state fMRI. *Neuroimage*. 2014. http://www.sciencedirect.com/science/article/pii/S1053811913009117. Accessed August 20, 2017.

529. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*. 2012;59(3):2142-2154. doi:10.1016/j.neuroimage.2011.10.018.

530. Lewis J, Essen D Van. Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey. *J Comp Neurol*. 2000. http://onlinelibrary.wiley.com/doi/10.1002/1096-9861(20001204)428:1%3C112::AID-CNE8%3E3.0.CO;2-9/full. Accessed August 20, 2017.

531. Markov NT, Gomes ARR, Lamy C, Magrou L, Vezoli J, Misery P. A Weighted and Directed Interareal Connectivity Matrix for Macaque Cerebral Cortex. 2014;(January):17-36. doi:10.1093/cercor/bhs270.

532. Styner M, Knickmeyer R, Joshi S, Coe C, Short SJ, Gilmore J. Automatic brain segmentation in rhesus monkeys. In: Pluim JPW, Reinhardt JM, eds. Vol 6512. International Society for Optics and Photonics; 2007:65122L. doi:10.1117/12.710027.

533. Paxinos G, Huang X, Toga A. The rhesus monkey brain in stereotaxic coordinates. 2000. http://ro.uow.edu.au/hbspapers/3613/. Accessed August 20, 2017.

534. Saleem K, Logothetis N. *A Combined MRI and Histology Atlas of the Rhesus Monkey Brain in Stereotaxic Coordinates*.; 2012. https://books.google.com/books?hl=en&lr=&id=tuVyU2-s8MUC&oi=fnd&pg=PP2&dq=a+combined+MRI+and+histology+atlas+of+the+rhesus+monkey&ots=yGEoYRne4c&sig=nFCd03dx7CBfgQf6fvntbAvAXeA. Accessed August 20, 2017.

535. Altmann S a. A field study of the Sociobiology of Rhesus Monkeys. *Ann N Y Acad Sci*. 1962;102(hlF 9005):338–435.

536. Maestripieri D, McCormack K, Lindell S. Influence of parenting style on the offspring's behaviour and CSF monoamine metabolite levels in crossfostered and noncrossfostered female rhesus macaques. *Behav brain*. 2006. http://www.sciencedirect.com/science/article/pii/S0166432806004451. Accessed August 21, 2017.

537. Graves F, Wallen K. Androgen-induced yawning in rhesus monkey females is reversed with a nonsteroidal anti-androgen. *Horm Behav*. 2006. http://www.sciencedirect.com/science/article/pii/S0018506X05001728. Accessed August 21, 2017.

538. Kalin NH, Shelton SE, Davidson RJ. Role of the Primate Orbitofrontal Cortex in Mediating Anxious Temperament. *Biol Psychiatry*. 2007;62(10):1134-1139. doi:10.1016/j.biopsych.2007.04.004.

539. Fox AS, Shelton SE, Oakes TR, Davidson RJ, Kalin NH. Trait-like brain activity during

adolescence predicts anxious temperament in primates. *PLoS One*. 2008;3(7). doi:10.1371/journal.pone.0002570.

540. Grand A, McCormack K, Maestripieri D, Sanchez M. Effects of infant maltreatment on emotional and HPA axis reactivity in juvenile rhesus macaques. *35th Annu Meet*. 2005. https://scholar.google.com/scholar?q=+Effects+of+infant+maltreatment+on+emotional+and+HPA+axis+reactivity+in+juvenile+rhesus+macaques&btnG=&hl=en&as_sdt=0%2C11. Accessed August 21, 2017.

541. Machado CJ, Kazama AM, Bachevalier J. Impact of amygdala, orbital frontal, or hippocampal lesions on threat avoidance and emotional reactivity in nonhuman primates. *Emotion*. 2009;9(2):147-163. doi:10.1037/a0014539.

542. Williamson D, Coleman K, Bacanu S, Devlin B. Heritability of fearful-anxious endophenotypes in infant rhesus macaques: a preliminary report. *Biological*. 2003. http://www.sciencedirect.com/science/article/pii/S0006322302016013. Accessed August 21, 2017.

543. Feise RJ. Do multiple outcome measures require p-value adjustment? *BMC Med Res Methodol*. 2002;2:8. doi:10.1186/1471-2288-2-8.

544. Wilson ME, Gordon TP, Collins DC. Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys. *Endocrinology*. 1986;118(1):293-301. doi:10.1210/endo-118-1-293.

545. Lomniczi A, Ojeda SR. The Emerging Role of Epigenetics in the Regulation of Female Puberty. In: *Endocrine Development*. Vol 29. ; 2016:1-16. doi:10.1159/000438840.

546. Plant TM. Neurobiological bases underlying the control of the onset of puberty in the rhesus monkey: a representative higher primate. *Front Neuroendocrinol*. 2001;22:107-

139. doi:10.1006/frne.2001.0211.

547. Stephens SBZ, Wallen K. Environmental and social influences on neuroendocrine puberty and behavior in macaques and other nonhuman primates. *Horm Behav*. 2013;64(2):226-239. doi:10.1016/j.yhbeh.2013.05.003.

548. Frisch RE, McArthur JW. Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science*. 1974;185(4155):949-951. http://www.ncbi.nlm.nih.gov/pubmed/4469672. Accessed January 11, 2018.

549. Hariri A, Mattay V, Tessitore A, Fera F. Neocortical modulation of the amygdala response to fearful stimuli. *Biological*. 2003. http://www.sciencedirect.com/science/article/pii/S0006322302017869. Accessed August 9, 2017.

550. Lévesque J, Eugène F, Joanette Y, Paquette V. Neural circuitry underlying voluntary suppression of sadness. *Biological*. 2003. http://www.sciencedirect.com/science/article/pii/S0006322302018176. Accessed August 9, 2017.

551. Davidson R, Pizzagalli D, Nitschke J. Depression: perspectives from affective neuroscience. *Annu Rev*. 2002. http://www.annualreviews.org/doi/abs/10.1146/annurev.psych.53.100901.135148. Accessed August 13, 2017.

552. Elliott R, Baker SC, Rogers RD, et al. Prefrontal dysfunction in depressed patients performing a complex planning task: a study using positron emission tomography. *Psychol Med*. 1997;27(4):931-942. http://www.ncbi.nlm.nih.gov/pubmed/9234470. Accessed October 28, 2017.

553. Davidson RJ, Pizzagalli D, Nitschke JB, Putnam K. Depression: perspectives from affective neuroscience. *Annu Rev Psychol*. 2002;53(1):545-574. doi:10.1146/annurev.psych.53.100901.135148.

554. Siegle GJ, Thompson W, Carter CS, Steinhauer SR, Thase ME. Increased Amygdala and Decreased Dorsolateral Prefrontal BOLD Responses in Unipolar Depression: Related and Independent Features. *Biol Psychiatry*. 2007;61(2):198-209. doi:10.1016/j.biopsych.2006.05.048.

555. Vasic N, Wolf RC, Walter H. [Executive functions in patients with depression. The role of prefrontal activation]. *Nervenarzt*. 2007;78(6):628, 630-2, 634-6 passim. doi:10.1007/s00115-006-2240-6.

556. Erk S, Mikschl A, Stier S, et al. Acute and Sustained Effects of Cognitive Emotion Regulation in Major Depression. *J Neurosci*. 2010;30(47):15726-15734. doi:10.1523/JNEUROSCI.1856-10.2010.

557. Lu Q, Li H, Luo G, et al. Neuroscience Letters Impaired prefrontal – amygdala effective connectivity is responsible for the dysfunction of emotion process in major depressive disorder : A dynamic causal modeling study on MEG. *Neurosci Lett*. 2012;523(2):125-130. doi:10.1016/j.neulet.2012.06.058.

558. Quaedflieg CWEM, Ven V Van De, Meyer T, Siep N, Merckelbach H, Smeets T. Temporal Dynamics of Stress-Induced Alternations of Intrinsic Amygdala Connectivity and Neuroendocrine Levels. 2015:1-16. doi:10.1371/journal.pone.0124141.

559. Edmiston EE, Wang F, Mazure CM, et al. Corticostriatal-limbic gray matter morphology in adolescents with self-reported exposure to childhood maltreatment. *Arch Pediatr Adolesc Med*. 2011;165(12):1069-1077. doi:10.1001/archpediatrics.2011.565.

560. Watzka M, Bidlingmaier F, Beyenburg S, Henke R. Corticosteroid receptor mRNA expression in the brains of patients with epilepsy. *Steroids*. 2000. http://www.sciencedirect.com/science/article/pii/S0039128X00002051. Accessed August 13, 2017.

561. McEwen B, Morrison J. The Brain on Stress: Vulnerability and Plasticity of the Prefrontal Cortex over the Life Course. *Neuron*. 2013;79(1):16-29. doi:10.1016/j.neuron.2013.06.028.

562. Ongür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex*. 2000;10(3):206-219. doi:10.1093/cercor/10.3.206.

563. Dalley J, Cardinal R, Robbins T. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav*. 2004. http://www.sciencedirect.com/science/article/pii/S014976340400096X. Accessed August 13, 2017.

564. Schulz K, Molenda-Figueira H, Sisk C. Back to the future: the organizational–activational hypothesis adapted to puberty and adolescence. *Horm Behav*. 2009. http://www.sciencedirect.com/science/article/pii/S0018506X0900066X. Accessed August 8, 2017.

565. Gunnar MR, Wewerka S, Frenn K, Long JD, Griggs C. Developmental changes in hypothalamus-pituitary-adrenal activity over the transition to adolescence: Normative changes and associations with puberty. *Dev Psychopathol*. 2009;21(1):69-85. doi:10.1017/S095457940999037X.

566. Silk J, Siegle G, Whalen D. Pubertal changes in emotional information processing:

Pupillary, behavioral, and subjective evidence during emotional word identification. *Dev*. 2009. https://www.cambridge.org/core/journals/development-and-psychopathology/article/pubertal-changes-in-emotional-information-processing-pupillary-behavioral-and-subjective-evidence-during-emotional-word-identification/AA1414C299CC25D7A79A8957F2185533. Accessed August 18, 2017.

567. Walker EF, Sabuwalla Z, Huot R. Pubertal neuromaturation, stress sensitivity, and psychopathology. *Dev Psychopathol*. 2004;16(4):807-824. http://www.ncbi.nlm.nih.gov/pubmed/15704816. Accessed January 12, 2018.

568. Juraska JM, Willing J. Pubertal onset as a critical transition for neural development and cognition. *Brain Res*. 2017;1654:87-94. doi:10.1016/j.brainres.2016.04.012.

569. Kuhn C, Johnson M, Thomae A, et al. The emergence of gonadal hormone influences on dopaminergic function during puberty. *Horm Behav*. 2010;58(1):122-137. doi:10.1016/j.yhbeh.2009.10.015.

570. Gottfried J, Dolan R. Human orbitofrontal cortex mediates extinction learning while accessing conditioned representations of value. *Nat Neurosci*. 2004. http://search.proquest.com/openview/51bb311f76ce75e95c6fba4e510d5bb9/1?pq-origsite=gscholar&cbl=44706. Accessed August 19, 2017.

571. Shiba Y, Santangelo AM, Roberts AC. Beyond the Medial Regions of Prefrontal Cortex in the Regulation of Fear and Anxiety. *Front Syst Neurosci*. 2016;10(February):12. doi:10.3389/fnsys.2016.00012.

572. Stein MB, Simmons AN, Feinstein JS, Paulus MP. Increased amygdala and insula activation during emotion processing in anxiety-prone subjects. *Am J Psychiatry*. 2007;164(2):318-327. doi:10.1176/appi.ajp.164.2.318.

573. Phan K, Orlichenko A, Boyd E, Angstadt M. Preliminary evidence of white matter abnormality in the uncinate fasciculus in generalized social anxiety disorder. *Biological*. 2009. http://www.sciencedirect.com/science/article/pii/S0006322309002959. Accessed August 19, 2017.

574. Woolley CS. Acute Effects of Estrogen on Neuronal Physiology. 2007. doi:10.1146/annurev.pharmtox.47.120505.105219.

575. Verdi JM, Campagnoni AT. Translational regulation by steroids. Identification of a steroid modulatory element in the 5'-untranslated region of the myelin basic protein messenger RNA. *J Biol Chem*. 1990;265(33):20314-20320. http://www.ncbi.nlm.nih.gov/pubmed/1700788. Accessed October 27, 2017.

576. Jung-Testas I, Renoir M, Bugnard H, Greene GL, Baulieu EE. Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells. *J Steroid Biochem Mol Biol*. 1992;41(3-8):621-631. http://www.ncbi.nlm.nih.gov/pubmed/1562533. Accessed October 27, 2017.

577. Garcia-Segura LM, Chowen JA, Naftolin F. Endocrine glia: roles of glial cells in the brain actions of steroid and thyroid hormones and in the regulation of hormone secretion. *Front Neuroendocrinol*. 1996;17(2):180-211. doi:10.1006/frne.1996.0005.

578. Hirahara Y, Matsuda K-I, Gao W, Arvanitis DN, Kawata M, Boggs JM. The localization and non-genomic function of the membrane-associated estrogen receptor in oligodendrocytes. *Glia*. 2009;57(2):153-165. doi:10.1002/glia.20742.

579. Shansky RM, Hamo C, Hof PR, Lou W, McEwen BS, Morrison JH. Estrogen Promotes Stress Sensitivity in a Prefrontal Cortex-Amygdala Pathway. *Cereb Cortex*. 2010;20(11):2560-2567. doi:10.1093/cercor/bhq003.

580. Warren MP, Brooks-Gunn J. Mood and behavior at adolescence: Evidence for hormonal factors. *J Clin Endocrinol Metab*. 1989;69(1):77-83. doi:10.1210/jcem-69-1-77.

581. Forbes EE, Dahl RE. Pubertal development and behavior : Hormonal activation of social and motivational tendencies. *Brain Cogn*. 2010;72(1):66-72. doi:10.1016/j.bandc.2009.10.007.

582. Eccles J. The development of children ages 6 to 14. *Futur Child*. 1999. http://www.jstor.org/stable/1602703. Accessed August 18, 2017.

583. Ehardt CL, Bernstein IS. Patterns of affiliation among immature rhesus monkeys (Macaca mulatta). *Am J Primatol*. 1987;13(3):255-269. doi:10.1002/ajp.1350130304.

584. Insel TR. Postpartum increases in brain oxytocin binding. *Neuroendocrinology*. 1986;44(4):515-518. http://www.ncbi.nlm.nih.gov/pubmed/3029617. Accessed January 12, 2018.

585. de Kloet ER, Voorhuis DA, Boschma Y, Elands J. Estradiol modulates density of putative "oxytocin receptors" in discrete rat brain regions. *Neuroendocrinology*. 1986;44(4):415-421. http://www.ncbi.nlm.nih.gov/pubmed/3029614. Accessed January 12, 2018.

586. Tribollet E, Charpak S, Schmidt A, Dubois-Dauphin M, Dreifuss JJ. Appearance and transient expression of oxytocin receptors in fetal, infant, and peripubertal rat brain studied by autoradiography and electrophysiology. *J Neurosci*. 1989;9(5):1764-1773. http://www.ncbi.nlm.nih.gov/pubmed/2542479. Accessed January 12, 2018.

587. Gordon I, Martin C, Feldman R, Leckman JF. Oxytocin and social motivation. *Dev Cogn Neurosci*. 2011;1(4):471-493. doi:10.1016/j.dcn.2011.07.007.

588. Mora M de la, Gallegos-Cari A. Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: structural and functional analysis. *Prog*. 2010.

http://www.sciencedirect.com/science/article/pii/S0301008209001610. Accessed August 20, 2017.

589. Cardinal R, Parkinson J, Hall J, Everitt B. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. *Biobehav Rev*. 2002. http://www.sciencedirect.com/science/article/pii/S0149763402000076. Accessed August 20, 2017.

590. Ulrich-Lai YM, Herman JP. Neural regulation of endocrine and autonomic stress responses. *Nat Rev Neurosci*. 2009;10(6):397-409. doi:10.1038/nrn2647.

591. Cressman VL, Balaban J, Steinfeld S, et al. Prefrontal cortical inputs to the basal amygdala undergo pruning during late adolescence in the rat. *J Comp Neurol*. 2010;518(14):NA-NA. doi:10.1002/cne.22359.

592. Anderson S, Dallal G, Must A. Relative weight and race influence average age at menarche: results from two nationally representative surveys of US girls studied 25 years apart. *Pediatrics*. 2003. http://pediatrics.aappublications.org/content/111/4/844.short. Accessed August 20, 2017.

593. Shi Y, Budin F, Yapuncich E, et al. UNC-Emory Infant Atlases for Macaque Brain Image Analysis : Postnatal Brain Development through 12 Months. 2017;10(January):1-12. doi:10.3389/fnins.2016.00617.

594. Charmandari E, Kino T, Souvatzoglou E, Chrousos GP. Pediatric stress: hormonal mediators and human development. *Horm Res*. 2003;59(4):161-179. doi:69325.

595. Bremne JD, Vermetten E. Stress and development: behavioral and biological consequences. *Dev Psychopathol*. 2001;13(3):473-489. http://www.ncbi.nlm.nih.gov/pubmed/11523844. Accessed October 15, 2017.

596. Cameron JL. Interrelationships between hormones, behavior, and affect during adolescence: Complex relationships exist between reproductive hormones, stress-related hormones, and the activity of neural systems that regulate behavioral affect - Comments on part III. *Ann N Y Acad Sci*. 2004;1021:134-142. doi:10.1196/annals.1308.015.

597. Mason SM, Bryn Austin S, Bakalar JL, et al. Child Maltreatment's Heavy Toll: The Need for Trauma-Informed Obesity Prevention. *Am J Prev Med*. 2016;50(5):646-649. doi:10.1016/j.amepre.2015.11.004.

598. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of Obesity and Trends in Body Mass Index Among US Children and Adolescents, 1999-2010. *JAMA*. 2012;307(5):483. doi:10.1001/jama.2012.40.

599. Finkelstein EA, Graham WCK, Malhotra R. Lifetime Direct Medical Costs of Childhood Obesity. *Pediatrics*. 2014;133(5):854-862. doi:10.1542/peds.2014-0063.

600. Guo SS, Chumlea WC. Tracking of body mass index in children in relation to overweight in adulthood. *Am J Clin Nutr*. 1999;70(1):145S-8S. http://www.ncbi.nlm.nih.gov/pubmed/10419418. Accessed October 15, 2017.

601. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The Relation of Childhood BMI to Adult Adiposity: The Bogalusa Heart Study. *Pediatrics*. 2005;115(1):22-27. doi:10.1542/peds.2004-0220.

602. Benjamin RM. The Surgeon General's Vision for a Healthy and Fit Nation. *Public Health Rep*. 2010;125(4):514-515. doi:10.1177/003335491012500402.

603. Adam TC, Epel ES. Stress, eating and the reward system. *Physiol Behav*. 2007;91(4):449-458. doi:10.1016/j.physbeh.2007.04.011.

604. la Fleur SE, Luijendijk MCM, van Rozen AJ, Kalsbeek A, Adan RAH. A free-choice

high-fat high-sugar diet induces glucose intolerance and insulin unresponsiveness to a glucose load not explained by obesity. *Int J Obes (Lond)*. 2011;35(4):595-604. doi:10.1038/ijo.2010.164.

605. Levin BE, Dunn-Meynell AA, Balkan B, Keesey RE. Selective breeding for diet-induced obesity and resistance in Sprague-Dawley rats. *Am J Physiol*. 1997;273(2 Pt 2):R725-30. http://www.ncbi.nlm.nih.gov/pubmed/9277561. Accessed October 15, 2017.
606. Greeno CG, Wing RR. Stress-induced eating. *Psychol Bull*. 1994;115(3):444-464. http://www.ncbi.nlm.nih.gov/pubmed/8016287. Accessed October 15, 2017.
607. Hagan MM, Chandler PC, Wauford PK, Rybak RJ, Oswald KD. The role of palatable food and hunger as trigger factors in an animal model of stress induced binge eating. *Int J Eat Disord*. 2003;34(2):183-197. doi:10.1002/eat.10168.
608. Foster MT, Solomon MB, Huhman KL, Bartness TJ. Social defeat increases food intake, body mass, and adiposity in Syrian hamsters. *AJP Regul Integr Comp Physiol*. 2005;290(5):R1284-R1293. doi:10.1152/ajpregu.00437.2005.
609. Solomon MB, Foster MT, Bartness TJ, Huhman KL. Social defeat and footshock increase body mass and adiposity in male Syrian hamsters. *Am J Physiol Regul Integr Comp Physiol*. 2007;292(1):R283-90. doi:10.1152/ajpregu.00330.2006.
610. Arce M, Michopoulos V, Shepard KN, Ha QC, Wilson ME. Diet choice, cortisol reactivity, and emotional feeding in socially housed rhesus monkeys. *Physiol Behav*. 2010;101(4):446-455. doi:10.1016/j.physbeh.2010.07.010.
611. Grunberg NE, Straub RO. The role of gender and taste class in the effects of stress on eating. *Heal Psychol*. 1992;11(2):97-100. doi:10.1037/0278-6133.11.2.97.
612. Greene GW, Schembre SM, White AA, et al. Identifying clusters of college students at

elevated health risk based on eating and exercise behaviors and psychosocial determinants of body weight. *J Am Diet Assoc*. 2011;111(3):394-400. doi:10.1016/j.jada.2010.11.011.

613. Joëls M, Karst H, Alfarez D, et al. Effects of Chronic Stress on Structure and Cell Function in Rat Hippocampus and Hypothalamus. *Stress*. 2004;7(4):221-231. doi:10.1080/10253890500070005.

614. Cerqueira JJ, Pêgo JM, Taipa R, Bessa JM, Almeida OFX, Sousa N. Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors. *J Neurosci*. 2005;25(34):7792-7800. doi:10.1523/JNEUROSCI.1598-05.2005.

615. Dias-Ferreira E, Sousa JC, Melo I, et al. Chronic stress causes frontostriatal reorganization and affects decision-making. *Science*. 2009;325(5940):621-625. doi:10.1126/science.1171203.

616. Soares JM, Sampaio A, Ferreira LM, et al. Stress Impact on Resting State Brain Networks. Zhan W, ed. *PLoS One*. 2013;8(6):e66500. doi:10.1371/journal.pone.0066500.

617. Craig AD. Interoception: The sense of the physiological condition of the body. *Curr Opin Neurobiol*. 2003;13(4):500-505. doi:10.1016/S0959-4388(03)00090-4.

618. Davis M, Whalen PJ. The amygdala: vigilance and emotion. *Mol Psychiatry*. 2001;6(1):13-34. http://www.ncbi.nlm.nih.gov/pubmed/11244481. Accessed October 16, 2017.

619. LeDoux J. The amygdala. *Curr Biol*. 2007;17(20):R868-R874. doi:10.1016/j.cub.2007.08.005.

620. Phillips ML, Ladouceur CD, Drevets WC. A neural model of voluntary and automatic emotion regulation: implications for understanding the pathophysiology and neurodevelopment of bipolar disorder. *Mol Psychiatry*. 2008;13(9):829, 833-857.

doi:10.1038/mp.2008.65.

621. Roy AK, Fudge JL, Kelly C, et al. Intrinsic functional connectivity of amygdala-based networks in adolescent generalized anxiety disorder. *J Am Acad Child Adolesc Psychiatry*. 2013;52(3):290-299.e2. doi:10.1016/j.jaac.2012.12.010.

622. Hare TA, Tottenham N, Galvan A, Voss HU, Glover GH, Casey BJ. Biological Substrates of Emotional Reactivity and Regulation in Adolescence During an Emotional Go-Nogo Task. *Biol Psychiatry*. 2008;63(10):927-934. doi:10.1016/j.biopsych.2008.03.015.

623. Russo SJ, Nestler EJ. The brain reward circuitry in mood disorders. *Nat Rev Neurosci*. 2013;14(9):609-625. doi:10.1038/nrn3381.

624. Lodge DJ, Grace AA. Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity. *J Pharmacol Exp Ther*. 2005;314(1):201-206. doi:10.1124/jpet.105.084913.

625. Wanat MJ, Hopf FW, Stuber GD, Phillips PEM, Bonci A. Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. *J Physiol*. 2008;586(8):2157-2170. doi:10.1113/jphysiol.2007.150078.

626. Haber SN, Knutson B. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology*. 2010;35(1):4-26. doi:10.1038/npp.2009.129.

627. Spanagel R, Weiss F. The dopamine hypothesis of reward: past and current status. *Trends Neurosci*. 1999;22(11):521-527. http://www.ncbi.nlm.nih.gov/pubmed/10529820. Accessed October 16, 2017.

628. Craig ADB. Human feelings: why are some more aware than others? *Trends Cogn Sci*. 2004;8(6):239-241. doi:10.1016/j.tics.2004.04.004.

629. Cauda F, D'Agata F, Sacco K, Duca S, Geminiani G, Vercelli A. Functional connectivity of the insula in the resting brain. *Neuroimage*. 2011;55(1):8-23. doi:10.1016/j.neuroimage.2010.11.049.

630. Nummenmaa L, Hirvonen J, Hannukainen JC, et al. Dorsal Striatum and Its Limbic Connectivity Mediate Abnormal Anticipatory Reward Processing in Obesity. 2012;7(2). doi:10.1371/journal.pone.0031089.

631. Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. *Am J Psychiatry*. 2007;164(8):1149-1159. doi:10.1176/appi.ajp.2007.05030503.

632. Koob G, Le Moal M. Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*. 2001;24(2):97-129. doi:10.1016/S0893-133X(00)00195-0.

633. Kenny PJ. Reward Mechanisms in Obesity: New Insights and Future Directions. *Neuron*. 2011;69(4):664-679. doi:10.1016/j.neuron.2011.02.016.

634. Michaelides M, Thanos PK, Volkow ND, Wang G-J. Dopamine-related frontostriatal abnormalities in obesity and binge-eating disorder: emerging evidence for developmental psychopathology. *Int Rev Psychiatry*. 2012;24(3):211-218. doi:10.3109/09540261.2012.679918.

635. Hajnal A, Smith GP, Norgren R. Oral sucrose stimulation increases accumbens dopamine in the rat. *AJP Regul Integr Comp Physiol*. 2003;286(1):31R-37. doi:10.1152/ajpregu.00282.2003.

636. Frank GKW, Oberndorfer TA, Simmons AN, et al. Sucrose activates human taste pathways differently from artificial sweetener. *Neuroimage*. 2008;39(4):1559-1569. doi:10.1016/j.neuroimage.2007.10.061.

637. DelParigi A, Chen K, Salbe AD, Reiman EM, Tataranni PA. Sensory experience of food and obesity: a positron emission tomography study of the brain regions affected by tasting a liquid meal after a prolonged fast. *Neuroimage*. 2005;24(2):436-443. doi:10.1016/j.neuroimage.2004.08.035.

638. Lips MA, Wijngaarden MA, Grond J Van Der, et al. Resting-state functional connectivity of brain regions involved in cognitive control , motivation , and reward is enhanced in obese. *Am J Clin Nutr*. 2014;100:524-531. doi:10.3945/ajcn.113.080671.1.

639. Anisman H, Zaharia MD, Meaney MJ, Merali Z. Do early-life events permanently alter behavioral and hormonal responses to stressors? *Int J Dev Neurosci*. 16(3-4):149-164. http://www.ncbi.nlm.nih.gov/pubmed/9785112. Accessed January 16, 2018.

640. Sánchez MM, Noble PM, Lyon CK, et al. Alterations in diurnal cortisol rhythm and acoustic startle response in nonhuman primates with adverse rearing. *Biol Psychiatry*. 2005;57(4):373-381. doi:10.1016/j.biopsych.2004.11.032.

641. Knickmeyer RC, Styner M, Short SJ, et al. Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging. *Cereb Cortex*. 2010;20(5):1053-1063. doi:10.1093/cercor/bhp166.

642. Hall BS, Moda RN, Liston C. Neurobiology of Stress Glucocorticoid mechanisms of functional connectivity changes in stress-related neuropsychiatric disorders. 2015;1:174-183.

643. Miller GE, Blackwell E. Turning Up the Heat. *Curr Dir Psychol Sci*. 2006;15(6):269-272. doi:10.1111/j.1467-8721.2006.00450.x.

644. Cohen S, Janicki-Deverts D, Doyle WJ, et al. Chronic stress, glucocorticoid receptor

resistance, inflammation, and disease risk. *Proc Natl Acad Sci*. 2012;109(16):5995-5999. doi:10.1073/pnas.1118355109.

645. Felger JC, Li Z, Haroon E, et al. Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry in depression. *Mol Psychiatry*. 2016;21(10):1358-1365. doi:10.1038/mp.2015.168.

646. Marsland AL, Kuan DC-H, Sheu LK, et al. Systemic inflammation and resting state connectivity of the default mode network. *Brain Behav Immun*. 2017;62:162-170. doi:10.1016/j.bbi.2017.01.013.

647. Rice D, Barone S. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ Health Perspect*. 2000;108 Suppl 3:511-533. http://www.ncbi.nlm.nih.gov/pubmed/10852851. Accessed January 13, 2018.

648. Hayashi M. Ontogeny of some neuropeptides in the primate brain. *Prog Neurobiol*. 1992;38(3):231-260. http://www.ncbi.nlm.nih.gov/pubmed/1312244. Accessed January 13, 2018.

649. Rakic P, Bourgeois JP, Eckenhoff MF, Zecevic N, Goldman-Rakic PS. Concurrent overproduction of synapses in diverse regions of the primate cerebral cortex. *Science*. 1986;232(4747):232-235. http://www.ncbi.nlm.nih.gov/pubmed/3952506. Accessed January 14, 2018.

650. Lidow MS, Goldman-Rakic PS, Rakic P. Synchronized overproduction of neurotransmitter receptors in diverse regions of the primate cerebral cortex. *Proc Natl Acad Sci U S A*. 1991;88(22):10218-10221. http://www.ncbi.nlm.nih.gov/pubmed/1658799. Accessed January 14, 2018.

651. Bourgeois JP, Goldman-Rakic PS, Rakic P. Synaptogenesis in the prefrontal cortex of

rhesus monkeys. *Cereb Cortex*. 4(1):78-96. http://www.ncbi.nlm.nih.gov/pubmed/8180493. Accessed January 14, 2018.

652. Rakic P, Nowakowski RS. The time of origin of neurons in the hippocampal region of the rhesus monkey. *J Comp Neurol*. 1981;196(1):99-128. doi:10.1002/cne.901960109.

653. Bachevalier J, Mishkin M. An early and a late developing system for learning and retention in infant monkeys. *Behav Neurosci*. 1984;98(5):770-778. http://www.ncbi.nlm.nih.gov/pubmed/6541498. Accessed January 14, 2018.

654. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol*. 1997;387(2):167-178. http://www.ncbi.nlm.nih.gov/pubmed/9336221. Accessed January 14, 2018.

655. Kawai M. On the rank system in a natural group of Japanese monkey (II). *Primates*. 1958;1(2):131-148. doi:10.1007/BF01813700.

656. Casabiell X, Piñeiro V, Tomé MA, Peinó R, Dieguez C, Casanueva FF. Presence of Leptin in Colostrum and/or Breast Milk from Lactating Mothers: A Potential Role in the Regulation of Neonatal Food Intake. *J Clin Endocrinol Metab*. 1997;82(12):4270-4273. doi:10.1210/jcem.82.12.4590.

657. Hart S, Boylan LM, Border B, Carroll SR, McGunegle D, Lampe RM. Breast milk levels of cortisol and Secretory Immunoglobulin A (SIgA) differ with maternal mood and infant neuro-behavioral functioning. *Infant Behav Dev*. 2004;27(1):101-106. doi:10.1016/J.INFBEH.2003.06.002.

658. Innis SM. Human milk: maternal dietary lipids and infant development. *Proc Nutr Soc*. 2007;66(3):397-404. doi:10.1017/S0029665107005666.

659. Scott J a., Grayson D, Fletcher E, et al. Longitudinal analysis of the developing rhesus

monkey brain using magnetic resonance imaging: birth to adulthood. *Brain Struct Funct*. 2015. doi:10.1007/s00429-015-1076-x.

660. Howell BR, Mcmurray MS, Guzman DB, et al. Maternal buffering beyond glucocorticoids : impact of early life stress on corticolimbic circuits that control infant responses to novelty. *Soc Neurosci*. 2016;0(0):1-15. doi:10.1080/17470919.2016.1200481.

661. Drury S, Howell B, Jones CR, et al. Shaping long-term primate development: telomere length trajectory as an indicator of early maternal maltreatment and predictor of future physiologic regulation. *Dev Psychopathol*. 2017;29:1539-1551.

662. Michopoulos V, Moore C, Wilson ME. Psychosocial Stress and Diet History Promote Emotional Feeding in Female Rhesus Monkeys. In: ; 2013:109-125. doi:10.1007/978-1-62703-104-2_8.

663. Moore CJ, Michopoulos V, Johnson ZP, Toufexis D, Wilson ME. Dietary variety is associated with larger meals in female rhesus monkeys. *Physiol Behav*. 2013;119:190-194. doi:10.1016/j.physbeh.2013.06.014.

664. Stroud FC, Appt SE, Wilson ME, Franke AA, Adams MR, Kaplan JR. Concentrations of isoflavones in macaques consuming standard laboratory monkey diet. *J Am Assoc Lab Anim Sci*. 2006;45(4):20-23.

665. Machado CJ, Bachevalier J. The effects of selective amygdala, orbital frontal cortex or hippocampal formation lesions on reward assessment in nonhuman primates. *Eur J Neurosci*. 2007;25(9):2885-2904. doi:10.1111/j.1460-9568.2007.05525.x.

666. Kalin NH, Shelton SE, Takahashi LK. Defensive behaviors in infant rhesus monkeys: ontogeny and context-dependent selective expression. *Child Dev*. 1991;62(5):1175-1183.

http://www.ncbi.nlm.nih.gov/pubmed/1756661. Accessed October 13, 2017.

667. McCormack K, Newman TK, Higley JD, Maestripieri D, Sanchez MM. Serotonin transporter gene variation, infant abuse, and responsiveness to stress in rhesus macaque mothers and infants. *Horm Behav*. 2009;55(4):538-547. doi:10.1016/j.yhbeh.2009.01.009.

668. Sanchez MM, McCormack K, Grand AP, Fulks R, Graff A, Maestripieri D. Effects of sex and early maternal abuse on adrenocorticotropin hormone and cortisol responses to the corticotropin-releasing hormone challenge during the first 3 years of life in group-living rhesus monkeys. *Dev Psychopathol*. 2010;22(1):45-53. doi:10.1017/S0954579409990253.

669. Raper J, Bachevalier J, Wallen K, Sanchez M. Neonatal amygdala lesions alter basal cortisol levels in infant rhesus monkeys. *Psychoneuroendocrinology*. 2013;38(6):818-829. doi:10.1016/j.psyneuen.2012.09.004.

670. Blank MS, Gordon TP, Wilson ME. Effects of capture and venipuncture on serum levels of prolactin, growth hormone and cortisol in outdoor compound-housed female rhesus monkeys (Macaca mulatta). *Acta Endocrinol (Copenh)*. 1983;102(2):190-195. http://www.ncbi.nlm.nih.gov/pubmed/6829259. Accessed October 13, 2017.

671. Davenport MD, Lutz CK, Tiefenbacher S, Novak MA, Meyer JS. A rhesus monkey model of self-injury: effects of relocation stress on behavior and neuroendocrine function. *Biol Psychiatry*. 2008;63(10):990-996. doi:10.1016/j.biopsych.2007.10.025.

672. Davenport MD, Tiefenbacher S, Lutz CK, Novak MA, Meyer JS. Analysis of endogenous cortisol concentrations in the hair of rhesus macaques. *Gen Comp Endocrinol*. 2006;147(3):255-261. doi:10.1016/j.ygcen.2006.01.005.

673. Meyer J, Novak M, Hamel A, Rosenberg K. Extraction and Analysis of Cortisol from Human and Monkey Hair. *J Vis Exp*. 2014;(83):e50882. doi:10.3791/50882.

674. Andersson JLR, Skare S, Ashburner J. How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging. *Neuroimage*. 2003;20(2):870-888. doi:10.1016/S1053-8119(03)00336-7.

675. Power J, Barnes K, Snyder A, Schlaggar B. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage*. 2012. http://www.sciencedirect.com/science/article/pii/S1053811911011815. Accessed August 20, 2017.

676. Mesulam M, Mufson EJ. Insula of the Old World Monkey . 111 : Efferent Cortical Output and Comments on Function. 1982;52:38-52.

677. Markov N, Ercsey-Ravasz M. A weighted and directed interareal connectivity matrix for macaque cerebral cortex. *Cerebral*. 2012. https://academic.oup.com/cercor/article-abstract/24/1/17/272931. Accessed August 20, 2017.

678. Sanchez MM, McCormack KM, Howell BR. Social buffering of stress responses in nonhuman primates: Maternal regulation of the development of emotional regulatory brain circuits. *Soc Neurosci*. 2015;10(5):512-526. doi:10.1080/17470919.2015.1087426.

679. Weingarten HP. Conditioned cues elicit feeding in sated rats: a role for learning in meal initiation. *Science*. 1983;220(4595):431-433. http://www.ncbi.nlm.nih.gov/pubmed/6836286. Accessed October 18, 2017.

680. MORGANE PJ, KOSMAN AJ. Relationship of the middle hypothalamus to amygdalar hyperphagia. *Am J Physiol*. 1960;198(6):1315-1318. http://www.ncbi.nlm.nih.gov/pubmed/14424127. Accessed October 18, 2017.

681. Green E, Jacobson A, Haase L, Murphy C. Reduced nucleus accumbens and caudate nucleus activation to a pleasant taste is associated with obesity in older adults. *Brain Res*.

2011;1386:109-117. doi:10.1016/j.brainres.2011.02.071.

682. Rolls ET. Taste, olfactory, and food reward value processing in the brain. *Prog Neurobiol*. 2015;127-128:64-90. doi:10.1016/j.pneurobio.2015.03.002.

683. Critchley HD, Rolls ET. Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. *J Neurophysiol*. 1996;75(4):1673-1686. http://www.ncbi.nlm.nih.gov/pubmed/8727405. Accessed October 18, 2017.

684. Grabenhorst F, Rolls ET. Selective attention to affective value alters how the brain processes taste stimuli. *Eur J Neurosci*. 2008;27(3):723-729. doi:10.1111/j.1460-9568.2008.06033.x.

685. Kadohisa M, Verhagen J V, Rolls ET. The primate amygdala: Neuronal representations of the viscosity, fat texture, temperature, grittiness and taste of foods. *Neuroscience*. 2005;132(1):33-48. doi:10.1016/j.neuroscience.2004.12.005.

686. Kadohisa M, Rolls ET, Verhagen J V. Neuronal representations of stimuli in the mouth: the primate insular taste cortex, orbitofrontal cortex and amygdala. *Chem Senses*. 2005;30(5):401-419. doi:10.1093/chemse/bji036.

687. Sanghera MK, Rolls ET, Roper-Hall A. Visual responses of neurons in the dorsolateral amygdala of the alert monkey. *Exp Neurol*. 1979;63(3):610-626. http://www.ncbi.nlm.nih.gov/pubmed/428486. Accessed October 18, 2017.

688. Bruce AS, Holsen LM, Chambers RJ, et al. Obese children show hyperactivation to food pictures in brain networks linked to motivation, reward and cognitive control. *Int J Obes (Lond)*. 2010;34(10):1494-1500. doi:10.1038/ijo.2010.84.

689. Schilman EA, Klavir O, Winter C, Sohr R, Joel D. The Role of the Striatum in Compulsive Behavior in Intact and Orbitofrontal-Cortex-Lesioned Rats: Possible

Involvement of the Serotonergic System. *Neuropsychopharmacology*. 2010;35(4):1026-1039. doi:10.1038/npp.2009.208.

690. Foland-Ross LC, Altshuler LL, Bookheimer SY, et al. Amygdala reactivity in healthy adults is correlated with prefrontal cortical thickness. *J Neurosci*. 2010;30(49):16673-16678. doi:10.1523/JNEUROSCI.4578-09.2010.

691. Hariri AR, Tessitore A, Mattay VS, Fera F, Weinberger DR. The amygdala response to emotional stimuli: a comparison of faces and scenes. *Neuroimage*. 2002;17(1):317-323. http://www.ncbi.nlm.nih.gov/pubmed/12482086. Accessed October 18, 2017.

692. Monk CS, Telzer EH, Mogg K, et al. Amygdala and ventrolateral prefrontal cortex activation to masked angry faces in children and adolescents with generalized anxiety disorder. *Arch Gen Psychiatry*. 2008;65(5):568-576. doi:10.1001/archpsyc.65.5.568.

693. Stein MB, Goldin PR, Sareen J, Zorrilla LTE, Brown GG. Increased amygdala activation to angry and contemptuous faces in generalized social phobia. *Arch Gen Psychiatry*. 2002;59(11):1027-1034. http://www.ncbi.nlm.nih.gov/pubmed/12418936. Accessed October 18, 2017.

694. Birn RM, Patriat R, Phillips ML, Germain A, Herringa RJ. Childhood maltreatment and combat posttraumatic stress differentially predict fear-related fronto-subcortical connectivity. *Depress Anxiety*. 2014;31(10):880-892. doi:10.1002/da.22291.

695. Brown VMV, Labar KSKKS, Haswell CCC, et al. Altered Resting-State Functional Connectivity of Basolateral and Centromedial Amygdala Complexes in Posttraumatic Stress Disorder. …. 2013;39(2):361-369. doi:10.1038/npp.2013.197.

696. KATO EA, RAY-SEA HSU B, RAYMOURE WJ, KUHN RW. Evidence for the Direct Transfer of Corticosteroid-Binding Globulin from Plasma to Whey in the Guinea Pig*.

Endocrinology. 1985;117(4):1404-1408. doi:10.1210/endo-117-4-1404.

697. Hamosh M. Bioactive factors in human milk. *Pediatr Clin North Am*. 2001;48(1):69-86. http://www.ncbi.nlm.nih.gov/pubmed/11236734. Accessed January 15, 2018.

698. Patacchioli FR, Cigliana G, Cilumbriello A, et al. Maternal plasma and milk free cortisol during the first 3 days of breast-feeding following spontaneous delivery or elective cesarean section. *Gynecol Obstet Invest*. 1992;34(3):159-163. http://www.ncbi.nlm.nih.gov/pubmed/1427417. Accessed January 15, 2018.

699. Yeh KY. Corticosterone concentrations in the serum and milk of lactating rats: parallel changes after induced stress. *Endocrinology*. 1984;115(4):1364-1370. doi:10.1210/endo-115-4-1364.

700. Angelucci L, Patacchioli FR, Scaccianoce S, Di Sciullo A, Cardillo A, Maccari S. A model for later-life effects of perinatal drug exposure: maternal hormone mediation. *Neurobehav Toxicol Teratol*. 7(5):511-517. http://www.ncbi.nlm.nih.gov/pubmed/4080068. Accessed January 15, 2018.

701. Boulanger LM. Immune Proteins in Brain Development and Synaptic Plasticity. *Neuron*. 2009;64(1):93-109. doi:10.1016/j.neuron.2009.09.001.

702. Carpentier PA, Palmer TD. Immune Influence on Adult Neural Stem Cell Regulation and Function. *Neuron*. 2009;64(1):79-92. doi:10.1016/j.neuron.2009.08.038.

703. Deverman BE, Patterson PH. Cytokines and CNS Development. *Neuron*. 2009;64(1):61-78. doi:10.1016/j.neuron.2009.09.002.

704. Garay PA, McAllister AK. Novel roles for immune molecules in neural development: implications for neurodevelopmental disorders. *Front Synaptic Neurosci*. 2010;2:136. doi:10.3389/fnsyn.2010.00136.

705. Yang S, Liu Z-W, Wen L, Qiao H-F, Zhou W-X, Zhang Y-X. Interleukin-1beta enhances NMDA receptor-mediated current but inhibits excitatory synaptic transmission. *Brain Res*. 2005;1034(1-2):172-179. doi:10.1016/j.brainres.2004.11.018.

706. Stellwagen D, Malenka RC. Synaptic scaling mediated by glial TNF-α. *Nature*. 2006;440(7087):1054-1059. doi:10.1038/nature04671.

707. Lee PC. Early infant development and maternal care in free-ranging vervet monkeys. *Primates*. 1984;25(1):36-47. doi:10.1007/BF02382294.

708. Amir N, Klumpp H, Elias J, Bedwell JS, Yanasak N, Miller LS. Increased activation of the anterior cingulate cortex during processing of disgust faces in individuals with social phobia. *Biol Psychiatry*. 2005;57(9):975-981. doi:10.1016/j.biopsych.2005.01.044.

709. Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry*. 2007;164(10):1476-1488. doi:10.1176/appi.ajp.2007.07030504.

710. Evans JSBT. Dual-processing accounts of reasoning, judgment, and social cognition. *Annu Rev Psychol*. 2008;59:255-278. doi:10.1146/annurev.psych.59.103006.093629.

711. Gorka SM, Fitzgerald DA, Labuschagne I, et al. Oxytocin modulation of amygdala functional connectivity to fearful faces in generalized social anxiety disorder. *Neuropsychopharmacology*. 2015;40(2):278-286. doi:10.1038/npp.2014.168.

712. Geng H, Li X, Chen J, Li X, Gu R. Decreased Intra- and Inter-Salience Network Functional Connectivity is Related to Trait Anxiety in Adolescents. *Front Behav Neurosci*. 2016;9(January):1-10. doi:10.3389/fnbeh.2015.00350.

713. Seeley WW, Menon V, Schatzberg AF, et al. Dissociable intrinsic connectivity networks for salience processing and executive control. *J Neurosci*. 2007;27(9):2349-2356.

doi:10.1523/JNEUROSCI.5587-06.2007.

714. Sridharan D, Levitin DJ, Menon V. A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc Natl Acad Sci*. 2008;105(34):12569-12574. doi:10.1073/pnas.0800005105.

715. Cornier M-A, Salzberg AK, Endly DC, Bessesen DH, Tregellas JR. Sex-based differences in the behavioral and neuronal responses to food. *Physiol Behav*. 2010;99(4):538-543. doi:10.1016/j.physbeh.2010.01.008.

716. Baylis LL, Rolls ET, Baylis GC. Afferent connections of the caudolateral orbitofrontal cortex taste area of the primate. *Neuroscience*. 1995;64(3):801-812. http://www.ncbi.nlm.nih.gov/pubmed/7715788. Accessed October 21, 2017.

717. Rolls ET, Sienkiewicz ZJ, Yaxley S. Hunger Modulates the Responses to Gustatory Stimuli of Single Neurons in the Caudolateral Orbitofrontal Cortex of the Macaque Monkey. *Eur J Neurosci*. 1989;1(1):53-60. http://www.ncbi.nlm.nih.gov/pubmed/12106174. Accessed October 21, 2017.

718. Rolls ET, Yaxley S, Sienkiewicz ZJ. Gustatory responses of single neurons in the caudolateral orbitofrontal cortex of the macaque monkey. *J Neurophysiol*. 1990;64(4):1055-1066. http://www.ncbi.nlm.nih.gov/pubmed/2258734. Accessed October 21, 2017.

719. Janes AC, Pizzagalli DA, Richardt S, et al. Neural Substrates of Attentional Bias for Smoking-Related Cues: An fMRI Study. *Neuropsychopharmacology*. 2010;35(12):2339-2345. doi:10.1038/npp.2010.103.

720. Bonson K, Grant SJ, Contoreggi CS, et al. Neural Systems and Cue-Induced Cocaine Craving,. *Neuropsychopharmacology*. 2002;26(3):376-386. doi:10.1016/S0893-

133X(01)00371-2.

721. Sinha R, Li CSR. Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug Alcohol Rev*. 2007;26(1):25-31. doi:10.1080/09595230601036960.

722. Naqvi NH, Rudrauf D, Damasio H, Bechara A. Damage to the Insula Disrupts Addiction to Cigarette Smoking. *Science (80-)*. 2007;315(5811):531-534. doi:10.1126/science.1135926.

723. Pelchat ML, Johnson A, Chan R, Valdez J, Ragland JD. Images of desire: food-craving activation during fMRI. *Neuroimage*. 2004;23(4):1486-1493. doi:10.1016/j.neuroimage.2004.08.023.

724. Killgore WDS, Yurgelun-Todd DA. Body mass predicts orbitofrontal activity during visual presentations of high-calorie foods. *Neuroreport*. 2005;16(8):859-863. http://www.ncbi.nlm.nih.gov/pubmed/15891585. Accessed October 21, 2017.

725. Schoenbaum G, Shaham Y. The Role of Orbitofrontal Cortex in Drug Addiction: A Review of Preclinical Studies. *Biol Psychiatry*. 2008;63(3):256-262. doi:10.1016/j.biopsych.2007.06.003.

726. Goldstein RZ, Volkow ND. Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat Rev Neurosci*. 2011;12(11):652-669. doi:10.1038/nrn3119.

727. Claus ED, Blaine SK, Filbey FM, Mayer AR, Hutchison KE. Association Between Nicotine Dependence Severity , BOLD Response to Smoking Cues , and Functional Connectivity. *Neuropsychopharmacology*. 2013;38(12):2363-2372. doi:10.1038/npp.2013.134.

728. Field CJ. The immunological components of human milk and their effect on immune development in infants. *J Nutr*. 2005;135(1):1-4. http://www.ncbi.nlm.nih.gov/pubmed/15623823. Accessed October 22, 2017.

729. Bertino M. Effects of high fat, protein supplemented diets on maternal behavior in rats. *Physiol Behav*. 1982;29(6):999-1005. http://www.ncbi.nlm.nih.gov/pubmed/7163403. Accessed January 17, 2018.

730. Purcell RH, Sun B, Pass LL, Power ML, Moran TH, Tamashiro KLK. Maternal stress and high-fat diet effect on maternal behavior, milk composition, and pup ingestive behavior. *Physiol Behav*. 2011;104(3):474-479. doi:10.1016/j.physbeh.2011.05.012.

731. Francis D, Diorio J, Liu D, Meaney MJ. Nongenomic transmission across generations of maternal behavior and stress responses in the rat. *Science*. 1999;286(5442):1155-1158. http://www.ncbi.nlm.nih.gov/pubmed/10550053. Accessed January 17, 2018.

732. Weaver ICG, Meaney MJ, Szyf M. Maternal care effects on the hippocampal transcriptome and anxiety-mediated behaviors in the offspring that are reversible in adulthood. *Proc Natl Acad Sci*. 2006;103(9):3480-3485. doi:10.1073/pnas.0507526103.

733. Horstmann A, Busse FP, Mathar D, et al. Obesity-Related Differences between Women and Men in Brain Structure and Goal-Directed Behavior. *Front Hum Neurosci*. 2011;5(June):58. doi:10.3389/fnhum.2011.00058.

734. Steele CC, Pirkle JRA, Kirkpatrick K. Diet-induced impulsivity: Effects of a high-fat and a high-sugar diet on impulsive choice in rats. Abe K, ed. *PLoS One*. 2017;12(6):e0180510. doi:10.1371/journal.pone.0180510.

735. Roy AK, Shehzad Z, Margulies DS, et al. Functional connectivity of the human amygdala using resting state fMRI. *Neuroimage*. 2009;45(2):614-626.

doi:10.1016/j.neuroimage.2008.11.030.

736. Taylor KS, Seminowicz DA, Davis KD. Two systems of resting state connectivity between the insula and cingulate cortex. *Hum Brain Mapp*. 2009;30(9):2731-2745. doi:10.1002/hbm.20705.

737. Ahmad SO, Park J-H, Radel JD, Levant B. Reduced numbers of dopamine neurons in the substantia nigra pars compacta and ventral tegmental area of rats fed an n-3 polyunsaturated fatty acid-deficient diet: a stereological study. *Neurosci Lett*. 2008;438(3):303-307. doi:10.1016/j.neulet.2008.04.073.

738. Delion S, Chalon S, Guilloteau D, Besnard JC, Durand G. alpha-Linolenic acid dietary deficiency alters age-related changes of dopaminergic and serotoninergic neurotransmission in the rat frontal cortex. *J Neurochem*. 1996;66(4):1582-1591. http://www.ncbi.nlm.nih.gov/pubmed/8627314. Accessed October 22, 2017.

739. Martinez M. Abnormal profiles of polyunsaturated fatty acids in the brain, liver, kidney and retina of patients with peroxisomal disorders. *Brain Res*. 1992;583(1-2):171-182. http://www.ncbi.nlm.nih.gov/pubmed/1504825. Accessed October 22, 2017.

740. Grayson DS, Kroenke CD, Neuringer M, Fair DA. Dietary Omega-3 Fatty Acids Modulate Large-Scale Systems Organization in the Rhesus Macaque Brain. 2014;34(6):2065-2074. doi:10.1523/JNEUROSCI.3038-13.2014.