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:

_____ _____

[Jodi R. Godfrey] Date

**Experiential and Developmental Factors Affecting Brain and Behavior**

**in Female Rhesus Macaques**

By

Jodi R. Godfrey

Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences

Neuroscience

_____

Mark E. Wilson, Ph.D.
Advisor

_____

Mar M. Sanchez, Ph.D.
Advisor

_____

Jocelyne Bachevalier, Ph.D.
Committee Member

_____

Patricia Brennan, Ph.D.
Committee Member

_____

Todd Preuss, Ph.D.
Committee Member

Accepted:

_____

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

_____

Date

**Experiential and Developmental Factors Affecting Brain and Behavior in Female Rhesus Macaques**

By

Jodi R. Godfrey

B.S., College of Charleston, 2005

Advisor: Mark E. Wilson, Ph.D.

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

2016

**Abstract**

**Experiential and Developmental Factors Affecting Brain and Behavior in Female Rhesus Macaques**

By

Jodi R. Godfrey

Exposure to chronic social stressors during childhood, adolescence and adulthood leads to alterations in brain structure and circuitry. Importantly, these stress-induced alterations can lead to emotional dysregulation and psychopathology, particularly in women. However, the mechanisms underlying the emergence of, and the factors contributing to, these stress-induced alterations are poorly understood. Therefore, the primary goal of this thesis was to investigate what factors underlie and modify chronic stressor-induced changes in brain structure and function and resulting behavior. Social subordination in female rhesus macaque monkeys is an established translational animal model that produces a number of stress-related phenotypes, including stress-induced over-eating of an obesogenic diet. Using this social subordination model of chronic stressor exposure, we found that developmental suppression of estradiol (E2) and consumption of an obesogenic diet both modify chronic stressor alterations in brain structure, functional connectivity (FC) and behavior. In one study, chronic social subordination across pre- and peripuberty resulted in larger amygdala (AMYG) volume compared to dominant subjects, but experimental suppression of E2 neutralized this effect. In the next study, the effects of social subordination on regional brain volumes in adults were modified by dietary environment, where the availability of an obesogenic diet reversed the impact of social rank on bilateral prefrontal cortex white matter (PFC WM). In the final study, the effects of social subordination on brain FC in adults were also modified by dietary environment. These results are important as they provide evidence that (1) exposure to developmental increases in E2 modify the consequences of social subordination on the volume of cortico-limbic regions involved in emotional and stress regulation during maturation, and (2) exposure to an obesogenic diet in adulthood modifies the consequences of social subordination on the volume and FC of cortico-limbic regions involved in stress regulation, in addition to emotional and motivational behavior in female rhesus macaques.

**Experiential and Developmental Factors Affecting Brain and Behavior in Female Rhesus Macaques**

By

Jodi R. Godfrey

B.S., College of Charleston, 2005

Advisor: Mark E. Wilson, Ph.D.

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

2016

**Acknowledgements**

I am exceedingly grateful to Dr. Mark Wilson and Dr. Mar Sanchez, who have provided exceptional guidance and support over the last several years. Drs. Wilson and Sanchez have been superior role models, not only in my professional life, but in my personal life as well. Thank you to my thesis committee members, Dr. Jocelyne Bachevalier, Dr. Patricia Brennan and Dr. Todd Preuss, for their investment in my graduate studies and professional development.

The studies presented in this dissertation would not have been possible without the expert technical assistance of Shannon Bounar, Natalie Brutto, Rebecca Herman, Brandon Hughes, Jessica Johnson, Jonathan Lowe, Christine Marsteller, Erin Olmoguez, Angela Tripp, Patrick Ulam and Jennifer Whitley, to whom I am extremely grateful. For both personal and professional support across my graduate career, I owe endless thanks to Dr. Amanda Mummert Anixter, Dr. Zsofia Kovacs Balint, Dr. Kathy Cole, Dr. Brittany Howell, Dr. Vasiliki Michopoulos, Elyse Morin and Melanie Pincus. Special thanks to our collaborators, Dr. Martin Styner and Yundi Shi 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, as well as the YNPRC animal husbandry and veterinary staff. The studies were supported by the following grants: DK096983, F31HD080404, HD05300, HD055255, IBN-9876754, MH100029, MH079100, MH078105, MH078105-S1, MH078105-04S1, MH078105-01S1, MH086633, and ORIP/OD P51OD011132.

## Table of Contents

**List of Tables and Figures**

# Chapter 1. Introduction

## 1.1 Low social status as a psychosocial stressor

### 1.1.1 Public health relevance of chronic social stress

In modern societies, chronic stress has become a significant public health concern. Adults reporting chronic stress are more likely to suffer from a myriad of diseases, including (but not limited to) obstructive pulmonary disease, heart disease, diabetes, depression and obesity (Baum and Posluszny 1999, Weissman, Pratt et al. 2015). These adverse consequences of chronic social stress are more prevalent in females than in males at every stage in life (Becker, Monteggia et al. 2007, Weissman, Pratt et al. 2015) and they often emerge during adolescence (Seeman 1997, Kessler, Avenevoli et al. 2001, Reardon, Leen-Feldner et al. 2009). The cumulative negative impact of adverse social experiences has been demonstrated in human studies of low socioeconomic status (SES), which is characterized by relatively greater social stress exposure and relatively poor access to material and societal resources such as appropriate nutrition, health care, or education (Bradley and Corwyn 2002, Hackman and Farah 2009). However, even in countries where universal healthcare is available, the relationship between low SES and adverse health outcomes persists (Wardle, Waller et al. 2002, Banks, Marmot et al. 2006). Indeed, reports of psychological distress in humans increases as income decreases (Weissman, Pratt et al. 2015). There is emerging evidence that neurobiological correlates of low SES may contribute to these adverse outcomes, particularly in brain regions that regulate executive function, emotional regulation and social cognition, such as the prefrontal cortex (PFC), and amygdala (AMYG) (Hackman and Farah 2009, Gianaros and Manuck 2010). Despite the evidence that chronic social stress is a strong predictor of numerous

adverse health outcomes in low SES humans, research on the mechanisms underlying these outcomes is scarce. Therefore, a more complete understanding of the etiology underlying these effects of stressor exposure and subsequent adverse outcomes is highly warranted.

### 1.1.2 Limbic-hypothalamic-pituitary-adrenal (LHPA) axis overview

The term stress has multiple definitions, but a common one views stress as state of perceived or actual threat to an organism's homeostasis, whereas the term stressor is defined a stimulus that disrupts homeostasis. Under normal conditions, the AMYG (one of the limbic structures representing the "L" in LHPA) is activated and initiates a downstream stimulation of the HPA axis in response to psychogenic stressors (Chrousos and Gold 1992, Johnson, Kamilaris et al. 1992), causing the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus (PVN) of the hypothalamus. CRH then binds to receptors in the pituitary, which results in the secretion of adrenocorticotropic hormone (ACTH) into systemic circulation. ACTH then binds to receptors in the adrenal glands, an action which causes an increase in circulating glucocorticoids (GCs; e.g. cortisol in primates) (Ulrich-Lai and Herman 2009, Myers, McKlveen et al. 2012). Under conditions of acute stress, GCs bind to glucocorticoid receptors (GR) in limbic structures, the hypothalamus and the pituitary (Herman, McKlveen et al. 2012) to shut down the axis and suppress further GC release through a negative feedback mechanism. When stress becomes prolonged or chronic, however, this negative feedback mechanism is impaired and results in excess and prolonged GC release (Raadsheer, Hoogendijk et al. 1994, Makino, Smith et al. 1995, Makino, Hashimoto et al.

2002, Myers, McKlveen et al. 2012) which can lead to an array of consequences for other physiological systems, and even the brain, due to the effects of these steroid hormones on gene expression. In the case of the brain, GCs can bind to receptors expressed widely throughout the brain (Sanchez, Young et al. 2000), potentially leading to alterations in gene expression that can cause changes in many systems, including corticolimbic circuits involved in cognitive and emotional processes. For example, in rodents, increased GR binding in the central nucleus of the AMYG leads to greater expression of CRH in that nucleus (Shepard, Barron et al. 2000, Kolber, Roberts et al. 2008) resulting in enhanced fear and anxiety responses that are sustained over time. Indeed, increased anxiety-like and depressive-like behaviors have been associated with CRH overexpression in the central nucleus of the AMYG in rats (Keen-Rhinehart, Michopoulos et al. 2009). Additionally, CRH interacts with neurotransmitter systems to affect behavioral output of the LHPA axis (Dunn and Berridge 1990, Owens and Nemeroff 1991, Valentino, Foote et al. 1993). For example, serotonergic neurons in the dorsal raphe nucleus express CRH receptors and synthesize and release serotonin (5HT) in response to CRH, providing a means by which stress may influence 5HT synthesis and release (Meloni, Reedy et al. 2008). In addition to affecting emotional behavior, increased CRH can also affect motivational and appetitive behaviors (Krahn, Gosnell et al. 1990, Glowa and Gold 1991, Shade, Blair-West et al. 2002, Keen-Rhinehart, Michopoulos et al. 2009).

### 1.1.3 Macaque model of social subordination

A useful translational model to determine the adverse effects of chronic social stress is social subordination in rhesus and other macaques (Shively 1998, Sapolsky 2005,

Shively, Register et al. 2005, Michopoulos, Higgins et al. 2012, Michopoulos, Reding et al. 2012, Michopoulos, Toufexis et al. 2012, Reding, Michopoulos et al. 2012, Shively and Willard 2012, Wilson, Bounar et al. 2013). Macaques share many similarities to humans in both brain and behavior (Byrne and Whiten 1988, Petrides 2005, Petrides, Tomaiuolo et al. 2012), such as protracted development of PFC (Knickmeyer, Styner et al. 2010), its reciprocal connections with the AMYG (Ghashghaei, Hilgetag et al. 2007), and a rich and complex repertoire of maternal and social behavior (Byrne and Whiten 1988). These prolonged periods of brain development are thought to contribute to the "biological embedding" of early life experience, where some brain regions and circuits (and resulting behavioral outcomes) are more sensitive to experience and are sculpted differently by them. However, it is also thought that these open windows of development make an individual more susceptible to the effects of more enduring environmental insults such as continual social stress (Hertzman and Wiens 1996, Hertzman 1999, McEwen 2012).

Social subordination in adult macaques has been well established over decades to investigate the adverse effects of psychosocial stress on a number of adult health outcomes including reproductive dysfunction (Kaplan and Manuck 2004, Zehr, Van Meter et al. 2005, Wilson and Kinkead 2008, Michopoulos, Berga et al. 2009, Kaplan, Chen et al. 2010), cardiovascular disease (Kaplan, Adams et al. 1996), psychostimulant self-administration (Morgan, Grant et al. 2002), monoamine and behavior dysfunction (Grant, Shively et al. 1998, Shively 1998, Shively, Friedman et al. 2006, Embree, Michopoulos et al. 2013), immune compromise (Gust, Gordon et al. 1991, Paiardini,

Hoffman et al. 2009, Tung, Barreiro et al. 2012), emotional feeding (Michopoulos, Toufexis et al. 2012) and alterations in emotional regulation (Shively, Register et al. 2005). Although the health outcomes are not as well-characterized during development, female juvenile and adolescent subordinate macaques also seem to exhibit increased emotional reactivity (Wilson, Bounar et al. 2013) and reproductive alterations (Wilson, Gordon et al. 1986, Zehr, Van Meter et al. 2005, Wilson, Bounar et al. 2013).

Rhesus monkey (*M. mulatta*) social groups are structured by a matrilineal dominance hierarchy that functions to maintain stability within the group, regardless of its size (Bernstein and Gordon 1974, Bernstein 1976). This provides ethological validity to the use of this model organism, as social subordination in rhesus macaques occurs naturally in not only free-ranging, but also captive groups (Bernstein and Gordon 1977). This is also true of other macaque species, such as cynomolgus monkeys (*M. fascicularis*) (Shively, Laber-Laird et al. 1997, Shively, Register et al. 2005). Moreover, social ranks within the groups are generally stable over long periods of time, which allows for long-term investigation of the effects of social experience reflected as a gradient of rank (Kaplan 2008). Subordinate rhesus macaques receive significantly more aggression from higher-ranking animals in addition to receiving lower rates of affiliation, including grooming, from group mates (Silk 2002, Abbott, Keverne et al. 2003, Sapolsky 2005). Subordinate animals terminate the aggression received from higher-ranking group mates by emitting submissive behavior, a defining feature of subordination in macaques (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Bernstein 1976, Shively and Kaplan 1984, Michopoulos, Higgins et al. 2012). Interestingly, these two behaviors

(increased aggression and decreased affiliation) significantly predict increased cortisol in several NHP species (Abbott, Keverne et al. 2003, Sapolsky 2005), including rhesus macaques. Subordinate macaques have less control over their environment, as agonistic behavior from higher-ranking animals is unpredictable and often unprovoked (Abbott, Keverne et al. 2003). Thus, due to the uncertainty of onset, timing and duration of this stressor, the subordinate animal is unable to initiate a preemptive physiological response in order to assuage the effect of the stressor (Silk 2002). Additionally, maintenance of the appropriate stress response is compromised as the subordinate monkey is unable to predict how long this response must be sustained (Silk 2002). This lack of control and predictability over the social and physical environment results in repeated activations of stress systems (Silk 2002), which can lead to other alterations, such as emotional dysregulation in subordinates (Abbott, Keverne et al. 2003). Indeed, when examining the set of anxiety-like behaviors exhibited by macaques in response to stress-eliciting situations (Schino, Troisi et al. 1991, Troisi, Schino et al. 1991, Troisi 2002, Kalin and Shelton 2003), they occur at higher rates in subordinates than in dominant animals (Shively, Register et al. 2005, Wilson, Bounar et al. 2013).

Infant and juvenile macaques born to subordinate mothers are also exposed to the effects of social subordination in early life, as they assume their mother's rank (Sade 1967, Bernstein 1970). Infants are buffered by their mothers from the effects of social subordination during the first few weeks after birth, but begin to learn their place in the hierarchy at approximately three months of age, when they increase their social interactions with non-family group members and begin to wean from their mother

(Spencer-Booth 1968, Hinde and Spencer-Booth 1971, Berman 1980). By the time they are juveniles, subordinate animals already receive more aggression from higher-ranking group members and terminate these interactions with submissive behaviors (Bernstein and Ehardt 1985). Thus, all females assume the mother's rank in the group throughout their development and adulthood. Although the subordinate phenotype has not been as well characterized as in adults, social subordination stress has developmental consequences, including delayed puberty onset (Schwartz, Wilson et al. 1985, Wilson, Gordon et al. 1986, Zehr, Van Meter et al. 2005, Wilson, Bounar et al. 2013), which have been associated with increased emotional reactivity in these animals (Wilson, Bounar et al. 2013).

### 1.1.4 Effects of social subordination on the LHPA axis

One notable effect of this social subordination experience of unpredictable and continual harassment accompanied by decreased availability of social buffering (e.g. grooming) is dysregulation of the LHPA neuroendocrine axis, evidenced by hypercortisolemia and impaired GC negative feedback, at least in adult females (Shively, Laber-Laird et al. 1997, Shively 1998, Wilson, Pazol et al. 2005, Jarrell, Hoffman et al. 2008, Wilson, Fisher et al. 2008, Paiardini, Hoffman et al. 2009). Consistent with the impaired negative feedback reported in adult subordinate female macaques (Shively, Laber-Laird et al. 1997, Shively 1998, Wilson, Pazol et al. 2005, Jarrell, Hoffman et al. 2008, Wilson, Fisher et al. 2008, Paiardini, Hoffman et al. 2009), the stress associated with social subordination in other animal models has been linked with down-regulation of GRs in the brain. For example, using the visible burrow system and a model of chronic social stress in rats, subordinate rats showed reduced mRNA levels of mineralocorticoid receptors

(MRs) and GRs in the hippocampus (HC), relative to dominant rats (Chao, Blanchard et al. 1993). In macaques exposed to a sustained stressor (destabilized social groups), dexamethasone resistance was associated with decreased GRs in the HC (Brooke, de Haas-Johnson et al. 1994). Given that GCs interact with and regulate the expression of a number of other neurotransmitters (Mora, Segovia et al. 2012), 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.

### 1.1.5 Chronic stress and role of immune system function

Chronic stress has also been associated with persistent inflammation (Miller and Blackwell 2006, Cohen, Janicki-Deverts et al. 2012) and resulting adverse behavioral outcomes, such as depression (Dunn, Swiergiel et al. 2005, Hryhorczuk, Sharma et al. 2013). During an acute stress response, glucocorticoids function in an anti-inflammatory manner in order to maintain homeostatic processes (McEwen, Biron et al. 1997, Sapolsky, Romero et al. 2000, Silverman and Sternberg 2012). Under conditions of chronic stress-related hypercortisolism, however, glucocorticoid resistance can develop in innate immune cells which prevents GC-induced suppression of inflammation (Reader, Jarrett et al. 2015). This can occur through various mechanisms, some of which are impaired GR signaling and function (Barnes 2006, De Bosscher, Vanden Berghe et al. 2006, Pace, Hu et al. 2007), as well as decreases in GR expression (Pace, Hu et al. 2007). In humans, low socio-economic status has been associated with increased levels of C-reactive protein (CRP) and interleukin-6 (IL6) (Pollitt, Kaufman et al. 2008, Loucks,

Pilote et al. 2010). In female rhesus macaques, higher mRNA gene expression (measured in peripheral blood mononuclear cells) of pro-inflammatory cytokines is observed in adult subordinate females (Tung, Barreiro et al. 2012). There is evidence that this peripheral inflammation can result in neuroinflammation and affect brain structure and function. For example, microglia can be activated by stressor exposure (Frank, Baratta et al. 2007) and greater microglia density and activation in the PFC has been associated with stress-induced psychopathology (Steiner, Bielau et al. 2008, Setiawan, Wilson et al. 2015). Furthermore, decreases in neuronal size, glial density or cell loss (presumably as a result of activated microglia) have been observed in postmortem studies in humans with depression (Rajkowska 2000, Cotter, Mackay et al. 2002), a disorder often associated with chronic stress (McEwen 2012). Therefore, in addition to LHPA axis signaling, chronic stress-induced proinflammatory cytokines could also affect neurobehavioral outcomes.

## 1.2 Effects of social subordination on limbic brain regions across the life span

### 1.2.1 Postnatal development of amygdala, hippocampus and prefrontal cortex

Primate cortical maturation occurs earlier in low-order processing regions (e.g. primary visual or somatosensory cortices) than in association cortices that integrate multimodal sensory inputs, like prefrontal and temporal cortices (Gogtay, Giedd et al. 2004). Cortical maturation also occurs earlier in phylogenetically older cortical regions, such as the piriform and entorhinal cortex, compared to phylogenetically newer regions such as the inferior temporal cortex and PFC (Giedd 2004, Gogtay, Giedd et al. 2004, Shaw, Kabani

et al. 2008). Specifically, normative development of PFC gray matter (GM) volume in both males and females follows an inverted U-shaped curve from childhood until adulthood, reaching a peak during the peripubertal period (Giedd, Blumenthal et al. 1999, Giedd 2004, Giedd and Rapoport 2010). The decrease in volume following this period seems due, in part, to synaptic pruning (Anderson, Classey et al. 1995, Huttenlocher and Dabholkar 1997, Markham, Mullins et al. 2013) and even apoptosis in the PFC (Nunez, Lauschke et al. 2001, Markham, Morris et al. 2007). In macaques, axonal and synaptic pruning occurs postnatally in a region-specific manner that matches the emergence of related behavioral functions (LaMantia and Rakic 1990).

The volumetric structural development of cortical white matter (WM) follows a different, mostly linearly increasing, trajectory than GM (Jernigan and Tallal 1990, Giedd, Blumenthal et al. 1999, Giedd 2004, Lenroot and Giedd 2006)**.** Cortical myelination in both humans and NHP precocial species begins prenatally in some regions (e.g. motor cortex, to support some level of infant motoric independence right after birth) but occurs postnatally in other cortical regions, continuing into early adulthood in association areas like the PFC, temporal and parietal cortices (Gibson 1991, Inder and Huppi 2000, Volpe 2000, Lenroot and Giedd 2006).

On the other hand, the basic architecture of the AMYG is present at birth in humans and monkeys (Ulfig, Setzer et al. 2003) and follows rapid regional-specific structural development patterns that makes some nuclei (and the functions they underlie) more vulnerable than others to postnatal experience. Most of this evidence comes from studies

in rhesus monkeys, where the lateral and basal nuclei, involved in emotional processing and learning, show rapid volume increases from birth through three 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 (Chareyron, Lavenex et al. 2012). These nuclei-specific changes result in an overall increase in total AMYG volume, with the highest rates evident during the first 4 postnatal months, which stabilize at about 8 months of age and reach adult volume at 7 years of age in macaques (Payne, Machado et al. 2010). The increase in AMYG volume results from structural changes in both neurons and glia, although after three months of age it is mostly due to increased number of oligodendrocytes and myelination, while neuronal size/number and astrocyte number do not change (Chareyron, Lavenex et al. 2012). Thus, after three months of age the AMYG volume increases are driven by glia that produce the myelin in WM, suggesting a particular vulnerability of AMYG WM tracts/circuits that mature around and after that age to social experiences [e.g. AMYG projections to orbitofrontal cortex -OFC-, which do not become mature until two months of age, when curiosity and frustration emerge in the infants (Machado and Bachevalier 2003)]. Functionally, the AMYG is active early in life, but undergoes continued refinement, largely through connections with cortical areas [For review, see (Tottenham, Hare et al. 2009, Saygin, Osher et al. 2015)].

The HC is also functionally active early in life (Robinson and Pascalis 2004) and shows consistent increase in volume through two years of age in monkeys, with the most drastic increases observed during the first two postnatal weeks (Payne, Machado et al. 2010). As

most of the neurons in the HC are generated prior to birth (Nowakowski and Rakic 1981, Eckenhoff and Rakic 1991, Arnold and Trojanowski 1996), these increases in volume are likely the result of synaptogenesis, myelination and dendritic arborization (Duffy and Rakic 1983, Eckenhoff and Rakic 1991, Seress 1992). In macaques, the HC reaches adult volumes between 7-13 years of age (Lavenex, Banta Lavenex et al. 2007).

### 1.2.2 Effects of social subordination on brain structure and connectivity in adult non-human primates

In addition to the neurochemical changes that result from social stress, in particular from social subordination, recent neuroimaging work demonstrates that social subordination also alters brain structure and functional connectivity (FC), particularly of corticolimbic circuits and social brain networks. In a magnetic resonance imaging (MRI) study of adult, mostly male, rhesus macaques, the gray matter (GM) volume in bilateral AMYG had was positively associated with the social rank of the monkey, where more subordinate monkeys had smaller AMYG volume (Noonan, Sallet et al. 2014). This positive relationship between GM volume and rank was also found for a region in the bilateral brain stem, which included the 5HT-containing raphe nucleus and the hypothalamus. Using measures of FC in the same study, Noonan et al. (2014) also found differences in connectivity 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. Another study also found that social network size was positively associated with brain morphology in the superior temporal sulcus, inferior temporal gyrus, temporal pole and AMYG (Sallet, Mars et al. 2011). The rank-dependent

changes in GM volume and intrinsic FC of the AMYG are consistent with findings of a relationship between AMYG structure and function and social status in other NHP and human studies (Bauman, Lavenex et al. 2004, Zink, Tong et al. 2008, Kumaran, Melo et al. 2012). The AMYG is very sensitive to the effects of stress and GCs, and it also plays a critical role in social behavior, evaluation of stimulus salience, threat detection and fear and safety learning. Thus, rank-related differences in AMYG structure, function, and connectivity with other regions may reflect both the effects of social rank on the perception/salience of social stimuli and the impact of stress-induced load on this region in subordinate monkeys.

Noonan et al., (2014) also found structural and functional differences in the basal ganglia associated with social rank. Extent of GM in the striatum significantly increased with more subordinate status, 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 receives dopaminergic innervation from the mesocortical limbic and mesostriatal systems, these findings along with studies demonstrating differences in dopamine D2 receptor expression and binding in the striatum for subordinate compared to dominant monkeys (Grant, Shively et al. 1998, Morgan, Grant et al. 2002) suggest that social stress in rhesus macaques may alter corticolimbic reward circuitry and motor pathways.

The PFC is a brain region critical for regulating socioemotional behavior (Quirk and Beer 2006) and mediating social cognition (Amodio and Frith 2006), and is also structurally

and functionally affected by social rank and social group size in rhesus macaques (Sallet, Mars et al. 2011, Noonan, Sallet et al. 2014). In a sample of mostly adult male rhesus macaques, GM volume in the rostral PFC and superior temporal sulcus (STS) was found to increase with social rank and social network size (Noonan, Sallet et al. 2014). 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 rodent models demonstrates that chronic stress leads to structural changes in the PFC. Specifically, chronic restraint stress is associated with decreased dendritic arborization in the medial PFC (Shansky and Morrison 2009) and increased neuronal excitability (Jackson and Moghaddam 2006). These findings suggest that the structural and functional changes associated with social rank in rhesus monkeys are, at least in part, the result of social stress experienced by the subordinate animals.

### 1.2.3 Effects of social subordination on brain structure and neurotransmission in juvenile non-human primates

Despite the extensive literature investigating the adverse effects of social stress on various health outcomes in adults, it is surprising that few studies have taken a developmental approach in order to investigate how social subordination affects macaques during infancy, adolescence and throughout development. A few recent studies are trying to uncover how the developmental consequences of this experience emerge. One of those investigated the effects of social rank on the pubertal timing of female

rhesus macaques (Wilson, Bounar et al. 2013). Relative rank within the social group (calculated by the ratio of a subject's rank to the total number of animals in the group, except for animals less than one year of age) significantly predicted age at menarche and age at first ovulation, with more dominant animals reaching menarche and first ovulation earlier than more subordinate animals. Additionally, other factors such as increased emotional reactivity and slower weight gain were also strong predictors of delayed puberty. To our knowledge, only two recent studies using female rhesus macaques have investigated the relation between subordinate status and neurobiological developmental outcomes.

Using Diffusion Tensor Imaging (DTI), Howell et al. (2014) investigated the effects of social subordination on the development of brain WM in pre-pubertal female macaques and the consequences for behavior and stress physiology. Briefly, DTI is an *in vivo* neuroimaging technique that measures water diffusion in the brain and provides measures of WM tract integrity, from which we can infer connectivity. This study found that, in pre-pubertal female rhesus macaques, subordinates had greater fractional anisotropy (FA; a measure of water diffusion directionality which is used to infer information about WM structural integrity, including level of myelination and axonal packing density or organization, where higher myelination and axon packing density/organization result in higher FA) than dominants in left medial PFC WM and along the left dorsal medial wall of the brain (Howell, Godfrey et al. 2014). Increased FA in medial PFC was correlated with increased fearful behavior in the Human Intruder paradigm, an ethologically relevant paradigm where fear and anxiety responses are measured in response to

threatening stimuli of varying intensity (Kalin and Shelton 1989, Meunier, Bachevalier et al. 1999, Wilson, Bounar et al. 2013, Howell, Grand et al. 2014), as well as with increased fearful and submissive social behavior. Increased FA in the left dorsal medial wall was also positively correlated with submissive behavior in the subject's social group. Altogether, the increased WM tract structural integrity detected, and its association with higher submissive behavior and emotional reactivity in subordinate animals, was interpreted by the authors as neural adaptations to facilitate socially adaptive behaviors to a high pressure environment. In another study exploring the effects of social subordination stress on neural development, Embree et al., 2013 investigated how social status and serotonin transporter (5HTT) polymorphisms affect the development of brain 5HT systems longitudinally during the pubertal transition in female rhesus macaques. 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 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 develop also more adult-like patterns of social and emotional behaviors, with increased receptor and transporter availability being associated with increased levels of emotional reactivity. Together, these findings are important because they revealed that differences in social rank are associated with differences in brain neurotransmission and circuitry in juvenile macaques.

## 1.3 Modifiers of stress-induced effects on neurobehavioral outcomes

### 1.3.1 Importance of estradiol on neurobehavioral outcomes

Estradiol (E2) is a gonadal steroid important for mood regulation (Pfaff, Vasudevan et al. 2000, Stoppe and Doren 2002) and for brain organization/development, including structural and neurochemical changes in systems controlling emotional responses (Pfaff, Vasudevan et al. 2000, Bethea, Mirkes et al. 2002, Stoppe and Doren 2002, Dreher, Schmidt et al. 2007, Bethea and Reddy 2008, Martel, Klump et al. 2009, Zadran, Qin et al. 2009, Galvin and Ninan 2014) even during the pubertal transition (Ahmed, Zehr et al. 2008, Schulz, Molenda-Figueira et al. 2009). E2 and other gonadal steroids have prenatal organizational effects on the brain, but also influence brain maturation and behavioral development postnatally, particularly during puberty (Ahmed, Zehr et al. 2008, Schulz, Molenda-Figueira et al. 2009). E2 can exert both activational and organizational effects on the brain (Arnold and Breedlove 1985, McCarthy 2010), through remodeling and refinement of neuronal circuits via various mechanisms [for review see (Goldstein, Kurz et al. 1990, Benes, Taylor et al. 2000, Nunez, Lauschke et al. 2001, Cunningham, Bhattacharyya et al. 2002, Sisk and Zehr 2005, Schulz, Molenda-Figueira et al. 2009)], resulting in persistent changes to gross brain morphology and synaptic organization. In humans, some, but not all, of the neurodevelopmental changes reported during adolescence have been associated with elevations in gonadal hormones. For example, higher levels of circulating E2 have been associated with lower GM density in frontal and parietal lobes (Brouwer, Koenis et al. 2015). Additionally, greater levels of E2 have also been associated with smaller anterior cingulate cortex (ACC) cortex GM, after

controlling for age (Koolschijn, Peper et al. 2014) and greater thinning of the left middle temporal gyrus (Herting, Gautam et al. 2015). Although the effects of pubertal increase in E2 are recognized, the pre-pubertal ovary, under the influence of gonadotropins, is also able to secrete biologically active, albeit low, concentrations of E2 prior to menarche in both girls (Apter, Butzow et al. 1993, Norjavaara, Ankarberg et al. 1996) and female macaques (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004, Wilson, Bounar et al. 2013). These prepubertal low circulating concentrations of E2 (Norjavaara, Ankarberg et al. 1996, Veldhuis, Roemmich et al. 2000) are able to regulate a number of systems in females, including luteinizing hormone (LH) secretion (Rapisarda, Bergman et al. 1983, Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), insulin-like growth factor 1 (IGF-1) and growth hormone (GH) (Wilson, Schwartz et al. 1984).

The neuroprotective effects of E2 have been long recognized (Engler-Chiurazzi, Singh et al. 2016). E2 interfaces with many neurotransmitter and neuropeptide systems in corticolimbic circuits to influence behavior (Bos, Panksepp et al. 2012) and responsivity to stressors (Luine 2016). Indeed, estrogen receptors (ERα and ERβ) are distributed in corticolimbic brain regions, including the AMYG, HC and cortex in rats (Shughrue, Lane et al. 1997, Shughrue, Scrimo et al. 1998, Shughrue and Merchenthaler 2000), macaques (Pau, Pau et al. 1998, Register, Shively et al. 1998, Blurton-Jones, Roberts et al. 1999, Perez, Sendera et al. 2004, Bao, Ni et al. 2006) and humans (Osterlund, Gustafsson et al. 2000, Osterlund, Keller et al. 2000). E2 influences emotionality in animal models (Vaillancourt, Cyr et al. 2002) and humans (Swerdlow, Hartman et al. 1997), and is thought to play a role in the etiology of depression and anxiety in female humans and

NHPs (Angold, Costello et al. 1998). Stress-related disorders like depression and anxiety emerge during adolescence and are more prevalent in females than in males (Forbes, Williamson et al. 2004) and increases in risk are coincident with changes in reproductive system function across the lifetime, such as premenstrual cycle, pregnancy, post-partum, perimenopause (Steiner, Dunn et al. 2003). E2 has been shown to directly influence the LPHA axis, for example by increasing CRH expression in the hypothalamus of female rhesus macaques (Roy, Reid et al. 1999) and exacerbating stress hormone responsivity in socially subordinate adult female rhesus monkeys (Wilson, Pazol et al. 2005). While chronic exposure to stressors may inhibit ovarian function in women (Berga and Loucks 2005) and monkeys (Pope, Gordon et al. 1986, Kaplan, Chen et al. 2010), ovarian activity and E2 release can continue in the presence of a chronic stressor (Adams, Kaplan et al. 1985, Pope, Gordon et al. 1986, Herod, Dettmer et al. 2011). However, in those circumstances chronic adverse social experience appears to attenuate the activational effects of E2 on behavior (Wallen 1990, Uphouse, Selvamani et al. 2005, Reding, Michopoulos et al. 2012). In summary, the effects of chronic stress on neurobehavioral development warrant elucidation, especially during developmental increases in E2.

### 1.3.2 Importance of dietary environment on brain and behavior and non-homeostatic brain regions involved in food intake

The availability and overconsumption of calorically dense diets have become significant worldwide health problems and result in increased risk for several adverse health outcomes, including type 2 diabetes, cancer, heart disease, and others (Must, Spadano et al. 1999, Visscher and Seidell 2001, Hill 2006). Globally, the number of overweight and

obese people has risen from 921 million in 1980 to an astounding 2.1 billion in 2013 (Ng, Fleming et al. 2014), which results in substantial increased healthcare costs and economic burden (Tsai, Williamson et al. 2011, Withrow and Alter 2011). In the United States, rates of diet-induced obesity have continued to rise over the last 20 years to greater than 30% of the population and are expected to increase to 50% by 2030 (Flegal, Carroll et al. 2010). Given these alarming statistics, it is becoming increasingly essential to understand the etiology underlying excessive consumption of obesogenic diets before frank obesity is evident, in order to develop effective treatment strategies.

Animal models have proven to be exceptionally informative in defining neuropeptide regulation of appetite and energy homeostasis (Berthoud 2012, Williams and Elmquist 2012). However, food intake is regulated by more than just satiety and hunger signals, including influences by reward and sensory circuits. The nucleus accumbens (NAcc), AMYG, insula (INS), HC and PFC are often studied due to their anatomical connections and involvement in appetitive behavior and reward process regulation (Ghashghaei, Hilgetag et al. 2007, Warne 2009, Haber and Knutson 2010, Volkow, Wang et al. 2012, Tomasi and Volkow 2013). The PFC has direct reciprocal anatomical connections with striatum, where vmPFC and OFC have stronger projections to ventral striatum, whereas dlPFC has stronger projections to dorsal striatum (Haber and Knutson 2010). The AMYG and INS are also reciprocally connected with the NAcc (Haber and Knutson 2010). Although much of the PFC has connections with the AMYG, the densest projections from PFC to AMYG originate in the ACC/mPFC, especially in Brodmann areas (BA) 24 and 25, whereas the densest projections back to the PFC from AMYG terminate in caudal

OFC (Ghashghaei, Hilgetag et al. 2007). The NAcc is involved in reward processing (Cohen, Asarnow et al. 2010), the AMYG is involved in evaluation of food reward salience (Baxter and Murray 2002) as well as fear and anxiety (LeDoux 2000), the INS integrates interoceptive, socio-emotional, sensorimotor, olfacto-gustatory and cognitive information (Kurth, Zilles et al. 2010) and the HC is important for memory and associative learning in control of appetitive behavior, including food-seeking and energy regulation (Henderson, Smith et al. 2013). The PFC can be divided into distinct sub-regions, including (but not limited to) the ventromedial PFC (vmPFC), the medial PFC (mPFC), the OFC and the dlPFC. The vmPFC is involved in emotional and motivational salience assessment, prosocial behavior and emotional regulation (Ma, Liu et al. 2010) (Rilling, Gutman et al. 2002, Rilling, Sanfey et al. 2004). The mPFC is involved in immediacy and anticipation of the benefit/risk ratio of rewards (Knutson, Taylor et al. 2005, Yacubian, Glascher et al. 2006, Haber and Knutson 2010) as well as integration of value across different stimuli (Blair, Marsh et al. 2006). The OFC is involved in goal-directed behavior, reward coding, impulse control and salience/value of food (Kuhnen and Knutson 2005, Rolls and McCabe 2007, Grabenhorst, Rolls et al. 2008) and prosocial behavior (Rilling, Gutman et al. 2002, Rilling, Sanfey et al. 2004). Finally, the dlPFC is involved in working memory for evaluation, comparison and selection of incentive-based behavioral responses (Haber and Knutson 2010).

### 1.3.3 Evidence of diet-induced obesity effects on brain structure

Consumption of obesogenic diets and resulting elevated body mass index (BMI) have also been linked to alterations in brain structure. The general findings are that diet-

induced obesity, resulting from consumption of obesogenic diets, is associated with smaller total brain volume and with reduced volume of cortical and subcortical structures. For example, several studies have reported smaller total brain volume associated with higher BMI, compared to controls (Ward, Carlsson et al. 2005, Gunstad, Paul et al. 2008, Debette, Beiser et al. 2010, Bobb, Schwartz et al. 2014). These differences may be driven by global decreases in GM (Figley, Asem et al. 2016), WM (Cole, Boyle et al. 2013) or both (Figley, Asem et al. 2016). Additionally, diet-induced obesity has also been associated with GM reductions in cortical regions (such as PFC and INS), as well as sub-cortical regions including the HC and AMYG. Specifically, increased visceral adipose tissue has been associated with decreased cortical thickness of the right INS (Veit, Kullmann et al. 2014) and decreased INS GM (Shott, Cornier et al. 2015). Diet-induced obesity is also negatively associated with volume of PFC subdivisions, such as dlPFC, OFC and mPFC (Pannacciulli, Del Parigi et al. 2006, Walther, Birdsill et al. 2010, Brooks, Benedict et al. 2013, Marques-Iturria, Pueyo et al. 2013, Shott, Cornier et al. 2015, Figley, Asem et al. 2016). Increased BMI has also been associated with decreased HC (Raji, Ho et al. 2010, Walther, Birdsill et al. 2010) and AMYG (Figley, Asem et al. 2016) volumes. While most studies focus on BMI or visceral adiposity, it is important to understand how consumption of calorically dense diets impact brain structure prior to the onset of frank obesity. One study investigated how different diets affected HC volume, where consumption of a prudent diet was associated with larger HC volume and consumption of a high caloric diet (HCD) was associated with smaller HC volume (Jacka, Cherbuin et al. 2015). Although most studies report decreases in brain volume associated with diet-induced obesity, a few studies have also reported increases in brain

volume. For example, one study in older women reported increased frontal WM volume (Walther, Birdsill et al. 2010) and another study reported positive correlations between BMI with OFC (Horstmann, Busse et al. 2011). What remains unclear, however, is whether these impairments in brain structure emerge prior to the onset of frank obesity, due to the obesogenic dietary environment.

### 1.3.4 Evidence of diet-induced obesity effects on brain functional connectivity

In recent years, investigators have begun to employ resting state-functional magnetic resonance imaging (rs-fMRI) techniques to investigate diet-induced obesity related alterations in brain FC, primarily in stress and reward networks. Rs-fMRI, a method used to examine temporal correlations of activity between different brain regions, as measured by blood oxygen level dependent -BOLD-related activity, has been used in studies of obese humans to demonstrate altered brain connectivity in PFC-AMYG-INS-NAcc circuitry. For example, women with high BMI have stronger connectivity between NAcc and ACC and vmPFC (Coveleskie, Gupta et al. 2015). Another rs-fMRI study showed that obese women have higher activity within the INS compared to lean women (Hogenkamp, Zhou et al. 2016). Excess weight has also been associated with increased connectivity between the ventral striatum and mPFC (Contreras-Rodriguez, Martin-Perez et al. 2015). In another study looking at FC during fasting, obese subjects exhibited positive connectivity between AMYG and INS, whereas lean individuals showed negative connectivity between these regions (Lips, Wijngaarden et al. 2014). Thus, resting state FC in obese humans seems to be stronger between brain regions involved in emotion and reward processing compared to lean individuals. Although several theories

exist to explain the physiological source of these differences, one idea is that this dysfunctional connectivity reflects impaired top-down inhibitory control of feeding behavior (Nummenmaa, Hirvonen et al. 2012). Because negative FC is thought to reflect increased activity of one ROI and decreased activity in another, whereas positive FC is thought to reflect simultaneous activity in both ROIs (Fox and Raichle 2007), the positive hyper-connectivity seen between prefrontal, striatal and limbic regions in obese humans may reflect an overactive reward circuit, where typical top-down inhibitory control over feeding (potentially represented as negative connectivity) and reward is impaired (Nummenmaa, Hirvonen et al. 2012). For example, negative FC can be mathematically induced by a preprocessing step, called global signal regression (Fox, Zhang et al. 2009, Murphy, Birn et al. 2009). Nevertheless, it is accepted that negative FC has a biological basis, as it has been recognized without the application of global signal regression (Chang and Glover 2009, Fox, Zhang et al. 2009, Chai, Castanon et al. 2012). Therefore, the physiological mechanisms underlying positive vs. negative FC are extremely complex and not fully understood, and are still a topic of intense debate in the field (Cole, Smith et al. 2010, Goelman, Gordon et al. 2014). As discussed in the previous section, it is important to identify whether these obesity-related changes reported in human studies before frank obesity is evident, as a consequence of a complex dietary environment, where calorically dense foods are available.

### 1.3.5 Effects of dietary environment on social and emotional behavior

Data suggest that obesity resulting from stress-induced overeating calorically dense diets may compromise brain structure and function (Berthoud, Lenard et al. 2011, Tryon,

Carter et al. 2013, Ulrich-Lai, Fulton et al. 2015, Michopoulos, Diaz et al. 2016), potentially leading to emotional dysregulation and social and behavioral impairment in humans (Onyike, Crum et al. 2003, Galvez, Bauer et al. 2014). Indeed, mood disorders and anxiety are frequently co-morbid with diet-induced obesity (Ayensa and Calderon 2011, Capuron, Lasselin et al. 2016), especially in females (Zender and Olshansky 2009, Hillman, Dorn et al. 2010). Social behavior may also be affected by a complex dietary environment. For example, one study in cynomolgus macaques showed that a high fat, high cholesterol diet decreased social contact aggression compared to monkeys fed a more prudent diet (Kaplan, Manuck et al. 1991). Another study in rhesus macaques also showed decreases in emitted social aggressive behavior associated with increased consumption of a calorically dense diet (Michopoulos, Diaz et al. 2016). These data suggest an important role of calorically dense diets in impaired neurobehavioral function.

### 1.3.6 Signals linking diet to neurobehavioral outcomes

Diet-induced obesity is also characterized by hypercortisolemia (Pasquali, Ambrosi et al. 2002) and increased levels of GCs may be one mechanism by which these adverse effects arise in a rich dietary environment. This idea is supported by data showing elevations in serum cortisol associated with depression in humans (Parker, Schatzberg et al. 2003, Raison and Miller 2003, Stetler and Miller 2011). Chronic corticosterone administration in rats produces depressive-like behavior (Kalynchuk, Gregus et al. 2004, Gregus, Wintink et al. 2005), most likely through the actions of glucocorticoid receptors which are expressed in limbic brain regions known to emotional behavior, such as the AMYG

and HC (Sanchez, Young et al. 2000, Ulrich-Lai and Herman 2009, Arnett, Kolber et al. 2011, Solomon, Furay et al. 2012, Wang, Verweij et al. 2014).

In addition to altered GC signaling, chronic inflammation may be another mechanism linking consumption of a calorically dense diet to adverse neurobehavioral outcomes. Chronic inflammation is not only a hallmark of obesity (Gregor and Hotamisligil 2011, Miller and Spencer 2014), but consumption of obesogenic diets increases both peripheral and central measures of inflammation prior to the onset of obesity (Myles 2014, Vasconcelos, Cabral-Costa et al. 2016). Peripheral inflammation, caused by an increase in adipose tissue as a result of consuming a HCD, can affect the brain and alter astrocyte and glial function (Argente-Arizon, Freire-Regatillo et al. 2015). Independently of visceral fat accumulation, glial cells in rodents can also be activated directly in response to dietary nutrients, including saturated fatty acids (Milanski, Degasperi et al. 2009). HCDs have also been shown to increase expression of inflammation related genes in rodents, including IL6 and tumor necrosis factor alpha (TNFα) in the cortex and PFC (Jayaraman, Lent-Schochet et al. 2014, Carlin, Grissom et al. 2016, Kang, Koo et al. 2016). This neuroinflammation resulting from activated glia can alter the brain in several ways, including preventing neurogenesis (Monje, Toda et al. 2003), causing neurodegeneration (Campbell, Stalder et al. 1997), dendritic atrophy (Richwine, Parkin et al. 2008), apoptosis (Watson, Cai et al. 2000, Semmler, Okulla et al. 2005, Moraes, Coope et al. 2009) or decreasing number of astrocytes (Sofroniew and Vinters 2010). Thus, altered GC signaling and proinflammatory cytokines are likely important mediators of diet effects on neurobehavioral outcomes.

## 1.4 Overall goal and hypotheses

The primary overall goal of this thesis was to investigate what factors modify chronic stressor-induced changes in brain structure and function. The goals of chapter 2 were to (1) examine the impact of social subordination stress on female macaque neurobehavioral development, (2) determine whether pubertal timing and duration of E2 exposure modulate the impact of stress on structural development of corticolimbic regions involved in emotional and stress regulation (specifically AMYG, PFC and HC), disentangling it from the effects of chronological age, and (3) examine whether these brain structural differences were related to behavioral and physiological outcomes. It was hypothesized that these social status effects would be evident in the AMYG, the PFC and the HC which are involved in social and emotional processing and have high levels of both glucocorticoid and estrogen receptors. Specifically, based on available literature discussed above, we predicted that subordinate juvenile females would have larger AMYG, but smaller PFC and HC volumes compared to dominant subjects and that these alterations would result in increased fear and anxiety behavior and stress reactivity. Because prepubertal E2 is bioactive in female rhesus monkeys (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), it was also hypothesized that these status-induced brain and behavioral effects would be exacerbated in females with delayed exposure to E2. The goals of chapter 3 were to investigate how social status interacts with dietary environment to affect brain structure of corticolimbic regions, specifically, the AMYG, PFC, INS and HC, known affect emotional reactivity and motivated behavior in adult female rhesus macaques. The impact of diet on neurobehavioral outcomes were assessed

in females who had been maintained on a low fat, high sugar chow diet their entire lives and females who had been consuming this chow diet in combination of a high fat, high sugar diet for one year. Although some studies have reported increases in brain volume as a consequence of consumption of calorically dense diets, the data overall suggest that there is a reduction in overall and regional brain volumes. Therefore, it was hypothesized that excessive calorie intake, particularly of a HCD, would result in decreased volume of AMYG, PFC, INS and HC. Additionally, it was hypothesized that subordination stress would result in decreased PFC, INS and HC, but increased AMYG volume, and that these diet and stress-induced alterations would result in altered social and emotional behavior. Using the same female subjects, and experimental conditions from chapter 3, the goals of chapter 4 were to investigate how social status interacts with dietary environment to affect brain FC between prefrontal, striatal, and other limbic regions known to affect emotional reactivity and motivated behavior. It was hypothesized that excessive calorie intake, particularly of a HCD, would result in increased FC between NAcc, AMYG, INS and PFC, and that these alterations would result in altered social and emotional behavior.

# Chapter 2. Effects of Social Rank and Experimental Delay of Puberty on Brain Structure in Female Rhesus Macaques

## 2.1 Abstract

Exposure to social stressors during childhood and adolescence leads to alterations in emotional and stress regulation and the development of underlying brain circuits. In this study, we examined the consequences of subordinate status in socially-housed juvenile female rhesus monkeys, as an ethologically valid model of chronic social stressor exposure, on brain structural and behavioral development through the pubertal transition. Adolescence is a developmental period of extensive brain remodeling that takes place in parallel with increased emotional and stress reactivity. Puberty-induced increases in gonadal hormones, particularly estradiol (E2), are likely involved due to its organizational effects on the brain and behavior. Thus, we also examined the modulation of pubertal development on stress effects by experimentally delaying pubertal onset in a cohort of females by the administration of a GnRH agonist, Lupron. Using a longitudinal experimental design, structural MRI (sMRI) scans were collected on 46 socially housed juvenile female rhesus monkeys (20 dominant; 25 subordinate) at a typical prepubertal age (18-25 months) and again at a typical post menarche age (29-36 months). Half of the females from each social rank received monthly Lupron injections to experimentally delay the onset of puberty. We examined the effects of both social status and pubertal delay on overall structural brain development (i.e. intracranial, grey matter (GM) and white matter (WM) volumes), as well as on cortico-limbic regions involved in emotion and stress regulation: amygdala (AMYG), hippocampus (HC) and prefrontal cortex (PFC). Measures of stress physiology, social behavior and emotional reactivity were collected in order to examine functional correlates of the brain structural effects. In addition to expected developmental effects, subordinates had bigger AMYG volumes

than dominant animals (particularly in the right hemisphere), but pubertal delay with Lupron-treatment abolished those differences, suggesting a role of gonadal hormones potentiating the structural impact of social stress. Subordinates had elevated baseline cortisol, indicating activation of stress systems. In general, Lupron-treated subjects had smaller AMYG and HC volume than controls, but larger total PFC (due to more GM), and different, region-specific, developmental patterns dependent on age and social status. These findings highlight a region-specific effect of E2 on structural development during female adolescence, independent of those due to chronological age. Additionally, pubertal delay and smaller AMYG volume were associated with increased emotional reactivity during the Human Intruder task (HI) and decreased social behavior compared to controls. These data provide evidence that exposure to developmental increases in E2 modify the consequences of social stress on the volume of cortico-limbic regions involved in emotional and stress regulation during maturation. But, even more importantly, they support different brain structural effects of chronological age and pubertal developmental stage in females, which are very difficult to disentangle in human studies. These findings have additional relevance for young girls who experience prolonged pubertal delays or for those whose puberty is clinically arrested by pharmacological administration of Lupron.

## 2.2 Introduction

Adolescence is a critical developmental period of significant physical, emotional and social changes (Schulz, Molenda-Figueira et al. 2009) that begins with the onset of puberty (Graber and Brooks-Gunn 1996). It is also associated with complex brain reorganization and plasticity, and remodeling of neural circuits characterizing normal brain development. However, this period also opens window for maladaptive developmental patterns (Boyce and Ellis 2005, Steinberg 2005), increasing susceptibility to the influences of adverse social experiences [for review, see (Holder and Blaustein 2013)]. Some of the brain circuits that undergo intense reorganization during this developmental period are those involved in emotional and stress regulation, including the amygdala (AMYG) and prefrontal cortex (PFC) (Casey, Duhoux et al. 2010). This, coupled with the increased intensity of social and emotional pressures of adolescence, explains the increased reported stress reactivity during this phase, especially in girls (Silberg, Pickles et al. 1999, Nelson, Leibenluft et al. 2005). Indeed, adolescence is a critical time for emergence of stress-related psychopathology and girls are more vulnerable than boys (Dahl and Gunnar 2009), particularly to anxiety and mood disorders during the pubertal transition (Angold, Costello et al. 1999, Dahl and Gunnar 2009), likely contributing to the higher lifetime incidence of these disorders in women compared to men (Kessler, Berglund et al. 2005).

This vulnerability may in part be due to puberty-related increases in estradiol (E2) (Anisman and Zacharko 1992, Lee, Geracioti et al. 2005), a gonadal steroid important for mood regulation (Pfaff, Vasudevan et al. 2000, Stoppe and Doren 2002) and for brain

organization/development, including structural and neurochemical changes in systems controlling emotional responses (Pfaff, Vasudevan et al. 2000, Bethea, Mirkes et al. 2002, Stoppe and Doren 2002, Dreher, Schmidt et al. 2007, Bethea and Reddy 2008, Martel, Klump et al. 2009, Zadran, Qin et al. 2009, Galvin and Ninan 2014) even during the pubertal transition (Ahmed, Zehr et al. 2008, Schulz, Molenda-Figueira et al. 2009). E2 and other gonadal steroids have prenatal organizational effects on the brain, but also influence brain maturation and behavioral development postnatally, particularly during puberty (Ahmed, Zehr et al. 2008, Schulz, Molenda-Figueira et al. 2009). E2 can exert both activational and organizational effects on the brain (Arnold and Breedlove 1985, McCarthy 2010), through remodeling and refinement of neuronal circuits via various mechanisms [for review see (Goldstein, Kurz et al. 1990, Benes, Taylor et al. 2000, Nunez, Lauschke et al. 2001, Cunningham, Bhattacharyya et al. 2002, Sisk and Zehr 2005, Schulz, Molenda-Figueira et al. 2009)], resulting in persistent changes to gross brain morphology and synaptic organization. In humans, some, but not all, of the neurodevelopmental changes reported during adolescence have been associated with elevations in gonadal hormones. For example, higher levels of circulating E2 in 12 year old girls have been associated with lower GM density in frontal and parietal lobes (Brouwer, Koenis et al. 2015). Additionally, greater levels of E2 have also been associated with smaller anterior cingulate cortex GM, after controlling for age (Koolschijn, Peper et al. 2014), and greater thinning of the left middle temporal gyrus (Herting, Gautam et al. 2015). Although the effects of pubertal increase in E2 are recognized, the pre-pubertal ovary, under the influence of gonadotropins, is also able to secrete biologically active, albeit low, concentrations of E2 prior to menarche in both

girls (Apter, Butzow et al. 1993, Norjavaara, Ankarberg et al. 1996) and female macaques (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004, Wilson, Bounar et al. 2013). These prepubertal low circulating concentrations of E2 (Norjavaara, Ankarberg et al. 1996, Veldhuis, Roemmich et al. 2000) are able to regulate a number of systems in females, including luteinizing hormone (LH) secretion (Rapisarda, Bergman et al. 1983, Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), insulin-like growth factor 1 (IGF-1) and growth hormone (GH)(Wilson, Schwartz et al. 1984). What remains poorly understood, however, is whether these low, prepubertal levels of E2 modify the effects of stress on brain structure and emotional development in similar or different ways than during the pubertal transition. This is one of the questions addressed in this study.

As described above, cortico-limbic regions seem particularly sensitive to chronic stress, particularly early in life (Kaufman and Charney 2001, Lupien, McEwen et al. 2009), including the PFC (Sanchez, Ladd et al. 2001, Bremner 2003, Spinelli, Chefer et al. 2009), AMYG, and hippocampus (HC) (Joels, Karst et al. 2007, Tottenham and Sheridan 2009, Weems, Scott et al. 2013, Howell, Grand et al. 2014, Lyons-Ruth, Pechtel et al. 2016). These regions have a high density of glucocorticoid receptors (GR) (Sanchez, Young et al. 2000, Ulrich-Lai and Herman 2009), and have a protracted development (Andersen 2003, Knickmeyer, Styner et al. 2010), which can explain their high susceptibility to chronic stress. Although reports in the literature are not consistent, several studies investigating long-term effects of early life stress in humans have shown smaller HC (Bremner, Randall et al. 1997, Driessen, Herrmann et al. 2000, Andersen, Tomada et al. 2008) and larger AMYG volumes (Mehta, Golembo et al. 2009,

Tottenham, Hare et al. 2010) in stressed patients compared to healthy controls. Studies in nonhuman primate models of early life stress have reported similar HC and AMYG findings (Jackowski, Perera et al. 2011, Coplan, Fathy et al. 2014, Howell, Grand et al. 2014). Another study found that PFC WM was decreased in peer-reared compared to mother-reared monkeys (Sanchez, Hearn et al. 1998). AMYG, PFC and HC are also important in mediating variations in species-typical stress-related behaviors (Fox and Kalin 2014), including behavioral inhibition (defined in these studies as greater freezing, less cooing and increased cortisol in response to an acute stressor), which is associated with increased brain metabolism in AMYG, PFC and HC in juvenile rhesus monkeys (Kalin, Shelton et al. 2005, Oler, Fox et al. 2010) as measured by $^{18}$fluoro-deoxyglucose positron emission tomography imaging (FDG-PET). Furthermore, lesions of orbitofrontal cortex in rhesus monkeys decrease both freezing behavior and cortisol reactivity (Kalin, Shelton et al. 2007, Machado and Bachevalier 2008). These regions are also important in the processing of socioemotional information in adult rhesus monkeys, where AMYG and PFC GM volume as well as structural and functional connectivity have been positively correlated with social rank and social network size (Sallet, Mars et al. 2011, Howell, Godfrey et al. 2014, Noonan, Sallet et al. 2014).

Rodent studies have provided insights into the cellular mechanisms underlying the effects of chronic stress on cortico-limbic brain regions and their behavioral correlates in macaques and humans. Thus, while chronic stress (or prolonged glucocorticoid treatment) results in decreased dendritic arborization and synaptic complexity (e.g. length, branching, spine density) in HC (Watanabe, Gould et al. 1992, Magarinos and

McEwen 1995, Magarinos, Orchinik et al. 1998, McEwen 1999) and in the mPFC (Joels, Karst et al. 2007, Shansky and Morrison 2009, Radley, Anderson et al. 2013), it increases dendritic arborization in the AMYG (Rosenkranz, Venheim et al. 2010). However, these studies have largely focused on stress-induced alterations in adult males. Adult females do not show the male-typical dendritic retraction or spine/synaptic loss in the HC (McEwen and Milner 2007) or in mPFC neurons projecting to AMYG (Garrett and Wellman 2009, Shansky, Hamo et al. 2010), but the opposite effect, while sex differences are not observed in the absence of stress (Rubinow, Drogos et al. 2009). These sex differences in rodents could be explained by evidence in females suggesting that E2 modulates the effects of stressors on dendritic remodeling (McLaughlin, Baran et al. 2009, Shansky, Hamo et al. 2010). Given this evidence, stress-induced alterations during adolescence could be different than in adulthood, not only due to incomplete development of cortico-limbic circuits, but due to the modulatory effect of E2 on brain structure. In other words, in order to understand the neurobehavioral impact of stress during adolescence, we need to disentangle the effects due to chronological age from those due to pubertal stage.

As addressing these mechanistic questions using a prospective, longitudinal experimental design in human children would be fraught with ethical and logistical difficulties, animal models provide an excellent alternative to study the longitudinal effects of early life stress. Naturally occurring social subordination in female rhesus macaque social groups is a useful translational model to determine the adverse effects of chronic social stressor exposure on neurobehavioral development (Wilson, Bounar et al. 2013). Social

subordination is a known psychosocial stressor in adult females that impairs HPA regulation (Shively 1998, Michopoulos, Reding et al. 2012) and produces a number of stress-related phenotypes (Shively, Laber-Laird et al. 1997, Michopoulos, Toufexis et al. 2012). Importantly, the continual experience of subordination not only delays puberty and the developmental increases in E2 in female rhesus macaques (Zehr, Van Meter et al. 2005), but also impacts both behavioral development (Wilson, Bounar et al. 2013) and PFC white matter tracts (Howell, Godfrey et al. 2014). However, what is not known is how this developmental increase in E2, from low but biologically active levels prior to menarche to higher concentrations as first ovulation approaches, interacts with adverse social experience to affect brain maturation and emotional development during puberty. Therefore, the goals of the present study were: (1) to examine the impact of social subordination stress on female macaque neurobehavioral development, (2) to determine whether pubertal timing and duration of E2 exposure modulate the impact of stress on structural development of cortico-limbic regions involved in emotional and stress regulation, specifically AMYG, PFC and HC, disentangling it from the effects of chronological age, and (3) to examine whether these brain structural differences are related to behavioral and physiological outcomes. To accomplish this, we used structural magnetic resonance imaging (sMRI) techniques prior to and after typical puberty onset in female rhesus monkeys embedded in large social groups. Additionally, we experimentally delayed puberty in a subset of animals through administration of Lupron, a gonadotropin releasing hormone (GnRH) agonist. Lupron acts on pituitary GnRH receptors to suppress the developmental increase in gonadotropin secretion and subsequent E2 synthesis and release (Wilson, Meethal et al. 2007). We also examined the

functional correlates of the effects social subordination and E2 suppression, particularly on social behavior, emotional reactivity and stress physiology. We tested the hypothesis that these social status effects would be evident in AMYG, PFC and HC, which are involved in social and emotional processing and are particularly sensitive to stress due to high levels of both glucocorticoid and estrogen receptors. Specifically, based on available literature discussed above, we predicted that subordinates would have larger AMYG, but smaller PFC and HC volumes compared to dominant subjects and that these alterations would result in increased fear and anxiety behavior and stress reactivity. Because prepubertal E2 is bioactive in female rhesus monkeys (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), we also hypothesized that these status-induced brain and behavioral effects would be exacerbated in females with delayed exposure to E2. A portion of the behavioral and physiological data from control subjects has been reported previously (Wilson, Bounar et al. 2013).

## 2.3 Methods

### 2.3.1 Subjects

Subjects were 45 juvenile female rhesus macaques (*Macaca mulatta*) living in four social groups at the Yerkes National Primate Research Center (YNPRC) consisting of 2-3 adult males and 30-60 adult females with their sub-adult and juvenile offspring. Each group was housed in an outdoor enclosure measuring approximately one acre with attached indoor, climate-controlled quarters. Groups had been together for more than 5 years prior to the start of this study. Animals were fed low fat, high fiber Purina monkey chow (Ralston Purina, St. Louis, MO) two times per day ad libitum and had continuous access

to water. Their diet was supplemented daily with fresh fruit and vegetables. 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 Social Subordination

Macaque groups, regardless of size, are organized by a matrilineal dominance hierarchy that functions to maintain group stability (Bernstein and Gordon 1974, Bernstein 1976). Lower ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Bernstein 1976). Subordinates have less control over their environment (Abbott, Keverne et al. 2003). A consequence of this continual harassment is impaired HPA regulation, evidenced by reduced glucocorticoid (GC) negative feedback and hypercortisolemia in adult females (Shively, Laber-Laird et al. 1997, Shively 1998, Wilson, Pazol et al. 2005, Jarrell, Hoffman et al. 2008, Wilson, Fisher et al. 2008, Paiardini, Hoffman et al. 2009). In addition, macaques exhibit a specific set of behaviors in stress-eliciting situations that are considered anxiety-like (Schino, Troisi et al. 1991, Troisi, Schino et al. 1991, Troisi 2001, Troisi 2002, Kalin and Shelton 2003) and these occur more often in subordinates (Shively, Register et al. 2005, Wilson, Fisher et al. 2008).

The social status for each subject was assessed during monthly 30 minute focal observations each of the two imaging ages and informally during group checks as previously described (Embree, Michopoulos et al. 2013). Rank was determined using the

outcome of dyadic agonistic interactions (Bernstein 1976) with subordination defined as the subject who unequivocally emits submissive behavior (withdrawing) to the other animal. Each female's relative rank was calculated as the ratio of her rank to the total number of monkeys in her group. For example, a subject with a rank of 30 out of a total 100 animals in her group would receive a relative rank of 0.30. Subjects with a relative rank equal to or less than 0.30 were classified as dominant (n=20) and subjects with a relative rank greater than or equal to 0.70 were classified as subordinate (n=25). Subjects whose rank changed from prepuberty to peripuberty because of rank shifts among matrilines were excluded from the analysis.

### 2.3.3 Experimental Procedures

*Animal Training for Access.* Using procedures in place at the YNPRC (Walker, Gordon et al. 1982, Blank, Gordon et al. 1983) that do not impact reproductive physiology (Walker, Gordon et al. 1982) or development (Wilson, Gordon et al. 1986), subjects were trained to individually move from their outdoor enclosure into the caged area of the attached indoor quarters. From there they moved to a small transfer box so they could be placed in a holding cage for blood draw or transferred to a behavioral testing room (see below). Females were trained and habituated to place their leg through a small opening in the front of the holding cage so that a blood sample could be obtained from the saphenous vein without the use of anesthesia.

*Lupron Administration and Pubertal Timing.* Females were divided into one of two cohorts: a control group (n=22) that received no exogenous treatment and reached puberty spontaneously; the second group (n=23) was treated with the GnRH analog,

depot Lupron (Tap Pharmaceuticals) to suppress developmental increases in E2 as previously described (Golub, Styne et al. 1997). Lupron-treated females received an injection once monthly (0.25 μg/kg/mo, IM) from 16.38 ± 0.27 through 37.57 ± 0.21 months of age encompassing the period of the study (Figure 2.1), which reflects the interval from prepuberty through post-menarche in control subjects (Wilson, Bounar et al. 2013). At the time of the first sMRI scan, subjects had been on Lupron-treatment for 6.36 ± 0.09 months and at the time of the second sMRI scan, subjects had been on Lupron-treatment for 16.46 ± 0.12 months (Figure 2.1). Control subjects reached menarche (defined as initial perineal swelling and menses) in an average of 4.54 ± 0.85 months prior to the second MRI scan and had their first ovulation in an average of 7.89 ± 0.90 months prior to the second MRI scan at peripuberty. Prior to menarche, serum E2 levels averaged 22 ± 3 pg/ml. Following menarche, samples were variable above 54 ± 5 pg/ml. E2 concentrations for Lupron-treated females were consistently below 18 pg/ml (sensitivity of the assay).

*Structural MR Image Acquisition.* Subjects scans were collected at prepuberty (22.77± 0.19 months) and again at peripuberty (33.14 ± 0.21 months) (Figure 2.1). At the time of the peripubertal scan, no Lupron subjects had reached menarche or first ovulation, but 15 control subjects had reached menarche and 7 had already had their first ovulation. One day prior to the scan, each subject was transported from the YNPRC Field Station to the YNPRC Imaging Center. Using a 3T Siemens Magnetom TRIO system, and an 8-channel phase array coil, T1-MR Scans were acquired using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel imaging sequence (GRAPPA (R=2); TR/TE=3000/3.51ms; voxel size=0.5x0.5x0.5mm$^3$'; 6 averages). A T2-MR scan was

collected in the same direction as the T1 (TR/TE=7900/125ms, voxel size=0.5x0.5x1.0mm$^3$, 10 averages) in order to aid with delineation of regions of interest (ROIs) by improving the contrast of GM (Rapisarda, Bergman et al. , Knickmeyer, Styner et al. 2010), WM and CSF borders. Subjects were scanned under isoflurane anesthesia (1-1.2% to effect, inhalation) following induction with telazol (5mg/kg, I.M.) and intubation to ensure lack of motion artifacts. For physiological monitoring, animals were fitted with an oximeter, electrocardiograph, rectal thermometer and blood pressure monitor. Additionally, an I.V. catheter was placed to administer dextrose/NaCl (0.45%) in order to maintain hydration. All subjects were scanned in the same supine placement and orientation on an MRI-compatible heating pad, using a custom-made head holder with ear bars and a mouth piece. A vitamin E capsule was taped to the right temple to mark the right side of the brain. Subjects were returned to their social groups upon completion of the scan and full recovery from anesthesia.

*Structural MR Image Processing and Analysis.* Structural data were analyzed using AutoSeg (version 2.6.2), which is an open-source pipeline developed by members of the Neuro Image Research and Analysis Laboratories of University of North Carolina (NIRAL) (Wang, Vachet et al. 2014). AutoSeg is an atlas-based software pipeline that segments brain into probabilistic tissue maps, lobar parcellations and subcortical ROIs using an atlas-based automatic segmentation approach. AutoSeg was used to automatically segment brain tissue classes (WM, GM, CSF) and generate parcellations of cortical lobes (specifically for this study: the PFC), and subcortical structures (AMYG and HC) to compute their respective volumes in our subjects, following previously described methods (Knickmeyer, Styner et al. 2010, Howell, Grand et al. 2014, Wang,

Vachet et al. 2014). This is achieved by registering each subject's native image space into a population-based T1-MRI atlas (Styner, Knickmeyer et al. 2007, Knickmeyer, Styner et al. 2010, Short, Lubach et al. 2010, Howell, Grand et al. 2014, Wang, Vachet et al. 2014) using the tools BRAINSFit and ResampleVolume2, modules in the Slicer program (Fedorov, Beichel et al. 2012). Inhomogeneity correction using N4-ITK bias field correction (Tustison, Avants et al. 2010) is then applied and Atlas Based Classification (ABC) is then used to automatically classify regions in each subject's images into brain tissue (WM, GM, CSF) or non-brain tissue (e.g. skull, vessels, muscle) and to remove non-brain tissue (skull-stripping), by warping the atlas tissue priors into the subject using affine and fluid deformable registration of the atlas to each subject. These warp fields were then applied to generate cortical and subcortical parcellations that were manually adjusted to ensure accurate delineation of the neuroanatomy following neuroanatomical conventions (Amaral and Bassett 1989, Paxinos, Huang et al. 2000, Sallem and Logothetis 2006). To control for differences in total brain size, ROI volumes were corrected for total brain/intracranial volume (ICV), if applicable (see Results section). Volumes were calculated for total ICV, in as well as for our regions of interest (ROIs): AMYG, PFC (total, GM and WM) and HC (Figure 2.2). The AMYG was defined following macaque anatomical landmarks (Price, Russchen et al. 1987, Amaral and Bassett 1989) with the rostral periamygdaloid cortex as the anterior boundary, the CSF as the ventral border, WM as the ventrolateral boundary and the rhinal fissure as the ventromedial boundary (Howell, Grand et al. 2014). The HC was defined using the horn of the lateral ventricle as the dorsal and lateral boundary with the WM separating the HC from the entorhinal cortex as the ventral border (Rosene and Van Hoesen 1987). The PFC

was defined using CSF at the surface of the brain as the lateral and anterior boundaries, the interhemispheric fissure as the medial boundary and the arcuate sulcus as the posterior boundary (Knickmeyer, Styner et al. 2010). The inferior boundary, moving rostral to caudal, was defined by the CSF, the sylvian fissure and the arcuate sulcus.

*MRI 3T TRIO Scanner Upgrade to Tim: Validation Studies.* The YNPRC 3T TRIO scanner underwent an upgrade to the Tim system in the early phases of our studies. Although our post-upgrade T1- and T2-weighted sequences were identical to the ones used pre-upgrade (except for the T1 TE=3.31ms, instead of 3.51ms), we performed a validation/replicability study to rule out potential effects of the upgrade on our structural MRI volumetric data. For this, four adult macaques were scanned twice, 3 months apart (prior and right after the upgrade) using the T1- and T2-weighted sequences described above and the data processed identically through the AutoSeg 2.6.2 pipeline. Adult subjects were selected for this study instead of juveniles to avoid effects of normative developmental brain growth that could take place during the month period between the pre- and post-upgrade scans. A paired t-test was conducted for each ROI (Total ICV, and right and left AMYG, HC and PFC GM and WM), studied. No significant effects of scanner upgrade were detected for any of the structural measures focus of our studies in the paired t-test analyses, suggesting that the age and groups differences reported are not spurious. The specific statistical results are listed here: Total ICV ($t(3) = -1.165$, $p = 0.328$), right AMYG ($t(3) = -0.231$, $p = 0.832$), left AMYG ($t(3) = -1.916$, $p = 0.151$), right HC ($t(3) = -0.444$, $p = 0.687$), left HC ($t(3) = -1.522$, $p = 0.225$), right PFC GM ($t(3) = 0.822$, $p = 0.472$), left PFC GM ($t(3) = 1.043$, $p = 0.374$), right PFC WM ($t(3) = 0.362$, $p = 0.741$), left PFC WM ($t(3) = 1.185$, $p = 0.321$).

*Behavior*

*Behavioral Observations.* One 30-minute behavioral observation was collected monthly on each subject for the duration of the study and observations were averaged for each scan age. Observations were conducted from towers above the animals' social groups using a well-established rhesus monkey ethogram (Maestripieri, McCormack et al. 2006), which quantifies frequencies and durations of affiliative (groom, proximity, play wrestle), agonistic (attack, chase, display, threat), submissive (withdraw, fear grimace), anxiety-like (self-scratch, yawn, body shake, self explore and sit alone) and self play behaviors. Behavior was coded in real time, using a notebook computer with an in-house program – WinObs- (Graves and Wallen 2006), which records the actor, behavior, recipient and the time for each behavior. Inter-observer reliability was calculated as percent agreement on frequencies and durations of behaviors and was greater than 92%.

*Emotionality Testing: Human Intruder Task.* Females received a standardized test of emotionality at prepuberty (20.98 ± 0.12 months) and again at peripuberty (29.64 ± 0.10 months) (Figure 2.1). The HI task is an ethologically relevant assessment of behavioral reactivity to novel stimuli of varying threatening intensities, including anxiety and fear responses (Kalin and Shelton 1989, Meunier, Bachevalier et al. 1999), and has been used to quantify differences in fearful behaviors in rhesus monkeys with different 5HTT alleles (Bethea, Streicher et al. 2004) and early social experience conditions (Grand, McCormick et al. 2005, Howell, Godfrey et al. 2014, Howell, Grand et al. 2014). Animals were accessed as described above and immediately transported to a testing cage [0.7m x 0.6m x 0.8m (L x W x H)] in a nearby behavioral testing facility. Prior to

receiving any tests, animals received habituation periods in the testing cage on different days (one 30-minute and two 15-minute) and were then returned to their group. The testing cage had a clear Plexiglas side to maximize monkey visual contact with the HI and quality of video recording for identification of facial expressions and behaviors during the HI paradigm. Each test was recorded using a Sony digital video camera (model DCR-SR85) and white noise was played to minimize interference from extraneous sounds.

Each subject completed the HI task (Kalin and Shelton 1989) following the last habituation day. This paradigm consists of three consecutive ten-minute sessions, including an alone condition, an intruder profile condition (an unfamiliar experimenter enters the room and sits with his/her profile towards the subject) and an intruder stare condition (the experimenter makes direct and continuous eye contact for the entire ten minute session). The HI task is designed to measure defensive and emotional behavior responses specific to each condition, which pose different degrees of threat to rhesus monkeys. The alone condition elicits exploratory and distress responses, such as vocalizations and locomotion, whereas the intruder profile condition elicits behavioral inhibition, such as freezing and visually scanning of the environment. Finally, the intruder stare condition elicits aggressive and/or submissive behaviors directed towards the intruder, as direct eye contact is threatening for rhesus macaques. Monkeys do not habituate to this test (Kalin and Shelton 1998). Videos of each test were scored by trained observers using a modification of published ethograms (Machado and Bachevalier 2006, Howell, Godfrey et al. 2014, Howell, Grand et al. 2014) to capture and quantify

locomotive, aggressive, submissive, anxiety-like and fearful behaviors. Inter-observer reliability was greater than 92%.

*Stress Physiology Assessment: HPA axis function*

*Social Separation.* Serum cortisol was measured on the first day of habituation to the behavioral testing room at both prepuberty and peripuberty in order to assess stress cortisol reactivity. Removal of a monkey from her group to a novel environment is a stressor (Arce, Michopoulos et al. 2010). Awake blood samples were collected from the saphenous vein at baseline (pre-test) and immediately after the 30 min separation/habituation period. Stress-induced cortisol secretion was calculated as the change in cortisol from pre- to post-test.

*Dexamethasone Suppression Test.* Glucocorticoid negative feedback was tested at prepuberty (16.86 ± 0.27 months) and again at peripuberty (34.10 ± 0.28 months) (Figure 2.1), following previously published procedures (Jarrell, Hoffman et al. 2008). A baseline, morning blood draw was collected at 1000 hr and again at 1800 hr. Following the evening sample, dexamethasone was administered (0.25mg/kg I.M.). The following morning (1000- hr) another blood sample was obtained to measure the degree to which dexamethasone suppressed morning cortisol.

### 2.3.4 Hormone Assays

Cortisol assays were performed in the YNPRC Biomarkers Core Lab using radioimmunoassay with a commercially available kit (Beckman-Coulter/DSL, Webster

TX). Using 25 µl, the assay has a range from 0.50 to 60 µg/dl with an intra- and inter-assay CV of 4.9% and 8.7%, respectively. Serum E2 was analyzed by ELISA following a modification of a previously validated assay (Pazol, Kaplan et al. 2004) using commercially available reagents (DRG International). One 0.5 ml of serum (in duplicate) was extracted twice with 5 ml anesthesia grade ether. The aqueous layer was discarded and the evaporated ether layer was reconstituted in assay buffer (zero calibrator). Using this volume of serum, the sensitivity of the assay was 18 pg/ml. The intra- and inter-assay CV was 5.5% and 11.7%, respectively, though all samples for the E2 analyses were done in the same assay. Blood circulating levels of dexamethasone were also measured using a commercial ELISA kit (BioX Diagnostics).

### 2.3.5 Statistical Analyses

All data were square root transformed and are summarized as mean ± SEM. A repeated measures ANOVA was run on each subjects ROI's volumetric data with social status, Lupron-treatment and hemisphere (right, left) as fixed factors and scan age (prepuberty, peripuberty) as a repeated measures, except for total ICV (no hemisphere factor). Main and interaction effects were considered significant if $p<0.05$. Post-hoc analyses (Bonferroni) were run for pairwise comparisons following significant p values for interaction effects, using a Bonferroni correction for multiple comparisons. Effect sizes (partial eta squared - $\eta^2$) were calculated for each significant main effect or interaction.

Next, a repeated measures ANOVA was run for each subject's baseline cortisol and behavioral data (from the HI task and social behavior), with social status and Lupron-treatment as fixed factors, and age (prepuberty, peripuberty) as a repeated measure. Variables were excluded from the analysis if they were low occurrence (occurred in ≤10% of the subjects). Additionally, cortisol changes in response to social separation and dexamethasone suppression challenge were analyzed in another repeated measures ANOVA, separately for each age, with social status and Lupron-treatment as fixed factors, and time of sample collection (pre, post; acute separation stressor or dexamethasone suppression challenge) as a repeated measure.

Finally, a stepwise multiple regression analysis was conducted to determine variables that were predictive of behavioral and stress-physiology outcomes. As a data reduction technique, only the variables that emerged as being significantly affected by main effects or interactions of either rank or Lupron-treatment from the behavioral analysis (described in the previous paragraph) were then entered into the stepwise multiple regression analysis (Table 1). Each brain ROI (right and left AMYG, HC, PFC WM and PFC GM) in addition to Lupron-treatment and relative rank were entered into the multiple regression analysis as predictor variables. Prepubertal and peripubertal variables were analyzed independently for each age. Models that contained only significant rank and Lupron-treatment effects (without a brain ROI entering into the model) were excluded, to avoid duplication of the results of the repeated measures ANOVA conducted on behavior and stress physiology (described above). Each model was examined to assess statistical test assumptions of normality, linearity, homogeneity of variance and independence. If

more than one model was identified at a significance level of $p<0.05$, the model that accounted for the most variance was selected, provided the condition index did not exceed 30 (to exclude multicollinear models).

## 2.4 Results

### 2.4.1 sMRI Data

Intracranial Volume (ICV)

The analysis of total ICV (sum of GM, WM and CSF) revealed a main effect of age, where total volume increased from pre- to peripuberty [$F(1, 41)=50.492$, $p=1.169E-8$; $\eta^2_{partial}=0.552$]. An age by Lupron-treatment interaction effect was also detected [$F(1, 41)=148.683$, $p=3.203E-15$; $\eta^2_{partial}=0.784$] (Figure 2.3). Post-hoc analyses indicated that, although there were no significant differences between Lupron-treated subjects and controls at either pre- and peripuberty ages, Lupron-treated animals showed an increase ($p=5.708E-17$), while control subjects showed a decrease in total ICV with age ($p=0.001$). Since there were significant effects in the analysis of total brain volume, all ROI data were corrected for total ICV and the results from this analysis are presented below. *For a summary of the results, see Table 2.1*.

Amygdala Volume

The AMYG analysis revealed main effects of rank, where subordinates had larger AMYG volume than dominants [$F(1, 41)=8.511$, $p=0.006$; $\eta^2_{partial}=0.172$] (Figure 2.4), and of Lupron-treatment, with larger AMYG volumes in control compared with Lupron-treated subjects [$F(1, 41)=5.055$, $p=0.030$; $\eta^2_{partial}=0.110$]. There was also a main

hemisphere effect, with larger right than left AMYG volumes [$F(1, 41)=6.921$, $p=0.012$; $\eta^2_{partial=}0.144$]. Hemisphere significantly modified the main effects of rank and Lupron-treatment [$F(1, 41)=4.615$, $p=0.038$; $\eta^2_{partial=}0.101$] (Figure 2.4a), so that the rank effect was specific to the right AMYG in the control group ($p=0.009$), but was detected only in the left AMYG for the Lupron group ($p=0.020$). Interestingly, Lupron-treatment seemed to prevent the increased right AMYG volume related to social subordination, suggesting a role of E2 on the stress-induced neural impact on this limbic structure. Additional significant interaction effects are summarized in Table 2.1.

Although there was no significant main effect of age, there was an age by Lupron-treatment by hemisphere interaction effect ($F(1, 41)=8.220$, $p=0.007$; $\eta^2_{partial=}0.167$) and an age by hemisphere interaction effect ($F(1, 41)=4.595$, $p=0.038$; $\eta^2_{partial=}0.101$), as shown in Figure 2.4b, where Lupron-treated subjects had smaller AMYG volume than controls in the left hemisphere at prepuberty only ($p=0.002$).

Hippocampal Volume

A main effect of Lupron-treatment was detected in the HC volume analysis, with controls having larger volume than Lupron-treated subjects [$F(1, 41)=8.620$, $p=0.005$; $\eta^2_{partial=}0.174$] (Figure 2.5a). There was also a main age effect, where HC volume increased from pre- to peripuberty [$F(1, 41)=7.614$, $p=0.009$; $\eta^2_{partial=}0.157$], and a main hemisphere effect, where right HC volume was larger than left [$F(1, 41)=17.422$, $p=0.00015$; $\eta^2_{partial=}0.298$]. Hemisphere modified the age effect [$F(1, 41)=10.875$, $p=0.002$; $\eta^2_{partial=}0.210$] (Figure 2.5b), with larger right than left volume at prepuberty

($p$=0.000009), but no differences at peripuberty, as only the left hemisphere increased in volume from pre- to peripuberty ($p$=0.001). None of these age effects were modified by social status or Lupron-treatment.

Prefrontal Cortex Volume

*PFC Gray Matter*

Lupron-treated subjects had larger PFC GM volume compared to controls [$F$(1, 41)=13.981, $p$=0.0005; $\eta^2_{partial}$=0.254]. There was also a main effect of age, showing a decrease in PFC GM volume from pre- to peripuberty [$F$(1, 41)=9.028, $p$=0.005; $\eta^2_{partial}$=0.180] and a main hemisphere effect, revealing a larger right than left PFC GM volume [$F$(1, 41)=17.614, $p$= 0.0001; $\eta^2_{partial}$=0.301]. A Lupron by age interaction effect was also detected ([$F$(1, 41)=6.713, $p$= 0.013; $\eta^2_{partial}$=0.141], with Lupron-treated, but not control, subjects showing decreased PFC GM volume from pre- to peripuberty ($p$=0.0003). The age by Lupron-treatment interaction effect was modified by both hemisphere and rank [age by hemisphere by Lupron-treatment interaction, $F$(1, 41)=4.219, $p$= 0.046; $\eta^2_{partial}$=0.093; age by hemisphere by Lupron-treatment by rank interaction, $F$(1, 41)=4.434, $p$= 0.041; $\eta^2_{partial}$=0.098], with all significant results summarized in Table 2.1 and shown graphically with post-hoc results in Figure 2.6a), although the general pattern is that Dominant Lupron subjects had larger PFC GM volume both at pre- and peripuberty compared to dominant controls, whereas subordinates showed a similar effect but only at prepuberty.

*PFC White Matter*

The analysis of PFC WM revealed a main effect of age, with an increase in volume from pre- to peripuberty [$F(1, 41)=18.042$, $p=0.0001$; $\eta^2_{partial}=0.306$], as shown in Figure 2.6b. The left hemisphere had larger PFC WM volume than the right [$F(1, 41)=19.045$, $p=0.00008$; η2 partial=0.317], although age modified the hemisphere effect, [$F(1, 41)=13.693$, $p=0.0006$; $\eta^2_{partial}=0.250$], as shown in Table 2.1A Lupron by hemisphere interaction effect was also detected [$F(1, 41)=5.083$, $p=0.030$; $\eta^2_{partial}=0.110$], with smaller right, but not left, PFC WM volume in control than Lupron-treated subjects ($p=0.019$), as well as an age by Lupron by hemisphere interaction effect [$F(1, 41)=20.611$, $p=0.00005$; $\eta^2_{partial}=0.335$] (Figure 2.6b), with control subjects increasing PFC WM volume with age in both hemispheres (right: $p=0.000004$; left: $p=0.013$), whereas Lupron-treated subjects did not. Other significant results are summarized in Table 2.1.

*Total PFC*

As shown in Figure 2.6c, Lupron-treated subjects had larger total PFC volume than controls [$F(1, 41)=16.451$, $p= 0.0002$; $\eta^2_{partial}=0.286$]. There was also a main hemisphere effect where right was larger than left total PFC volume [$F(1, 41)=4.313$, $p= 0.044$; $\eta^2_{partial}=0.095$]. A Lupron by hemisphere interaction effect was also detected [$F(1, 41)=4.310$, $p= 0.044$; $\eta^2_{partial}=0.095$] with Lupron-treated subjects smaller left than right PFC volumes ($p=0.005$), but no laterality effects found for controls. Age modified Lupron-treatment and hemisphere effects (age by hemisphere interaction, $F(1, 41)=10.038$, $p= 0.003$; $\eta^2_{partial}=0.197$; age by Lupron-treatment interaction, $F(1, 41)=12.792$, $p= 0.0009$; $\eta^2_{partial}=0.238$; age by hemisphere by Lupron-treatment

interaction, $F(1, 41)=19.881$, $p= 0.00006$; $\eta^2_{partial=}0.327$] as shown in detail in Table 2.1. One of the most interesting results is that Lupron-treated subjects showed a decrease in total PFC volume with age ($p=0.002$), whereas controls did not (Figure 2.6c).

### 2.4.2 Behavior and Stress Physiology

The complete results of the repeated measures ANOVA to examine effects of age, Lupron-treatment, and social rank effects on behavior and stress neuroendocrine measures are discussed below and summarized in Table 2.2.

Social Behavior

As expected, subordinate subjects emitted significantly more submissive behavior [$F(1, 41)=18.203$, $p=1.142E-04$; $\eta^2_{partial=}0.307$] and emitted less aggressive behavior than dominants [$F(1, 41)=9.304$, $p=0.004$; $\eta^2_{partial=}0.185$], but also sat alone [$F(1, 40)=5.921$, $p=0.020$; $\eta^2_{partial=}0.129$] more often than dominants. Additionally, frequency of anxiety behavior increased from pre- to peripuberty [$F(1, 40)=9.124$, $p=0.004$; $\eta^2_{partial=}0.186$]. Two interactions emerged for social behavior. A Lupron-treatment by age interaction [$F(1, 41)=6.143$, $p=0.017$; $\eta^2_{partial}=0.130$] revealed that controls play wrestle more than Lupron-treated subjects, but only at prepuberty ($p=0.028$). A rank by Lupron-treatment by age interaction emerged [$F(1, 40)=4.324$, $p=0.044$; $\eta^2_{partial}=0.098$], where subordinate Lupron-treated subjects played alone more than subordinate controls, but only at prepuberty ($p=0.046$) and this behavior was significantly reduced by peripuberty ($p=0.009$).

Human Intruder Task

Two main effects of rank were detected, where in the stare condition, dominants froze more [$F(1, 41)=6.989$, $p=0.012$; $\eta^2_{partial}=0.146$] and threatened the intruder less [$F(1, 41)=5.136$, $p=0.029$; $\eta^2_{partial}=0.111$] compared to subordinates. Interestingly, Lupron-treated subjects averted their gaze from the intruder more often than controls during the stare condition [$F(1, 41)=21.185$, $p= 3.985E-05$; $\eta^2_{partial}=0.341$]. Several effects of age emerged independently of social status and Lupron treatment. Incidences of freezing in the alone [$F(1, 41)=30.088$, $p=2.33E-06$; $\eta^2_{partial}=0.423$] and stare conditions [$F(1, 41)=14.994$, $p=3.80E-04$; $\eta^2_{partial}=0.268$] and averting gaze in the profile condition [$F(1, 41)=5.365$, $p=0.026$; $\eta^2_{partial}=0.116$] increased with age, whereas incidences of cooing [$F(1, 41)=6.322$, $p=0.016$; $\eta^2_{partial}=0.134$] and lipsmacking [$F(1, 41)=10.724$, $p=0.002$; $\eta^2_{partial}=0.207$] in the stare condition and locomotion in the alone condition [$F(1, 41)=19.718$, $p=6.64E-05$; $\eta^2_{partial}=0.325$] decreased with age. Additionally, several interesting interactions emerged for HI behavior and they are reported in detail in Table 2.2.

HPA axis function

*Baseline cortisol*: An age effect revealed that baseline cortisol was higher at pre- compared to peripuberty [$F(1, 40)=25.937$, $p<0.001$; $\eta^2_{partial}=0.387$]. The age effect was modified by rank [$F(1, 40)=5.456$, $p=0.024$; $\eta^2_{partial}=0.117$], where subordinates had higher baseline cortisol than dominants, but only at peripuberty ($p=0.026$).

*Cortisol response to acute stressor*: Cortisol significantly increased in response to the social separation stressor at both prepuberty [$F(1, 40)=228.874$, $p<0.001$; $\eta^2_{partial}=0.848$] and peripuberty [$F(1, 41)=243.617$, $p<0.001$; $\eta^2_{partial}=0.856$]. At peripuberty, control subjects had higher cortisol levels than Lupron-treated subjects [$F(1, 41)=44.266$, $p<0.001$; $\eta^2_{partial}=0.519$], at both baseline and post-stress time points. At prepuberty, cortisol levels were modified by both rank and Lupron-treatment (rank by sample collection time point -baseline vs. post-stress- interaction effect: $F(1, 40)=6.698$, $p=0.13$; $\eta^2_{partial}=0.140$; rank by Lupron-treatment by sample time point interaction: $F(1, 40)=6.648$, $p=0.014$; $\eta^2_{partial}=0.140$), where subordinates had higher baseline cortisol compared to dominants ($p=0.009$) and this effect was driven by controls ($p=0.003$).

*Glucocorticoid negative feedback*: Cortisol was significantly suppressed in response to dexamethasone at both prepuberty [$F(1, 41)=31.918$, $p<0.001$; $\eta^2_{partial}=0.438$] and peripuberty [$F(1, 40)=45.096$, $p<0.001$; $\eta^2_{partial}=0.524$]. At peripuberty, a sample collection time by rank interaction [$F(1, 40)=4.966$, $p=0.031$; $\eta^2_{partial}=0.108$] was detected, where subordinates had higher cortisol than dominants, but only at baseline ($p=0.026$).

### 2.4.3 Multiple Regression Analyses

Described below and summarized in Table 2.3 are the results showing how rank, Lupron-treatment and brain ROIs predicts social and emotional behavior, as well as HPA axis function at pre- and peri-puberty.

Predictors of Stress Physiology

No significant models emerged.

Predictors of emotional reactivity in Human Intruder

*Significant Models at Prepuberty.* Left AMYG and Lupron-treatment explained 30.1% of the variance in freezing during the alone condition of the HI task, where smaller left AMYG volume and Lupron-treatment predicted increased duration of freezing ($F(2, 42)=10.480$, $p= 0.0002$, Adj. $R^2= 0.301$). Left AMYG and Lupron-treatment also entered into a model predicting 27.9% of the variance in averting gaze at the intruder during the stare condition of the HI task, where smaller L AMYG volume and Lupron-treatment predicted longer durations of averting gaze away from the intruder ($F(2, 42)=9.495$, $p= 0.0004$, Adj. $R^2= 0.279$). Both rank and right AMYG predicted 18.3% of the variance in frequency of threats toward the intruder during the stare condition of the HI, where subordinate status and smaller right AMYG volume predicted increased frequency of threats ($F(2, 42)=5.929$, $p= 0.005$, Adj. $R^2= 0.183$). Finally, Lupron-treatment and right AMYG predicted 24.1% of the variance in locomotion duration during the stare condition, where Lupron-treatment predicted reduced locomotion and right AMYG volume predicted more locomotion ($F(2, 42)=7.966$, $p= 0.001$, Adj. $R^2= 0.241$).

*Significant Models at Peripuberty.* Consistent with the findings at prepuberty, right AMYG also predicted a 8.8% of the variance in locomotion during the stare condition at peripuberty, where larger right AMYG volume was associated with increased locomotion ($F(1, 43)=5.253$, $p= 0.027$, Adj. $R^2= 0.088$).

Predictors of Social Behavior

*Significant Models at Prepuberty.* Larger left HC volume predicted increased duration of sitting alone ($F(1, 43)=14.103$, $p= 0.001$, Adj. $R^2= 0.229$).

*Significant Models at Peripuberty.* Smaller left PFC GM predicted increased aggressive behavior ($F(1, 43)=7.489$, $p= 0.009$, Adj. $R^2= 0.129$). One model was consistent between ages, where larger left HC volume predicted increased duration of sitting alone ($F(1, 43)=5.928$, $p= 0.019$, Adj. $R^2= 0.101$).

## 2.5 Discussion

The goals of the present study were: (1) to examine the impact of social subordination stress on female macaque neurobehavioral development, (2) to determine whether pubertal timing and duration of E2 exposure modulate the impact of stress on structural development of cortico-limbic regions involved in emotional and stress regulation, specifically AMYG, PFC and HC, disentangling it from the effects of chronological age, and (3) to examine whether these brain structural differences are related to behavioral and physiological outcomes. The main findings of this study suggest that social subordination, but more strikingly, Lupron-induced delay of puberty has robust effects on overall brain volume, AMYG, HC and PFC volumes during development and specific aspects of emotional behavior and HPA axis function. In addition to expected developmental effects due to chronological age (i.e. similar patterns in control and Lupron groups), subordinates had bigger AMYG volumes than dominant animals

(particularly in the right hemisphere), but pubertal delay with Lupron-treatment abolished those differences. Subordinates also had elevated baseline cortisol, indicating activation of stress systems. In general, Lupron-treated subjects had smaller AMYG and HC volume than controls, but larger total PFC, due to more GM, and different, region-specific, developmental patterns dependent on age and social status. Additionally, Lupron-treatment and smaller AMYG volume were associated with increased emotional reactivity in the HI task and decreased social behavior compared to controls. These data provide evidence that exposure to developmental increases in E2 modify the consequences of social stress on the volume of cortico-limbic regions involved in emotional and stress regulation during maturation. But, even more importantly, they support different brain structural effects of chronological age and pubertal developmental stage in females, which are very difficult to disentangle in human studies.

Social subordination resulted in elevated baseline cortisol, both at pre- and peri-puberty, suggesting activation of the HPA axis consistent with social stress. In addition, and confirming the social subordination model, dominant status predicted increased aggression, whereas subordinate status predicted increased submission and time sitting alone in the social group, hallmark behaviors in this NHP animal model (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Bernstein 1976, Shively and Kaplan 1984). Subordinate animals also had larger AMYG volumes than dominant animals, although this effect was driven by right AMYG volume. This bigger AMYG volume is consistent with reports of chronic stress impact on this limbic structure (Sanchez, Ladd et al. 2001, Tottenham and Sheridan 2009, Tottenham, Hare et al. 2010, Coplan, Fathy et al. 2014,

Howell, Grand et al. 2014). One possible mechanism for these volumetric increases could be explained by the increase in dendritic arborization and/or density of dendritic spines in the AMYG in response to chronic stress or direct administration of glucocorticoids into the AMYG in animal models (Vyas, Mitra et al. 2002, Vyas, Bernal et al. 2003, Mitra, Jadhav et al. 2005, Vyas, Jadhav et al. 2006, Mitra and Sapolsky 2008, Rosenkranz, Venheim et al. 2010, Eiland, Ramroop et al. 2012). Evidence supporting AMYG volume increases includes reports of early life stress in non-human primates and humans. For example, studies in non-human primates include variable foraging demand stress (Coplan, Fathy et al. 2014) and infant maltreatment (Howell, Grand et al. 2014). Human developmental studies include impact of traumatic stress (Weems, Scott et al. 2013) and institutional rearing, particularly evident on the right amygdala (Mehta, Golembo et al. 2009, Tottenham, Hare et al. 2010). Despite this evidence other human studies have produced inconsistent results on the impact of chronic stress during development on AMYG volume (increased, decreased or unaltered) both in adults (Bremner 2002, Bremner 2003, Bremner 2006, van Harmelen, van Tol et al. 2010, Dannlowski, Stuhrmann et al. 2012, Teicher, Anderson et al. 2012) and in children and adolescents (Tottenham and Sheridan 2009, Tottenham, Hare et al. 2010, Hanson, Nacewicz et al. 2015). These discrepant findings may be explained by differences in timing and/or duration of the stressor in addition to timing of and differences in amygdala maturation, or even methodology in measurement across different studies (Tottenham and Sheridan 2009). However, a potential mechanism proposed by recent human studies is the possibility that differential pubertal development explains the different impact of stress on AMYG development during adolescence (Weems, Scott et al. 2013), since pubertal

development also explains the curvilinear relationship between AMYG volume growth and age, which peaks around puberty, at least in humans (Uematsu, Matsui et al. 2012). Given the potential regulation of AMYG development by E2 during adolescence, which is bioactive even at low levels -before menarche- [in girls: (Apter, Butzow et al. 1993, Norjavaara, Ankarberg et al. 1996) and female macaques: (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004, Wilson, Bounar et al. 2013)], as well as the evidence that it modulates the impact of stress in female rodent dendritic remodeling (McLaughlin, Baran et al. 2009, Shansky, Hamo et al. 2010), we disentangled chronological age from pubertal stage in our experiments, to test the hypothesis that suppressing developmental increases in E2 (which has been proposed as a vulnerability factor for stress-induced neurodevelopmental impact during adolescence) would prevent stress effects. Our findings that the increased AMYG volume detected in subordinate animals was prevented in the group treated with Lupron (which produces experimental delay of puberty by suppressing E2), support a role of E2 modifying the stress impact during primate adolescence and explains some of the inconsistencies in the literature. This seems due to a Lupron treatment induced reduction of AMYG volume, based on the additional finding that control females had larger AMYG volumes than Lupron-treated subjects, and evidence that increases in AMYG volume in ovariectomized rats have also been shown in response to E2 injections (Fan, Hanbury et al. 2008). This increase in AMYG volume may be due to promotion of dendritic shaft synapse formation (Nishizuka and Arai 1981) via mechanisms such as E2-increases in brain derived neurotrophic factor -BDNF- (Zhou, Zhang et al. 2005), which potentiates neuronal growth and survival (Sohrabji and Lewis 2006). Another putative mechanism underlying reduced AMYG volume in Lupron-

treated subjects may be reduced neuronal nuclei and cell body volumes, as a previous study showed that ovariectomized rats had smaller neuronal nuclei and soma volumes in the medial AMYG compared to those treated with E2 or progesterone (de Castilhos, Hermel et al. 2010). Taken together, our results provide evidence that delayed puberty and E2 suppression may be protective against the impact of social stress on AMYG volume.

Although previous studies have shown that stress during childhood and adolescence can also alter the structure of PFC and HC (Bremner, Randall et al. 1997, Spinelli, Chefer et al. 2009), we did not find effects of social subordination on these structures. Since the PFC follows a protracted development in both humans and macaques, (Giedd 2004, Lenroot and Giedd 2006, Knickmeyer, Styner et al. 2010), it is possible that social subordination effects may not become evident in this region until after puberty or even adulthood. We did not find effects of social subordination on HC, either, although we are reporting developmental increases in HC volume consistent with those previously reported in macaques (Knickmeyer, Styner et al. 2010, Payne, Machado et al. 2010).

In contrast to the region-specific impact of social subordination stress, the effects of experimental delay of puberty on brain development were surprisingly profound. Lupron-treatment resulted in greater PFC GM and total PFC volume, but smaller AMYG and HC volume. Interestingly, the effects of Lupron were, in some cases, dependent on age and social status. For example, total ICV increased with age, as expected based on previous studies in macaques and humans (Malkova, Heuer et al. 2006, Knickmeyer, Styner et al.

2010, Payne, Machado et al. 2010, Scott, Grayson et al. 2015). However, the effect was explained largely by Lupron treatment, as control subjects actually showed decreased total ICV with age. Although previous studies describing normative rhesus macaque neurodevelopment have reported increases in total ICV at this age (Malkova, Heuer et al. 2006, Knickmeyer, Styner et al. 2010, Payne, Machado et al. 2010, Scott, Grayson et al. 2015), two studies in humans actually report that total ICV decreases during the comparable pre- to peripubertal ages described in this study (Lenroot and Giedd 2006, Brain Development Cooperative 2012). These studies support a potential reduction in brain volume due to puberty onset and the transition to early adolescence and a developmental delay in the Lupron-treated animals.

The HC volume increased with age, and Lupron-treatment resulted in smaller HC compared to controls. In normative development, after a drastic increase in volume during the first two postnatal weeks, the HC shows consistent increase in volume through two years of age in monkeys (Payne, Machado et al. 2010) and this is thought to be due to increased synaptogenesis, myelination and dendritic arborization (Duffy and Rakic 1983, Eckenhoff and Rakic 1991, Seress 1992, Jabes, Lavenex et al. 2011). This developmental increase in volume is consistent with our data, as HC volume increased from pre- to peripuberty. The Lupron-treatment volume reduction is likely due to protracted development in comparison to E2-related neurite growth and synaptogenesis/spines in the HC of controls, at least based on evidence in adult rat hippocampal CA1 pyramidal dendrites, where the number of dendritic spines and synapses are positively correlated with circulating levels of E2 (Woolley, Gould et al.

1990, Woolley and McEwen 1992). Additionally, ovariectomy in rats produces a decrease in HC spine density (Woolley and McEwen 1993). Although these studies were conducted in adult rats, this may be a potential mechanism also during adolescence. Another study investigated the effects of a GnRH agonist (Goserelin acetate) on gene expression in the HC (Nuruddin, Wojniusz et al. 2013). This study found that female sheep receiving GnRH agonist treatment (beginning just prior to puberty) showed upregulated HC gene expression in genes involved in neuroplasticity (among others). These changes in gene expression may be another putative mechanism explaining why Lupron-treated subjects had smaller HC than controls.

PFC GM in humans and macaques increases prior to puberty and begins to decline during peripuberty (Lenroot and Giedd 2006, Knickmeyer, Styner et al. 2010), following an inverted U-shaped curve from childhood until adulthood, reaching a peak during puberty (Giedd, Blumenthal et al. 1999, Giedd 2004, Giedd and Rapoport 2010). Although the cellular mechanisms underlying this reduction of GM are not completely understood, they seem due mostly to axonal/synaptic pruning (Anderson, Classey et al. 1995, Huttenlocher and Dabholkar 1997, Markham, Mullins et al. 2013) or even apoptosis in the PFC (Mor, Nilsen et al. 1999, Nunez, Lauschke et al. 2001, Markham, Morris et al. 2007). The normative developmental trajectory of PFC WM follows a different, linear, increase, which is thought to be the result of increased myelination in parallel GM pruning (Lenroot and Giedd 2006, Lenroot, Gogtay et al. 2007, Knickmeyer, Styner et al. 2010). E2 promotes myelination (Verdi and Campagnoni 1990, Jung-Testas, Renoir et al. 1992, Garcia-Segura, Chowen et al. 1996), but the role of E2 on normative GM

adolescent development is less understood, although some studies have shown that high levels of E2 during puberty in girls are linked to region-specific decreases in GM (Castellanos, Giedd et al. 1996, Gogtay, Giedd et al. 2004, Gogtay and Thompson 2010), notably, reduced volumes of PFC, anterior cingulate, occipitotemporal and parietal cortices (Nelson, Leibenluft et al. 2005, Koolschijn, Peper et al. 2014, Brouwer, Koenis et al. 2015, Herting, Gautam et al. 2015) likely due to E2-effects on synaptic pruning.

In this study, Lupron-treatment resulted in bigger PFC GM volumes than in controls, again supporting an E2 role on PFC synaptic pruning, and a sharp drop from pre- to peripuberty reflecting delayed development, while there was no significant difference in controls during this time period. An alternative mechanism underlying the decrease in PFC GM volume from pre- to peri-puberty could be hypoestrogenism-related decreases in dendritic arborization, as shown ovariectomized rodents (Kolb and Stewart 1991), although this evidence was generated in adult rats. An interesting alternative mechanism could be increased synaptic pruning regulated through E2 actions on microglia via estrogen receptors ERα and ERβ expressed in these glial cells (Takahashi, Tonchev et al. 2004, Garcia-Ovejero, Azcoitia et al. 2005, Liu, Fan et al. 2005, Sierra, Gottfried-Blackmore et al. 2008, Paolicelli, Bolasco et al. 2011, Wu, Tan et al. 2013)

WM volume in the PFC increased with age in control subjects, but not Lupron-treated subjects. As WM increases linearly with age (Lenroot and Giedd 2006, Lenroot, Gogtay et al. 2007, Knickmeyer, Styner et al. 2010) mainly explained by E2-promotion of myelination (Verdi and Campagnoni 1990, Jung-Testas, Renoir et al. 1992, Garcia-

Segura, Chowen et al. 1996), our findings may indicate that delayed puberty, induced by Lupron-treatment, may slow maturation of PFC WM due to hypoestrogenism-related attenuation of axonal myelination, as supported by evidence in the corpus callosum of ovariectomized adult mice (Patel, Moore et al. 2013). This study was conducted with adult mice, however, and hypoestrogensim may be affecting WM alterations differently prior to adulthood, during development.

While brain volumetric differences can be informative to understand the impact of stress and pubertal hormones on the development of cortico-limbic circuits, they are also meaningful in the context of associated functional outcomes. In this study, we found that smaller Left AMYG volume and Lupron-treatment were, in general, associated with increased emotional reactivity in the human intruder task, as evidenced by increased freezing, averting gaze and threats. The relation between AMYG volume and emotional reactivity may seem paradoxical, but we want to note here that it involves the Left and not the right AMYG, whose volume was bigger in subordinate animals. There is, indeed, literature highlighting the asymmetry of this structure, and lateralized effects of stress during its development, sometimes resulting in increased right AMYG volume, discussed above (Mehta, Golembo et al. 2009, Weems, Scott et al. 2013) (Tottenham, Hare et al. 2010), and sometimes in smaller left AMYG volume (Hanson, Nacewicz et al. 2015). Lupron-treated subjects also spent less time engaged in social play wrestle compared to controls. Few studies have investigated the effects of GnRH agonists on emotional behavior, but one previous study in sheep showed that, after prepubertal administration of a GnRH agonist (Goserelin acetate), females exhibited increased anxiety and avoidance

behavior compared to controls (Wojniusz, Vogele et al. 2011). Another study, in prepubertal female sheep treated with Goserelin acetate, showed alterations in AMYG gene expression, with several gene ontology groups emerging as being important. These included gene expression alterations in microtubules, immune response, ovarian cycle process, mitotic cell cycle, anti-apoptosis, ubiquitin binding and other gene ontology groups (Nuruddin, Krogenaes et al. 2013). These GnRH agonist-induced changes in both emotional behavior and gene expression are supported by the findings in this study, where Lupron-treatment resulted in smaller AMYG volume and increased emotional reactivity.

One of the most striking and unexpected results of this study were the effects of Lupron-treatment on brain and behavior before puberty. Although circulating levels of E2 are low yet contribute to the regulation of gonadotropin secretion in rhesus macaques prior to puberty (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), data from the present study indicate that these low levels are important for determining maturational changes in structural anatomy, given the differences observed in brain, PFC, AMYG and HC volumes between control and Lupron-treated females at prepuberty. These widespread Lupron-treatment effects are difficult to interpret due to the lack of studies investigating the developmental effects of pubertal delay with Lupron-treatment. Only one study has reported the effects of another GnRH agonist, Goserelin acetate, on peripubertal brain development, which reported an increase in AMYG volume in female sheep (Sex On Brain European Research Group, Nuruddin et al. 2013), which is the opposite effect to what is reported in this study. These discrepant findings may be due to methodological

differences between studies, the different drugs administered, the timing and duration of GnRH agonist treatment, the methods used to measure brain volume, and animal model. To our knowledge, no other studies have reported the effect of Lupron treatment on developing brain volume, so we propose several speculative mechanisms by which these alterations may occur. Lupron depot functions by binding to pituitary GnRH receptors, thereby downregulating GnRH receptors and desensitizing pituitary to endogenous GnRH to diminish gonadotropin secretion and subsequent ovarian secretion of E2 and ovarian maturation (Wilson, Meethal et al. 2007). Thus, as there is no evidence that Lupron crosses the blood brain barrier, indirect effects of Lupron (such as suppression of E2) may be impacting neuronal E2 receptors, which are expressed in each of the ROIs examined in this study (Gundlah, Kohama et al. 2000, McEwen 2001, Wang, Hara et al. 2010, Rettberg, Yao et al. 2014), potentially affecting myelination, axonal/synaptic pruning as well as neuronal soma or nucleus size (de Castilhos, Hermel et al. 2010). Alternatively, or in parallel, the effects of Lupron-treatment on brain structure may be occurring indirectly and in a region specific manner through actions on microglia –e.g. increasing synaptic pruning- (Takahashi, Tonchev et al. 2004, Garcia-Ovejero, Azcoitia et al. 2005, Liu, Fan et al. 2005, Sierra, Gottfried-Blackmore et al. 2008, Paolicelli, Bolasco et al. 2011, Wu, Tan et al. 2013, Ullewar and Umathe 2016) and oligodendrocytes -delaying PFC WM myelination- (Jung-Testas, Renoir et al. 1992, Marin-Husstege, Muggironi et al. 2004, Hirahara, Matsuda et al. 2009). While these central effects could be secondary to suppression of E2, there is also evidence that Lupron affects the peripheral immune system, independently of its reproductive effects (Ullewar and Umathe 2016). Taken together, Lupron-treatment and resulting

hypoestrogenism may lead to volumetric alterations in a number of direct and indirect ways, including altering neuronal soma and nucleus size, synaptogenesis or synaptic pruning, myelination, dendritic arborization, neuronal pruning and perhaps altering central or peripheral immune function.

It is important to discuss several limitations of this study. Using this particular study design, we are unable to rule out possible prenatal contributions, such as differences in maternal physiological function of dominant versus subordinate animals affecting neurobehavioral development of the offspring (Machado, Whitaker et al. 2014). Moreover, we need to be cautious when interpreting the developmental implications of these findings, as only two ages were examined. Lupron and rank-related differences reported here may (or may not) persist across development into adulthood, therefore, these effects need to be examined at later ages into adulthood. Additionally, several methodological issues need to be addressed. It is well established that the ROIs investigated in this paper can be divided into further functional sub-regions and nuclei. For example, the orbital PFC and the medial PFC are both included in what we define as the PFC ROI, and those two sub-divisions of the PFC have unique functional roles, characterized by differential connections with subcortical structures (Haber and Knutson 2010). The AMYG is also divided into functionally distinct sub-nuclei (Cardinal, Parkinson et al. 2002, Ulrich-Lai and Herman 2009, de la Mora, Gallegos-Cari et al. 2010), however, MRI scanning protocols lack the anatomical resolution necessary to clearly delineate these small regions. Lastly, causal effects of puberty at the peripubertal

scan cannot be determined, as some (n=15 out of 22) of the control subjects had begun the pubertal transition as measured by first menarche.

Although circulating levels of E2 are low prior to puberty, our findings indicate that even these low levels are important for maturational changes in brain structure and resulting stress physiology and behavior, because experimentally suppressing even low E2 levels resulted in profound structural and functional behavioral changes at pre-puberty. Our findings also suggest that delayed puberty may be protective against negative effects of subordination stress on several neurobehavioral outcomes (e.g. impact on AMYG volume). Future studies are necessary to describe additional brain regions affected by chronic developmental social stress and suppression of developmental increases in E2 and their underlying signals and mechanisms. These studies are important in a larger context, in particular given that the administration of Lupron to treat girls with precocious puberty may alter the developmental trajectory of these patients' brains with subsequent effects on behavioral and stress neuroendocrine phenotypes. Indeed, these findings may be particularly relevant for young girls who experience social stress, constitutional delays in puberty or whose puberty is arrested by pharmacological administration of Lupron. The overall phenotype of the Lupron-treated subjects warrants future elucidation, which may identify potential fundamental cellular mechanisms, perhaps independent of the HPA axis, underlying the structural brain effects. Finally, the neural and behavioral development of these subjects needs to be characterized past peripuberty, through puberty itself and into adulthood, to determine whether these functional alterations are enduring.

**Table 2.1 Summary of Results from rmANOVA on Brain ROIs.** Main and interaction effects of age, rank, Lupron-treatment and hemisphere are reported for each of the ROIs in the study: **Top:** Total ICV, AMYG and HC; **Bottom:** PFC (GM, WM, total volume). * and bold font indicate a significant *p* value at < 0.05.

| | **Total ICV** | | | | **Amygdala** | | | | **Hippocampus** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | Main Effect Direction |
| **Within Subjects Effects** | | | | | | | | | | | | |
| Age | 50.492 | **1.17E-08*** | 0.552 | **Pre < Peri** | 0.014 | 0.907 | 3.34E-04 | | 7.614 | **0.007*** | 0.157 | **Pre < Peri** |
| Age * Lupron Tx | 148.683 | **3.20E-15*** | 0.784 | | 0.034 | 0.855 | 0.001 | | 3.425 | 0.071 | 0.077 | |
| Age * Rank | 0.762 | 0.388 | 0.018 | | 0.340 | 0.563 | 0.008 | | 0.174 | 0.679 | 0.004 | |
| Age * Lupron Tx * Rank | 0.165 | 0.687 | 0.004 | | 3.172 | 0.082 | 0.072 | | 0.166 | 0.686 | 0.004 | |
| Hemisphere | | | | | 6.921 | **0.012*** | 0.144 | **Left < Right** | 17.422 | **1.52E-04*** | 0.298 | **Left < Right** |
| Hemisphere * Lupron Tx | | | | | 3.672 | 0.062 | 0.082 | | 2.991 | 0.091 | 0.068 | |
| Hemisphere * Rank | | | | | 0.005 | 0.944 | 1.20E-04 | | 0.725 | 0.399 | 0.017 | |
| Hemisphere * Lupron Tx * Rank | | | | | 4.615 | **0.038*** | 0.101 | | 0.382 | 0.540 | 0.009 | |
| Age * Hemisphere | | | | | 4.595 | **0.038*** | 0.101 | | 10.875 | **0.002*** | 0.210 | |
| Age * Hemisphere * Lupron Tx | | | | | 8.220 | **0.007*** | 0.167 | | 0.685 | 0.413 | 0.016 | |
| Age * Hemisphere * Rank | | | | | 0.006 | 0.938 | 1.51E-04 | | 0.672 | 0.417 | 0.016 | |
| Age * Hemisphere * Lupron Tx * Rank | | | | | 2.016 | 0.163 | 0.047 | | 0.469 | 0.497 | 0.011 | |
| **Between Subjects Effects** | | | | | | | | | | | | |
| Lupron Tx | 0.028 | 0.868 | 0.001 | | 5.055 | **0.030*** | 0.110 | **Control > Lupron** | 8.620 | **0.005*** | 0.174 | **Control > Lupron** |
| Rank | 0.021 | 0.885 | 0.001 | | 8.511 | **0.006*** | 0.172 | **Dom < Sub** | 2.163 | 0.149 | 0.050 | |
| Lupron Tx * Rank | 0.409 | 0.526 | 0.010 | | 0.001 | 0.973 | 2.85E-05 | | 0.817 | 0.371 | 0.020 | |

| | PFC GM | | | | PFC WM | | | | Total PFC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | Main Effect Direction |
| **Within Subjects Effects** | | | | | | | | | | | | |
| Age | 9.028 | **0.005*** | 0.180 | **Pre > Peri** | 18.042 | **1.21E-04*** | 0.306 | **Pre < Peri** | 1.078 | 0.305 | 0.026 | |
| Age * Lupron Tx | 6.713 | **0.013*** | 0.141 | | 3.239 | 0.079 | 0.073 | | 12.792 | **0.001*** | 0.238 | |
| Age * Rank | 0.641 | 0.428 | 0.015 | | 0.468 | 0.498 | 0.011 | | 0.246 | 0.622 | 0.006 | |
| Age * Lupron Tx * Rank | 0.634 | 0.431 | 0.015 | | 1.021 | 0.318 | 0.024 | | 0.110 | 0.742 | 0.003 | |
| Hemisphere | 17.614 | **1.42E-04*** | 0.301 | **Left < Right** | 19.045 | **8.40E-05*** | 0.317 | **Left > Right** | 4.313 | **0.044*** | 0.095 | **Left < Right** |
| Hemisphere * Lupron Tx | 2.339 | 0.134 | 0.054 | | 5.083 | **0.030*** | 0.110 | | 4.310 | **0.044*** | 0.095 | |
| Hemisphere * Rank | 1.200 | 0.280 | 0.028 | | 0.553 | 0.461 | 0.013 | | 1.412 | 0.242 | 0.033 | |
| Hemisphere * Lupron Tx * Rank | 1.115 | 0.297 | 0.026 | | 0.001 | 0.977 | 2.05E-05 | | 0.791 | 0.379 | 0.019 | |
| Age * Hemisphere | 1.329 | 0.256 | 0.031 | | 13.693 | **0.001*** | 0.250 | | 10.038 | **0.003*** | 0.197 | |
| Age * Hemisphere * Lupron Tx | 4.219 | **0.046*** | 0.093 | | 20.611 | **4.90E-05*** | 0.335 | | 19.881 | **6.30E-05*** | 0.327 | |
| Age * Hemisphere * Rank | 2.272 | 0.139 | 0.053 | | 0.012 | 0.915 | 2.83E-04 | | 1.987 | 0.166 | 0.046 | |
| Age * Hemisphere * Lupron Tx * Rank | 4.434 | **0.041*** | 0.098 | | 0.865 | 0.358 | 0.021 | | 1.808 | 0.186 | 0.042 | |
| **Between Subjects Effects** | | | | | | | | | | | | |
| Lupron Tx | 13.981 | **0.001*** | 0.254 | **Control < Lupron** | 3.406 | 0.072 | 0.077 | | 16.451 | **2.18E-04*** | 0.286 | **Control < Lupron** |
| Rank | 0.041 | 0.840 | 0.001 | | 0.192 | 0.663 | 0.005 | | 0.104 | 0.748 | 0.003 | |
| Lupron Tx * Rank | 0.571 | 0.454 | 0.014 | | 0.206 | 0.652 | 0.005 | | 0.322 | 0.573 | 0.008 | |

**Table 2.2 Summary of Results from rmANOVA on Cortisol and Behavioral Measures. (A)** Main and interaction) of sample collection time (pre, post), rank and Lupron-treatment, analyzed separately at pre- and peripuberty, are reported for cortisol changes in response to social separation and dexamethasone challenge. (**B).** Main and interaction effects of age, rank and Lupron-treatment are reported for baseline cortisol and behavioral measures (human intruder and social behavior). * and bold font indicate a significant *p* value at < 0.05. **Top:** Within subjects effects; **Bottom:** Between subjects effects.

| | A. | Within Subjects Effects | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sample Collection Time (Pre and Post) | | | | Sample Collection Time * Rank | | | Sample Collection Time * Lupron Tx | | | Sample Collection Time * Rank * Lupron Tx | | |
| | | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | F | *p* | $\eta^2_{partial}$ | F | *p* | $\eta^2_{partial}$ |
| Cortisol at Prepuberty | Dex Suppression | 31.918 | **1.36E-06*** | 0.438 | **Pre > Post** | 0.399 | 0.531 | 0.010 | 2.761 | 0.104 | 0.063 | 0.540 | 0.467 | 0.013 |
| | Social Separation | 228.874 | **2.24E-18*** | 0.848 | **Pre < Post** | 6.698 | **0.013*** | 0.140 | 0.007 | 0.932 | 0.000 | 6.648 | **0.014*** | 0.140 |
| Cortisol at Peripuberty | Dex Suppression | 45.096 | **4.16E-08*** | 0.524 | **Pre > Post** | 4.966 | **0.031*** | 0.108 | 0.395 | 0.533 | 0.010 | 0.001 | 0.974 | 2.61E-05 |
| | Social Separation | 243.617 | **7.49E-19*** | 0.856 | **Pre < Post** | 0.009 | 0.923 | 0.00023 | 0.001 | 0.975 | 2.48E-05 | 0.340 | 0.563 | 0.008 |

| | B. | Age | | | | Age * Rank | | | Age * Lupron Tx | | | Age * Rank * Lupron Tx | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | F | *p* | $\eta^2_{partial}$ | Main Effect Direction | F | *p* | $\eta^2_{partial}$ | F | *p* | $\eta^2_{partial}$ | F | *p* | $\eta^2_{partial}$ |
| Cortisol | Dex Cort Baseline | 25.937 | **8.32E-06*** | .387 | **Pre > Peri** | 5.456 | **0.024*** | .117 | 0.705 | 0.406 | 0.017 | 0.998 | 0.324 | 0.024 |
| Human Intruder | Freeze Alone | 30.088 | **2.33E-06*** | 0.423 | **Pre < Peri** | 0.258 | 0.614 | 0.006 | 4.584 | **0.038*** | 0.101 | 1.812 | 0.186 | 0.042 |
| | Freeze Profile | 0.461 | 0.501 | 0.011 | | 2.649 | 0.111 | 0.061 | 0.082 | 0.776 | 0.002 | 5.712 | **0.022*** | 0.122 |
| | Freeze Stare | 14.994 | **3.80E-04*** | 0.268 | **Pre < Peri** | 4.421 | **0.042*** | 0.097 | 9.347 | **0.004*** | 0.186 | 1.266 | 0.267 | 0.030 |
| | Avert Gaze Profile | 5.365 | **0.025*** | 0.116 | **Pre < Peri** | 0.048 | 0.828 | 0.001 | 1.522 | 0.224 | 0.036 | 0.298 | 0.588 | 0.007 |
| | Avert Gaze Stare | 0.046 | 0.831 | 0.001 | | 1.448 | 0.236 | 0.034 | 0.093 | 0.762 | 0.002 | 0.206 | 0.652 | 0.005 |
| | Coo Stare | 6.322 | **0.016*** | 0.134 | **Pre > Peri** | 4.50E-05 | 0.995 | 1.10E-06 | 5.46E-04 | 0.981 | 1.33E-05 | 0.019 | 0.890 | 4.71E-04 |
| | Threat Stare | 2.008 | 0.164 | 0.047 | | 2.644 | 0.112 | 0.061 | 0.079 | 0.780 | 0.002 | 0.028 | 0.867 | 6.89E-04 |
| | Lipsmack Stare | 10.724 | **0.002*** | 0.207 | **Pre > Peri** | 0.281 | 0.599 | 0.007 | 1.645 | 0.207 | 0.039 | 0.051 | 0.822 | 0.001 |
| | Locomote Alone | 19.718 | **6.64E-05*** | 0.325 | **Pre > Peri** | 0.077 | 0.783 | 0.002 | 0.178 | 0.675 | 0.004 | 1.512 | 0.226 | 0.036 |
| | Locomote Profile | 0.175 | 0.678 | 0.004 | | 0.001 | 0.975 | 2.51E-05 | 1.767 | 0.191 | 0.041 | 0.090 | 0.766 | 0.002 |
| | Locomote Stare | 2.520 | 0.120 | 0.058 | | 0.068 | 0.796 | 0.002 | 8.386 | **0.006*** | 0.170 | 0.002 | 0.962 | 5.48E-05 |
| | Crouch Alone | 0.178 | 0.675 | 0.004 | | 0.098 | 0.756 | 0.002 | 1.560 | 0.219 | 0.037 | 1.466 | 0.233 | 0.035 |
| | Crouch Profile | 2.375 | 0.131 | 0.055 | | 0.660 | 0.421 | 0.016 | 0.534 | 0.469 | 0.013 | 0.481 | 0.492 | 0.012 |
| | Crouch Stare | 0.710 | 0.404 | 0.017 | | 0.147 | 0.703 | 0.004 | 0.448 | 0.507 | 0.011 | 0.124 | 0.727 | 0.003 |
| Social Behavior | Affiliation | 1.990 | 0.166 | 0.046 | | 0.426 | 0.517 | 0.010 | 0.066 | 0.799 | 0.002 | 0.141 | 0.709 | 0.003 |
| | Agression | 0.858 | 0.360 | 0.020 | | 0.014 | 0.906 | 3.41E-04 | 0.266 | 0.609 | 0.006 | 1.184 | 0.283 | 0.028 |
| | Submission | 3.935 | 0.054 | 0.088 | | 0.646 | 0.426 | 0.016 | 0.873 | 0.355 | 0.021 | 0.047 | 0.830 | 0.001 |
| | Play Wrestle | 0.051 | 0.823 | 0.001 | | 0.113 | 0.739 | 0.003 | 6.143 | **0.017*** | 0.130 | 0.168 | 0.684 | 0.004 |
| | Self Play | 2.687 | 0.109 | 0.063 | | 0.718 | 0.402 | 0.018 | 0.505 | 0.482 | 0.012 | 4.324 | **0.044*** | 0.098 |
| | Anxiety | 9.124 | **0.004*** | 0.186 | **Pre < Peri** | 2.493 | 0.122 | 0.059 | 2.978 | 0.092 | 0.069 | 0.071 | 0.791 | 0.002 |
| | Sit Alone | 3.455 | 0.070 | 0.080 | | 1.829 | 0.184 | 0.044 | 1.63E-04 | 0.990 | 4.07E-06 | 0.214 | 0.646 | 0.005 |

| | | Between Subjects Effects | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Rank | | | | Lupron Tx | | | | Rank * Lupron Tx | | |
| | A. | F | *p* | $\eta^2$ *partial* | Main Effect Direction | F | *p* | $\eta^2$ *partial* | Main Effect Direction | F | *p* | $\eta^2$ *partial* |
| Cortisol at Prepuberty | Dex Suppression | 1.577 | 0.216 | 0.037 | | 2.894 | 0.096 | 0.066 | | 1.885 | 0.177 | 0.044 |
| | Social Separation | 0.001 | 0.972 | 3.10E-05 | | 0.043 | 0.836 | 0.001 | | 0.612 | 0.439 | 0.015 |
| Cortisol at Peripuberty | Dex Suppression | 1.269 | 0.267 | 0.030 | | 1.831 | 0.183 | 0.043 | | 2.126 | 0.152 | 0.049 |
| | Social Separation | 0.329 | 0.569 | 0.008 | | 44.266 | **5.10E-08*** | 0.519 | **Con > Lup** | 2.259 | 0.140 | 0.052 |
| | **B.** | **Rank** | | | | **Lupron Tx** | | | | **Rank * Lupron Tx** | | |
| | | F | *p* | $\eta^2$ *partial* | Main Effect Direction | F | *p* | $\eta^2$ *partial* | Main Effect Direction | F | *p* | $\eta^2$ *partial* |
| Cortisol | Dex Cort Baseline | 3.337 | 0.075 | 0.075 | | 0.650 | 0.425 | 0.016 | | 2.019 | 0.163 | 0.047 |
| Human Intruder | Freeze Alone | 0.756 | 0.390 | 0.018 | | 2.756 | 0.104 | 0.063 | | 0.305 | 0.584 | 0.007 |
| | Freeze Profile | 0.003 | 0.954 | 8.25E-05 | | 0.015 | 0.905 | 3.55E-04 | | 5.48E-05 | 0.994 | 1.34E-06 |
| | Freeze Stare | 6.989 | **0.012*** | 0.146 | **Dom > Sub** | 3.708 | 0.061 | 0.083 | | 5.009 | **0.031*** | 0.109 |
| | Avert Gaze Profile | 0.007 | 0.932 | 1.81E-04 | | 1.484 | 0.230 | 0.035 | | 1.398 | 0.244 | 0.033 |
| | Avert Gaze Stare | 0.082 | 0.776 | 0.002 | | 21.185 | **3.98E-05*** | 0.341 | **Con < Lup** | 0.019 | 0.890 | 4.70E-04 |
| | Coo Stare | 0.119 | 0.731 | 0.003 | | 0.087 | 0.769 | 0.002 | | 0.028 | 0.868 | 6.82E-04 |
| | Threat Stare | 5.136 | **0.029*** | 0.111 | **Dom < Sub** | 0.508 | 0.480 | 0.012 | | 1.216 | 0.276 | 0.029 |
| | Lipsmack Stare | 0.111 | 0.741 | 0.003 | | 2.229 | 0.143 | 0.052 | | 1.292 | 0.262 | 0.031 |
| | Locomote Alone | 0.288 | 0.595 | 0.007 | | 0.800 | 0.376 | 0.019 | | 0.104 | 0.749 | 0.003 |
| | Locomote Profile | 0.200 | 0.657 | 0.005 | | 0.016 | 0.900 | 3.93E-04 | | 0.383 | 0.539 | 0.009 |
| | Locomote Stare | 0.375 | 0.544 | 0.009 | | 2.389 | 0.130 | 0.055 | | 0.020 | 0.888 | 4.88E-04 |
| | Crouch Alone | 2.021 | 0.163 | 0.047 | | 0.209 | 0.650 | 0.005 | | 1.039 | 0.314 | 0.025 |
| | Crouch Profile | 0.504 | 0.482 | 0.012 | | 1.824 | 0.184 | 0.043 | | 0.001 | 0.973 | 2.84E-05 |
| | Crouch Stare | 1.525 | 0.224 | 0.036 | | 3.783 | 0.059 | 0.084 | | 3.116 | 0.085 | 0.071 |
| Social Behavior | Affiliation | 0.014 | 0.906 | 3.43E-04 | | 0.009 | 0.926 | 2.10E-04 | | 3.403 | 0.072 | 0.077 |
| | Agression | 9.304 | **0.004*** | 0.185 | **Dom > Sub** | 0.603 | 0.442 | 0.014 | | 1.274 | 0.266 | 0.030 |
| | Submission | 18.203 | **1.14E-04*** | 0.307 | **Dom < Sub** | 3.207 | 0.081 | 0.073 | | 2.484 | 0.123 | 0.057 |
| | Play Wrestle | 0.991 | 0.325 | 0.024 | | 2.643 | 0.112 | 0.061 | | 0.066 | 0.799 | 0.002 |
| | Self Play | 0.546 | 0.464 | 0.013 | | 1.043 | 0.313 | 0.025 | | 2.574 | 0.116 | 0.060 |
| | Anxiety | 0.046 | 0.832 | 0.001 | | 0.671 | 0.417 | 0.017 | | 0.894 | 0.350 | 0.022 |
| | Sit Alone | 5.921 | **0.020*** | 0.129 | **Dom < Sub** | 0.112 | 0.740 | 0.003 | | 1.658 | 0.205 | 0.040 |

**Table 2.3 Results from Stepwise Multiple Regression Analysis.** Dependent variables were stress-physiology (cortisol), behavioral reactivity to the HI and social behavior outcomes. Candidate predictor variables were right and left AMYG, HC, PFC GM, PFC WM, social rank and Lupron-treatment. Models that contained only rank and Lupron-treatment effects (without a brain ROI entering into the model) were excluded, as to not duplicate the results of the repeated measures ANOVA conducted on behavior and stress physiology. If more than one model was identified at a significance level of $p < 0.05$, the model that accounted for the most variance was selected, provided the condition index did not exceed 30 (to exclude multicollinear models). Measures at prepuberty are represented in white and measures at peripuberty are represented in gray. Models notated with [a] violated one or more statistical test assumptions of normality, linearity, homogeneity of variance.

| | | Dependent Variable | Overall Model Adj. $R^2$ | F | p | Retained Predictor Variables | Predictor Variables B | Std. Error | β | t | p |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cortisol | Age 1; Prepuberty | Δ Cortisol - SS | No brain regions were entered | | | | | | | | |
| | | Δ Cortisol - Dex Suppression | No variables were entered - non significant | | | | | | | | |
| | Age 2; Peripuberty | Δ Cortisol - SS | No variables were entered - non significant | | | | | | | | |
| | | Δ Cortisol - Dex Suppression | No brain regions were entered | | | | | | | | |
| Human Intruder | Age 1; Prepuberty | Freeze - Alone | 0.301 | 10.480 | 0.0002 | Left Amygdala | -693.346 | 258.650 | -0.371 | -2.681 | 0.010 |
| | | | | | | Lupron Treatment | 0.424 | 0.186 | 0.315 | 2.280 | 0.028 |
| | | Freeze - Profile | No variables were entered - non significant | | | | | | | | |
| | | Freeze - Stare | No variables were entered - non significant | | | | | | | | |
| | | Avert Gaze - Stare | 0.279 | 9.495 | 0.0004 | Lupron Treatment | 1.546 | 0.641 | 0.339 | 2.411 | 0.020 |
| | | | | | | Left Amygdala | -2065.379 | 891.836 | -0.325 | -2.316 | 0.026 |
| | | Threat - Stare | 0.183 | 5.929 | 0.005 | Rank | 4.600 | 1.453 | 0.450 | 3.165 | 0.003 |
| | | | | | | Right Amygdala | -5388.518 | 2454.857 | -0.312 | -2.195 | 0.034 |
| | | Locomote - Stare | 0.241 | 7.966 | 0.001 | Lupron Treatment | -0.309 | 0.093 | -0.436 | -3.317 | 0.002 |
| | | | | | | Right Amygdala | 353.135 | 158.094 | 0.293 | 2.234 | 0.031 |
| | Age 2; Peripuberty | Freeze - Alone | No variables were entered - non significant | | | | | | | | |
| | | Freeze - Profile | No variables were entered - non significant | | | | | | | | |
| | | Freeze - Stare | No brain regions were entered | | | | | | | | |
| | | Avert Gaze - Stare | No brain regions were entered | | | | | | | | |
| | | Threat - Stare | No variables were entered - non significant | | | | | | | | |
| | | Locomote - Stare | 0.088 | 5.253 | 0.027 | Right Amygdala | 382.583 | 166.925 | 0.330 | 2.292 | 0.027 |
| Social Behavior | Age 1; Prepuberty | Aggression | No brain regions were entered | | | | | | | | |
| | | Submission | No brain regions were entered | | | | | | | | |
| | | Play Wrestle | No brain regions were entered | | | | | | | | |
| | | Self Play | No variables were entered - non significant | | | | | | | | |
| | | Sit Alone | 0.229 | 14.103 | 0.001 | Left Hippocampus [a] | 1154.467 | 307.414 | 0.497 | 3.755 | 0.001 |
| | Age 2; Peripuberty | Aggression | 0.129 | 7.489 | 0.009 | Left PFC GM [a] | -107.662 | 39.341 | -0.385 | -2.737 | 0.009 |
| | | Submission | No brain regions were entered | | | | | | | | |
| | | Play Wrestle | No variables were entered - non significant | | | | | | | | |
| | | Self Play | No variables were entered - non significant | | | | | | | | |
| | | Sit Alone | 0.101 | 5.928 | 0.019 | Left Hippocampus [a] | 688.181 | 282.638 | 0.348 | 2.435 | 0.019 |

**Figure 2.1 Timeline of experimental design.** The diagram shows the measures collected in this study for both control (black) and Lupron-treated subjects (gray) and the mean age (in months) at data collection.

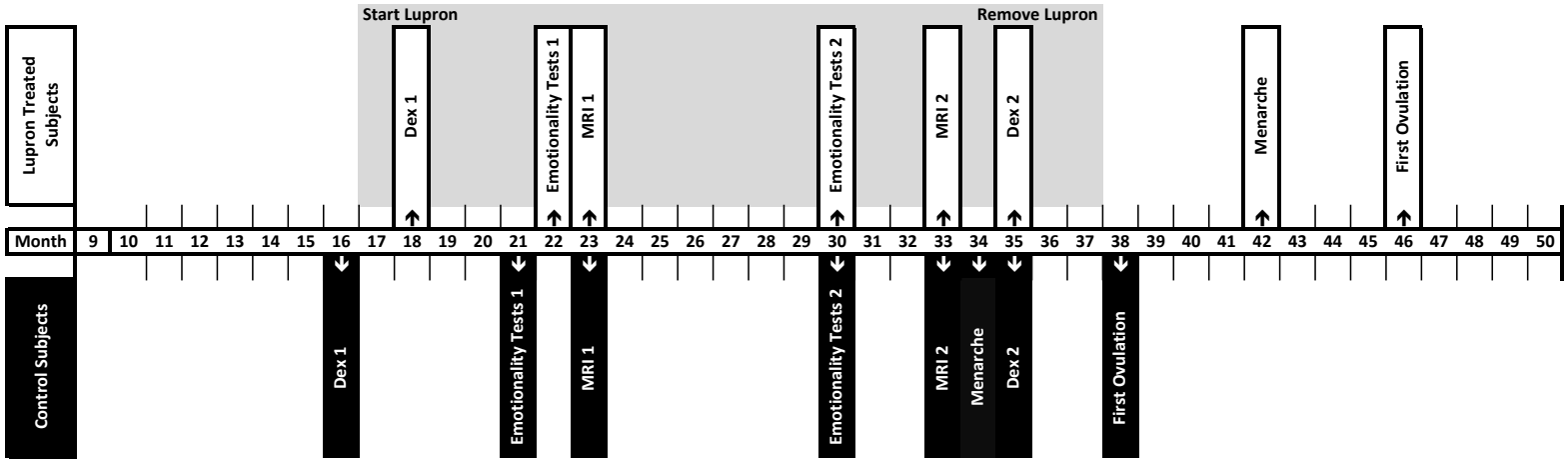
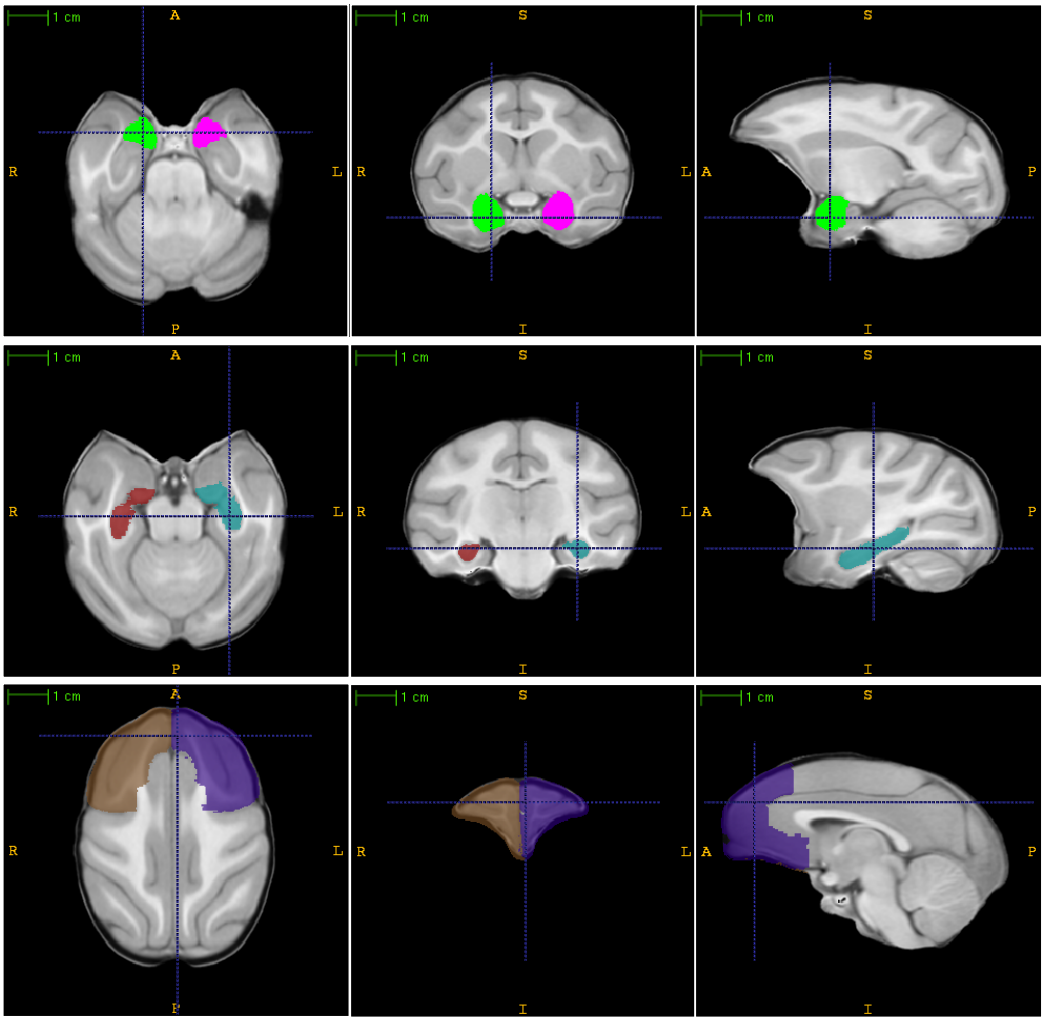


**Figure 2.2 (A) AMYG, HC and PFC regions of interest (ROIs), used in the Autoseg pipeline** (Styner, Knickmeyer et al. 2007, Howell, Grand et al. 2014)**.** From left to right: axial, coronal and saggital views. **(Top Row)** Right (green) and left (fuschia) AMYG ROIs; **(Middle Row)** Right (red) and left (blue) HC ROIs; **(Bottom Row)** Right (brown) and left (purple) PFC ROIs. **(B) Example of automatic segmentation of GM, WM and CSF tissue classes using the Autoseg pipeline.** From left to right: axial, coronal and saggital views. Green=GM, Red=WM and Blue=CSF.

**A.**



**B.**

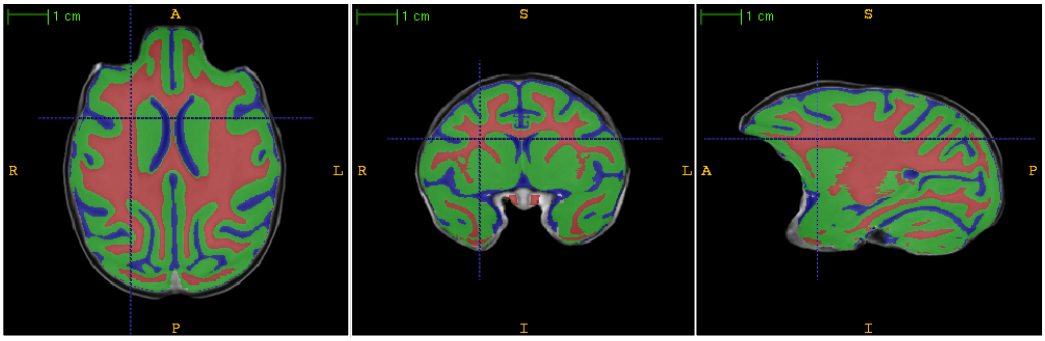


**Figure 2.3 Effects E2 suppression (Lupron-treatment) on total ICV at pre- and peripuberty.** An age by Lupron-treatment interaction [F(1, 41)=148.683, *p*=3.203E-15; η2 partial=0.784] emerged, which indicated that, despite the lack of post-hoc significant differences between Lupron-treated subjects and controls at either pre- or peripuberty, Lupron-treated animals showed an increase (*p*=5.708E-17), while control subjects showed a decrease in total ICV with age (*p*=0.001). **a**, significantly different from Lupron prepuberty, **b**, significantly different from Control prepuberty.

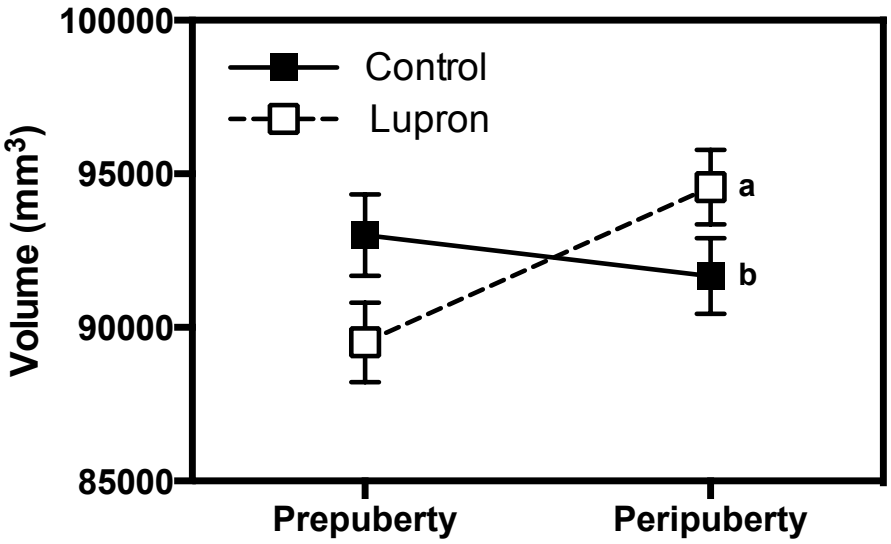


**Figure 2.4 Subordinates have larger AMYG volume than dominant subjects, but Lupron-treatment neutralizes this effect. (a)** Lupron-treatment by rank by hemisphere interaction effect [$F(1, 41)=4.615$, $p=0.038$; $\eta 2$ partial=0.101], where subordinates had larger AMYG volume compared to dominants, both in control and Lupron-treated groups, but depending on hemisphere (right: rank effect only in the control group; $p=0.009$; left: rank effect only in the Lupron group ($p=0.020$)). Interestingly, Lupron-treatment prevented social subordination related effects in the right AMYG. Additionally, dominant controls had larger AMYG volume than dominant Lupron-treated subjects, but only in the left hemisphere ($p=0.017$). **(b)** Age by Lupron-treatment by hemisphere interaction effect [$F(1, 41)=8.220$, $p=0.007$; $\eta 2$ partial=0.167], where Lupron-treated subjects had smaller AMYG volume in the left hemisphere, but only at pre-puberty ($p=0.002$). *, $p \leq 0.05$; **, $p \leq 0.01$.

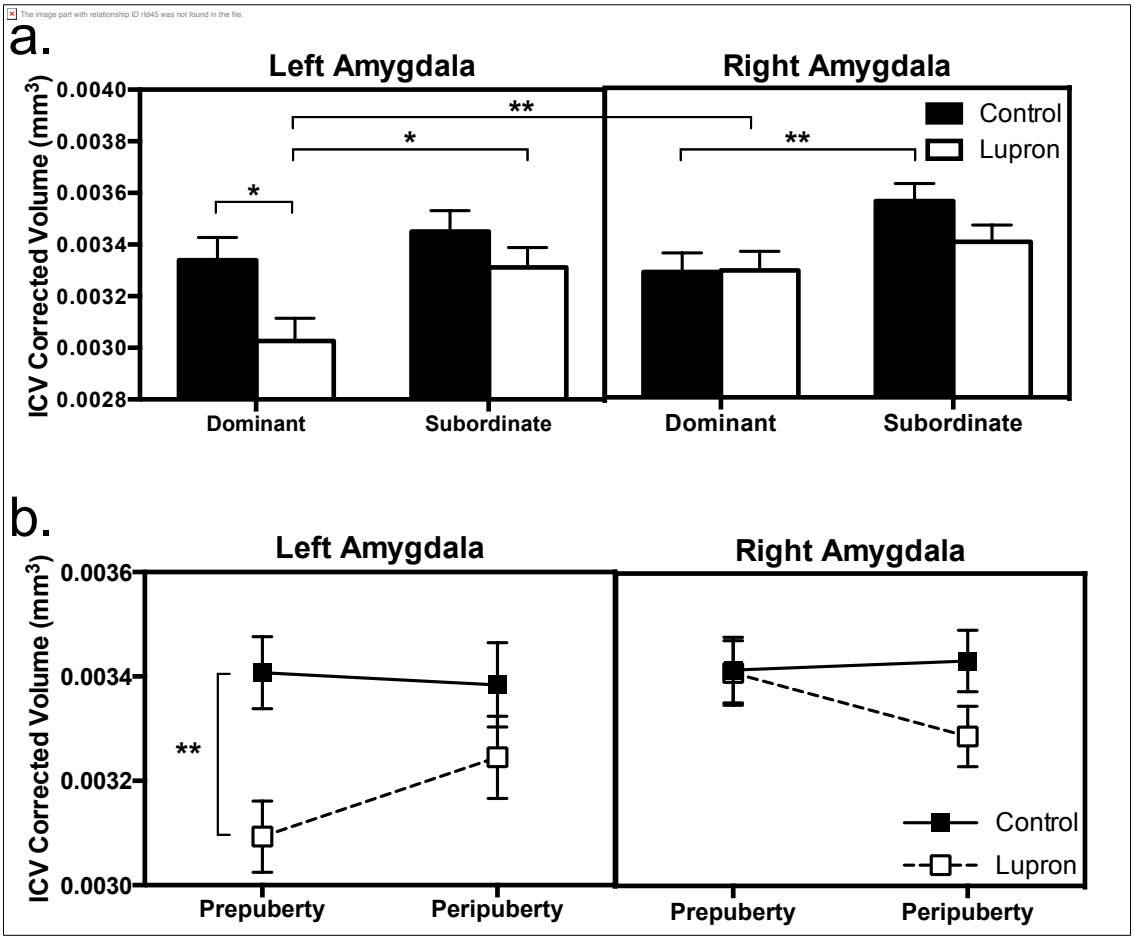

a.
Left Amygdala
Right Amygdala
ICV Corrected Volume (mm3)
0.0040
0.0038
0.0036
0.0034
0.0032
0.0030
0.0028
Control
Lupron
*
*
**
**
Dominant
Subordinate
Dominant
Subordinate
b.
Left Amygdala
Right Amygdala
ICV Corrected Volume (mm3)
0.0036
0.0034
0.0032
0.0030
**
Control
Lupron
Prepuberty
Peripuberty
Prepuberty
Peripuberty

**Figure 2.5 Controls have larger hippocampal volume than Lupron-treated subjects.** **(a)** Main effect of Lupron-treatment, with controls having larger volume than Lupron-treated subjects [F(1, 41)=8.620, $p$=0.005; η2 partial=0.174]. **(b)** There was also a main age effect, with HC volume increasing from pre- to peripuberty [$F$(1, 41)=7.614, $p$=0.009; $\eta^2_{partial}$=0.157], and an age by hemisphere interaction effect [F(1, 41)=10.875, $p$=0.002; η2 partial=0.210], with larger right than left volume at prepuberty only ($p$=0.000009). None of these age effects were affected by social status or Lupron-treatment. **, $p \leq 0.01$, ***, $p \leq 0.001$; ****, $p \leq 0.0001$.

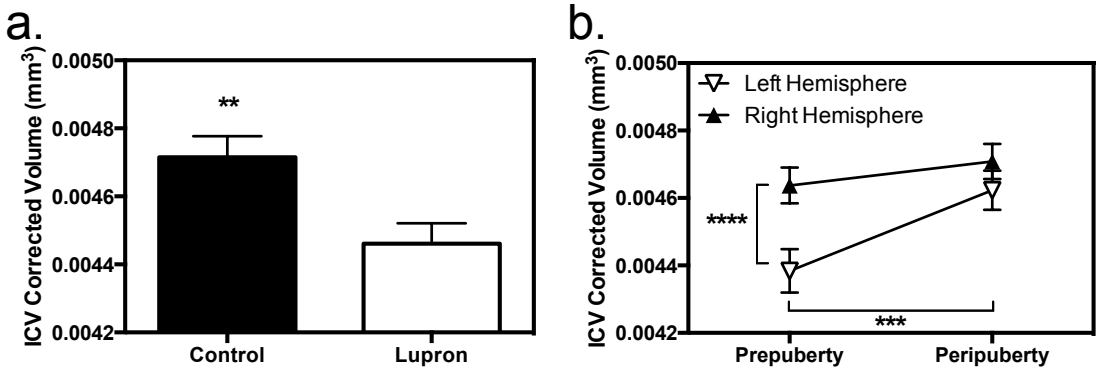
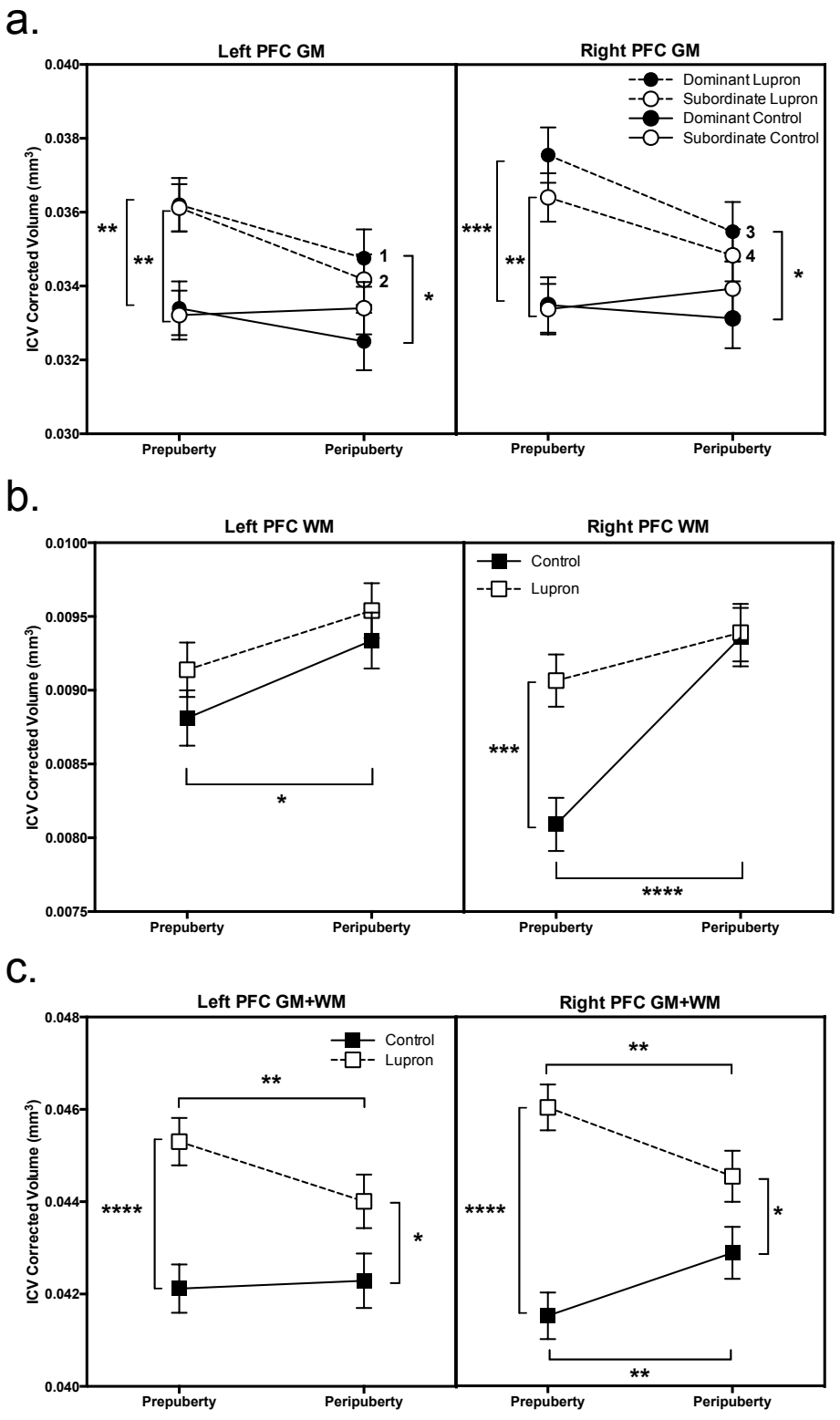


**Figure 2.6 (a) Lupron increases PFC GM volume in a hemisphere and rank dependent manner.** An age by hemisphere by Lupron-treatment by rank interaction [F(1, 41)=4.434, $p$= 0.041; η2 partial=0.098] revealed that dominant Lupron-treated subjects had larger PFC GM volume in both right hemisphere at pre- ($p$=0.0004) and peripuberty ($p$=0.045) and left hemisphere at pre- ($p$=0.009) and peripuberty ($p$=0.047) compared to dominant controls. Subordinate Lupron-treated subjects had larger PFC GM volume in right ($p$=0.003) and left ($p$=0.003) hemispheres, but only at prepuberty, compared to subordinate controls subjects. **(b) PFC WM increases with age, an effect driven by control subjects.** This age by hemisphere by Lupron-treatment interaction [F(1, 41)=20.611, $p$=0.00005; η2 partial=0.335] showed that control subjects had an increase in PFC WM volume with age in both hemispheres (right hemisphere, $p$=0.000004; left hemisphere, $p$=0.013), whereas Lupron-treated subjects did not (although left hemisphere Lupron-treated subjects showed a trend ($p$=0.051) in this direction). Additionally, Lupron-treated subjects had larger PFC WM volume in the right hemisphere and at prepuberty only ($p$=0.0004). **(c) Lupron increases total PFC volume.** In this graph illustrating the three-way interaction between age, hemisphere and Lupron-treatment, the main effect that emerged as significant is evident, with Lupron-treated subjects having larger total PFC volume than controls [$F$(1, 41)=16.451, $p$= 0.0002; η2 *partial*=0.286]. Additionally, both right and left hemisphere volume decreased with age in Lupron-treated subjects, whereas total PFC volume increased in control subjects, but only in the right hemisphere ($p$=0.006). *, $p \leq 0.05$; **, $p \leq 0.01$, ***, $p \leq 0.001$; ****, $p \leq 0.0001$. **1**, significantly different from left dominant Lupron at prepuberty, **2**, significantly different from left subordinate Lupron at prepuberty, **3**,

significantly different from right dominant Lupron at prepuberty, **4**, significantly different from right subordinate Lupron at prepuberty.



# Chapter 3. Chronic Social Stress and Consumption of a Palatable Diet Alter Brain Structure and Behavior in Female Rhesus Macaques

## 3.1 Abstract

High caloric diet-induced obesity has become a significant worldwide health problem and results in increased risk for numerous adverse health outcomes, including type 2 diabetes, cancer, heart disease, among other others. Additionally, chronic social stressor exposure can increase the drive to consume these palatable foods, further contributing to an obese phenotype. Data suggest that obesity resulting from stress-induced overeating may compromise brain structure and function, potentially leading to emotional dysregulation and behavioral impairment. However, these impairments in brain structural and functional outcomes may emerge before significant body fat accumulates, suggesting that consuming an obesogenic diet itself may have adverse effects on the neurobehavioral outcomes. Social subordination in female rhesus macaque monkeys is an established translational animal model to study a number of stress-related phenotypes, including stress-induced over eating of a palatable diet. Thus, we sought to investigate how social status interacts with dietary environment to affect brain structure of corticolimbic regions, specifically, the amygdala (AMYG), prefrontal cortex (PFC), insula (INS) and hippocampus (HC), known to regulate emotional reactivity and motivated behavior in adult female rhesus macaques. The impact of diet on neurobehavioral outcomes was assessed in females who had been maintained on a low fat, low sugar chow diet (low caloric diet only -LCD-only-) their entire lives and females who had been consuming this chow diet in combination with a high fat, high sugar diet (choice diet -CH-) for one year. We found a significant impact of the CH diet on regional brain structure, with number of calories consumed both positively and negatively predicting region-specific differences in brain volume, whereas calorie intake did not predict any variance in regional brain

volumes for females in the LCD diet condition. Specifically, in the CH subjects, social status positively predicted prefrontal cortex (PFC) white matter (WM) and dorsolateral PFC (dlPFC) volumes, whereas in the LCD-only subjects, social status negatively predicted PFC WM, ventromedial PFC (vmPFC), amygdala (AMYG), insular cortex gray matter (INS GM) and hippocampal (HC) volumes. Interestingly, exposure to the CH diet reversed the direction of the dominance rank effects in bilateral PFC WM. A functional outcomes analysis revealed that in the CH subjects, larger left dlPFC and smaller left PFC GM volumes predicted increased affiliative behavior. In the LCD-only subjects, more subordinate rank predicted larger left AMYG and left INS GM volumes, which resulted in decreased anxious-aggressive and increased submissive-fearful behavior, respectively. Overall, the results of the present study revealed that social status differentially affects regional brain volumes, depending on dietary condition. Additionally, the impact of diet on regional brain volumes only becomes evident when a CH dietary environment is available. Taken together, these data suggest that considerably different phenotypes emerge in rhesus macaques with access to either a prudent LCD or a more complex dietary environment where a choice between LCD and high sugar, high fat diet is available. These results underscore the view that calories derived from diets high in sugar and fat impact the brain and behavior differently than carioles derived from healthier low fat and sugar diets.

## 3.2 Introduction

Excess body weight and obesity have become significant worldwide health problems and result in increased risk for several adverse health outcomes, including type 2 diabetes, cancer, heart disease, and others (Must, Spadano et al. 1999, Visscher and Seidell 2001, Hill 2006). Globally, the number of overweight and obese people has risen from 921 million in 1980 to an astounding 2.1 billion in 2013 (Ng, Fleming et al. 2014), which results in substantial increased healthcare costs and economic burden (Tsai, Williamson et al. 2011, Withrow and Alter 2011). In the United States, rates of obesity have continued to rise over the last 20 years to greater than 30% of the population and are expected to increase to 50% by 2030 (Flegal, Carroll et al. 2010). Given these alarming statistics, it is becoming increasingly essential to understand the etiology of overweight and obesity. However, equally important is to determine how the consumption of obesogenic diets before frank obesity is evident, affects the neurobehavioral health of people.

The availability of highly palatable foods, rich in fat and sugar, can cause an individual's energy intake to exceed its energy expenditure, leading to excess weight gain, as evidenced by the vast literature on diet-induced obesity (Keenan, Wallig et al. 2013). Additionally, experiential factors, including chronic social stressor exposure, can increase the drive to consume these palatable foods, further contributing to an obese phenotype (Oliver, Wardle et al. 2000, Epel, Lapidus et al. 2001, Gibson 2006). Importantly, data suggest that obesity resulting from stress-induced overeating may compromise brain structure and function in humans, monkeys and rodents (Berthoud, Lenard et al. 2011,

Tryon, Carter et al. 2013, Ulrich-Lai, Fulton et al. 2015, Michopoulos, Diaz et al. 2016), potentially leading to emotional dysregulation and behavioral impairment in humans (Onyike, Crum et al. 2003, Galvez, Bauer et al. 2014). Indeed, mood disorders and anxiety are frequently co-morbid with obesity (Ayensa and Calderon 2011, Capuron, Lasselin et al. 2016), especially in females (Zender and Olshansky 2009, Hillman, Dorn et al. 2010), suggesting obesity-induced impaired neurobehavioral function. However, these impairments in brain structure and functional outcomes may emerge before significant body fat accumulates, suggesting that consuming an obesogenic diet itself may have adverse effects on the neurobehavioral outcomes (Beilharz, Maniam et al. 2015, Morris, Beilharz et al. 2015).

Because obesity is also characterized by hypercortisolemia (Pasquali, Ambrosi et al. 2002) and sustained intake of an obesogenic diet increases glucocorticoid (GC) secretion in response to a stressor (Pasquali, Ambrosi et al. 2002, Legendre and Harris 2006, Michopoulos, Toufexis et al. 2012), elevated GCs may be key biological signals mediating the adverse consequences of both stress and increased fat mass on neurobehavioral outcomes. In addition, proinflammatory cytokines are likely important mediators of both stress and diet on neurobehavioral outcomes. Chronic inflammation is not only a hallmark of obesity (Gregor and Hotamisligil 2011, Miller and Spencer 2014), but consumption of obesogenic diets increases both peripheral and central measures of inflammation prior to the onset of obesity (Myles 2014, Vasconcelos, Cabral-Costa et al. 2016). Furthermore, persistent inflammation is positively associated with chronic stress (Miller and Blackwell 2006, Cohen, Janicki-Deverts et al. 2012) and resulting adverse

behavioral outcomes such as depression (Dunn, Swiergiel et al. 2005, Hryhorczuk, Sharma et al. 2013).

Diet-induced obesity and resulting elevated BMI have also been linked to alterations in brain structure and function. The general findings include smaller total brain volume and reduced volume of cortical and subcortical structures. For example, several studies have reported smaller total brain volume in obese subjects with higher BMI, compared to controls (Ward, Carlsson et al. 2005, Gunstad, Paul et al. 2008, Debette, Beiser et al. 2010, Bobb, Schwartz et al. 2014). These differences may be driven by global decreases in gray matter (GM) (Figley, Asem et al. 2016), white matter (WM) (Cole, Boyle et al. 2013) or both (Figley, Asem et al. 2016), as well as CSF. Additionally, diet-induced obesity has also been associated with GM reductions in cortical regions [such as prefrontal cortex (PFC) and insular cortex (INS)], as well as sub-cortical regions [including the hippocampus (HC) and amygdala (AMYG)]. Specifically, increased visceral adipose tissue has been associated with decreased cortical thickness of the right INS (Veit, Kullmann et al. 2014) and decreased INS GM (Shott, Cornier et al. 2015). Moreover, diet-induced obesity is also negatively associated with volume of PFC subdivisions, such as dorsolateral (dlPFC), orbitofrontal (OFC) and medial PFC (mPFC) (Pannacciulli, Del Parigi et al. 2006, Walther, Birdsill et al. 2010, Brooks, Benedict et al. 2013, Marques-Iturria, Pueyo et al. 2013, Shott, Cornier et al. 2015, Figley, Asem et al. 2016). Increased BMI has also been associated with decreased HC (Raji, Ho et al. 2010, Walther, Birdsill et al. 2010) and AMYG (Figley, Asem et al. 2016) volumes. In contrast, a few studies have reported opposite directionality of effects of obesity on

corticolimbic regional volume. For example, one study in older women reported increased frontal WM volume (Walther, Birdsill et al. 2010). Another study reported positive correlations between BMI and OFC volume (Horstmann, Busse et al. 2011). While most studies focus on obesity-induced changes in brain structure, one study investigated how different diets affected HC volume in a mixed population of normal weight and overweight subjects. Results showed consumption of a prudent diet was associated with larger HC volume (Jacka, Cherbuin et al. 2015), similar to what is observed in normal weight vs. obese humans described above. Thus, the data suggest that obesity has an impact on brain GM and WM volume, but the impact of dietary condition prior to the onset of frank obesity is not fully understood.

Chronic stress (including social subordination stress in the macaque model) also alters brain structure, particularly of corticolimbic circuits and social brain networks, including the AMYG, PFC, INS and HC. Indeed, these limbic regions have a high density of glucocorticoid receptors (GR) (Sanchez, Young et al. 2000, Ulrich-Lai and Herman 2009), which can explain their high susceptibility to chronic stress. For example, the AMYG is sensitive to the effects of stress and GCs, and it also plays a critical role in social behavior (Bickart, Dickerson et al. 2014). Indeed, chronic stress-induced hypercortisolemia (Raadsheer, Hoogendijk et al. 1994, Makino, Smith et al. 1995, Makino, Hashimoto et al. 2002, Myers, McKlveen et al. 2012) or direct administration of glucocorticoids into the AMYG leads to hyper-excitability of the AMYG in rodents (Rosenkranz, Venheim et al. 2010) and increased dendritic arborization and/or density of dendritic spines within the AMYG, resulting in bigger AMYG volumes (Vyas, Mitra et

al. 2002, Vyas, Bernal et al. 2003, Mitra, Jadhav et al. 2005, Vyas, Jadhav et al. 2006, Mitra and Sapolsky 2008, Rosenkranz, Venheim et al. 2010, Eiland, Ramroop et al. 2012). Additionally, AMYG GM volume has been positively associated with social rank in rhesus macaques, where more subordinate monkeys had smaller AMYG volume in males (Noonan, Sallet et al. 2014). The PFC, which is critical for regulating socioemotional behavior (Quirk and Beer 2006) and mediating social cognition (Amodio and Frith 2006), is also structurally affected by social stress and social group size in humans, rhesus macaques and rodents (Sallet, Mars et al. 2011, Noonan, Sallet et al. 2014). For example, rostral PFC GM volume in macaques has been found to increase with social rank and social network size (Noonan, Sallet et al. 2014). In humans, reduced OFC volume has been observed in women experiencing chronic stress (Gianaros, Jennings et al. 2007) and lower subjective social status is associated with reduced GM volume in the anterior cingulate cortex (ACC) (Gianaros, Horenstein et al. 2007). In rodents, chronic restraint stress is associated with decreased dendritic arborization in the mPFC (Shansky and Morrison 2009) and increased neuronal excitability (Jackson and Moghaddam 2006). Chronic administration of dexamethasone or corticosterone in adrenalectomized rats results in both reduced volume and loss of neurons in the PFC, respectively (Cerqueira, Pego et al. 2005). The INS integrates interoceptive, socio-emotional, sensorimotor, olfacto-gustatory and cognitive information (Kurth, Zilles et al. 2010) and is an important component in salience and attention networks to detect and evaluate stimuli valence, including social stimuli (Menon and Uddin 2010). Studies have shown decreases in INS GM in patients with post-traumatic stress disorder (PTSD) (Chen, Xia et al. 2006, Pruessner, Dedovic et al. 2010), a stress-related psychiatric

disorder. Conversely, humans who have meditated for many years, which can decrease stress perception and responses (Tang, Holzel et al. 2015), have thicker cortical INS volume (Lazar, Kerr et al. 2005). The HC, which is critical for learning and memory (Kim and Diamond 2002, Lieberwirth, Pan et al. 2016), is particularly susceptible to stress alterations (Kim and Diamond 2002). For example, chronic stress or prolonged glucocorticoid treatment in rodents results in decreased dendritic arborization and synaptic complexity (e.g. length, branching, spine density) (Watanabe, Gould et al. 1992, Magarinos and McEwen 1995, Magarinos, Orchinik et al. 1998, McEwen 1999) and even apoptosis (Lucassen, Heine et al. 2006) in the HC. These morphological changes in rodents are consistent with the human literature, where humans reporting chronic stress or with stress-related psychiatric conditions have smaller HC volumes than controls (McEwen 2006, Gianaros, Jennings et al. 2007). These findings suggest that the structural and functional changes in the AMYG, HC, PFC and INS associated with social rank in rhesus monkeys could reflect differences in social stress experienced by animals across the hierarchy. Although the impact of chronic stressor exposure on brain structure and function is well accepted, it is not clear how these effects interact with those of an obesogenic diet, in contrast to a low fat, low sugar diet.

In human studies it is difficult to disentangle individual contributions of stress, diet and caloric content (e.g., saturated fats and sugars) on alterations in brain volume, due to factors like inaccuracies with self-reporting and lack of experimental control. Thus, a translational animal model is warranted that allows for stringent control over environmental and dietary factors, and permits a level of experimental control not present

in human studies. Social subordination in female rhesus macaque monkeys is a translational animal model to study a number of stress-related phenotypes (Michopoulos, Higgins et al. 2012, Shively and Willard 2012), including stress-induced over eating of an obesogenic diet (Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012, Michopoulos, Diaz et al. 2016). Macaque groups, regardless of size, are organized by a matrilineal dominance hierarchy that functions to maintain group stability (Bernstein and Gordon 1974, Bernstein 1976), where lower ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Bernstein 1976, Shively and Kaplan 1984). A consequence of this continual harassment is impaired limbic hypothalamic pituitary adrenal (LHPA) axis regulation, typically evidenced by reduced glucocorticoid (GC) negative feedback and hypercortisolemia in adult females (Shively, Laber-Laird et al. 1997, Shively 1998, Wilson, Pazol et al. 2005, Jarrell, Hoffman et al. 2008, Wilson, Fisher et al. 2008, Paiardini, Hoffman et al. 2009), a physiological phenotype similar to what has been described in some humans suffering from stress-induced psychopathology such as depression (Coryell, Fiedorowicz et al. 2008, Jokinen, Nordstrom et al. 2008). Using this model, we sought to investigate how the imposition of a social stressor, achieved by forming new social social groups, interacts with dietary environment to affect regional brain volume of limbic regions known to affect emotional reactivity and motivated behavior. Although some studies have reported increases in brain regional volume as a consequence of diet-induced obesity, the data overall suggest that there is a volumetric reduction. Therefore, we hypothesized that excessive calorie intake, particularly of a high

caloric diet (HCD), would result in decreased volume of AMYG, PFC, INS and HC. Additionally, we hypothesized that subordination stress would magnify some of those effects, except for the AMYG volume, which would be increased in subordinates, and that these diet and stress-induced alterations would result in potentiated socio-emotional behavior.

## 3.3 Methods

### 3.3.1 Subjects

Subjects were 16 adult female rhesus macaques (*Macaca mulatta*) living as members of one of in six social groups housed in indoor-outdoor pens consisting of 4-6 adult females (n = 34) each at the Yerkes National Primate Research Center (YNPRC) in Lawrenceville, Ga. 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". The social groups were formed following procedures previous described (Jarrell, Hoffman et al. 2008, Snyder-Mackler, Kohn et al. 2016). Briefly, females were removed from the natal breeding groups and sequentially introduced to their new groups over the course of two weeks. This process minimizes aggression towards newly introduced animals. Dominance ranks are established within this two-week group formation phase. The 16 animals chosen for the present analysis were the highest (n = 8) and lowest ranking females (n = 8) in each of the six groups based on rates ELO rankings described below.

Data for determining social rank were obtained from weekly 30-minute observations using an established ethogram (Jarrell, Hoffman et al. 2008) and assessed using the outcome of dyadic interactions (Bernstein 1976) to capture the frequency of submission emitted and of aggression received. Rank was calculated using the ELO rating method, in which higher scores correspond to higher status (Albers and de Vries 2001, Neumann, Duboscq et al. 2011, Snyder-Mackler, Kohn et al. 2016). Briefly, ELO rating updates an animal's score after each dominance interaction based on "winning" or "losing" the interaction. For example, if a lower-ranking female is defeated by a higher-ranking female, the scores for both females would only change slightly (the lower ranking female's score would decrease and the higher-ranking female's score would increase). If a higher-ranking female is defeated by a lower-ranking female, however, their scores would change considerably. One advantage to this method is that it distinguishes individuals that are similarly ranked based on rates of agonistic interactions.

### 3.3.2 Dietary Intervention

The availability of automated feeding stations at the YNPRC (Wilson, Fisher et al. 2008) allows the continual quantification of calories consumed from specific diets in socially-housed, free feeding rhesus monkeys. This feeding system is employed in order to assess how exposure to chronic social stress influences food intake in a complex dietary environment, in which animals can choose freely between a HCD and a low caloric diet (LCD) (Wilson, Fisher et al. 2008). Studies using this model in established female macaque social groups have shown that, although all animals prefer the HCD, subordinate animals consume significantly more calories and gain more weight compared

to more dominant group mates (Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012, Michopoulos, Diaz et al. 2016). This model has also been used to detect alterations in brain dopamine (DA) neurochemistry. Specifically, decreased dopamine D2 receptor binding potential (D2R-BP) in the OFC predicts increased caloric intake, as measured by positron emission tomography (PET) (Michopoulos, Diaz et al. 2016).

For the present study, half of the 16 subjects (LCD-only subjects) had *ad libitum* access to the standard monkey chow [LCD; 3.45 kcal/g, Purina #5038] and half had *ad libitum* access to a choice dietary environment (Choice -CH- subjects), wherein both the LCD and a calorically dense diet or HCD [4.47 kcal/g, D07091204S Research Diets, New Brunswick NJ) were available. The caloric composition of the LCD was 12% fat, 18% protein, 4.14% sugar carbohydrate and 65.9% fiber carbohydrate. The calories of the HCD were distributed as 36% fat, 18% protein, 16.4% sugar carbohydrate and 29.6% fiber-starch carbohydrate. The rationale for providing access to both the LCD and HCD is based on well-established data showing that dietary choice sustains intake of HCDs (la Fleur, van Rozen et al. 2010) and more closely models the dietary environment available to humans. Food access was controlled using previously validated automated feeders that allow for quantification of caloric intake in socially housed, free feeding monkeys (Wilson, Fisher et al. 2008, Arce, Michopoulos et al. 2010, Michopoulos, Diaz et al. 2016). Briefly, subjects have radio-frequency identification chips inserted within each of their wrists, so when they place either of their hands in the feeder, the chip is scanned and signals a computer to dispense a single pellet of food via a pellet dispenser. Each social group had access to two different automated feeders and a computer recorded each

feeding event in a log. This method has been previously validated and ensures that high-ranking females rarely (<1%) take the pellet from subordinate animals and pellets are never discarded (Wilson, Fisher et al. 2008).

### 3.3.3 Behavior

*Social Behavior.* Behavioral observations were collected weekly for 30 minutes using an established ethogram (Jarrell, Hoffman et al. 2008) that quantifies frequencies and durations of agonistic (aggression (threat, chase, slap, bite); affiliative (proximity and grooming) and anxiety-like behaviors (yawn, scratch or groom self, body shake). Behavior was coded in real time, using a notebook computer with an in-house program WinObs (Graves and Wallen 2006), which records the actor, behavior, recipient and the time for each behavior. Inter-observer reliability was calculated as percent agreement on frequencies and durations of behaviors and was greater than 92%. Behavioral observations were initiated once all of the females had been introduced into their new groups.

*Human Intruder (HI) Task.* Each subject received the HI test, a standardized test of emotionality (Kalin and Shelton 1989), 7.70 ± 0.11 months following group formation and 4.44 ± 0.07 months prior to the rs-fMRI scans. The test was administered as previously described (Embree, Michopoulos et al. 2013, Wilson, Bounar et al. 2013). Briefly, this paradigm consists of three consecutive ten-minute sessions, including an alone condition, an intruder profile condition (an unfamiliar experimenter enters the room and sits with his/her profile towards the subject) and an intruder stare condition (the

experimenter makes direct and continuous eye contact for the entire 10 minute session). The HI task is designed to measure defensive and emotional behavior responses specific to each condition, which pose different degrees of threat to rhesus monkeys. The alone condition elicits exploratory and distress responses, such as vocalizations and locomotion, whereas the intruder profile condition elicits behavioral inhibition, such as freezing and visually scanning of the environment. Finally, the intruder stare condition elicits aggressive and/or submissive behaviors directed towards the intruder, as direct eye contact is threatening for rhesus macaques. Videos of each test were scored by trained observers using a modification of published ethograms (Machado and Bachevalier 2006, Howell, Godfrey et al. 2014, Howell, Grand et al. 2014) to capture and quantify locomotion, aggressive, submissive, anxiety-like and fearful behaviors. Inter-observer reliability was greater than 92%.

*Behavioral data reduction analysis.* To reduce the number of behavioral variables, we performed a principal components analysis (PCA) using a rotation method of Varimax with Kaiser Normalization on social and HI behavior outcomes and diet condition (no choice = 1; choice = 2) in all 16 subjects. Variables were entered into the PCA and were chosen based on their relevance to rhesus macaque social behavior and emotional reactivity (Social behavior: proximity duration, groom frequency and duration, aggression and submission frequency; HI behavior: freeze duration in the alone, profile and stare conditions, look away frequency in the profile and stare conditions, threat and anxiety frequency in the stare condition). Dietary condition (1 for LCD only; 2 for CH) was included as preliminary analyses revealed that the dietary choice condition was

associated with significantly reduced rates of initiating proximity ($r_{32}$=-0.36, $p$=0.03) and grooming ($r_{32}$=-0.37, $p$=0.03) towards group mates. This PCA method was chosen in order to consolidate behaviors into similar patterns of responses that represent the most prominent behavioral phenotypes. PCAs have previously been used to identify clusters of behaviors during the HI task in macaques (Williamson, Coleman et al. 2003, Wilson, Bounar et al. 2013). Behaviors that had a loading score of 0.45 or less were excluded. Four components emerged from the PCA analysis (Table 3.1). The first component was labeled "affiliation" as both social groom frequency and duration loaded positively, in addition to proximity duration (time spent sitting near another monkey). Grooming is a prosocial behavior in rhesus macaques and functions to maintain social alliances (Lindburg 1973). Dietary condition loaded negatively into this factor, suggesting that CH subjects were less sociable, which is consistent with our preliminary correlational analysis. The second component was labeled "anxious-aggression", where rates of aggression directed at groups mates as well as three behaviors from the stare condition of the HI, looking away from the intruder, threatening the intruder, and anxiety-like behavior, loaded positively. Threat is a non-contact aggressive behavior in rhesus macaques (Altmann 1962). Since a monkey cannot move away from the intruder during the HI task, looking away during the stare condition may reflect increased anxiety and avoidance of the intruder (Pelphrey, Viola et al. 2004, Patterson 2008). In addition, freezing in the stare condition, a behavior indicative of fear (Kalin and Shelton 1998), loaded negatively on component 2. Behaviors that loaded positively on the third component were threat in the stare condition, freeze in the alone condition and submissive behavior directed towards group mates. In contrast, aggression directed at

group mates loaded negatively. Because higher rates of rates of submissive and lower rates of aggressive behaviors are characteristic of subordinate monkeys, this component was labeled "submissive-fearful". Although threat in the stare condition is being interpreted as aggressive in the anxious-aggression component described above, in the context of the other behaviors in this component it may also be indicative of non-contact, defensive aggression, as previously reported (Meunier, Bachevalier et al. 1999). The fourth component was labeled "anxious-avoidant" because looking away from the intruder in the profile condition and anxiety in the stare condition loaded positively, while freezing in the profile condition loaded negatively. Together, these four components account for 82.7% of the variance for the included variables.

### 3.3.4 Stress Physiology and Immune Markers

For all animal accesses and blood draws, animals were habituated to being removed from their group for conscious venipuncture, using previously described procedures (Walker, Gordon et al. 1982). Blood samples were obtained within 10 minutes of entering the animal area to minimize arousal (Blank, Gordon et al. 1983).

*Cortisol response to acute stressor.* Plasma samples were collected immediately before and after the 30 minute HI task to determine acute stress-induced cortisol increases. Additional plasma samples were collected 60 and 240 minutes after the completion of the HI task to assess recovery to baseline cortisol levels.

*Basal Cortisol and Dexamethasone Suppression Test.* Glucocorticoid negative feedback was tested at 14.40 ± 0.08 months following group formation and 2.34 ± 0.08 months following the rs-fMRI scans, according to previously published procedures (Jarrell, Hoffman et al. 2008). A baseline, morning plasma sample was collected 1 hour after sunrise and an additional sample was collected again 4 hours later. Another plasma sample was collected 1 hour before sunset, immediately followed by dexamethasone administration (0.25mg/kg I.M.). The following morning (1 hour after sunrise) another blood sample was obtained to measure the degree to which dexamethasone suppressed morning cortisol. All Plasma levels of cortisol were measured by LC-ESI-tandem mass spectrometry using a Discovery 5 cm × 2.1 mm C18 column (Supelco, PA) eluted at flow rate of 0.5 ml/min at the YNPRC Biomarkers Core. The intra- and inter-assay coefficient of variation (CV%) was 1.21% and 5.78%, respectively.

### 3.3.5 Cytokine/Immune Markers

Proinflammatory cytokine concentrations [C-reactive protein (CRP), tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6)] in plasma were measured in duplicate using using the following immunoassays: the V-Plex Human CRP Kit; V-Plex custom NHP Proinflammatory Panel (IL-6); and rhesus specific TNFα kit (Meso Scale Discovery, Rockville, MD, USA). For measurement of CRP, samples were diluted 1:200. For all other assays, samples were measured undiluted. Samples were collected within two weeks of the MRI scan for each female.

### 3.3.6 Structural MRI

*MR Image Acquisition.* Neuroimaging scans were collected 52.74 ± 0.39 weeks after group formation. One day prior to the scan, each subject was transported from the YNPRC Field Station to the YNPRC Imaging Center. Using a 3T Siemens Magnetom TRIO system, and an 8-channel phase array coil, T1-MR Scans were acquired using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel imaging sequence (GRAPPA (R=2); TR/TE=2600/3.38ms; Averages=1; voxel size=0.5x0.5x0.5mm$^3$). A T2-MR scan was collected in the same direction as the T1 (TR/TE=3200/373ms, voxel size=0.5x0.5x1.0mm$^3$, 1 average) in order to aid with delineation of regions of interest (ROIs) by improving the contrast of GM (Rapisarda, Bergman et al. 1983, Knickmeyer, Styner et al. 2010), WM and CSF borders. Subjects were scanned under isoflurane anesthesia (1% to effect, inhalation) following induction with telazol (15-35mg, I.M.) and intubation to ensure lack of motion artifacts. For physiological monitoring, animals were fitted with an oximeter, electrocardiograph, rectal thermometer and blood pressure monitor. Additionally, an I.V. catheter was placed to administer dextrose/NaCl (0.45%) in order to maintain hydration. All subjects were scanned in the same supine placement and orientation on an MRI-compatible heating pad, using a custom-made head holder with ear bars and a mouth piece. A vitamin E capsule was taped to the right temple to mark the right side of the brain. Subjects were returned to their social groups upon completion of the scan and full recovery from anesthesia.

*sMRI Processing and Analysis.* Structural data was analyzed using AutoSeg (version 3.0.2) which is an open-source pipeline developed by members of the Neuro Image

Research and Analysis Laboratories of University of North Carolina (NIRAL) (Wang, Vachet et al. 2014). AutoSeg is an atlas-based software pipeline that segments brain into probabilistic tissue maps, cortical lobar parcellations and subcortical ROIs using an atlas-based automatic segmentation approach. AutoSeg was used to automatically segment brain tissue classes (WM, GM, CSF) and generate parcellations of cortical lobes [specifically for this study: the PFC –which was also further parcellated into dorsolateral, medial, ventromedial and orbitofrontal subdivisions, as detailed below (Ghashghaei, Hilgetag et al. 2007, Petrides and Pandya 2007, Yeterian, Pandya et al. 2012)- , INS and cingulate cortex], and subcortical structures (AMYG and HC) to compute their respective volumes in our subjects, following previously described methods (Knickmeyer, Styner et al. 2010, Howell, Grand et al. 2014, Wang, Vachet et al. 2014). This is achieved by registering each subject's native space image into a population-based T1-MRI atlas (Styner, Knickmeyer et al. 2007, Short, Lubach et al. 2010, Howell, Grand et al. 2014, Wang, Vachet et al. 2014) using the tools BRAINSFit and ResampleVolume2, modules in the Slicer program (Fedorov, Beichel et al. 2012). Inhomogeneity correction N4-ITK bias field correction (Tustison, Avants et al. 2010) is then applied and Atlas Based Classification (ABC) is then used to automatically classify regions in each subject's images into brain tissue (WM, GM, CSF) or non-brain tissue (e.g. skull, vessels, muscle) and to remove non-brain tissue (skull-stripping), by warping the atlas tissue priors into the subject using affine and fluid deformable registration of the atlas to each subject using the ANTS (Advanced Normalization Tools) registration tool (Tustison and Avants 2013). These warp fields were then applied to generate cortical and subcortical parcellations that were manually adjusted to ensure accurate delineation of the neuroanatomy following

neuroanatomical conventions (Amaral and Bassett 1989, Paxinos, Huang et al. 2000, Sallem and Logothetis 2006). Volumes were calculated for total ICV, as well as right and left hemisphere volumes for regions of interest (ROIs): PFC [total GM and WM, in addition to subdivision volumes: ACC (BA24), mPFC (BAs 10 and 32), ventromedial PFC (vmPFC; BA 25), dlPFC (BAs 8, 9 and 46) and ventrolateral PFC (vlPFC; BA 45)], INS (total GM and WM), cingulate (total GM and WM), AMYG and HC (Figure 3.1). The AMYG was defined following macaque anatomical landmarks (Price, Russchen et al. 1987, Amaral and Bassett 1989) with the rostral periamygdaloid cortex as the anterior boundary, the CSF as the ventral border, WM as the ventrolateral boundary and the rhinal fissure as the ventromedial boundary (Howell, Grand et al. 2014). The HC was defined using the horn of the lateral ventricle as the dorsal and lateral boundary with the WM separating the HC from the entorhinal cortex as the ventral border (Rosene and Van Hoesen 1987). The PFC was defined using CSF at the surface of the brain as the lateral and anterior boundaries, the interhemispheric fissure as the medial boundary and the arcuate sulcus as the posterior boundary (Knickmeyer, Styner et al. 2010). The inferior boundary, moving rostral to caudal, was defined by the CSF, the sylvian fissure and the arcuate sulcus. The different PFC subdivisions (ACC, dlPFC, vmPFC, vlPFC and mPFC) were anatomically defined based on: (Ghashghaei, Hilgetag et al. 2007, Petrides and Pandya 2007, Yeterian, Pandya et al. 2012). The INS was defined using the CSF within the sylvian fissure as the lateral boundary, the lateral edge of the extreme capsule as the medial boundary and the center of the insular sulcus as the superior, inferior, anterior and posterior boundaries (Knickmeyer, Styner et al. 2010).

### 3.3.7 Statistical Analyses

To determine how caloric intake of specific diets, ELO rank, stress and proinflammatory markers affect volume of AMYG, PFC, HC and INS, stepwise multiple regression analyses were conducted separately for subjects in both the CH and LCD conditions. For these regressions, the following were entered as dependent variables: sMRI ROIs; PFC [total GM and WM, in addition to subdivision volumes: BA 24 (ACC), BA 25 (vmPFC), BAs 8, 9 and 46 (dlPFC), BAs 10 and 32 (mPFC) and BA 45 (vlPFC)], INS (total GM and WM), cingulate (total GM and WM), AMYG and HC. Predictor variables entered into the stepwise regression were the following: (1) For LCD-only subjects: ELO rank and average weekly LCD consumed (kcal) across the entire study; (2) For CH subjects, ELO rank, average weekly HCD consumed (kcal) and average weekly total kcal consumed (LCD+HCD), across the entire study; (3) Additional predictors for all subjects included cortisol (baseline cortisol, change in response to dexamethasone suppression and change from prior to and immediately after the HI test) and proinflammatory markers (circulating CRP, TNFα and IL6).

Following this analysis, another stepwise multiple regression analysis was conducted separately for subjects in both the CH and LCD conditions to investigate what functional outcomes in behavior emerge as a result of the observed diet and rank induced alterations in resting state functional brain connectivity. For this analysis, we used each of the following predictor variables: ELO rank and sMRI ROIs; PFC [total GM and WM, in addition to subdivision volumes: BA 24 (ACC), BA 25 (vmPFC), BAs 8, 9 and 46 (dlPFC), BAs 10 and 32 (mPFC) and BA 45 (vlPFC)], INS (total GM and WM),

cingulate (total GM and WM), AMYG and HC. The four components of behavior determined from the PCA (affiliation, anxious-aggression, submissive-fearful and anxious-avoidant) were entered as dependent variables.

## 3.4 Results

### 3.4.1 Predictors of alterations in ROI volumes

For a summary of the results, see Table 3.3A. For descriptive statistics of weight, BMI and predictor variables, see Table 3.2.

*Predictors of PFC volume*

CH subjects: Lower IL6 and lower total kcal consumed predicted larger left BA 32 [ACC; $F(2, 7)=98.952$, $p<0.001$, Adj. $R^2=0.966$] volume. Larger right BA 9 (dlPFC) volume was predicted by lower IL6, lower HCD consumed and higher TNFα [$F(3, 7)=78.787$, $p=0.001$, Adj. $R^2=0.971$]. Dominance rank also predicted differences in select PFC ROIs for females in the CH diet condition. Higher ELO score (more dominant females) predicted larger left BA 46 [dlPFC; $F(1, 7)=9.761$, $p=0.020$, Adj. $R^2=0.556$] volume. In addition, higher ELO scores also predicted greater PFC WM volume in both the left [$F(1, 7)=13.519$, $p=0.010$, Adj. $R^2=0.641$] and right [$F(1, 7)=12.237$, $p=0.013$, Adj. $R^2=0.616$] hemispheres.

LCD-only subjects: For females in the no choice, LCD-only diet condition, calorie intake did not predict differences in volume of any of the ROIs studied. In contrast, dominance rank had numerous effects on specific volumetric measures of PFC subdivisions and tissue classes (Table 3.3A). Lower ELO score (more subordinate rank) predicted larger

left [F(1, 7)=8.652, $p$=0.026, Adj. $R^2$=0.522] and right [F(1, 7)=9.254, $p$=0.023, Adj. $R^2$=0.541] BA 25 (vmPFC) volumes. More subordinate ELO rank also predicted larger left [F(1, 7)=7.480, $p$=0.034, Adj. $R^2$=0.481] and right [F(1, 7)=6.653, $p$=0.042, Adj. $R^2$=0.447] PFC WM volumes.

*Predictors of AMYG volume*

CH subjects: Social status was not predictive of AMYG volume in the CH subjects, but feeding behavior and proinflammatory cytokines emerged as significant. Greater total caloric intake and greater CRP, but lower IL6, predicted larger right AMYG volume in the CH diet subjects. [F(3, 7)=36.909, $p$=0.002, Adj. $R^2$=0.939].

LCD-only subjects: Whereas calorie intake predicted AMYG volume in the CH subjects, ELO status predicted AMYG volume in the LCD-only animals. Lower ELO score (more subordinate status) predicted larger left [F(1, 7)=8.195, $p$=0.029, Adj. $R^2$=0.507] and right [F(1, 7)=11.351, $p$=0.015, Adj. $R^2$=0.597] AMYG volumes.

*Predictors of INS volume*

CH subjects: There were no significant predictors of INS volume for subjects in the CH diet condition.

LCD-only subjects: In contrast to subjects in the CH diet condition, where no significant predictors of INS volume emerged, ELO score predicted INS GM volume in the LCD-only subjects. Lower ELO score (more subordinate females) predicted larger left [F(1, 7)=10.170, $p$=0.019, Adj. $R^2$=0.567] INS GM volume. Additionally, lower ELO rank also

entered into a model in combination with greater IL6, which together, predicted larger right INS GM [F(2, 7)=27.511, $p$=0.002, Adj. $R^2$=0.883] volume.

*Predictors of HC volume*

CH subjects: Social status was not predictive of HC volume in the CH subjects, but feeding behavior and proinflammatory cytokines were. Greater total caloric intake, CRP and TNFα, but lower IL6 predicted larger left HC volume [F(4, 7)=60.396, $p$=0.003, Adj. $R^2$=0.971] for the subjects in the CH diet condition.

LCD-only subjects: Lower ELO score (more subordinate status) predicted larger HC volume in both the left [F(1, 7)=8.465, $p$=0.027, Adj. $R^2$=0.516] and right [F(1, 7)=8.032, $p$=0.030, Adj. $R^2$=0.501] hemispheres in the LCD-only subjects.

### 3.4.2 Functional behavioral outcomes

For a summary of the results, see Table 3.3B.

*Affiliation Component*

CH subjects: Although none of the ROIs that were significantly predicted by feeding behavior, inflammation or ELO score in the CH subjects were significant in this analysis, two models emerged with different ROIs. These included larger left BA 8 (dlPFC) and smaller left PFC GM volumes, which predicted greater component scores for affiliation in the CH diet condition [F(2, 7)=21.90, $p$=0.003, Adj. $R^2$=0.857].

LCD-only subjects: Although two ROIs were predictive of differences in the affiliation component in the CH subjects, no significant predictors emerged in the LCD-only subjects.

*Anxious-Aggression Component*

CH subjects: Unlike the LCD-only subjects, the anxious-aggression PCA component did not have any significant predictors for the CH diet condition.

LCD-only subjects: In contrast to the CH subjects, where no significant predictors of the anxious-aggression component emerged, smaller left AMYG volume predicted greater component scores for anxious-aggression [$F(1, 7)=6.684$, $p=0.041$, Adj. $R^2=0.448$] in the LCD-only subjects.

*Submissive-Fearful Component*

CH subjects: Unlike the LCD-only subjects, no significant predictors emerged of the submissive-fearful component in the CH diet condition.

LCD-only subjects: Although there were no significant ROI predictors of the submissive-fearful component in the CH subjects, one ROI was significant in the LCD-only subjects, where larger left INS GM volume predicted greater component scores of submissive-fearful [$F(1, 7)=43.288$, $p=0.001$, Adj. $R^2=0.858$].

*Anxious-Avoidant Component*

CH subjects: No significant predictors arose for the anxious-avoidant component in the CH subjects.

LCD-only subjects: Similarly to the CH diet condition, there were no significant predictors of the anxious-avoidant component in the LCD-only subjects.

## 3.5 Discussion

The results of the present study revealed a significant effect of diet (prudent LCD versus a more complex dietary environment where a choice between LCD and HCD is available) on neurobehavioral phenotypes of adult female primates. In general, all calorie-intake variables significantly predicted models in the CH subjects, but calorie-intake in the LCD-only subjects did not affect any of the regional brain volumes measured (Table 3.4). In the CH subjects, ELO status positively predicted dlPFC and PFC WM volumes, whereas in the LCD-only subjects, ELO status negatively predicted vmPFC, PFC WM, AMYG, INS GM and HC volumes. Interestingly, exposure to the CH diet reversed the direction of the dominance rank effects in bilateral PFC WM. In the CH subjects, lower IL6, but greater TNFα and CRP significantly predicted larger volumes of PFC (left ACC and right dlPFC) and right AMYG, whereas in the LCD-only subjects, IL6 positively predicted one model. Cortisol did not significantly predict any variance in the outcomes in either the CH or the LCD-only subjects. For the functional outcomes analysis in the CH subjects, larger left dlPFC and smaller left PFC GM volumes predicted increased affiliative behavior, although neither of these regions were significantly predicted by diet, ELO rank, cortisol or inflammatory markers. For the functional outcomes analysis in the LCD-only subjects, more subordinate rank predicted larger left AMYG and left INS GM volumes, which resulted in decreased component scores for anxious-aggression and increased component scores or submissive-fearful,

respectively. Overall, the results of the present study revealed that social status differentially affects regional brain volumes, depending on dietary condition. Additionally, the impact of diet on regional brain volumes only becomes evident when a CH dietary environment is available. Taken together, these data suggest that considerably different phenotypes emerge in rhesus macaques with access to either a prudent LCD or a more complex dietary environment where a choice between LCD and high sugar, high fat diet is available. These results underscore the view that calories derived from diets high in sugar and fat impact the brain differently than carioles derived from healthier low fat diets.

Several significant models emerged in the CH subjects where number of calories consumed predicted differences in regional brain volumes. In contrast, calorie intake did not significantly predict any variance in regional volumetrics for females in the prudent LCD-only diet condition, where only lab chow was available. In the CH subjects, greater HCD consumption predicted smaller right dlPFC (BA 9) volume. Additionally, greater number of total calories consumed (from both diets) predicted smaller left mPFC (BA 32). These results are consistent with previous literature describing diet-induced obesity related decreases in PFC volumes (Pannacciulli, Del Parigi et al. 2006, Walther, Birdsill et al. 2010, Brooks, Benedict et al. 2013, Marques-Iturria, Pueyo et al. 2013). A potential mediator of these morphological changes may be inflammation. Indeed, higher IL6 also predicted decreased volumes of both right dlPFC and left mPFC. Peripheral inflammation, caused by an increase in adipose tissue as a result of consuming a HCD, can affect the brain and alter astrocyte and glial function (Argente-Arizon, Freire-

Regatillo et al. 2015). Glial cells can also be activated directly in response to dietary nutrients, including saturated fatty acids (Milanski, Degasperi et al. 2009). Neuroinflammation resulting from activated microglia can result in decreases in regional brain volume in several ways, including preventing neurogenesis –specifically in the hippocampus(Monje, Toda et al. 2003)-, causing neurodegeneration (Campbell, Stalder et al. 1997), dendritic atrophy (Richwine, Parkin et al. 2008) and apoptosis (Watson, Cai et al. 2000, Semmler, Okulla et al. 2005, Moraes, Coope et al. 2009). Although most studies have investigated these effects in the HC, a few have focused on PFC specifically. HCD has been shown to increase expression of inflammation related genes, including IL6 and TNFα in cortical regions, such as the PFC, in mice and rats (Jayaraman, Lent-Schochet et al. 2014, Carlin, Grissom et al. 2016, Kang, Koo et al. 2016). Decreased dlPFC volume could also be explained by decreases in the number of astrocytes, as astrocytes make up a significant portion of GM and even outnumber neurons (Sofroniew and Vinters 2010). This idea is supported in studies of multiple sclerosis, where the severity of inflammation is positively correlated with the magnitude of GM loss (Haider, Simeonidou et al. 2014) and one previous study speculates that astrocyte loss underlies these decreases in GM (Morris, Berk et al. 2015). Importantly, the dlPFC is involved in working memory for evaluation, comparison and selection of incentive-based behavioral responses (Haber and Knutson 2010, Genovesio, Wise et al. 2014). In task-evoked fMRI studies, the dlPFC shows less activation in response to appetizing foods in obese than control subjects (Nummenmaa, Hirvonen et al. 2012), which could be explained through inflammation-mediated decreases in volume potentially resulting in impaired top-down inhibitory control over feeding behavior. The mPFC is involved in immediacy, expected value and

anticipation of the benefit/risk ratio of rewards (Knutson, Taylor et al. 2005, Haber and Knutson 2010). Thus, smaller mPFC may reflect impaired reward processing of dietary information and cues (such as the availability of a HCD) and excessive feeding behavior.

The HC is important for memory and associative learning in control of appetitive behavior, including food-seeking and energy regulation (Henderson, Smith et al. 2013). Interestingly, greater total calorie consumption predicted larger left HC volume in the CH subjects. This finding is not consistent with previous studies, where HCD exposure in juvenile and adult mice caused decreased HC volume due to reduced neurogenesis (Vinuesa, Pomilio et al. 2016). Another study in rats showed that a high-fat diet decreased both the number of pyramidal cells and dendritic spine density in the HC (Wang, Fan et al. 2016). In humans, HCD consumption also results in decreased volume (Jacka, Cherbuin et al. 2015), which has been associated with cognitive impairment (Kanoski and Davidson 2011, Martin and Davidson 2014) and emotional dysregulation, such as depression (Videbech and Ravnkilde 2004). Future studies are necessary to identify the discrepancy between the current findings (and their functional relevance) and what is reported in the literature.

In the CH subjects, greater total kcal intake predicted larger right AMYG volume. Although decreases in AMYG volume are generally found in obese humans (Janowitz, Wittfeld et al. 2015, Figley, Asem et al. 2016, Masouleh, Arelin et al. 2016), some studies report volume increases. For example, one study found that obese subjects had larger AMYG volume in older adults (Widya, de Roos et al. 2011). Again, inflammation

may be mediating this effect as CRP positively predicted AMYG volume in our statistical models. Although most previous studies have focused on inflammation-induced decreases in regional brain volume, these studies have focused mainly on the HC. There is evidence to suggest that inflammation can affect brain regions differentially. For example, inflammation was inversely related to HC, but not AMYG volume in one study (Marsland, Gianaros et al. 2015) and astrocytes display heterogeneous morphology, density and activation responses, depending on their location (Chaboub and Deneen 2012, Hawrylycz, Lein et al. 2012). Thus, the AMYG volume increase associated with increased total kcal consumption may reflect a different cellular mechanism, perhaps increases in the number of microglia and astrocytes, which has been observed in the HC after exposure to a HCD in rats (Graham, Harder et al. 2016, Kang, Koo et al. 2016). Further supporting this possibility is the finding that HCD exposure in juvenile mice causes increased AMYG activity, evidenced by a greater number of c-Fos positive nuclei (Vinuesa, Pomilio et al. 2016). Another potential mediator of increased AMYG volume with greater total kcal intake may be glucocorticoids. As previously discussed, diet-induced obesity is also characterized by hypercortisolemia resulting from synthesis of cortisol by adipocytes (Pasquali, Ambrosi et al. 2002). Chronic stress, which is also characterized by hypercortisolemia (Raadsheer, Hoogendijk et al. 1994, Makino, Smith et al. 1995, Makino, Hashimoto et al. 2002, Myers, McKlveen et al. 2012), leads to hyper-excitability of the AMYG in rodents (Rosenkranz, Venheim et al. 2010) and increased dendritic arborization within the AMYG (Vyas, Mitra et al. 2002). Therefore, increased glucocorticoids as a result of diet-induced obesity may be affecting the AMYG similarly as in chronic stress, independently of stressor exposure. As the AMYG is involved in the

regulation of food reward (Baxter and Murray 2002), through connections with both the ventral striatum (nucleus accumbens, in particular) and PFC, increased volume may represent an overactive AMYG in response to a CH dietary condition which, in combination with reduced top-down inhibitory control due to reduced dlPFC volume in these subjects, could result in a hyperactive reward circuit evidenced by impaired top-down inhibitory control over feeding behavior.

These findings underscore the important effect of a dietary choice environment on brain and behavior, as caloric intake was only significant within the CH, but not the LCD-only subjects. Further highlighting the importance of dietary environment was the finding that ELO score was a significant predictor for all of the models in LCD-only subjects, but only for a few in the CH subjects. One model emerged in the CH (but not the LCD-only) subjects with ELO as a predictor, where more subordinate status predicted smaller left dlPFC (BA 46). This finding is consistent with previous studies reporting dlPFC volume decreases in response to stress in adult (Ansell, Rando et al. 2012) and adolescent humans (Edmiston, Wang et al. 2011). These volumetric changes may be explained by decreased neuronal size, glial density or cell loss, which was has been seen in postmortem studies in humans with depression (Rajkowska 2000, Cotter, Mackay et al. 2002), which is often associated with chronic stress (McEwen 2012).

Interestingly, higher ELO score predicted larger PFC WM and dlPFC volumes in the CH subjects, whereas in the LCD-only subjects, lower ELO predicted larger PFC WM, vmPFC, AMYG, INS GM and HC volumes. One direct comparison emerged, where right

and left PFC WM were significantly predicted by ELO in both the CH and LCD-only subjects. In the LCD-only subjects, more subordinate status predicted larger PFC WM volume. Intriguingly, this effect was reversed in the CH subjects, where more subordinate status predicted smaller PFC WM volume, underscoring the impact of consuming calories from a HCD on PFC WM. Although not directly tested in this study, these results may be explained by alterations in DA neurochemistry. Previous studies in cynomolgus macaques have shown that CSF homovanillac acid (HVA), a metabolite of DA, is higher in subordinates (compared to dominants) consuming a LCD (Nader, Nader et al. 2012). However, when animals are consuming a HCD, dominants have higher CSF HVA than subordinates (Kaplan, Manuck et al. 2002). Higher CSF HVA in the subordinates consuming an LCD and in the dominants consuming a HCD may indicate increased extracellular DA in these subjects. There is some evidence that oligodendrocytes express DA receptors in vitro (Bongarzone, Howard et al. 1998) and in vivo in humans (Sovago, Makkai et al. 2005), but their function is poorly understood (Butt, Fern et al. 2014). However, exogenous agonist activation of these receptors may have a protective effect, as this has been shown to reduce oligodendrocyte damage by oxidative glutamate toxicity and oxygen/glucose deprivation injury (Rosin, Colombo et al. 2005). Although speculative, the increased WM observed in the subordinate LCD (compared to the dominant LCD) and the dominant CH (compared to the subordinate CH) may be due to protective effects on oligodendrocytes by higher levels of extracellular DA. This hypothesis will have to be tested in future studies.

Several additional models emerged in the LCD-only subjects with ELO as a predictor, where more subordinate status consistently predicted larger bilateral vmPFC (BA 25), AMYG, INS GM (which also loaded positively with IL6) and HC. Increased volume in response to stress or glucocorticoids has been previously demonstrated with regards to AMYG volume (Rosenkranz, Venheim et al. 2010, Coplan, Fathy et al. 2014), but the findings that more subordinate status caused larger vmPFC, INS GM and HC are inconsistent with previous studies that show decreased volume of these regions in response to chronic stress (mPFC and vmPFC: (Joels, Karst et al. 2007, Hains, Vu et al. 2009, Shansky and Morrison 2009, Ansell, Rando et al. 2012, Radley, Anderson et al. 2013); INS GM: (Ansell, Rando et al. 2012); HC: (Watanabe, Gould et al. 1992, Magarinos and McEwen 1995, Magarinos, Orchinik et al. 1998, McEwen 1999). As these social groups have only been formed for about a year, perhaps the typical effects on brain structure associated with social subordination stress in the LCD-only subjects need more time to progress into the typical phenotype consistent with previous studies of chronic stress. These findings further highlight the importance of dietary environment, as these rank effects are obscured by diet in the CH subjects.

Despite a number of significant models where ELO, caloric intake and inflammatory signals significantly predicted variance in regional volumes, only a few of the regional brain volumes in this analysis predicted variance in behavior measures reflected in the PCA scores. For brain regions predicting behavior, larger left dlPFC (BA 8) and smaller left PFC GM in the CH subjects predicted increased component scores of affiliation. The PFC has been previously implicated in social affiliative behavior. For example, one study

in humans showed that increased activation of the dlPFC predicted subsequent prosocial behavior (Majdandzic, Amashaufer et al. 2016). Moreover, prefrontal regions, including vmPFC and OFC, are activated in humans during a prosocial mutual cooperation task (Rilling, Gutman et al. 2002, Rilling, Sanfey et al. 2004) and also when making social judgments (O'Doherty, Winston et al. 2003). In non-human primates, lesions of the OFC result in impaired social behavior, including increased aggressive interactions and decreased affiliative personality qualities (Machado and Bachevalier 2006). Although it is difficult to draw direct conclusions from these previous studies (which investigated BOLD response and lesions, not brain volume) regarding functional outcomes of the volume of PFC regions, these data highlight the importance of the PFC in regulating affiliative social behavior.

Interestingly, larger left AMYG volume (which was negatively predicted by ELO rank) in the LCD-only subjects predicted decreased component scores of anxious-aggression. Thus, more subordinate status predicts larger left AMYG volume, which leads to decreased anxious-aggression behavior. This is consistent with the social subordination model, as subordinates exhibit reduced rates of aggression compared to more dominant animals. Larger left INS GM volume (which was negatively predicted by ELO) in the LCD-only subjects predicted increased component scores of submissive-fearful. Therefore, more subordinate status predicts larger left INS GM volume, which leads to increased submissive-fearful behavior. Taken together, the decreased scores of anxious-aggression mediated by AMYG in the subordinates in the LCD-only condition may be because these subjects are engaging in more submissive-fearful behavior, which is

associated with larger INS GM. Previous studies in humans have shown that increased AMYG volumes are associated with increased negative emotionality (including anxiety) (Baur, Hanggi et al. 2012, Mincic 2015) and increased INS volumes are reported as being both positively (Uchida, Del-Ben et al. 2008) and negatively (Asami, Yamasue et al. 2009) associated with panic disorder. Although the directionality of the relationship between AMYG and INS volume and resulting emotional behavior are inconsistent with previous studies (potentially due to a species difference), these findings underscore their importance in fear and anxiety.

Several limitations need to be addressed in the present study. Due to timing constraints, we were unable to include additional brain regions known to regulate both feeding and emotional behavior. For example, previous studies have shown positive relationships between BMI and GM volume in both the NAcc (Horstmann, Busse et al. 2011, Coveleskie, Gupta et al. 2015) and the hypothalamus (Horstmann, Busse et al. 2011). Additionally, sex differences have previously been reported when comparing brain volumes of obese men compared to women (Horstmann, Busse et al. 2011). As such, differences in brain volume as a consequence of dietary environment or social rank may emerge differently in male subjects, which we did not address in this study. Future studies are necessary to investigate additional brain region volumes that may be sensitive to dietary environment or social status and whether they are mediated by gender. Additionally, causality cannot be determined with the current study design, as scans were not obtained prior to study initiation. Future studies are necessary with baseline measures, collected prior to diet intervention, in order to determine whether diet-induced changes in

cortisol, immune markers, total and regional brain volume and behavior are directly caused by consumption of a HCD. Also, since our sample size was small (n=8 per group), we may have been underpowered to detect statistically significant effects. Therefore, future studies are necessary to replicate these findings with a larger sample size. Nevertheless, the differences in regional brain volumes and associated behavior between the CH and LCD-only females suggest a significant impact of the dietary environment on regional brain volumes.

In conclusion, the results of the present study revealed that social status differentially affects regional brain volumes, depending on dietary condition. Additionally, the impact of diet on regional brain volumes only becomes evident when a CH dietary environment is available. Taken together, these data suggest that considerably different phenotypes emerge in rhesus macaques with access to either a prudent LCD or a more complex dietary environment where a choice between LCD and HCD is available. The interactions, and underlying mechanisms, between how a complex dietary environment and chronic social stress (resulting from subordinate social status) affect brain and behavior highly warrant future elucidation and underscore the necessity for considering dietary environment in any animal model of human health outcomes. These studies are essential in order to successfully prevent and treat stress and diet-induced obesity related illness in humans.

**Table 3.1 Principal component analysis (PCA) factor loading of human intruder and social behavior.** Four components emerged and were labeled affiliation, anxious-aggression, submissive-fear and anxious avoidant.

| | Factor Loading | | | |
|---|---|---|---|---|
| | Component 1 | Component 2 | Component 3 | Component 4 |
| **Variable** | **Affiliation** | **Anxious-Aggression** | **Sumissive-Fearful** | **Anxious-Avoidant** |
| Groom Dur. | 0.935 | | | |
| Groom Freq. | 0.922 | | | |
| Proximity Dur. | 0.870 | | | |
| Diet | -0.568 | | | |
| Look Away Freq.-Stare | | 0.963 | | |
| Freeze Dur.-Stare | | -0.945 | | |
| Threat Freq.-Stare | | 0.693 | 0.630 | |
| Freeze Dur.-Alone | | | 0.870 | |
| Submission Freq. | | | 0.849 | |
| Aggression Freq. | | 0.560 | -0.583 | |
| Freeze Dur.-Profile | | | | -0.857 |
| Look Away Freq.-Profile | | | | 0.688 |
| Anxiety Freq.-Stare | | 0.550 | | 0.624 |

**Table 3.2 Descriptive statistics.** Descriptive statistics of body weight, BMI and predictor variables used in regression analyses in the CH and LCD-only subjects.

| | CH Subjects | | | | LCD-only Subjects | | | |
|---|---|---|---|---|---|---|---|---|
| | **Minimum** | **Maximum** | **Mean** | **SEM** | **Minimum** | **Maximum** | **Mean** | **SEM** |
| **BMI** | 11.282 | 19.364 | 16.313 | 0.982 | 11.242 | 16.870 | 14.373 | 0.700 |
| **Weight** | 8.180 | 13.780 | 11.473 | 0.685 | 7.440 | 11.030 | 9.525 | 0.488 |
| **Avg. Weekly HCD Consumed (kcal)** | 3626.000 | 9962.000 | 6715.810 | 762.010 | n/a | | | |
| **Avg. Weekly LCD Consumed (kcal)** | 4174.527 | 10118.635 | 6601.941 | 804.222 | 5313.757 | 13680.232 | 9730.659 | 896.618 |
| **Avg. Weekly Total Consumed (kcal)** | 8400.954 | 20081.034 | 13317.746 | 1407.779 | | | | |
| **CRP** | 13920.249 | 117141.714 | 57610.088 | 13872.672 | 6366.685 | 130380.635 | 37714.541 | 14343.221 |
| **TNFα** | 4.444 | 62.089 | 24.179 | 6.952 | 0.610 | 59.068 | 16.206 | 6.902 |
| **IL6** | 0.496 | 1.293 | 0.981 | 0.094 | 0.364 | 2.757 | 1.064 | 0.292 |
| **Baseline Cortisol** | 15.100 | 29.258 | 19.898 | 1.763 | 13.934 | 24.313 | 17.662 | 1.265 |
| **Δ Cortisol Dex Suppression** | -28.533 | -11.415 | -17.165 | 1.969 | -22.053 | -13.012 | -16.217 | 1.091 |
| **Δ Cortisol Pre-Post HI** | -0.050 | 5.200 | 2.875 | 0.690 | 0.550 | 5.300 | 3.406 | 0.613 |

**Table 3.3 Summary of significant models from stepwise multiple stepwise regression for both CH and LCD experimental groups. (A)** Predictors of alterations brain ROI volume; and **(B)** Functional behavioral outcomes of ROI brain volumes.

**A.**

| | | | Overall Model | | | | Predictor Variables | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Dependent Variable | *Adj. $R^2$* | *F* | *Sig.* | Retained Predictor Variables | B | Std. Error | β | *t* | *Sig.* |
| Choice | PFC | BA 32 (mPFC) Left | 0.966 | 98.952 | 9.50E-05 | IL6 | -62.712 | 4.654 | -0.949 | -13.476 | 4.00E-05 |
| | | | | | | Avg. Weekly Total Consumed (kcal) | -0.001 | 0.000311 | -0.212 | -3.013 | 0.03 |
| | | BA 9 (dlPFC) Right | 0.971 | 78.787 | 0.001 | IL6 | -213.170 | 15.834 | -0.950 | -13.463 | 1.76E-04 |
| | | | | | | Avg. Weekly HCD Consumed (kcal) | -0.011 | 0.002 | -0.406 | -5.988 | 0.004 |
| | | | | | | TNFα | 0.868 | 0.222 | 0.286 | 3.903 | 0.017 |
| | | BA 46 (dlPFC) Left | 0.556 | 9.761 | 0.020 | ELO Rank | 0.069 | 0.022 | 0.787 | 3.124 | 0.020 |
| | | PFC WM Left | 0.641 | 13.519 | 0.010 | ELO Rank | 0.154 | 0.042 | 0.832 | 3.677 | 0.010 |
| | | PFC WM Right | 0.616 | 12.237 | 0.013 | ELO Rank | 0.134 | 0.038 | 0.819 | 3.498 | 0.013 |
| | AMYG | AMYG Right | 0.939 | 36.909 | 0.002 | Avg. Weekly Total Consumed (kcal) | 0.006 | 0.001 | 0.752 | 7.947 | 0.001 |
| | | | | | | IL6 | -82.394 | 12.317 | -0.674 | -6.689 | 0.003 |
| | | | | | | CRP | 2.72E-04 | 8.45E-05 | 0.328 | 3.224 | 0.032 |
| | HC | HC Left | 0.971 | 60.396 | 0.003 | Avg. Weekly Total Consumed (kcal) | 0.006 | 0.001 | 0.639 | 9.417 | 0.003 |
| | | | | | | IL6 | -103.155 | 9.753 | -0.783 | -10.577 | 0.002 |
| | | | | | | CRP | 3.39E-04 | 6.20E-05 | 0.379 | 5.437 | 0.012 |
| | | | | | | TNFα | 0.427 | 0.131 | 0.239 | 3.255 | 0.047 |
| LCD-Only | PFC | BA 25 (vmPFC) Left | 0.522 | 8.652 | 0.026 | ELO Rank | -0.015 | 0.005 | -0.768 | -2.941 | 0.026 |
| | | BA 25 (vmPFC) Right | 0.541 | 9.254 | 0.023 | ELO Rank | -0.012 | 0.004 | -0.779 | -3.042 | 0.023 |
| | | PFC WM Left | 0.481 | 7.480 | 0.034 | ELO Rank | -0.162 | 0.059 | -0.745 | -2.735 | 0.034 |
| | | PFC WM Right | 0.447 | 6.653 | 0.042 | ELO Rank | -0.162 | 0.063 | -0.725 | -2.579 | 0.042 |
| | AMYG | AMYG Left | 0.507 | 8.195 | 0.029 | ELO Rank | -0.049 | 0.017 | -0.760 | -2.863 | 0.029 |
| | | AMYG Right | 0.597 | 11.351 | 0.015 | ELO Rank | -0.048 | 0.014 | -0.809 | -3.369 | 0.015 |
| | INS | INS GM Left | 0.567 | 10.170 | 0.019 | ELO Rank | -0.051 | 0.016 | -0.793 | -3.189 | 0.019 |
| | | INS GM Right | 0.883 | 27.511 | 0.002 | ELO Rank | -0.058 | 0.009 | -0.866 | -6.705 | 0.001 |
| | | | | | | IL6 | 31.659 | 9.337 | 0.438 | 3.391 | 0.019 |
| | HC | HC Left | 0.516 | 8.465 | 0.027 | ELO Rank | -0.048 | 0.017 | -0.765 | -2.909 | 0.027 |
| | | HC Right | 0.501 | 8.032 | 0.030 | ELO Rank | -0.040 | 0.014 | -0.757 | -2.834 | 0.030 |

**B.**

| | Dependent Variable | Overall Model | | | Retained Predictor Variables | Predictor Variables | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Adj. $R^2$* | *F* | *Sig.* | | B | Std. Error | β | *t* | *Sig.* |
| **Choice** | Affiliation | 0.857 | 21.9 | 0.003 | BA 8 (dlPFC) Left | 0.03 | 0.006 | 3.069 | 5.363 | 0.003 |
| | | | | | PFC GM Left | -0.004 | 0.001 | -2.416 | -4.222 | 0.008 |
| | Anxious-Aggression | | | | *No Significant Predictors* | | | | | |
| | Submissive-Fearful | | | | *No Significant Predictors* | | | | | |
| | Anxious-Avoidant | | | | *No Significant Predictors* | | | | | |
| **LCD-Only** | Affiliation | | | | *No Significant Predictors* | | | | | |
| | Anxious-Aggression | 0.448 | 6.684 | 0.041 | AMYG Left | -0.012 | 0.004 | -0.726 | -2.585 | 0.041 |
| | Submissive-Fearful | 0.858 | 43.288 | 0.001 | INS GM Left | 0.014 | 0.002 | 0.937 | 6.579 | 0.001 |
| | Anxious-Avoidant | | | | *No Significant Predictors* | | | | | |

**Table 3.4 Direct comparison of results from Table 3.3A.** ROI predictors in both the CH (red) and LCD-only (blue) subjects.

| | Dependent Variable | Significant Predictors | |
|---|---|---|---|
| | | CHOICE | LCD Only |
| PFC | BA 32 (mPFC) Left | ↓ IL6<br>↓ Avg. Weekly Total Consumed (kcal) | n/a |
| | BA 9 (dlPFC) Right | ↓ IL6<br>↓ Avg. Weekly HCD Consumed (kcal)<br>↑ TNFα | n/a |
| | BA 25 (vmPFC) Left | n/a | ↓ ELO Rank |
| | BA 25 (vmPFC) Right | n/a | ↓ ELO Rank |
| | BA 46 (dlPFC) Left | ↑ ELO Rank | n/a |
| | PFC WM Left | ↑ ELO Rank | ↓ ELO Rank |
| | PFC WM Right | ↑ ELO Rank | ↓ ELO Rank |
| AMYG | AMYG Left | n/a | ↓ ELO Rank |
| | AMYG Right | ↑ Avg. Weekly Total Consumed (kcal)<br>↓ IL6<br>↑ CRP | ↓ ELO Rank |
| INS | INS GM Left | n/a | ↓ ELO Rank |
| | INS GM Right | n/a | ↓ ELO Rank<br>↑ IL6 |
| HC | HC Left | ↑ Avg. Weekly Total Consumed (kcal)<br>↓ IL6<br>↑ CRP<br>↑ TNFα | ↓ ELO Rank |
| | HC Right | n/a | ↓ ELO Rank |

**Figure 3.1 AMYG, HC and PFC ROIs used in the Autoseg pipeline** (Styner, Knickmeyer et al. 2007, Howell, Grand et al. 2014). From left to right: axial, coronal and saggital views. (Top Row) Right (green) and left (fuschia) AMYG ROIs; (Middle Row) Right (red) and left (blue) HC ROIs; (Bottom Row) Right and left PFC ROIs: ACC (BAs 24) in yellow text, mPFC (BA 10, 32) in red text, vmPFC (BA 25) in light blue text, vlPFC (BA 45) in white text, dlPFC (BAs 8, 9, 46) in dark blue text in red text.

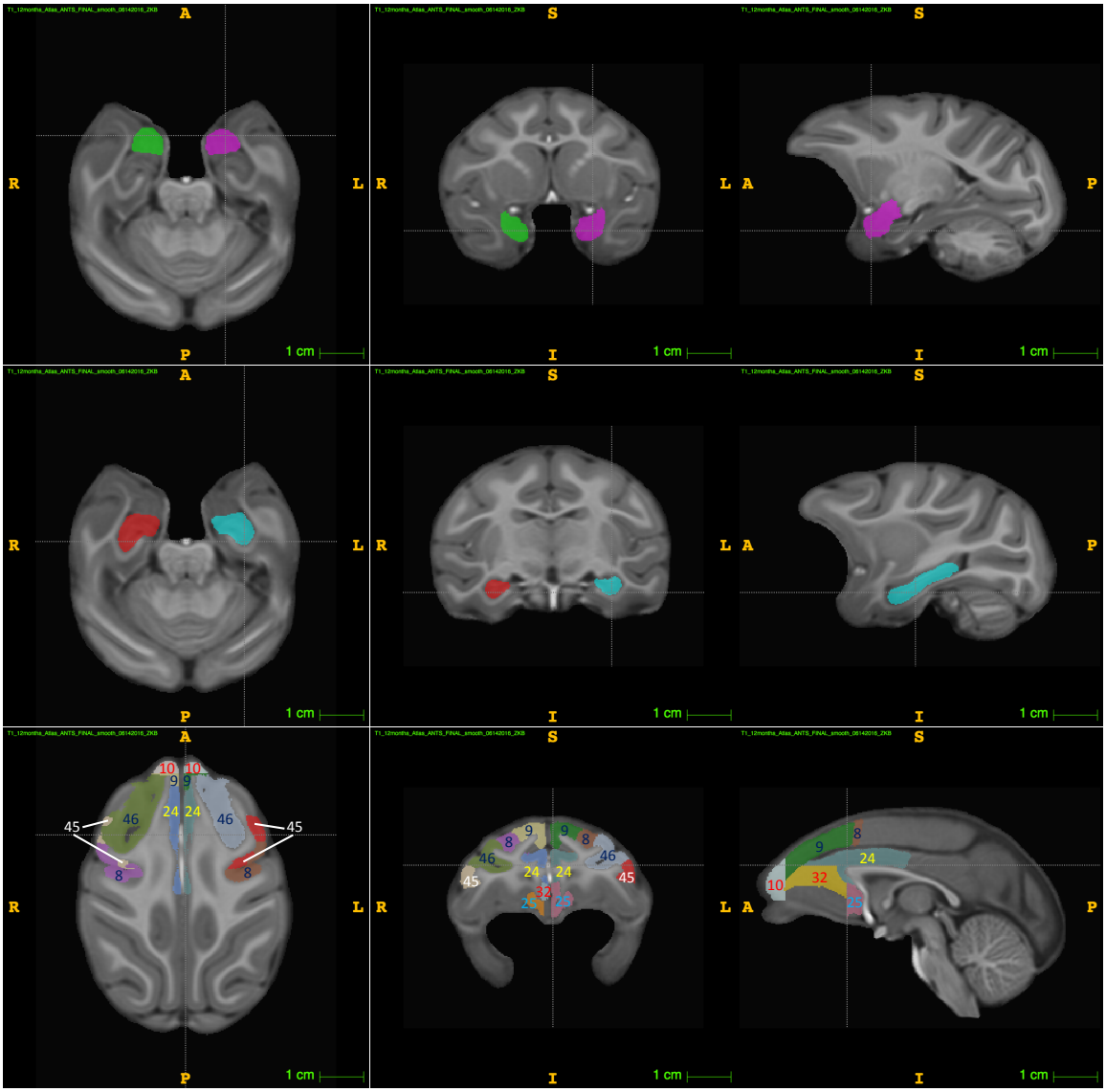
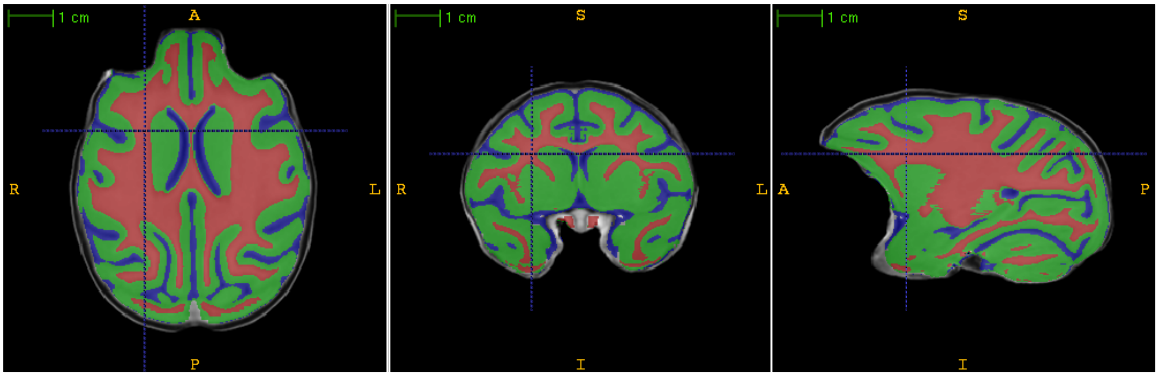


**Figure 3.2 Example of automatic segmentation of GM, WM and CSF tissue classes using the Autoseg pipeline.** From left to right: axial, coronal and saggital views. Green=GM, Red=WM and Blue=CSF.



# Chapter 4. Cortico-Limbic-Striatal Functional Connectivity and Behavior Are Impacted by Dietary Environment and Exposure to Social Stressors in Female Rhesus Macaques

## 4.1 Abstract

Diet-induced obesity has become a significant worldwide health problem and results in increased risk for numerous adverse health outcomes, including type 2 diabetes, cancer, heart disease. Additionally, chronic social stressor exposure can increase the drive to consume these palatable foods, further contributing to an obese phenotype. Data suggest that obesity resulting from stress-induced overeating may compromise brain structure and function, potentially leading to emotional dysregulation and behavioral impairment. However, these impairments in brain structural and functional outcomes may emerge before significant body fat accumulates, suggesting that consuming an obesogenic diet itself may have adverse effects on the neurobehavioral outcomes. Social subordination in female rhesus macaque monkeys is an established translational animal model to study a number of stress-related phenotypes, including stress-induced over eating of a palatable diet. Thus, we sought to investigate how the imposition of a social stressor, achieved by forming new social social groups, interacts with dietary environment to affect brain functional connectivity (FC) between prefrontal, striatal, and other limbic regions known to affect emotional reactivity and motivated behavior. The impact of diet on neurobehavioral outcomes was assessed in females who had been maintained on a low fat, low sugar chow diet (low caloric diet only -LCD-only-) their entire lives and females who had been consuming this chow diet in combination with a high fat, high sugar diet (choice diet -CH-) for one year. Overall, we found that variance in region-specific FC for each experimental group was predicted differentially by food intake, social status, inflammatory factors and cortisol. In the CH subjects, number of calories consumed positively predicted FC in the left hemisphere between amygdala (AMYG) and

dorsolateral prefrontal cortex (dlPFC), in addition to AMYG and insular cortex (INS) FC. This effect was reversed in the right hemisphere in the CH subjects, where total kcal intake was negatively predictive of FC between AMYG and nucleus accumbens (NAcc) with dlPFC. In the LCD subjects, food intake positively predicted right AMYG FC with INS and negatively predicted right AMYG FC with orbitofrontal cortex (OFC). In the CH subjects, social status negatively predicted right INS and OFC FC, whereas social status positively predicted FC between left AMYG and OFC in the LCD subjects. Pro-inflammatory factors entered into several models, which consistently predicted more positive FC between ROIs. Cortisol significantly predicted FC in two models, where lower baseline cortisol predicted more positive FC between right AMYG and dlPFC in the CH subjects and greater stress-induced cortisol predicted stronger and more positive FC between right AMYG and OFC in the LCD subjects. HCD consumption was associated with decreased affiliative and submissive-fearful behavior in the CH subjects, whereas more subordinate social status was associated with increased submissive-fearful and decreased anxious-avoidant behavior in social group behavior and the Human Intruder task. Taken together, the finding that greater caloric intake results in specific altered FC in dlPFC-NAcc-AMYG-INS circuitry and resulting behavior, particularly within the CH dietary condition, may suggest that the incentive salience of and motivational drive to consume palatable foods is increased, while the ability to regulate food intake is decreased in these subjects. The results of the present study revealed that considerably different phenotypes emerge in rhesus macaques with access to either a prudent LCD or a more complex dietary environment where a choice between LCD and high sugar, high fat diet is available. These results underscore the view that calories

derived from diets high in sugar and fat impact the brain and behavior differently than calories derived from healthier low fat and sugar diets.

## 4.2 Introduction

Obesity is a significant public health concern and economic burden (Tsai, Williamson et al. 2011, Withrow and Alter 2011) and results in an increased risk for numerous adverse health outcomes (Must, Spadano et al. 1999, Visscher and Seidell 2001, Hill 2006). Rates of obesity have continued to rise over the last 20 years to greater than 30% of the United States population and are expected to increase to 50% by 2030 (Flegal, Carroll et al. 2010). Given these concerning trends, there is concerted effort and resources dedicated to understanding the etiology of and developing strategies to treat overweight and obesity are essential. However, equally important is to determine pathophysiological impact of consuming an obesogenic diet before frank obesity is evident.

The availability of highly palatable foods, rich in fat and sugar, can cause an individual's energy intake to exceed its energy expenditure, leading to excess weight gain, as evidenced by the vast literature on diet-induced obesity (Keenan, Wallig et al. 2013). Additionally, experiential factors, including chronic social stressor exposure, can increase the drive to consume these palatable foods, further contributing to an obese phenotype (Oliver, Wardle et al. 2000, Epel, Lapidus et al. 2001, Gibson 2006). Importantly, data suggest that obesity resulting from stress-induced overeating may compromise brain structure and function in humans, monkeys and rodents (Berthoud, Lenard et al. 2011, Tryon, Carter et al. 2013, Ulrich-Lai, Fulton et al. 2015, Michopoulos, Diaz et al. 2016), potentially leading to emotional dysregulation and behavioral impairment in humans (Onyike, Crum et al. 2003, Galvez, Bauer et al. 2014). Indeed, mood disorders and anxiety are frequently co-morbid with obesity (Ayensa and Calderon 2011, Capuron,

Lasselin et al. 2016), especially in females (Zender and Olshansky 2009, Hillman, Dorn et al. 2010), suggesting obesity-induced impaired neurobehavioral function. However, these impairments in brain structure and functional outcomes may emerge before significant body fat accumulates, suggesting that consuming an obesogenic diet itself may have adverse effects on the neurobehavioral outcomes (Beilharz, Maniam et al. 2015, Morris, Beilharz et al. 2015).

Because obesity is also characterized by hypercortisolemia (Pasquali, Ambrosi et al. 2002) and sustained intake of an obesogenic diet increases glucocorticoid (GC) secretion in response to a stressor (Pasquali, Ambrosi et al. 2002, Legendre and Harris 2006, Michopoulos, Toufexis et al. 2012), elevated GCs may be key biological signals mediating the adverse consequences of both stress and increased fat mass on neurobehavioral outcomes. In addition, proinflammatory cytokines are likely important mediators of both stress and diet on neurobehavioral outcomes. Chronic inflammation is not only a hallmark of obesity (Gregor and Hotamisligil 2011, Miller and Spencer 2014), but consumption of obesogenic diets increases both peripheral and central measures of inflammation prior to the onset of obesity (Myles 2014, Vasconcelos, Cabral-Costa et al. 2016). Furthermore, persistent inflammation is positively associated with chronic stress (Miller and Blackwell 2006, Cohen, Janicki-Deverts et al. 2012) and resulting adverse behavioral outcomes such as depression (Dunn, Swiergiel et al. 2005, Hryhorczuk, Sharma et al. 2013).

Several brain regions have been implicated in the consumption of obesogenic diets and resulting diet-induced obesity (Warne 2009, Tomasi and Volkow 2013). The nucleus accumbens (NAcc), amygdala (AMYG), insula (INS) and prefrontal cortex (PFC) are often studied due to their involvement in both stress response and reward process regulation (Ghashghaei, Hilgetag et al. 2007, Haber and Knutson 2010, Volkow, Wang et al. 2012). The NAcc is involved in reward processing (Cohen, Asarnow et al. 2010), the AMYG is involved in regulation of food reward (Baxter and Murray 2002) as well as fear and anxiety (LeDoux 2000) and the INS integrates interoceptive, socio-emotional, sensorimotor, olfacto-gustatory and cognitive information (Kurth, Zilles et al. 2010). In recent years, investigators have begun to employ resting state-functional magnetic resonance imaging (rs-fMRI) techniques to investigate obesity related alterations in brain functional connectivity (FC), primarily in stress and reward networks. Rs-fMRI FC, which measures temporal correlations in blood oxygen level dependent -BOLD-related activity between regions, has been used in studies in obese humans to demonstrate altered brain connectivity in PFC-AMYG-INS-NAcc circuitry. For example, women with high body mass index (BMI) have stronger connectivity between NAcc and anterior cingulate cortex (ACC) and ventromedial PFC (vmPFC) (Coveleskie, Gupta et al. 2015). Another rs-fMRI study showed that obese women have higher activity within the INS compared to lean women (Hogenkamp, Zhou et al. 2016). Excess weight has also been associated with increased connectivity between the ventral striatum (which consists of the NAcc) and medial PFC (mPFC) (Contreras-Rodriguez, Martin-Perez et al. 2015). In another study looking at FC during fasting, obese subjects exhibited positive connectivity between AMYG and INS, whereas lean individuals showed negative connectivity

between these regions (Lips, Wijngaarden et al. 2014). Thus, resting state FC in obese humans seems to be stronger between brain regions involved in emotion and reward processing compared to lean individuals. Although several theories exist to explain the physiological source of these differences, one idea is that this dysfunctional connectivity reflects impaired top-down inhibitory control of feeding behavior (Nummenmaa, Hirvonen et al. 2012). Because negative FC is thought to reflect increased activity of one ROI and decreased activity in another, whereas positive FC is thought to relect simultaneous activity in both ROIs (Fox and Raichle 2007), the positive hyper-connectivity seen between prefrontal, striatal and limbic regions in obese humans may reflect an overactive reward circuit, where typical top-down inhibitory control over feeding (potentially represented as negative connectivity) and reward is impaired (Nummenmaa, Hirvonen et al. 2012). This theory, however, is most likely an oversimplification. For example, negative FC can be mathematically induced by a preprocessing step, called global signal regression (Fox, Zhang et al. 2009, Murphy, Birn et al. 2009). Nevertheless, it is accepted that negative FC has a biological basis, as it has been recognized without the application of global signal regression (Chang and Glover 2009, Fox, Zhang et al. 2009, Chai, Castanon et al. 2012). Therefore, the physiological mechanisms underlying positive vs. negative FC are extremely complex and not fully understood, and are still a topic of intense debate in the field (Cole, Smith et al. 2010, Goelman, Gordon et al. 2014).

Exposure to chronic stress also affects brain structure and function (Joels, Karst et al. 2004, Cerqueira, Pego et al. 2005, Dias-Ferreira, Sousa et al. 2009, Soares, Sampaio et al.

2013). However, relatively few studies have specifically investigated the effects of chronic stress on resting state FC. One study in humans found that chronic work-related stress was associated with weaker FC within clusters in the PFC and stronger FC within INS clusters in stressed compared to control subjects (Golkar, Johansson et al. 2014). This study also found that FC between AMYG and PFC was weaker, whereas FC between AMYG and INS was stronger in stressed compared to control subjects (Golkar, Johansson et al. 2014). During a task-based fMRI study, FC was decreased between PFC and INS in stressed individuals compared to controls (Liston, McEwen et al. 2009). Thus, these limited data suggest that exposure to chronic stressors differentially affects brain FC in a region specific manner.

Our focus on brain circuitry impacted by stress and diet-induced obesity has an established anatomical basis. The PFC has direct reciprocal anatomical connections with striatum, where vmPFC and orbitofrontal cortex (OFC) have stronger projections to ventral striatum, and dorsolateral PFC (dlPFC) has stronger projections to dorsal striatum (Haber and Knutson 2010). The AMYG and INS also reciprocally project to the NAcc (Haber and Knutson 2010). Although much of the PFC has connections with the AMYG, the densest projections from PFC to AMYG originate in the ACC and mPFC, especially in Brodmann areas (BA) 24 and 25. The densest projections back to the PFC from AMYG terminate in caudal OFC (Ghashghaei, Hilgetag et al. 2007). The vmPFC is involved in emotional and motivational salience assessment, and emotional regulation (Ma, Liu et al. 2010). The OFC is involved in goal-directed behavior, reward coding, impulse control and salience/value of food (Kuhnen and Knutson 2005, Rolls and

McCabe 2007, Grabenhorst, Rolls et al. 2008). The dlPFC is involved in working memory for evaluation, comparison and selection of incentive-based behavioral response (Haber and Knutson 2010). Finally, mPFC is involved in immediacy and anticipation of the benefit/risk ratio of rewards (Knutson, Taylor et al. 2005, Yacubian, Glascher et al. 2006, Haber and Knutson 2010) as well as integration of value across different stimuli (Blair, Marsh et al. 2006).

In human studies it is difficult to disentangle individual contributions of stress, diet and caloric content (e.g., saturated fats and sugars) on alterations in brain FC, due to factors like inaccuracies with self-reporting and lack of experimental control. Thus, a translational animal model is warranted that allows a level of experimental control not present in human studies (i.e social and dietary environment). Social subordination in female rhesus macaque monkeys is a translational animal model to study a number of stress-related phenotypes (Michopoulos, Higgins et al. 2012, Shively and Willard 2012), including stress-induced over eating of an obesogenic diet (Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012, Michopoulos, Diaz et al. 2016). Macaque groups, regardless of size, are organized by a matrilineal dominance hierarchy that functions to maintain group stability (Bernstein and Gordon 1974, Bernstein 1976), where lower-ranking animals receive more aggression from higher-ranking group mates and terminate these interactions by emitting submissive behavior, a defining feature of subordination (Bernstein and Gordon 1974, Bernstein, Gordon et al. 1974, Bernstein 1976, Shively and Kaplan 1984). A consequence of this continual harassment is impaired limbic hypothalamic pituitary adrenal (LHPA) axis regulation, typically evidenced by

reduced glucocorticoid (GC) negative feedback and hypercortisolemia in adult females (Shively, Laber-Laird et al. 1997, Shively 1998, Wilson, Pazol et al. 2005, Jarrell, Hoffman et al. 2008, Wilson, Fisher et al. 2008, Paiardini, Hoffman et al. 2009), a physiological phenotype similar to what has been described in some humans suffering from stress-induced psychopathology, such as depression (Coryell, Fiedorowicz et al. 2008, Jokinen, Nordstrom et al. 2008). Using this model, we sought to investigate how the imposition of a social stressor, achieved by forming new social social groups, interacts with dietary environment to affect brain FC between prefrontal, striatal, and other limbic regions known affect emotional reactivity and motivated behavior. Based on the evidence from the obesity literature summarized above, we hypothesized that excessive calorie intake, particularly of a high caloric diet (HCD), would result in increased FC between NAcc, AMYG, INS and PFC, and that these alterations would result in altered socio-emotional behavior.

## 4.3 Methods

### 4.3.1 Subjects

Subjects were 16 adult female rhesus macaques (*Macaca mulatta*) embedded as members of one of in six social groups housed in indoor-outdoor pens consisting of 4-6 adult females each at the Yerkes National Primate Research Center (YNPRC) in Lawrenceville, Ga. 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”. The social groups were formed following procedures previous described

(Jarrell, Hoffman et al. 2008, Snyder-Mackler, Kohn et al. 2016). Briefly, females were removed from the natal breeding groups and sequentially introduced to their new groups over the course of two weeks. This process minimizes aggression towards newly introduced animals. Dominance ranks are established within this two-week group formation phase. The 16 animals chosen for the present analysis were the highest (n = 8) and lowest ranking females (n = 8) in each of the six groups based on ELO rankings described below.

Data for determining social rank were obtained from weekly 30-minute observations using an established ethogram (Jarrell, Hoffman et al. 2008) and assessed using the outcome of dyadic interactions (Bernstein 1976) to capture the frequency of submission emitted and of aggression received. Rank was calculated using the ELO rating method, in which higher scores correspond to higher status (Albers and de Vries 2001, Neumann, Duboscq et al. 2011, Snyder-Mackler, Kohn et al. 2016). Briefly, ELO rating updates an animal's score after each dominance interaction based on "winning" or "losing" the interaction. For example, if a lower-ranking female is defeated by a higher-ranking female, the scores for both females would only change slightly (the lower ranking female's score would decrease and the higher-ranking female's score would increase). If a higher-ranking female is defeated by a lower-ranking female, however, their scores would change considerably. One advantage to this method is that it distinguishes individuals that are similarly ranked based on rates of agonistic interactions.

### 4.3.2 Dietary Intervention

The automated feeding stations at the YNPRC (Wilson, Fisher et al. 2008) allows the continual quantification of calories consumed from specific diets in socially-housed, free feeding rhesus monkeys. This feeding system is employed in order to assess how exposure to chronic social stress influences food intake in a complex dietary environment, in which animals can choose freely between a HCD and a low caloric diet (LCD) (Wilson, Fisher et al. 2008). Studies using this model in established female macaque social groups have shown that although all animals prefer the HCD, subordinate animals consume significantly more calories and gain more weight compared to more dominant group mates (Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012, Michopoulos, Diaz et al. 2016). This model has also been used to detect alterations in brain dopamine (DA) neurochemistry. Specifically, decreased dopamine D2 receptor binding potential (D2R-BP) in the OFC predicts increased caloric intake, as measured by positron emission tomography (PET) (Michopoulos, Diaz et al. 2016).

For the present study, half of the 16 subjects (LCD-only subjects) had *ad libitum* access to the standard monkey chow [LCD; 3.45 kcal/g, Purina #5038] and half had *ad libitum* access to a choice dietary environment (Choice -CH- subjects), wherein both the LCD and a calorically dense diet or HCD [4.47 kcal/g, D07091204S Research Diets, New Brunswick NJ) were available. The caloric composition of the LCD was 12% fat, 18% protein, 4.14% sugar carbohydrate and 65.9% fiber carbohydrate. The calories of the HCD were distributed as 36% fat, 18% protein, 16.4% sugar carbohydrate and 29.6% fiber-starch carbohydrate. The rationale for providing access to both the LCD and HCD is

based on well-established data showing that dietary choice sustains intake of HCDs (la Fleur, van Rozen et al. 2010) and more closely models the dietary environment available to humans. Food access was controlled using previously validated automated feeders that allow for quantification of caloric intake in socially housed, free feeding monkeys (Wilson, Fisher et al. 2008, Arce, Michopoulos et al. 2010, Michopoulos, Diaz et al. 2016). Briefly, subjects have radio-frequency identification chips inserted within each of their wrists, so when they place either of their hands in the feeder, the chip is scanned and signals a computer to dispense a single pellet of food via a pellet dispenser. Each social group had access to two different automated feeders and a computer recorded each feeding event in a log. This method has been previously validated and ensures that high-ranking females rarely (<1%) take the pellet from subordinate animals and pellets are never discarded (Wilson, Fisher et al. 2008).

### 4.3.3 Behavior

*Social Behavior.* Behavioral observations were collected weekly for 30 minutes using an established ethogram (Jarrell, Hoffman et al. 2008) that quantifies frequencies and durations of agonistic (aggression (threat, chase, slap, bite); affiliative (proximity and grooming) and anxiety-like behaviors (yawn, scratch or groom self, body shake). Behavior was coded in real time, using a notebook computer with an in-house program WinObs (Graves and Wallen 2006), which records the actor, behavior, recipient and the time for each behavior. Inter-observer reliability was calculated as percent agreement on frequencies and durations of behaviors and was greater than 92%. Behavioral

observations were initiated once all of the females had been introduced into their new groups.

*Human Intruder (HI) Test.* Each subject received the HI test, a standardized test of emotionality (Kalin and Shelton 1989), 7.70 ± 0.11 months following group formation and 4.44 ± 0.07 months prior to the rs-fMRI scans. The test was administered as previously described (Embree, Michopoulos et al. 2013, Wilson, Bounar et al. 2013). Briefly, this paradigm consists of three consecutive ten-minute sessions, including an alone condition, an intruder profile condition (an unfamiliar experimenter enters the room and sits with his/her profile towards the subject) and an intruder stare condition (the experimenter makes direct and continuous eye contact for the entire 10 minute session). The HI task is designed to measure defensive and emotional behavior responses specific to each condition, which pose different degrees of threat to rhesus monkeys. The alone condition elicits exploratory and distress responses, such as vocalizations and locomotion, whereas the intruder profile condition elicits behavioral inhibition, such as freezing and visually scanning of the environment. Finally, the intruder stare condition elicits aggressive and/or submissive behaviors directed towards the intruder, as direct eye contact is threatening for rhesus macaques. Videos of each test were scored by trained observers using a modification of published ethograms (Machado and Bachevalier 2006, Howell, Godfrey et al. 2014, Howell, Grand et al. 2014) to capture and quantify locomotion, aggressive, submissive, anxiety-like and fearful behaviors. Inter-observer reliability was greater than 92%.

*Behavioral data reduction analysis.* To reduce the number of behavioral variables, we performed a principal components analysis (PCA) using a rotation method of Varimax with Kaiser Normalization on social and HI behavior outcomes and diet condition (no choice = 1; choice = 2) in all 16 subjects. Variables were entered into the PCA and were chosen based on their relevance to rhesus macaque social behavior and emotional reactivity (Social behavior: proximity duration, groom frequency and duration, aggression and submission frequency; HI behavior: freeze duration in the alone, profile and stare conditions, look away frequency in the profile and stare conditions, threat and anxiety frequency in the stare condition). Dietary condition (1 for LCD only; 2 for CH) was included as preliminary analyses revealed that the dietary choice condition was associated with significantly reduced rates of initiating proximity ($r_{32}$=-0.36, $p$=0.03) and grooming ($r_{32}$=-0.37, $p$=0.03) towards group mates. This PCA method was chosen in order to consolidate behaviors into similar patterns of responses that represent the most prominent behavioral phenotypes. PCAs have previously been used to identify clusters of behaviors during the HI task in macaques (Williamson, Coleman et al. 2003, Wilson, Bounar et al. 2013). Behaviors that had a loading score of 0.45 or less were excluded. Four components emerged from the PCA analysis (Table 4.1). The first component was labeled "affiliation" as both social groom frequency and duration loaded positively, in addition to proximity duration (time spent sitting near another monkey). Grooming is a prosocial behavior in rhesus macaques and functions to maintain social alliances (Lindburg 1973). Dietary condition loaded negatively into this factor, suggesting that CH subjects were less sociable, which is consistent with our preliminary correlational analysis. The second component was labeled "anxious-aggression", where rates of

aggression directed at groups mates as well as three behaviors from the stare condition of the HI, looking away from the intruder, threatening the intruder, and anxiety-like behavior, loaded positively. Threat is a non-contact aggressive behavior in rhesus macaques (Altmann 1962). Since a monkey cannot move away from the intruder during the HI task, looking away during the stare condition may reflect increased anxiety and avoidance of the intruder (Pelphrey, Viola et al. 2004, Patterson 2008). In addition, freezing in the stare condition, a behavior indicative of fear (Kalin and Shelton 1998), loaded negatively on component 2. Behaviors that loaded positively on the third component were threat in the stare condition, freeze in the alone condition and submissive behavior directed towards group mates. In contrast, aggression directed at group mates loaded negatively. Because higher rates of rates of submissive and lower rates of aggressive behaviors are characteristic of subordinate monkeys, this component was labeled "submissive-fearful". Although threat in the stare condition is being interpreted as aggressive in the anxious-aggression component described above, in the context of the other behaviors in this component it may also be indicative of non-contact, defensive aggression, as previously reported (Meunier, Bachevalier et al. 1999). The fourth component was labeled "anxious-avoidant" because looking away from the intruder in the profile condition and anxiety in the stare condition loaded positively, while freezing in the profile condition loaded negatively. Together, these four components account for 82.7% of the variance for the included variables.

### 4.3.4 Stress Physiology and Immune Markers

For all animal accesses and blood draws, animals were habituated to being removed from their group for conscious venipuncture, using previously described procedures (Walker, Gordon et al. 1982). Blood samples were obtained within 10 minutes of entering the animal area to minimize arousal (Blank, Gordon et al. 1983).

*Cortisol response to acute stressor.* Plasma samples were collected immediately before and after the 30 minute HI task to determine acute stress-induced cortisol increases. Additional plasma samples were collected 60 and 240 minutes after the completion of the HI task to assess recovery to baseline cortisol levels.

*Basal Cortisol and Dexamethasone Suppression Test.* Glucocorticoid negative feedback was tested at 14.40 ± 0.08 months following group formation and 2.34 ± 0.08 months following the rs-fMRI scans, according to previously published procedures (Jarrell, Hoffman et al. 2008). A baseline, morning plasma sample was collected 1 hour after sunrise and an additional sample was collected again 4 hours later. Another plasma sample was collected 1 hour before sunset, immediately followed by dexamethasone administration (0.25mg/kg I.M.). The following morning (1 hour after sunrise) another blood sample was obtained to measure the degree to which dexamethasone suppressed morning cortisol. All plasma levels of cortisol were measured by LC-ESI-tandem mass spectrometry using a Discovery 5 cm × 2.1 mm C18 column (Supelco, PA) eluted at flow rate of 0.5 ml/min at the YNPRC Biomarkers Core. The intra- and inter-assay coefficient of variation (CV%) was 1.21% and 5.78%, respectively.

### 4.3.5 Cytokine/Immune Markers

Proinflammatory cytokine concentrations [C-reactive protein (CRP), tumor necrosis factor alpha (TNFα) and interleukin 6 (IL6)] in plasma were measured in duplicate using the following immunoassays: the V-Plex HumanCRP Kit; V-Plex custom NHP Proinflammatory Panel (IL-6); and rhesus specific TNFα kit (Meso Scale Discovery, Rockville, MD, USA). For measurement of CRP, samples were diluted 1:200. For all other assays, samples were measured undiluted. Samples were collected within two weeks of the MRI scan for each female.

### 4.3.6 Rs-fMRI

*Rsf-MRI acquisition.* Neuroimaging scans were collected 52.74 ± 0.39 weeks after group formation, using a 3T Siemens Magnetom Trio Tim scanner (Siemens Med. Sol., Malvern, PA, USA), and an 8-channel phase array coil. Subjects were transported from their social groups at the YNPRC Field Station in Lawrenceville, GA to the YNPRC Imaging Center in Atlanta, GA, either the morning of the scan or the day prior to the scan. Two 15 minute rs-fMRI (T2*-weighted) scans were acquired during a single session, which included T1- and T2-weighted structural MRI scans for registration purposes, in order to measure temporal changes in blood-oxygen-level dependent (BOLD) signal. Subjects were scanned under isoflurane anesthesia (1% to effect, inhalation) following induction with telazol (15-35mg, I.M.) and intubation to ensure lack of motion artifacts. This dose (and higher doses) of isoflurane has previously been used

to report coherent patterns of BOLD fluctuations in rhesus macaques, including sensory, motor, visual and cognitive-task related systems (Vincent, Patel et al. 2007, Hutchison, Womelsdorf et al. 2013, Li, Patel et al. 2013, Tang and Ramani 2016). Importantly, these patterns are similar to those observed in awake, behaving monkeys (Vincent, Patel et al. 2007). For physiological monitoring, animals were fitted with an oximeter, electrocardiograph, rectal thermometer and blood pressure monitor. Additionally, an I.V. catheter was placed to administer dextrose/NaCl (0.45%) in order to maintain hydration. All subjects were scanned in the same supine placement and orientation on an MRI-compatible heating pad, using a custom-made head holder with ear bars and a mouth piece. A vitamin E capsule was taped to the right temple to mark the right side of the brain. Subjects were returned to their social groups upon completion of the scan and full recovery from anesthesia. BOLD-weighted functional images were collected using a T2*-weighted gradient-echo echoplanar imaging (EPI) sequence (400 volumes, TR/TE = 2290/25msec, 2x15min, voxel size: 1.5mm$^3$ isotropic) to analyze FC between brain regions. The rs-fMRI scans were collected following the T1-MRI scan (which lasted about 30 min) to standardize time from initial anesthesia to 45 minutes for all subjects. An additional short reverse-phase encoding scan was also acquired for unwarping distortions in the EPI scans, using previously published methods (Andersson, Skare et al. 2003). The first 3 volumes were removed from each scan to allow for scanner equilibrium, resulting in a total of 794 concatenated volumes.

*Structural MRI acquisition.* High-resolution T1-weighted structural MRI scans were acquired using a 3D magnetization prepared rapid gradient echo (3D-MPRAGE) parallel

image sequence (TR/TE = 2600/3.38msec, FoV: 128mm, voxel size: 0.5mm$^3$ isotropic, 1 average, GRAPPA, R=2). T2-weighted MRI scans were also collected in the same direction as the T1 (TR/TE = 3200/373msec, FoV: 128mm, voxel size: 0.5mm$^3$ isotropic, 1 average, GRAPPA, R=2) to aid with registration and delineation of anatomical borders.

*Rs-fMRI data preprocessing.* Rs-fMRI is a useful tool to investigate FC between brain regions, based on evidence that blood oxygen level dependent (BOLD)-related activity in neural circuits during resting state recapitulates task-evoked activations in humans (Cordes, Haughton et al. 2000), even in anesthetized macaques (Vincent, Patel et al. 2007, Margulies, Vincent et al. 2009). All raw data were preprocessed using the FMRIB Software Library [FSL, Oxford, UK; (Smith, Jenkinson et al. 2004, Woolrich, Jbabdi et al. 2009)], in addition 4dfp tools (ftp://ftp.*imaging*.wustl.edu/pub/raichlab/4dfp_tools/) and an in-house built Nipype pipeline [based on (Gorgolewski, Burns et al. 2011)] with modifications of previously published methods (Fair, Dosenbach et al. 2007, Fair, Cohen et al. 2009, Fair, Nigg et al. 2012, Iyer, Shafran et al. 2013). Some methods were adapted specifically for the rhesus monkey brain (Miranda-Dominguez, Mills et al. 2014). After file conversion, in order to reduce noise and artifact, functional imaging series were 1.) unwarped using a reverse phase-encoding distortion correction method (Andersson, Skare et al. 2003), 2.) slice-time corrected (for the even vs. odd slice intensity differences due to interleaved acquisition), 3.) motion-corrected (rigid body motion correction within-run, linear registration from EPI to T1, and nonlinear registration from T1 to template applied all in one resampling step), 4.) Normalized (signal normalization to a whole brain mode value gradient of 1000). Next, the EPI functional time series were concatenated and rigid-

body co-registered to the subject's averaged T1-weighted structural image. This was then transformed to the 112RM-SL (RM: Rhesus Macaque; SL: Saleem-Logothetis coordinate space) atlas (McLaren, Kosmatka et al. 2009, McLaren, Kosmatka et al. 2010), using rigid-body registration followed by linear (FLIRT) and non-linear registration (FNIRT) methods in FSL. This 112RM-SL atlas is an average of 112 male and female adult monkeys (McLaren, Kosmatka et al. 2009, McLaren, Kosmatka et al. 2010), in F99 space, and the EPI images were transformed into F99 space in one interpolation step for the region of interest (ROI) analysis described below. Additional pre-processing steps included: 6.) functional signal detrending, 7.) removing nuisance regressor (i.e. regression of rigid body head motion parameters in 6 directions, global whole-brain signal, ventricular and white matter functional signal -averaged from a ventricle and a white matter mask, respectively-, and first-order derivatives for the whole brain, ventricular and white matter signals) and 8.) temporal low-pass filtering ($f < 0.1Hz$) via a second order Butterworth filter (Fair, Dosenbach et al. 2007, Fair, Cohen et al. 2009, Fair, Nigg et al. 2012, Miranda-Dominguez, Mills et al. 2014). Analyses were conducted with the removal of frames having displacement (FD) value greater than 0.2 mm (Power, Barnes et al. 2012, Power, Mitra et al. 2014). Additionally, all imaging data were visually inspected upon preprocessing completion, to determine if any series had unsatisfactory co-registration or significant blood-oxygen-level dependent (BOLD) signal dropout.

FC between NAcc, AMYG, INS and PFC sub-regions was analyzed. PFC ROIs were defined based on previously published anatomical parcellations (Lewis and Van Essen 2000, Markov, Ercsey-Ravasz et al. 2014), mapped onto the cortical surface of the

112RM-SL atlas (registered to F99 space). The AMYG ROIs were manually drawn by experts using cytoarchitectonic maps in an existing UNC-Wisconsin adolescent atlas [RRID: SCR_002570 (Styner, Knickmeyer et al. 2007)] and then warped to the 112RM-SL atlas (in F99 space) using deformable registration via ANTS [for details see (Shi, Budin et al. under review)]. Each ROI was manually edited in both the 112RM-SL atlas and each of the subjects to exclude overlapping ROIs, non-brain tissue or signal dropout). The resting state BOLD time series were then correlated ROI by ROI for each subject. For this, the time course of the BOLD signal was averaged across the voxels within each ROI, and then correlated with the time course of all other ROIs. Correlation coefficients (r-values) between ROIs were extracted from these correlation matrices using Matlab (MathWorks Inc., Natick, MA, RRID: SCR_001622).

### 4.3.7 Statistical Analyses

To determine how caloric intake of specific diets, ELO rank, stress and proinflammatory markers affect functional brain connectivity between PFC, NAcc, INS and AMYG, stepwise multiple regression analyses were conducted separately for subjects in both the CH and LCD conditions. For these regressions, the following were entered as dependent variables: resting state connectivity correlations (ipsilateral) between right and left AMYG, NAcc and INS, in addition to connectivity from AMYG, Nacc and INS to dlPFC (BA 9, 46), ACC (BA 24, 32), vmPFC (BA 14, 25) and OFC (BA11, 13). Predictor variables entered into the stepwise regression were the following: (1) For LCD-only subjects: ELO rank and average weekly LCD consumed (kcal) across the entire study; (2) For CH subjects, ELO rank, average weekly HCD consumed (kcal) and average weekly

total kcal consumed (LCD+HCD), across the entire study; (3) Additional predictors for all subjects included cortisol (baseline cortisol, change in response to dexamethasone suppression and change from prior to and immediately after the HI test) and proinflammatory markers (circulating CRP, TNFα and IL6).

Following this analysis, another stepwise multiple regression analysis was conducted separately for subjects in both the CH and LCD conditions to investigate what functional outcomes in behavior emerge as a result of the observed diet and rank induced alterations in resting state functional brain connectivity. For this analysis, we used each of the following predictor variables: ELO rank and resting state FC correlations (ipsilateral) between right and left AMYG, NAcc and INS, as well as connectivity from AMYG, Nacc and INS to BA 9, 46 (dlPFC), 24, 32 (ACC), 14, 25 (vmPFC) and 11, 13 (OFC). The four components of behavior determined from the PCA (affiliation, anxious-aggression, submissive-fearful and anxious-avoidant) were entered as dependent variables.

## 4.4 Results

### 4.4.1 Predictors of alterations in ROI-ROI FC

For descriptive statistics of weight, BMI and predictor variables, see Table 4.2. For a summary of the results, see Table 4.3A, for descriptive statistics of FC data, see Table 4.4 and for a graphic of the results, see Figure 4.1.

*AMYG-dlPFC models*

CH subjects: Greater HCD consumption and greater CRP predicted more positive FC between left AMYG and BA 9 [F=(2, 7)=47.099, $p$=0.001, Adj. $R^2$=0.929]. Lower total kcal consumed, lower baseline cortisol and higher IL6 predicted more positive FC between right AMYG and BA 9 [F=(3, 7)=128.160, $p$<0.001, Adj. $R^2$=0.982)].

LCD subjects: There were no significant AMYG-dlPFC FC models in the LCD subjects.

*AMYG-OFC models*

CH subjects: There were no significant AMYG-OFC FC models in the CH subjects.

LCD subjects: Higher ELO rank and greater TNFα predicted stronger and more positive FC between left AMYG and BA 13 [F=(2, 7)=29.880, $p$=0.002, Adj. $R^2$=0.892)]. Greater change in cortisol in response to the HI task and lower LCD consumed predicted stronger and more positive FC between right AMYG and BA 13 [F=(2, 7)=32.928, $p$=0.001, Adj. $R^2$=0.901)].

*AMYG-INS models*

CH subjects: Greater HCD intake predicted stronger positive connectivity between left AMYG and INS [F=(1, 7)=9.926, $p$=0.02, Adj. $R^2$=0.560)].

LCD subjects: Greater LCD consumed and greater CRP predicted stronger, more positive FC between right AMYG and INS [F=(2, 7)=18.547, $p$=0.005, Adj. $R^2$=0.834)].

*INS-OFC models*

CH subjects: Higher ELO rank (more dominant) predicted weaker and more negative connectivity between right INS and BA 11 [F=(1, 7)=10.061, $p$=0.019, Adj. $R^2$=0.564)].

LCD subjects: There were no significant INS-OFC FC models in the LCD subjects.

*NAcc-dlPFC models*

CH subjects: Greater total kcal intake predicted more negative FC between right NAcc and BA 46 [F=(1, 7)=11.101, $p$=0.016, Adj. $R^2$=0.591)].

LCD subjects: There were no significant NAcc-dlPFC FC models in the LCD subjects.

**4.4.2 Functional behavioral outcomes**

The complete results from the stepwise multiple regression are summarized in Table 4.3B. The results reported here are the specific behavioral outcomes predicted by diet and rank induced alterations in resting state functional brain connectivity.

*Affiliation Component*

CH subjects: Connectivity between left AMYG and BA 9(dlPFC) negatively predicted the variance in the component score for affiliation [F(4, 3)=1614.93, $p$<0.001, Adj. $R^2$=0.999].

LCD subjects: No significant resting state FC correlations between brain regions predicted the PCA component affiliation.

*Anxious-Aggression Component*

No pairs of ROI FC, that were previously predicted by diet and rank induced alterations in resting state functional brain connectivity, entered into these models in either the CH or the LCD-only subjects.

*Submissive-Fearful Component*

CH subjects: The PCA component submissive-fearful was negatively predicted by connectivity between left AMYG and BA 9(dlPFC) [$F(4, 3)=1159.27$, $p<0.001$, Adj. $R^2=0.998$].

LCD subjects: Left AMYG FC with BA13(OFC) positively predicted the variance in this model [$F(2, 5)=27.20$, $p=0.002$, Adj. $R^2=0.882$].

*Anxious-Avoidant Component*

CH subjects: No resting state FC correlations between brain regions predicted the PCA component anxious-avoidant.

LCD subjects: Left AMYG FC with BA 13(OFC) negatively predicted the variance in this model [$F(5, 2)=1754.93$, $p=0.001$, Adj. $R^2=0.999$].

## 4.5 Discussion

The results of the present study revealed strikingly different phenotypes in rhesus macaques with access to a LCD or a more complex dietary environment where a choice between LCD and HCD was available, underscoring the notion that calories derived from diets high in sugar and fat impact the brain differently than calories derived from healthier low sugar and fat diets. Overall, the significant ROI-ROI FC in the CH and LCD-only subjects was predicted by food intake, inflammatory factors, social status and cortisol. However, no significant FC pairs emerged in both the CH and the LCD-only subjects, thus, different variables were responsible for predicting FC in each dietary

environment. In the CH subjects, HCD consumption positively predicted FC in the left hemisphere between AMYG and dlPFC, in addition to AMYG and INS FC. The direction of these effects was reversed in the right hemisphere, where total kcal intake was negatively predictive of FC between AMYG and NAcc with dlPFC. In the CH subjects, ELO rank negatively predicted right INS and OFC FC, whereas ELO rank positively predicted FC between left AMYG and OFC in the LCD subjects. In the LCD subjects, food intake positively predicted right AMYG FC with INS and negatively predicted right AMYG FC with OFC. Pro-inflammatory signals entered into several models, where greater CRP, IL6 and TNFα consistently predicted more positive FC between ROIs. Cortisol significantly predicted FC in two models, where lower baseline cortisol predicted more positive FC between right AMYG and dlPFC in the CH subjects and greater stress-induced cortisol predicted stronger and more postitive FC between right AMYG and OFC in the LCD subjects. HCD consumption was associated with decreased affiliative and submissive-fearful behavior in the CH subjects, whereas more subordinate social status was associated with increased submissive-fearful and decreased anxious-avoidant behavior in social group behavior and the Human Intruder task. Taken together, the findings that greater caloric intake result in specific altered FC in dlPFC-NAcc-AMYG-INS circuits and resulting behavior, particularly within the CH subjects, may suggest that the incentive salience of and motivational drive to consume palatable foods is increased, while the ability to regulate food intake is decreased in these subejcts (Figure 4.1).

In the CH subjects, greater intake of the HCD predicted more positive connectivity between left AMYG and dlPFC. Interestingly, this relationship was reversed in the right hemisphere for females in the CH condition, where right AMYG and dlPFC FC was negatively predicted by total caloric intake. Although these two regions have weak direct anatomical connectivity (Ghashghaei, Hilgetag et al. 2007), they have both been implicated in executive function, emotional regulation, social cognition, craving and conditioned responses to food (Haber and Knutson 2010, Volkow, Wang et al. 2012, Bickart, Dickerson et al. 2014). This result suggests an effect of laterality in FC between AMYG and dlPFC in the right and left hemispheres. There is some evidence of laterality in both AMYG and dlPFC function. Previously, in both task-evoked and rs-fMRI studies, the left dlPFC has been implicated in approach behaviors for potentially rewarding outcomes (Spielberg, Miller et al. 2011, Krmpotich, Tregellas et al. 2013). For example, greater rs-fMRI BOLD signal in the executive control network (which comprises the dlPFC) in the left hemisphere was associated with potentiated behavioral approach tendencies, which was interpreted as contributing to drug seeking behavior in substance-dependent subjects (Krmpotich, Tregellas et al. 2013). Additionally, AMYG FC with middle frontal gyrus (which contains the dlPFC subdivision) shows a laterality effect in healthy adult humans, where both right and left hemispheres exhibited a negative correlation, but right hemisphere was more strongly negative than left hemisphere (Roy, Shehzad et al. 2009). Although the literature is limited, one task-evoked fMRI study (in response to pictures of appetizing food) suggests a functional relevance to these observed difference in AMYG laterality. In this task-evoked fMRI study, greater right AMYG activation was associated with lack of ability to regulate and adapt behavior (interpreted

as subject prone to respond reflexively to external triggers of eating) and greater left AMYG activation in subjects who reported greater ability to successfully regulate food intake (Grimm, Jacob et al. 2012). The results of these studies are difficult to synthesize, however, because some measure network connectivity during rs-fMRI and others measure task-evoked BOLD activation in specific regions. Additionally, the described literature in the executive control network, involving the dlPFC, does no include the AMYG. Although speculative, the literature described here may provide a theoretical framework in which to understand the laterality in the findings from the present study. Perhaps the positive connectivity in the CH subjects (associated with greater HCD consumption) in the left hemisphere reflects a greater drive to obtain environmental rewards [palatable food, for example, supported by the findings from (Krmpotich, Tregellas et al. 2013)] and the negative connectivity [associated with greater total food intake, supported by the findings from (Grimm, Jacob et al. 2012)] seen in the right hemisphere between the AMYG and dlPFC is reflective of impaired behavioral regulation/cessation of food intake, as suggested by the human literature described above. This hypothesis is consistent with theories underlying the development of addictions, where a loss of control over drug intake is reported (Koob and Volkow 2016). As this hypothesis was not directly tested in the current study, future research is necessary to better understand functional relevance of laterality in AMYG and dlPFC FC. Interestingly, left AMYG FC with BA 9 (dlPFC) was the only significant ROI pair in the behavioral analysis for the CH subjects, which showed that these regions negatively predicted the PCA components affiliation and submissive-fearful, providing a valuable insight to the functional outcomes of the diet and inflammation associated effects. Thus,

the more positive FC between these regions in the left hemisphere predicted by higher HCD consumption leads to decreased affiliative and submissive-fearful behavior in the CH subjects. Perhaps the decreased affilitive behavior in these subjects is because they forgo social interacitons in favor of consuming the HCD. The finding of decreased submissive-fearful behavior may be consistent with the proposed increased drive to consume food in these subjects (described above), because decreased fear of potential negative consequences of feeding behavior (such as being displaed from the feeder by a more dominant animal) is potentiated, facilitating the increased consumption of food.

Ingestion of the HCD by CH females was predictive of stronger and more positive connectivity between left AMYG and INS. The AMYG and INS are anatomically reciprocally connected (Mesulam and Mufson 1982, Ghashghaei and Barbas 2002, Reynolds and Zahm 2005, Hoistad and Barbas 2008) and have both been implicated in a salience network which is thought to function to identify the most relevant internal and external stimuli in order to select appropriate behavioral responses (Seeley, Menon et al. 2007). These findings are consistent with one previous study in obese humans that showed positive connectivity between AMYG and INS during a fasting state, whereas lean individuals exhibited negative connectivity between these regions (Lips, Wijngaarden et al. 2014). One interpretation of the increased FC between the AMYG and INS in subjects eating more of the HCD could be that in those subjects, the HCD is more salient, palatable and rewarding, which has been argued in the case of obese humans (Rolls 2011). Indeed, the INS has been implicated in gustatory processing and interoception in humans (Craig 2009). Additionally, information about the emotional

valence and rewarding properties of a stimulus may be conveyed to the INS through it's direct reciprocal connections with the AMYG and NAcc (Mesulam and Mufson 1982, Ghashghaei and Barbas 2002, Reynolds and Zahm 2005, Hoistad and Barbas 2008). Furthermore, activation of the INS in task-evoked fMRI studies in humans positively correlates with the subjective pleasantness of taste (Grabenhorst and Rolls 2008, Grabenhorst, Rolls et al. 2008).

Greater total total caloric intake predicted more negative FC between right NAcc and dlPFC in the CH subjects. While some previous studies show increased FC between NAcc and other PFC regions in both obesity and drug addiction (Ma, Liu et al. 2010, Contreras-Rodriguez, Martin-Perez et al. 2015, Coveleskie, Gupta et al. 2015), other studies in drug addiction show decreased FC (Sutherland, McHugh et al. 2012). To our knowledge, no studies have specifically investigated dlPFC connectivity with AMYG and NAcc in the context of obesogenic feeding, but the addiction literature has interpreted this decreased FC as decreased functioning of reward circuitry, which precipitates the transition from using drugs into the development of addiction (Koob and Le Moal 2008, Sutherland, McHugh et al. 2012). FC between these regions in healthy humans, without a history of neuropsychiatric disease, is positive (Di Martino, Scheres et al. 2008). Therefore, the negative FC between right NAcc and dlPFC in the CH subjects may reflect decreased rewarding properties of the HCD. Although speculative and not directly measured in the present study, these findings may be mediated by decreased D2R-BP in the NAcc. Calorically dense foods elicit DA release in the NAcc (Spanagel and Weiss 1999, Hajnal, Smith et al. 2004), which is interconnected with cortico-limbic

regions including the PFC and serves as a critical region integrating reward processes (Lodge and Grace 2005, Wanat, Hopf et al. 2008). Sustained DA release in the NAcc, as a result of sustained HCD consumption, results in D2R down regulation in rats (Johnson and Kenny 2010). Indeed, obese people not only show decreased D2R availability in the striatum but also exhibit reduced PFC activity compared to lean individuals (Volkow, Wang et al. 2008). The PFC, which also receives DA innervation (Haber and Knutson 2010), plays a critical role in top-down inhibitory control of the NAcc (e.g., inhibiting impulsive eating, drug intake), reducing impulsive behavior. As such, decreases in PFC D2R have been linked with increased vulnerability to cocaine addiction in women (Volkow, Tomasi et al. 2011). Thus, the finding that greater total total caloric intake predicted more negative FC between right NAcc and dlPFC in the CH subjects may be, in part, due to mechanisms involving changes in DA signaling.

In the CH subjects, more subordinate status predicted stronger and more positive connectivity between right INS and OFC. The INS and OFC have direct anatomical connections (Mesulam and Mufson 1982, Mufson and Mesulam 1982) and, as previously discussed, also comprise part of the salience network, particularly the INS, which is involved in the identification and processing of the saliency of social and environmental simuli (Menon and Uddin 2010). Both regions have also been associated with social decision making (Lee 2008). Thus, stronger connectivity between these regions in more subordinate CH subjects may reflect hyperactive processing of salient social environmental stimuli, although this was not directly tested in the present study. This putative neural adaptation to social stress would be necessary for a subordinate monkey

to successfully navigate social environments, which require recognizing one's social status in relation to others and selecting behaviors accordingly (for example, withdrawing from a more dominant animal).

In the LCD-only subjects, two models were predicted by caloric intake, where higher LCD consumption predicted increased FC between right AMYG and INS and decreased FC between right AMYG and OFC. As previously discussed, the AMYG and INS have been implicated in a salience network which is thought to function to identify the most relevant internal and external stimuli in order to select appropriate behavioral responses (Seeley, Menon et al. 2007). The result that FC between INS and AMYG was increased with greater kcal intake, in both the LCD and the CH subjects, indicates that circuitry between these regions becomes hyperactive with increased food inake, regardless of caloric content, potentially through increasing the salience of food. Decreased FC between right AMYG and OFC was also predicted by greater LCD intake. As yet, no studies have examined resting state FC between AMYG and OFC in diet-induced obesity specifically, however, the orbitofrontal cortex is important in regulating control over food intake (Batterham, ffytche et al. 2007, Rolls 2008) and decreased connectivity between AMYG and OFC may reflect lack of control over caloric consumption, even when only a LCD is available.

More dominant rank also predicted increased FC between AMYG and OFC in the LCD subjects, but in the left hemisphere. These regions have been implicated in a social perception network in macaques and humans, responsible for identification and

interpretation of relevant social stimuli, especially facial expressions (Azzi, Sirigu et al. 2012, Bickart, Dickerson et al. 2014). Importantly, FC between left AMYG and OFC was the only ROI pair to enter into the behavioral analysis, where increased FC between these regions predicted greater submissive-fearful behavior and less anxious-avoidant behavior. While this functional outcome may seem contentious (more dominant status predicts increased AMYG-OFC FC, which leads to increased submissive-fearful behavior), some of behaviors which make up the PCA component submissive-fearful are derived from the HI task. As dominant monkeys are rarely presented with direct social threats in their social group, they may be more reactive to the HI task than more subordinate animals who have adapted to continual threats and harassment by other monkeys.

Interestingly, circulating pro-inflammatory cytokines entered into several models, where greater CRP, IL6 and TNFα always predicted more positive FC between ROI pairs. CRP, IL6 and TNFα all were predictive of models including feeding behavior and ELO rank, suggesting an important role for inflammation in mediating FC between the ROI pairs investigated in the current study. Peripheral pro-inflammatory cytokines, resulting from increased adipose tissue, may be affecting FC by recruiting peripheral macrophages into the brain, thereby activating microglia and facilitating neuroinflammation (Quan and Banks 2007, Miller, Maletic et al. 2009). Additionally, microglia can also be activated by stressor exposure (Frank, Baratta et al. 2007), which may help to explain the cytokine predictors in the LCD models where ELO rank negatively predicted FC. Furthermore, greater microglia density and activation in the PFC has been associated with stress-induced psychopathology (Steiner, Bielau et al. 2008, Setiawan, Wilson et al. 2015).

Indeed, decreased connectivity between ventral striatum and vmPFC has been associated with increased CRP in depressed patients (Felger, Li et al. 2015).

Cortisol significantly predicted FC in both the CH and LCD subjects. In the CH subjects, lower baseline cortisol predicted more positive FC between right AMYG and dlPFC and in the LCD subjects, greater stress-induced cortisol (change in cortisol in response to the HI task) predicted stronger and more postitive FC between right AMYG and OFC. Negative feedback of glucocorticoids is regulated by PFC and other regions (Herman 2013). Glucocorticoids have known actions in mediating energy homeostasis (Uchoa, Aguilera et al. 2014) and acute stress response has been linked to increased feeding behavior in previous studies, particularly when palatable food is available. For example, injection of dexamethasone into the brain in rats stimulates food intake and subsequent body weight gain (Zakrzewska, Cusin et al. 1999, Cusin, Rouru et al. 2001). Importantly, this result underlies a critical interplay between glucocorticoids and feeding behavior evident in both the CH and LCD subjects.

It is important to acknowledge several limitations in the present study. A network-based approach would add complementary information to this ROI-focused analysis, as it is possible that the impact of social status and dietary environment have generalized effects on global brain circuitry and behavioral outcomes, and it will be interesting how the affected circuits identified here are part of even bigger affected networks, as no two brain regions function in isolation (Miranda-Dominguez, Mills et al. 2014, Paolini, Laurienti et al. 2014). With a network approach, other important circuits not selected as ROIs in the

present study may emerge as importantly affected by social status and dietary environment. For example, the executive control network is comprised of many subdivisions of the PFC (including dlPFC) and INS, but also includes lateral parietal cortex (Seeley, Menon et al. 2007), which was not included in the present study. Next, care must be taken when making strong inferences about anti-correlated results, as global signal regression (used in the preprocessing of the resting state data) has been shown to induce negative correlations (Murphy, Birn et al. 2009). Additionally, causality cannot be determined with the current study design, as baseline scans were not obtained prior to study initiation. Future studies are necessary with baseline measures, collected prior to diet intervention, in order to determine whether diet-induced changes in cortisol, immune markers, brain FC and behavior are directly caused by consumption of a HCD. Also, since our sample size was small (n=8 per group), we may have been underpowered to detect statistically significant effects. Therefore, future studies are necessary to replicate these findings with a larger sample size. Nevertheless, the differences in FC and associated behavior between the CH and LCD-only females suggest a significant impact of the dietary environment on brain function.

In summary, the present study revealed robust phenotypic differences between rhesus macaques consuming either a standard laboratory chow, LCD or a complex diet comprised of both a LCD and HCD. Overall, the significant ROI-ROI FC in the CH and LCD-only subjects was predicted by food intake, inflammatory factors, social status and cortisol. However, no significant FC pairs emerged in both the CH and the LCD-only subjects, thus, different variables were responsible for predicting FC in each dietary

environment. In the CH subjects, greater caloric intake positively predicted FC in the left hemisphere between AMYG and dlPFC, in addition to AMYG and INS FC. This effect was reversed in the right hemisphere, where total kcal intake was negatively predictive of FC between AMYG and NAcc with dlPFC. These effects were also associated with increased inflammatory factors, decreased baseline cortisol, in addition to decreased affiliative and submissive-fearful behavior. Conversely, in the LCD subjects, different connectivity patterns were predicted by caloric intake, where food intake positively predicted right AMYG FC with INS and negatively predicted right AMYG FC with OFC. These findings in the LCD-only subjects were associated with greater change in cortisol in response to an acute stressor and increased inflammation. There were no functional outcomes of diet-induced changes in brain FC in the LCD-only subjects. ELO rank also predicted different patterns of FC in the CH compared to the LCD subjects. In the CH subjects, ELO rank negatively predicted right INS and OFC FC, while ELO rank positively predicted FC between left AMYG and OFC in the LCD subjects. The functional outcomes predicted by social status were only evident in the LCD-only subjects, where they exhibited increased submissive-fearful and decreased anxious-avoidant behavior. Taken together, the findings that greater caloric intake result in specific altered FC in dlPFC-NAcc-AMYG-INS circuits and resulting behavior, particularly within the CH subjects, suggest that the incentive salience of and motivational drive to consume palatable foods is increased, while the ability to regulate food intake is decreased in these subjects. The complex interactions, and underlying mechanisms, between how dietary environment and chronic stress affect brain and

behavior highly warrant future elucidation, in order to successfully prevent and treat stress and diet-induced obesity related illness.

**Table 4.1 Principal component analysis (PCA) factor loading of human intruder and social behavior.** Four components emerged and were labeled affiliation, anxious-aggression, submissive-fear and anxious avoidant.

| | Factor Loading | | | |
|---|---|---|---|---|
| | Component 1 | Component 2 | Component 3 | Component 4 |
| **Variable** | **Affiliation** | **Anxious-Aggression** | **Sumissive-Fearful** | **Anxious-Avoidant** |
| Groom Dur. | 0.935 | | | |
| Groom Freq. | 0.922 | | | |
| Proximity Dur. | 0.870 | | | |
| Diet | -0.568 | | | |
| Look Away Freq.-Stare | | 0.963 | | |
| Freeze Dur.-Stare | | -0.945 | | |
| Threat Freq.-Stare | | 0.693 | 0.630 | |
| Freeze Dur.-Alone | | | 0.870 | |
| Submission Freq. | | | 0.849 | |
| Aggression Freq. | | 0.560 | -0.583 | |
| Freeze Dur.-Profile | | | | -0.857 |
| Look Away Freq.-Profile | | | | 0.688 |
| Anxiety Freq.-Stare | | 0.550 | | 0.624 |

**Table 4.2 Descriptive statistics of BMI, weight and predictor variables.** Descriptive statistics of body weight, BMI and predictor variables used in regression analyses in the CH and LCD-only subjects.

| | CH Subjects | | | | LCD-only Subjects | | | |
|---|---|---|---|---|---|---|---|---|
| | **Minimum** | **Maximum** | **Mean** | **SEM** | **Minimum** | **Maximum** | **Mean** | **SEM** |
| **BMI** | 11.282 | 19.364 | 16.313 | 0.982 | 11.242 | 16.870 | 14.373 | 0.700 |
| **Weight** | 8.180 | 13.780 | 11.473 | 0.685 | 7.440 | 11.030 | 9.525 | 0.488 |
| **Avg. Weekly HCD Consumed (kcal)** | 3626.000 | 9962.000 | 6715.810 | 762.010 | n/a | | | |
| **Avg. Weekly LCD Consumed (kcal)** | 4174.527 | 10118.635 | 6601.941 | 804.222 | 5313.757 | 13680.232 | 9730.659 | 896.618 |
| **Avg. Weekly Total Consumed (kcal)** | 8400.954 | 20081.034 | 13317.746 | 1407.779 | | | | |
| **CRP** | 13920.249 | 117141.714 | 57610.088 | 13872.672 | 6366.685 | 130380.635 | 37714.541 | 14343.221 |
| **TNFα** | 4.444 | 62.089 | 24.179 | 6.952 | 0.610 | 59.068 | 16.206 | 6.902 |
| **IL6** | 0.496 | 1.293 | 0.981 | 0.094 | 0.364 | 2.757 | 1.064 | 0.292 |
| **Baseline Cortisol** | 15.100 | 29.258 | 19.898 | 1.763 | 13.934 | 24.313 | 17.662 | 1.265 |
| **Δ Cortisol Dex Suppression** | -28.533 | -11.415 | -17.165 | 1.969 | -22.053 | -13.012 | -16.217 | 1.091 |
| **Δ Cortisol Pre-Post HI** | -0.050 | 5.200 | 2.875 | 0.690 | 0.550 | 5.300 | 3.406 | 0.613 |

**Table 4.3 Summary of significant models from stepwise multiple stepwise regression for both CH and LCD experimental groups. (A)** Predictors of alterations in ROI-ROI resting state FC; and **(B)** Functional behavioral outcomes of ROI-ROI resting state FC. ROI-ROI resting state FC pairs in bold were significant in both multiple stepwise regressions.

**A.**

| | | Overall Model | | | Retained Predictor Variables | Predictor Variables | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **Dependent Variable** | ***Adj. $R^2$*** | ***F*** | ***Sig.*** | | **B** | **Std. Error** | **β** | ***t*** | ***Sig.*** |
| **Choice** | AMYG-INS Left | 0.560 | 9.926 | 0.020 | Avg. Weekly HCD Consumed (kcal) | 5.15E-05 | 1.63E-05 | 0.789 | 3.151 | 0.020 |
| | **AMYG-9(dlPFC) Left** | 0.929 | 47.099 | 0.001 | Avg. Weekly HCD Consumed (kcal) | 6.10E-05 | 7.34E-06 | 0.843 | 8.309 | 4.12E-04 |
| | | | | | CRP | 1.53E-06 | 4.03E-07 | 0.385 | 3.793 | 0.013 |
| | AMYG-9(dlPFC) Right | 0.982 | 128.160 | 1.98E-04 | IL6 | 0.315 | 0.020 | 0.810 | 15.879 | 9.19E-05 |
| | | | | | Avg. Weekly Total Consumed (kcal) | -1.44E-05 | 1.38E-06 | -0.553 | -10.400 | 4.83E-04 |
| | | | | | Baseline Cortisol | -0.004 | 0.001 | -0.170 | -3.202 | 0.033 |
| | NAcc-46(dlPFC) Right | 0.591 | 11.101 | 0.016 | Avg. Weekly Total Consumed (kcal) | -1.75E-05 | 5.24E-06 | -0.806 | -3.332 | 0.016 |
| | INS-11(OFC) Right | 0.564 | 10.061 | 0.019 | ELO Rank | -1.03E-04 | 3.24E-05 | -0.791 | -3.172 | 0.019 |
| **LCD-Only** | AMYG-INS Right | 0.834 | 18.547 | 0.005 | CRP | 2.38E-06 | 4.26E-07 | 0.865 | 5.586 | 0.003 |
| | | | | | Avg. Weekly LCD Consumed (kcal) | 2.01E-05 | 6.82E-06 | 0.456 | 2.943 | 0.032 |
| | **AMYG-13(OFC) Left** | 0.892 | 29.880 | 0.002 | TNFα | 0.011 | 0.001 | 1.135 | 7.731 | 0.001 |
| | | | | | ELO Rank | 1.28E-04 | 3.12E-05 | 0.605 | 4.120 | 0.009 |
| | AMYG-13(OFC) Right | 0.901 | 32.928 | 0.001 | Δ Cortisol Pre-Post HI | 0.057 | 0.007 | 1.217 | 7.687 | 0.001 |
| | | | | | Avg. Weekly LCD Consumed (kcal) | -1.58E-05 | 5.05E-06 | -0.495 | -3.129 | 0.026 |

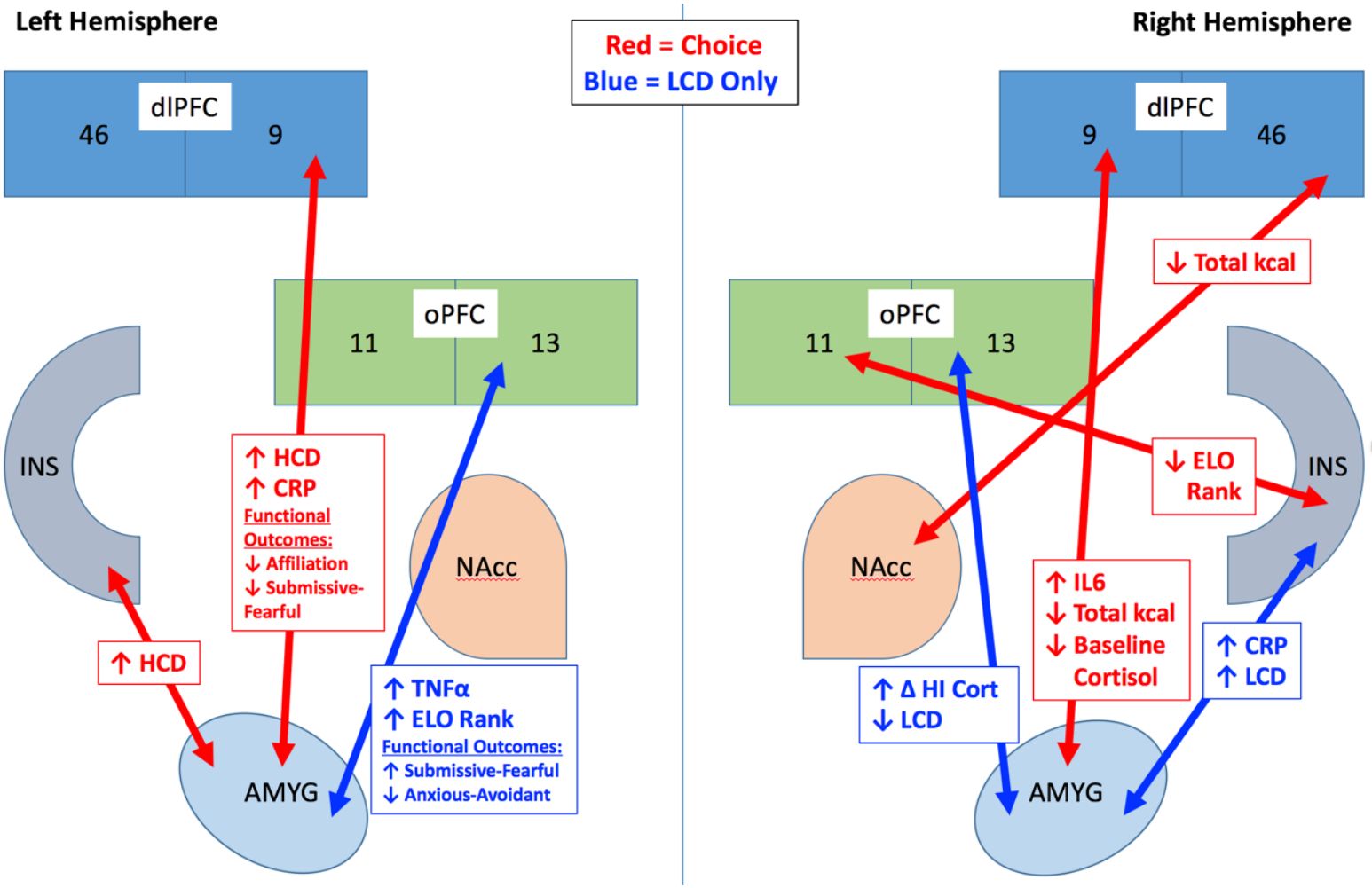
**B.**

| | Dependent Variable | Overall Model *Adj. $R^2$* | *F* | *Sig.* | Retained Predictor Variables | Predictor Variables B | Std. Error | β | *t* | *Sig.* |
|---|---|---|---|---|---|---|---|---|---|---|
| **Choice** | Affiliation | 0.999 | 1614.93 | 2.50E-05 | **AMYG-9(dlPFC) Left** | -1.118 | 0.046 | -0.338 | -24.473 | 1.50E-04 |
| | | | | | NAcc-14(vmPFC) Left | 8.137 | 0.11 | 1.004 | 73.646 | 6.00E-06 |
| | | | | | NAcc-25(vmPFC) Right | 0.802 | 0.052 | 0.253 | 15.314 | 0.001 |
| | | | | | NAcc-32(mPFC) Right | -0.549 | 0.096 | -0.082 | -5.725 | 0.011 |
| | Anxious-Aggression | 1.000 | 4.52E+07 | 1.14E-04 | AMYG-46(dlPFC) Right | 5.003 | 0.001 | 0.532 | 7454.913 | 8.50E-05 |
| | | | | | AMYG-24(ACC) Left | 0.02 | 3.70E-04 | 0.004 | 54.37 | 0.012 |
| | | | | | NAcc-11(OFC) Right | 3.102 | 0.002 | 0.192 | 1976.115 | 3.20E-04 |
| | | | | | NAcc-14(vmPFC) Left | -7.411 | 0.002 | -0.423 | -4156.4 | 1.50E-04 |
| | | | | | INS-13(OFC) Left | -5.736 | 0.001 | -0.608 | -5180.78 | 1.20E-04 |
| | | | | | ELO Rank | -3.49E-05 | 1.15E-07 | -0.031 | -302.364 | 0.002 |
| | Submissive-Fearful | 0.998 | 1159.271 | 4.10E-05 | **AMYG-9(dlPFC) Left** | -0.548 | 0.123 | -0.071 | -4.463 | 0.021 |
| | | | | | NAcc-INS Right | -3.383 | 0.268 | -0.218 | -12.624 | 0.001 |
| | | | | | INS-46(dlPFC) Right | 6.814 | 0.106 | 1.013 | 64.326 | 8.00E-06 |
| | | | | | INS-14(vmPFC) Left | -5.389 | 0.202 | -0.409 | -26.627 | 1.20E-04 |
| | Anxious-Avoidant | | | | *No Significant Models* | | | | | |
| **LCD-Only** | Affiliation | | | | *No Significant Models* | | | | | |
| | Anxious-Aggression | 1.000 | 3.41E+07 | 1.31E-04 | NAcc-AMYG Left | -1.738 | 0.001 | -0.134 | -1233.970 | 0.001 |
| | | | | | NAcc-INS Right | -7.573 | 0.002 | -1.012 | -4269.750 | 0.0001 |
| | | | | | NAcc-13(OFC) Left | -0.784 | 0.0004 | -0.151 | -1752.970 | 0.0004 |
| | | | | | INS-11(OFC) Right | 3.809 | 0.001 | 0.506 | 3273.951 | 0.0002 |
| | | | | | INS-46(dlPFC) Right | 0.293 | 0.001 | 0.091 | 454.691 | 0.001 |
| | | | | | INS-9(dlPFC) Right | 0.034 | 0.001 | 0.008 | 41.791 | 0.015 |
| | Submissive-Fearful | 0.882 | 27.203 | 0.002 | **AMYG-13(OFC) Left** | 3.308 | 0.727 | 0.753 | 4.550 | 0.006 |
| | | | | | NAcc-AMYG Left | 14.315 | 1.941 | 1.221 | 7.376 | 0.001 |
| | Anxious-Avoidant | 0.999 | 1754.926 | 0.001 | AMYG-9(dlPFC) Right | -4.541 | 0.120 | -0.518 | -37.720 | 0.001 |
| | | | | | **AMYG-13(OFC) Left** | -7.104 | 0.142 | -0.994 | -50.054 | 0.0004 |
| | | | | | NAcc-AMYG Left | -2.950 | 0.284 | -0.155 | -10.392 | 0.009 |
| | | | | | NAcc-14(vmPFC) Left | 2.951 | 0.090 | 0.550 | 32.684 | 0.001 |
| | | | | | NAcc-9(dlPFC) Left | 0.385 | 0.080 | 0.057 | 4.824 | 0.040 |

**Table 4.4 Descriptive statistics of ROI-ROI resting state FC, which were significantly predicted by inflammatory factors, cortisol, feeding behavior and/or ELO rank.** Minimum, median, maximum, range and mean of ROI-ROI resting state FC pairs are reported.

| | ROI-ROI FC | Minimum | Median | Maximum | Range | Mean |
|---|---|---|---|---|---|---|
| Choice (LCD+HCD) | AMYG-INS Left | -0.0863 | 0.1697 | 0.3508 | 0.4371 | 0.1464 |
| | AMYG-9(dlPFC) Left | -0.3016 | -0.1112 | 0.2311 | 0.5327 | -0.0762 |
| | AMYG-9(dlPFC) Right | -0.1799 | -0.0622 | 0.0858 | 0.2658 | -0.0465 |
| | NAcc-46(dlPFC) Right | -0.1475 | 0.0482 | 0.0865 | 0.2339 | 0.0138 |
| | INS-11(OFC) Right | -0.0887 | 0.0259 | 0.3174 | 0.4061 | 0.0502 |
| LCD Only | AMYG-INS Right | -0.0840 | 0.0967 | 0.3229 | 0.4069 | 0.1006 |
| | AMYG-13(OFC) Left | -0.2547 | 0.0064 | 0.4210 | 0.6757 | 0.0410 |
| | AMYG-13(OFC) Right | -0.0066 | 0.1334 | 0.1974 | 0.2040 | 0.1079 |

**Figure 4.1** Illustration of significant models predicting FC in the Choice (red lines) and LCD (blue lines) groups and their functional outcomes.



# Chapter 5. General Discussion

## 5.1 Summary of results

### 5.1.1 Summary of Chapter 2

The goals of chapter 2 were to (1) examine the impact of social subordination stress on female macaque neurobehavioral development, (2) determine whether pubertal timing and duration of estradiol (E2) exposure modulate the impact of stress on structural development of corticolimbic regions involved in emotional and stress regulation (specifically amygdala -AMYG-, prefrontal cortex -PFC- and hippocampus -HC-), disentangling it from the effects of chronological age, and (3) examine whether these brain structural differences were related to behavioral and physiological outcomes. It was hypothesized that these social status effects would be evident in the AMYG, the PFC and the HC which are involved in social and emotional processing and have high levels of both glucocorticoid and estrogen receptors. Specifically, based on available literature discussed above, we predicted that subordinate juvenile females would have larger AMYG, but smaller PFC and HC volumes compared to dominant subjects and that these alterations would result in increased fear and anxiety behavior and stress reactivity. Because prepubertal E2 is bioactive in female rhesus monkeys (Pohl, deRidder et al. 1995, Wilson, Fisher et al. 2004), it was also hypothesized that these status-induced brain and behavioral effects would be exacerbated in females with delayed exposure to E2. We found that in addition to expected developmental effects, subordinates had larger AMYG volumes than dominant animals (particularly in the right hemisphere), but pubertal delay with Lupron-treatment abolished those differences, suggesting a role of gonadal hormones potentiating the structural impact of social stress. Subordinates had elevated

baseline cortisol, indicating activation of stress systems. In general, Lupron-treated subjects had smaller AMYG and HC volume than controls, but larger total PFC (due to more gray matter -GM-), and different, region-specific, developmental patterns dependent on age and social status. These findings highlight a region-specific effect of E2 on structural development during female adolescence, independent of those due to chronological age. Additionally, pubertal delay and smaller AMYG volume were associated with increased emotional reactivity during the Human Intruder task (HI) and decreased social behavior compared to controls. These data provide evidence that exposure to developmental increases in E2 modify the consequences of social stress on the volume of cortico-limbic regions involved in emotional and stress regulation during maturation. Also, even more importantly, they support different brain structural effects of chronological age and pubertal developmental stage in females, which are very difficult to disentangle in human studies. These findings have additional relevance for young girls who experience prolonged pubertal delays or for those whose puberty is clinically arrested by pharmacological administration of Lupron.

### 5.1.2 Summary of Chapter 3

The goals of chapter 3 were to investigate how social status interacts with dietary environment to affect brain structure of corticolimbic regions, specifically, the AMYG, PFC, insular cortex -INS- and HC, known to affect emotional reactivity and motivated behavior in adult female rhesus macaques. The impact of diet on neurobehavioral outcomes were assessed in females who had been maintained on a low fat, high sugar chow diet their entire lives and females who had been consuming this chow diet in

combination of a high fat, high sugar diet for one year. Although some studies have reported increases in brain volume as a consequence of consumption of calorically dense diets, the data overall suggest that there is a reduction in overall and regional brain volumes. Therefore, it was hypothesized that excessive calorie intake, particularly of a high caloric diet (HCD), would result in decreased volume of AMYG, PFC, INS and HC. Additionally, it was hypothesized that subordination stress would result in decreased PFC, INS and HC, but increased AMYG volume, and that these diet and stress-induced alterations would result in altered social and emotional behavior. We found a significant impact of the choice (CH) dietary condition on regional brain structure, with number of calories consumed both positively and negatively predicting region-specific differences in brain volume, while calorie intake did not predict any variance in regional brain volumes for females in the LCD-only diet condition. Additionally, in the CH subjects, social status positively predicted PFC white matter (WM) and dorsolateral PFC (dlPFC) volumes, whereas in the low caloric diet only (LCD-only) subjects, social status negatively predicted PFC WM, ventromedial PFC (vmPFC), AMYG, INS GM and HC volumes. Interestingly, exposure to the CH diet reversed the direction of the dominance rank effects in bilateral PFC WM. A functional outcomes analysis revealed that in the CH subjects, larger left dlPFC and smaller left PFC GM volumes predicted increased affiliative behavior. In the LCD-only subjects, more subordinate rank predicted larger left AMYG and left INS GM volumes, which resulted in decreased anxious-aggressive and increased submissive-fearful behavior, respectively. The results of the present study revealed that considerably different phenotypes emerge in rhesus macaques with access to either a prudent LCD or a more complex dietary environment where a choice between

LCD and high sugar, high fat diet is available. These results underscore the idea that calories derived from diets high in sugar and fat impact the brain and behavior differently than carioles derived from healthier low fat and sugar diets.

### 5.1.3 Summary of Chapter 4

Using the same females and experimental conditions for chapter 3, the goals of chapter 4 were to investigate how social status interacts with dietary environment to affect brain functional connectivity (FC) between prefrontal, striatal, and other limbic regions known affect emotional reactivity and motivated behavior. It was hypothesized that excessive calorie intake, particularly of a HCD, would result in increased FC between nucleus accumbens (NAcc), AMYG, INS and PFC, and that these alterations would result in altered social and emotional behavior. We found that in the CH subjects, number of calories consumed positively predicted FC in the left hemisphere between AMYG and dlPFC, in addition to AMYG and INS FC. This effect was reversed in the right hemisphere, where total kcal intake was negatively predictive of FC between AMYG and NAcc with dlPFC. In the CH subjects, social status negatively predicted right INS and orbitofrontal (OFC) FC, while social status positively predicted FC between left AMYG and OFC in the LCD subjects. In the LCD subjects, right AMYG with both INS and OFC FC was positively predicted by food intake. Pro-inflammatory factors entered into several models, which consistently predicted more positive FC between ROIs. Cortisol significantly predicted FC in two models, where lower baseline cortisol predicted more positive FC between right AMYG and dlPFC in the CH subjects and greater stress-induced cortisol predicted stronger and more positive FC between right AMYG and OFC

in the LCD subjects. Finally, social and emotional reactivity behaviors were predicted from different pairs of region of interest (ROI)-ROI correlations in the CH compared to the LCD-only subjects. As in chapter 3, these results underscore the idea that calories derived from diets high in sugar and fat impact neurobehavioral outcomes differently than calories derived from more prudent, low fat and sugar diets.

## 5.2 Integration of findings

Exposure to chronic social stressors during childhood, adolescence and adulthood leads to alterations in brain structure and circuitry. Importantly, these stress-induced alterations can lead to emotional dysregulation and psychopathology, particularly in women. However, the mechanisms underlying the emergence of, and the factors contributing to, these stress-induced alterations are poorly understood. Therefore, the primary, overall goal of this thesis was to investigate what factors underlie and modify chronic stressor-induced changes in brain structure and function and resulting behavior. Social subordination in female rhesus macaque monkeys is an established translational animal model that produces a number of stress-related phenotypes (Shively, Laber-Laird et al. 1997, Michopoulos, Toufexis et al. 2012), including stress-induced over eating of an obesogenic diet (Arce, Michopoulos et al. 2010, Michopoulos, Toufexis et al. 2012, Michopoulos, Diaz et al. 2016). Using this social subordination model of chronic stressor exposure, we found that developmental suppression of E2 and consumption of an obesogenic diet both modify chronic stressor alterations in brain structure, FC and function. In chapter 2, the effects of social subordination on regional brain volumes across pre- and peripuberty were modified by Lupron-treatment (suppression of E2). For

example, we found that subordinates had larger AMYG volume than dominant subjects, but Lupron-treatment neutralized this effect. In chapter 3, the effects of social subordination on regional brain volumes in adults were modified by dietary environment. For example, the availability of an obesogenic diet reversed the direction of the dominance rank effects in bilateral PFC WM, where in the LCD-only subjects, more subordinate status predicted larger PFC WM volume, although in the CH subjects, more subordinate status predicted smaller PFC WM volume. In chapter 4, the effects of social subordination on brain FC were also modified by dietary environment, where social status predicted different pairs of ROI-ROI functional connectivity. These results are important as they provide evidence that (1) exposure to developmental increases in E2 modify the consequences of social subordination on the volume of cortico-limbic regions involved in emotional and stress regulation during maturation (specifically, delayed puberty and E2 suppression may be protective against the impact of social stress on AMYG volume), and (2) exposure to an obesogenic diet in adulthood modifies the consequences of social subordination on the volume and FC of cortico-limbic regions involved in stress regulation, in addition to emotional and motivational behavior in female rhesus macaques. Specifically, consumption of a HCD overrides the effects of social subordination on regional brain volume and FC, although the exact functional significance of these alterations is yet to be elucidated.

An important and consistent finding was that social subordination affected brain structure and FC in all three studies. Social subordination resulted in increased volume of AMYG during the pre- and peripubertal ages studied and also in adulthood. Additionally, social

status positively predicted FC between left AMYG and OFC in the adult subjects fed a LCD, an effect which may be driven by the increased left AMYG volume observed in these subjects. Interestingly this effect was not observed in subjects with access to a choice between a HCD and a LCD, where social status negatively predicted right INS and OFC FC, providing evidence that the effects of social status on ROI-ROI brain FC were modified by dietary environment and the availability of a HCD. The finding that social subordination was associated with an increase in AMYG volume is likely due to increases in dendritic arborization and/or density of dendritic spines, as has been demonstrated in rodent models in response to chronic stress or direct administration of glucocorticoids into the AMYG (Vyas, Mitra et al. 2002, Vyas, Bernal et al. 2003, Mitra, Jadhav et al. 2005, Vyas, Jadhav et al. 2006, Mitra and Sapolsky 2008, Rosenkranz, Venheim et al. 2010, Eiland, Ramroop et al. 2012). Our findings are also consistent with studies reporting AMYG volume increases in response to stress in non-human primates and humans. For example, studies in non-human primates include variable foraging demand stress (Coplan, Fathy et al. 2014) and infant maltreatment (Howell, Grand et al. 2014) and human developmental studies include impact of traumatic stress (Weems, Scott et al. 2013) and institutional rearing, particularly evident in the right amygdala, which is also consistent with our findings (Mehta, Golembo et al. 2009, Tottenham, Hare et al. 2010).

Functional outcomes also emerged to help explain the relevance of these alterations in AMYG volume and FC. In chapter 2, larger left AMYG volume was associated with decreased emotional reactivity in the HI task, as evidenced by increased freezing,

averting gaze and threats. For this chapter, we argued that the relation between AMYG volume and emotional reactivity may seem paradoxical, it is important to note that it involves the left and not the right AMYG, whose volume was bigger in subordinate animals. There is, indeed, literature highlighting the asymmetry of this structure and lateralized effects of stress during its development, sometimes resulting in increased right AMYG volume, as previously discussed (Mehta, Golembo et al. 2009, Tottenham, Hare et al. 2010, Weems, Scott et al. 2013), and sometimes in smaller left AMYG volume (Hanson, Nacewicz et al. 2015). In chapter 3, larger left AMYG volume, which was predicted by more subordinate status, also significantly predicted decreased anxious-aggressive behavior. Therefore, another result which was consistent across studies (chapters 2 and 3) was that larger AMYG volume, associated with more subordinate status, resulted in decreased emotionally reactive behavior [primarily measured in the HI task]. Subordinate monkeys have to cope with high social pressures and successfully navigate social environments, which require recognizing one's social status in relation to others and selecting behaviors accordingly. Thus, this finding suggests that subordinate animals have adapted (and maybe even habituated) to continual threats and harassment by other monkeys and consequently react less strongly compared dominant monkeys in response to an acute social stressor.

## 5.3 Conclusions and future directions

Overall, this dissertation provides evidence that (1) exposure to developmental increases in E2 modify the consequences of social subordination on the volume of cortico-limbic

regions involved in emotional and stress regulation during maturation (specifically, delayed puberty and E2 suppression may be protective against the impact of social stress on AMYG volume), and (2) exposure to an obesogenic diet in adulthood modifies the consequences of social subordination on the volume and FC of cortico-limbic regions involved in stress regulation, in addition to emotional and motivational behavior in female rhesus macaques. Specifically, consumption of a HCD overrides the effects of social subordination on regional brain volume and FC, although the exact functional significance of these alterations is yet to be elucidated.

It is difficult to draw all-encompassing conclusions from these different studies, but a few consistent findings emerged. In all three studies, social subordination was associated with larger AMYG volumes. Additionally, the larger AMYG volume associated with subordinate status resulted in decreased emotionally reactive behavior, primarily measured in the HI task. Future studies are necessary to answer several remaining questions, some of which include: What prenatal effects, such as differences in maternal physiological function of dominant versus subordinate animals, affect neurobehavioral development of offspring? Do Lupron-related differences reported in chapter 2 persist across development into adulthood, after Lupron-treatment is discontinued? What specific nuclei in the AMYG are responsible for the social subordination-induced increases in volume? What additional brain regions, not examined in this dissertation, are affected by social subordination and availability of an obesogenic diet? As only females were examined in this dissertation, how would social subordination and availability of an obesogenic diet affect brain structure, FC and behavior in male rhesus macaques? In

conclusion, the results of the present study provide translational evidence to help understand what factors underlie and modify chronic stressor-induced changes in brain structure and function and resulting behavior, which may assist in understanding the etiology of and treating stress and diet-induced disease and psychopathology in humans.

# Chapter 6. References

Abbott, D. H., E. B. Keverne, F. B. Bercovitch, C. a. Shively, S. P. Mendoza, W. Saltzman, C. T. Snowdon, T. E. Ziegler, M. Banjevic, T. Garland and R. M. Sapolsky (2003). "Are subordinates always stressed? a comparative analysis of rank differences in cortisol levels among primates." Hormones and Behavior **43**: 67-82.

Adams, M. R., J. R. Kaplan and D. R. Koritnik (1985). "Psychosocial influences on ovarian endocrine and ovulatory function in Macaca fascicularis." Physiol Behav **35**(6): 935-940.

Ahmed, E. I., J. L. Zehr, K. M. Schulz, B. H. Lorenz, L. L. DonCarlos and C. L. Sisk (2008). "Pubertal hormones modulate the addition of new cells to sexually dimorphic brain regions." Nat Neurosci **11**(9): 995-997.

Albers, P. C. H. and H. de Vries (2001). "Elo-rating as a tool in the sequential estimation of dominance strengths." Animal Behaviour **61**(2): 489-495.

Altmann, S. A. (1962). "A field study of the sociobiology of rhesus monkeys, Macaca mulatta." Ann N Y Acad Sci **102**: 338-435.

Amaral, D. G. and J. L. Bassett (1989). "Cholinergic innervation of the monkey amygdala: an immunohistochemical analysis with antisera to choline acetyltransferase." J Comp Neurol **281**(3): 337-361.

Amodio, D. M. and C. D. Frith (2006). "Meeting of minds: the medial frontal cortex and social cognition." Nat Rev Neurosci **7**(4): 268-277.

Andersen, S. L. (2003). "Trajectories of brain development: point of vulnerability or window of opportunity?" Neurosci Biobehav Rev **27**(1-2): 3-18.

Andersen, S. L., A. Tomada, E. S. Vincow, E. Valente, A. Polcari and M. H. Teicher (2008). "Preliminary evidence for sensitive periods in the effect of childhood sexual abuse on regional brain development." J Neuropsychiatry Clin Neurosci **20**(3): 292-301.

Anderson, S. A., J. D. Classey, F. Conde, J. S. Lund and D. A. Lewis (1995). "Synchronous development of pyramidal neuron dendritic spines and parvalbumin-immunoreactive chandelier neuron axon terminals in layer III of monkey prefrontal cortex." Neuroscience **67**(1): 7-22.

Andersson, J. L., S. Skare and J. Ashburner (2003). "How to correct susceptibility distortions in spin-echo echo-planar images: application to diffusion tensor imaging." Neuroimage **20**(2): 870-888.

Angold, A., E. J. Costello, A. Erkanli and C. M. Worthman (1999). "Pubertal changes in hormone levels and depression in girls." Psychol Med **29**(5): 1043-1053.

Angold, A., E. J. Costello and C. M. Worthman (1998). "Puberty and depression: the roles of age, pubertal status and pubertal timing." Psychol Med **28**(1): 51-61.

Anisman, H. and R. M. Zacharko (1992). "Depression as a consequence of inadequate neurochemical adaptation in response to stressors." Br J Psychiatry Suppl(15): 36-43.

Ansell, E. B., K. Rando, K. Tuit, J. Guarnaccia and R. Sinha (2012). "Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions." Biol Psychiatry **72**(1): 57-64.

Apter, D., T. L. Butzow, G. A. Laughlin and S. S. Yen (1993). "Gonadotropin-releasing hormone pulse generator activity during pubertal transition in girls: pulsatile and diurnal patterns of circulating gonadotropins." J Clin Endocrinol Metab **76**(4): 940-949.

Arce, M., V. Michopoulos, K. N. Shepard, Q.-C. Ha and M. E. Wilson (2010). "Diet choice, cortisol reactivity, and emotional feeding in socially housed rhesus monkeys." Physiology & behavior **101**: 446-455.

Argente-Arizon, P., A. Freire-Regatillo, J. Argente and J. A. Chowen (2015). "Role of non-neuronal cells in body weight and appetite control." Front Endocrinol (Lausanne) **6**: 42.

Arnett, M. G., B. J. Kolber, M. P. Boyle and L. J. Muglia (2011). "Behavioral insights from mouse models of forebrain--and amygdala-specific glucocorticoid receptor genetic disruption." Mol Cell Endocrinol **336**(1-2): 2-5.

Arnold, A. P. and S. M. Breedlove (1985). "Organizational and activational effects of sex steroids on brain and behavior: a reanalysis." Horm Behav **19**(4): 469-498.

Arnold, S. E. and J. Q. Trojanowski (1996). "Human fetal hippocampal development: I. Cytoarchitecture, myeloarchitecture, and neuronal morphologic features." J Comp Neurol **367**(2): 274-292.

Asami, T., H. Yamasue, F. Hayano, M. Nakamura, K. Uehara, T. Otsuka, T. Roppongi, N. Nihashi, T. Inoue and Y. Hirayasu (2009). "Sexually dimorphic gray matter volume reduction in patients with panic disorder." Psychiatry Res **173**(2): 128-134.

Ayensa, J. I. and M. J. Calderon (2011). "[Psychopathological comorbidity of obesity]." An Sist Sanit Navar **34**(2): 253-261.

Azzi, J. C., A. Sirigu and J. R. Duhamel (2012). "Modulation of value representation by social context in the primate orbitofrontal cortex." Proc Natl Acad Sci U S A **109**(6): 2126-2131.

Banks, J., M. Marmot, Z. Oldfield and J. P. Smith (2006). "Disease and disadvantage in the United States and in England." JAMA **295**(17): 2037-2045.

Bao, J. Z., C. R. Ni and W. Q. Zheng (2006). "Age-related effects of estrogen on the expression of estrogen receptor alpha and beta mRNA in the ovariectomized monkey hypothalamus." Neurosci Bull **22**(2): 97-102.

Barnes, P. J. (2006). "Corticosteroid effects on cell signalling." Eur Respir J **27**(2): 413-426.

Batterham, R. L., D. H. ffytche, J. M. Rosenthal, F. O. Zelaya, G. J. Barker, D. J. Withers and S. C. Williams (2007). "PYY modulation of cortical and hypothalamic brain areas predicts feeding behaviour in humans." Nature **450**(7166): 106-109.

Baum, A. and D. M. Posluszny (1999). "Health psychology: mapping biobehavioral contributions to health and illness." Annu Rev Psychol **50**: 137-163.

Bauman, M. D., P. Lavenex, W. A. Mason, J. P. Capitanio and D. G. Amaral (2004). "The development of social behavior following neonatal amygdala lesions in rhesus monkeys." J Cogn Neurosci **16**(8): 1388-1411.

Baur, V., J. Hanggi and L. Jancke (2012). "Volumetric associations between uncinate fasciculus, amygdala, and trait anxiety." BMC Neurosci **13**: 4.

Baxter, M. G. and E. A. Murray (2002). "The amygdala and reward." Nat Rev Neurosci **3**(7): 563-573.

Becker, J. B., L. M. Monteggia, T. S. Perrot-Sinal, R. D. Romeo, J. R. Taylor, R. Yehuda and T. L. Bale (2007). "Stress and disease: is being female a predisposing factor?" J Neurosci **27**(44): 11851-11855.

Beilharz, J. E., J. Maniam and M. J. Morris (2015). "Diet-Induced Cognitive Deficits: The Role of Fat and Sugar, Potential Mechanisms and Nutritional Interventions." Nutrients **7**(8): 6719-6738.

Benes, F. M., J. B. Taylor and M. C. Cunningham (2000). "Convergence and plasticity of monoaminergic systems in the medial prefrontal cortex during the postnatal period: implications for the development of psychopathology." Cereb Cortex **10**(10): 1014-1027.

Berga, S. L. and T. L. Loucks (2005). "The diagnosis and treatment of stress-induced anovulation." Minerva Ginecol **57**(1): 45-54.

Berman, C. M. (1980). "Mother-infant relationships among free-ranging rhesus monkeys on Cayo Santiago: a comparison with captive pairs." Animal Behaviour **28**(3): 860-873.

Bernstein, I. S. (1970). "Primate status hierarchies." Primate behavior: Developments in field and laboratory research **1**: 71-109.

Bernstein, I. S. (1976). "Dominance, aggression and reproduction in primate societies." J Theor Biol **60**(2): 459-472.

Bernstein, I. S. and C. L. Ehardt (1985). "Age-sex differences in the expression of agonistic behavior in rhesus monkey (Macaca mulatta) groups." J Comp Psychol **99**(2): 115-132.

Bernstein, I. S. and T. P. Gordon (1974). "The function of aggression in primate societies." Am Sci **62**(3): 304-311.

Bernstein, I. S. and T. P. Gordon (1977). "Behavioral research in breeding colonies of Old World monkeys." Lab Anim Sci **27**(4): 532-540.

Bernstein, I. S., T. P. Gordon and R. M. Rose (1974). "Aggression and social controls in rhesus monkey (Macaca mulatta) groups revealed in group formation studies." Folia Primatol (Basel) **21**(2): 81-107.

Berthoud, H. R. (2012). "The neurobiology of food intake in an obesogenic environment." Proc Nutr Soc **71**(4): 478-487.

Berthoud, H. R., N. R. Lenard and A. C. Shin (2011). "Food reward, hyperphagia, and obesity." Am J Physiol Regul Integr Comp Physiol **300**(6): R1266-1277.

Bethea, C. L., S. J. Mirkes, A. Su and D. Michelson (2002). "Effects of oral estrogen, raloxifene and arzoxifene on gene expression in serotonin neurons of macaques." Psychoneuroendocrinology **27**(4): 431-445.

Bethea, C. L. and A. P. Reddy (2008). "Effect of ovarian hormones on survival genes in laser captured serotonin neurons from macaques." J Neurochem **105**(4): 1129-1143.

Bethea, C. L., J. M. Streicher, K. Coleman, F. K. Pau, R. Moessner and J. L. Cameron (2004). "Anxious Behavior and Fenfluramine-Induced Prolactin Secretion in Young Rhesus Macaques with Different Alleles of the Serotonin Reuptake Transporter Polymorphism (5HTTLPR)." Behav Genet **34**(3): 295-307.

Bickart, K. C., B. C. Dickerson and L. F. Barrett (2014). "The amygdala as a hub in brain networks that support social life." Neuropsychologia **63**: 235-248.

Blair, K., A. A. Marsh, J. Morton, M. Vythilingam, M. Jones, K. Mondillo, D. C. Pine, W. C. Drevets and J. R. Blair (2006). "Choosing the lesser of two evils, the better of two goods: specifying the roles of ventromedial prefrontal cortex and dorsal anterior cingulate in object choice." J Neurosci **26**(44): 11379-11386.

Blank, M. S., T. P. Gordon and M. E. Wilson (1983). "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) **102**(2): 190-195.

Blurton-Jones, M. M., J. A. Roberts and M. H. Tuszynski (1999). "Estrogen receptor immunoreactivity in the adult primate brain: neuronal distribution and association with p75, trkA, and choline acetyltransferase." J Comp Neurol **405**(4): 529-542.

Bobb, J. F., B. S. Schwartz, C. Davatzikos and B. Caffo (2014). "Cross-sectional and longitudinal association of body mass index and brain volume." Hum Brain Mapp **35**(1): 75-88.

Bongarzone, E. R., S. G. Howard, V. Schonmann and A. T. Campagnoni (1998). "Identification of the dopamine D3 receptor in oligodendrocyte precursors: potential role in regulating differentiation and myelin formation." J Neurosci **18**(14): 5344-5353.

Bos, P. A., J. Panksepp, R. M. Bluthe and J. van Honk (2012). "Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: a review of single administration studies." Front Neuroendocrinol **33**(1): 17-35.

Boyce, W. T. and B. J. Ellis (2005). "Biological sensitivity to context: I. An evolutionary-developmental theory of the origins and functions of stress reactivity." Dev Psychopathol **17**(2): 271-301.

Bradley, R. H. and R. F. Corwyn (2002). "Socioeconomic status and child development." Annu Rev Psychol **53**: 371-399.

Brain Development Cooperative, G. (2012). "Total and regional brain volumes in a population-based normative sample from 4 to 18 years: the NIH MRI Study of Normal Brain Development." Cereb Cortex **22**(1): 1-12.

Bremner, J. D. (2002). "Neuroimaging of childhood trauma." Semin Clin Neuropsychiatry **7**(2): 104-112.

Bremner, J. D. (2003). "Long-term effects of childhood abuse on brain and neurobiology." Child Adolesc Psychiatr Clin N Am **12**(2): 271-292.

Bremner, J. D. (2006). "Traumatic stress: effects on the brain." Dialogues Clin Neurosci **8**(4): 445-461.

Bremner, J. D., P. Randall, E. Vermetten, L. Staib, R. A. Bronen, C. Mazure, S. Capelli, G. McCarthy, R. B. Innis and D. S. Charney (1997). "Magnetic resonance imaging-based measurement of hippocampal volume in posttraumatic stress disorder related to childhood physical and sexual abuse--a preliminary report." Biol Psychiatry **41**(1): 23-32.

Brooke, S. M., A. M. de Haas-Johnson, J. R. Kaplan, S. B. Manuck and R. M. Sapolsky (1994). "Dexamethasone resistance among nonhuman primates associated with a selective decrease of glucocorticoid receptors in the hippocampus and a history of social instability." Neuroendocrinology **60**(2): 134-140.

Brooks, S. J., C. Benedict, J. Burgos, M. J. Kempton, J. Kullberg, R. Nordenskjold, L. Kilander, R. Nylander, E. M. Larsson, L. Johansson, H. Ahlstrom, L. Lind and H. B. Schioth (2013). "Late-life obesity is associated with smaller global and regional gray matter volumes: a voxel-based morphometric study." Int J Obes (Lond) **37**(2): 230-236.

Brouwer, R. M., M. M. Koenis, H. G. Schnack, G. C. van Baal, I. L. van Soelen, D. I. Boomsma and H. E. Hulshoff Pol (2015). "Longitudinal Development of Hormone Levels and Grey Matter Density in 9 and 12-Year-Old Twins." Behav Genet.

Butt, A. M., R. F. Fern and C. Matute (2014). "Neurotransmitter signaling in white matter." Glia **62**(11): 1762-1779.

Byrne, R. W. and A. Whiten (1988). Machiavellian intelligence: social expertise and the evolution of intellect in monkeys, apes, and humans, Clarendon Press.

Campbell, I. L., A. K. Stalder, C. S. Chiang, R. Bellinger, C. J. Heyser, S. Steffensen, E. Masliah, H. C. Powell, L. H. Gold, S. J. Henriksen and G. R. Siggins (1997). "Transgenic models to assess the pathogenic actions of cytokines in the central nervous system." Mol Psychiatry **2**(2): 125-129.

Capuron, L., J. Lasselin and N. Castanon (2016). "Role of Adiposity-Driven Inflammation in Depressive Morbidity." Neuropsychopharmacology.

Cardinal, R. N., J. A. Parkinson, J. Hall and B. J. Everitt (2002). "Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex." Neurosci Biobehav Rev **26**(3): 321-352.

Carlin, J. L., N. Grissom, Z. Ying, F. Gomez-Pinilla and T. M. Reyes (2016). "Voluntary exercise blocks Western diet-induced gene expression of the chemokines CXCL10 and CCL2 in the prefrontal cortex." Brain Behav Immun.

Casey, B. J., S. Duhoux and M. Malter Cohen (2010). "Adolescence: what do transmission, transition, and translation have to do with it?" Neuron **67**: 749-760.

Castellanos, F. X., J. N. Giedd, W. L. Marsh, S. D. Hamburger, A. C. Vaituzis, D. P. Dickstein, S. E. Sarfatti, Y. C. Vauss, J. W. Snell, N. Lange, D. Kaysen, A. L. Krain, G. F. Ritchie, J. C. Rajapakse and J. L. Rapoport (1996). "Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder." Arch Gen Psychiatry **53**(7): 607-616.

Cerqueira, J. J., J. M. Pego, R. Taipa, J. M. Bessa, O. F. Almeida and N. Sousa (2005). "Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviors." J Neurosci **25**(34): 7792-7800.

Chaboub, L. S. and B. Deneen (2012). "Developmental origins of astrocyte heterogeneity: the final frontier of CNS development." Dev Neurosci **34**(5): 379-388.

Chai, X. J., A. N. Castanon, D. Ongur and S. Whitfield-Gabrieli (2012). "Anticorrelations in resting state networks without global signal regression." Neuroimage **59**(2): 1420-1428.

Chang, C. and G. H. Glover (2009). "Effects of model-based physiological noise correction on default mode network anti-correlations and correlations." Neuroimage **47**(4): 1448-1459.

Chao, H. M., D. C. Blanchard, R. J. Blanchard, B. S. McEwen and R. R. Sakai (1993). "The effect of social stress on hippocampal gene expression." Mol Cell Neurosci **4**(6): 543-548.

Chareyron, L. J., P. B. Lavenex, D. G. Amaral and P. Lavenex (2012). "Postnatal development of the amygdala: A stereological study in macaque monkeys." J Comp Neurol **520**(9): 1965-1984.

Chen, S., W. Xia, L. Li, J. Liu, Z. He, Z. Zhang, L. Yan, J. Zhang and D. Hu (2006). "Gray matter density reduction in the insula in fire survivors with posttraumatic stress disorder: a voxel-based morphometric study." Psychiatry Res **146**(1): 65-72.

Chrousos, G. P. and P. W. Gold (1992). "The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis." JAMA **267**(9): 1244-1252.

Cohen, J. R., R. F. Asarnow, F. W. Sabb, R. M. Bilder, S. Y. Bookheimer, B. J. Knowlton and R. A. Poldrack (2010). "A unique adolescent response to reward prediction errors." Nat Neurosci **13**(6): 669-671.

Cohen, S., D. Janicki-Deverts, W. J. Doyle, G. E. Miller, E. Frank, B. S. Rabin and R. B. Turner (2012). "Chronic stress, glucocorticoid receptor resistance, inflammation, and disease risk." Proc Natl Acad Sci U S A **109**(16): 5995-5999.

Cole, D. M., S. M. Smith and C. F. Beckmann (2010). "Advances and pitfalls in the analysis and interpretation of resting-state FMRI data." Front Syst Neurosci **4**: 8.

Cole, J. H., C. P. Boyle, A. Simmons, S. Cohen-Woods, M. Rivera, P. McGuffin, P. M. Thompson and C. H. Fu (2013). "Body mass index, but not FTO genotype or major depressive disorder, influences brain structure." Neuroscience **252**: 109-117.

Contreras-Rodriguez, O., C. Martin-Perez, R. Vilar-Lopez and A. Verdejo-Garcia (2015). "Ventral and Dorsal Striatum Networks in Obesity: Link to Food Craving and Weight Gain." Biol Psychiatry.

Coplan, J. D., H. M. Fathy, A. P. Jackowski, C. Y. Tang, T. D. Perera, S. J. Mathew, J. Martinez, C. G. Abdallah, A. J. Dwork, G. Pantol, D. Carpenter, J. M. Gorman, C. B. Nemeroff, M. J. Owens, A. Kaffman and J. Kaufman (2014). "Early life stress and macaque amygdala hypertrophy: preliminary evidence for a role for the serotonin transporter gene." Front Behav Neurosci **8**: 342.

Cordes, D., V. M. Haughton, K. Arfanakis, G. J. Wendt, P. A. Turski, C. H. Moritz, M. A. Quigley and M. E. Meyerand (2000). "Mapping functionally related regions of brain with functional connectivity MR imaging." AJNR Am J Neuroradiol **21**(9): 1636-1644.

Coryell, W., J. Fiedorowicz, M. Zimmerman and E. Young (2008). "HPA-axis hyperactivity and mortality in psychotic depressive disorder: preliminary findings." Psychoneuroendocrinology **33**(5): 654-658.

Cotter, D., D. Mackay, G. Chana, C. Beasley, S. Landau and I. P. Everall (2002). "Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder." Cereb Cortex **12**(4): 386-394.

Coveleskie, K., A. Gupta, L. A. Kilpatrick, E. D. Mayer, C. Ashe-McNalley, J. Stains, J. S. Labus and E. A. Mayer (2015). "Altered functional connectivity within the central reward network in overweight and obese women." Nutr Diabetes **5**: e148.

Craig, A. D. (2009). "How do you feel--now? The anterior insula and human awareness." Nat Rev Neurosci **10**(1): 59-70.

Cunningham, M. G., S. Bhattacharyya and F. M. Benes (2002). "Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence." J Comp Neurol **453**(2): 116-130.

Cusin, I., J. Rouru and F. Rohner-Jeanrenaud (2001). "Intracerebroventricular glucocorticoid infusion in normal rats: induction of parasympathetic-mediated obesity and insulin resistance." Obes Res **9**(7): 401-406.

Dahl, R. E. and M. R. Gunnar (2009). "Heightened stress responsiveness and emotional reactivity during pubertal maturation: implications for psychopathology." Dev Psychopathol **21**(1): 1-6.

Dannlowski, U., A. Stuhrmann, V. Beutelmann, P. Zwanzger, T. Lenzen, D. Grotegerd, K. Domschke, C. Hohoff, P. Ohrmann, J. Bauer, C. Lindner, C. Postert, C. Konrad, V. Arolt, W. Heindel, T. Suslow and H. Kugel (2012). "Limbic scars: long-term consequences of childhood maltreatment revealed by functional and structural magnetic resonance imaging." Biol Psychiatry **71**(4): 286-293.

De Bosscher, K., W. Vanden Berghe and G. Haegeman (2006). "Cross-talk between nuclear receptors and nuclear factor kappaB." Oncogene **25**(51): 6868-6886.

de Castilhos, J., E. E. Hermel, A. A. Rasia-Filho and M. Achaval (2010). "Influence of substitutive ovarian steroids in the nuclear and cell body volumes of neurons in the posterodorsal medial amygdala of adult ovariectomized female rats." Neurosci Lett **469**(1): 19-23.

de la Mora, M. P., A. Gallegos-Cari, Y. Arizmendi-Garcia, D. Marcellino and K. Fuxe (2010). "Role of dopamine receptor mechanisms in the amygdaloid modulation of fear and anxiety: Structural and functional analysis." Prog Neurobiol **90**(2): 198-216.

Debette, S., A. Beiser, U. Hoffmann, C. Decarli, C. J. O'Donnell, J. M. Massaro, R. Au, J. J. Himali, P. A. Wolf, C. S. Fox and S. Seshadri (2010). "Visceral fat is associated with lower brain volume in healthy middle-aged adults." Ann Neurol **68**(2): 136-144.

Di Martino, A., A. Scheres, D. S. Margulies, A. M. Kelly, L. Q. Uddin, Z. Shehzad, B. Biswal, J. R. Walters, F. X. Castellanos and M. P. Milham (2008). "Functional

connectivity of human striatum: a resting state FMRI study." Cereb Cortex **18**(12): 2735-2747.

Dias-Ferreira, E., J. C. Sousa, I. Melo, P. Morgado, A. R. Mesquita, J. J. Cerqueira, R. M. Costa and N. Sousa (2009). "Chronic stress causes frontostriatal reorganization and affects decision-making." Science **325**(5940): 621-625.

Dreher, J. C., P. J. Schmidt, P. Kohn, D. Furman, D. Rubinow and K. F. Berman (2007). "Menstrual cycle phase modulates reward-related neural function in women." Proc Natl Acad Sci U S A **104**(7): 2465-2470.

Driessen, M., J. Herrmann, K. Stahl, M. Zwaan, S. Meier, A. Hill, M. Osterheider and D. Petersen (2000). "Magnetic resonance imaging volumes of the hippocampus and the amygdala in women with borderline personality disorder and early traumatization." Arch Gen Psychiatry **57**(12): 1115-1122.

Duffy, C. J. and P. Rakic (1983). "Differentiation of granule cell dendrites in the dentate gyrus of the rhesus monkey: a quantitative Golgi study." J Comp Neurol **214**(2): 224-237.

Dunn, A. J. and C. W. Berridge (1990). "Physiological and behavioral responses to corticotropin-releasing factor administration: is CRF a mediator of anxiety or stress responses?" Brain Res Brain Res Rev **15**(2): 71-100.

Dunn, A. J., A. H. Swiergiel and R. de Beaurepaire (2005). "Cytokines as mediators of depression: what can we learn from animal studies?" Neurosci Biobehav Rev **29**(4-5): 891-909.

Eckenhoff, M. F. and P. Rakic (1991). "A quantitative analysis of synaptogenesis in the molecular layer of the dentate gyrus in the rhesus monkey." Brain Res Dev Brain Res **64**(1-2): 129-135.

Edmiston, E. E., F. Wang, C. M. Mazure, J. Guiney, R. Sinha, L. C. Mayes and H. P. Blumberg (2011). "Corticostriatal-limbic gray matter morphology in adolescents with self-reported exposure to childhood maltreatment." Arch Pediatr Adolesc Med **165**(12): 1069-1077.

Eiland, L., J. Ramroop, M. N. Hill, J. Manley and B. S. McEwen (2012). "Chronic juvenile stress produces corticolimbic dendritic architectural remodeling and modulates emotional behavior in male and female rats." Psychoneuroendocrinology **37**(1): 39-47.

Embree, M., V. Michopoulos, J. R. Votaw, R. J. Voll, J. Mun, J. S. Stehouwer, M. M. Goodman, M. E. Wilson and M. M. Sanchez (2013). "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 **228**: 83-100.

Engler-Chiurazzi, E. B., M. Singh and J. W. Simpkins (2016). "From the 90's to now: A brief historical perspective on more than two decades of estrogen neuroprotection." Brain Res **1633**: 96-100.

Epel, E., R. Lapidus, B. McEwen and K. Brownell (2001). "Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior." Psychoneuroendocrinology **26**(1): 37-49.

Fair, D. A., A. L. Cohen, J. D. Power, N. U. Dosenbach, J. A. Church, F. M. Miezin, B. L. Schlaggar and S. E. Petersen (2009). "Functional brain networks develop from a "local to distributed" organization." PLoS Comput Biol **5**(5): e1000381.

Fair, D. A., N. U. Dosenbach, J. A. Church, A. L. Cohen, S. Brahmbhatt, F. M. Miezin, D. M. Barch, M. E. Raichle, S. E. Petersen and B. L. Schlaggar (2007). "Development of distinct control networks through segregation and integration." Proc Natl Acad Sci U S A **104**(33): 13507-13512.

Fair, D. A., J. T. Nigg, S. Iyer, D. Bathula, K. L. Mills, N. U. Dosenbach, B. L. Schlaggar, M. Mennes, D. Gutman, S. Bangaru, J. K. Buitelaar, D. P. Dickstein, A. Di Martino, D. N. Kennedy, C. Kelly, B. Luna, J. B. Schweitzer, K. Velanova, Y. F. Wang, S. Mostofsky, F. X. Castellanos and M. P. Milham (2012). "Distinct neural signatures detected for ADHD subtypes after controlling for micro-movements in resting state functional connectivity MRI data." Front Syst Neurosci **6**: 80.

Fan, L., R. Hanbury, S. C. Pandey and R. S. Cohen (2008). "Dose and time effects of estrogen on expression of neuron-specific protein and cyclic AMP response element-binding protein and brain region volume in the medial amygdala of ovariectomized rats." Neuroendocrinology **88**(2): 111-126.

Fedorov, A., R. Beichel, J. Kalpathy-Cramer, J. Finet, J. C. Fillion-Robin, S. Pujol, C. Bauer, D. Jennings, F. Fennessy, M. Sonka, J. Buatti, S. Aylward, J. V. Miller, S. Pieper and R. Kikinis (2012). "3D Slicer as an image computing platform for the Quantitative Imaging Network." Magn Reson Imaging **30**(9): 1323-1341.

Felger, J. C., Z. Li, E. Haroon, B. J. Woolwine, M. Y. Jung, X. Hu and A. H. Miller (2015). "Inflammation is associated with decreased functional connectivity within corticostriatal reward circuitry in depression." Mol Psychiatry.

Figley, C. R., J. S. Asem, E. L. Levenbaum and S. M. Courtney (2016). "Effects of Body Mass Index and Body Fat Percent on Default Mode, Executive Control, and Salience Network Structure and Function." Front Neurosci **10**: 234.

Flegal, K. M., M. D. Carroll, C. L. Ogden and L. R. Curtin (2010). "Prevalence and trends in obesity among US adults, 1999-2008." JAMA **303**(3): 235-241.

Forbes, E. E., D. E. Williamson, N. D. Ryan and R. E. Dahl (2004). "Positive and negative affect in depression: influence of sex and puberty." Ann N Y Acad Sci **1021**: 341-347.

Fox, A. S. and N. H. Kalin (2014). "A translational neuroscience approach to understanding the development of social anxiety disorder and its pathophysiology." Am J Psychiatry **171**(11): 1162-1173.

Fox, M. D. and M. E. Raichle (2007). "Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging." Nat Rev Neurosci **8**(9): 700-711.

Fox, M. D., D. Zhang, A. Z. Snyder and M. E. Raichle (2009). "The global signal and observed anticorrelated resting state brain networks." J Neurophysiol **101**(6): 3270-3283.

Frank, M. G., M. V. Baratta, D. B. Sprunger, L. R. Watkins and S. F. Maier (2007). "Microglia serve as a neuroimmune substrate for stress-induced potentiation of CNS pro-inflammatory cytokine responses." Brain Behav Immun **21**(1): 47-59.

Galvez, J. F., I. E. Bauer, M. Sanches, H. E. Wu, J. E. Hamilton, B. Mwangi, F. P. Kapczinski, G. Zunta-Soares and J. C. Soares (2014). "Shared clinical associations between obesity and impulsivity in rapid cycling bipolar disorder: a systematic review." J Affect Disord **168**: 306-313.

Galvin, C. and I. Ninan (2014). "Regulation of the mouse medial prefrontal cortical synapses by endogenous estradiol." Neuropsychopharmacology **39**(9): 2086-2094.

Garcia-Ovejero, D., I. Azcoitia, L. L. Doncarlos, R. C. Melcangi and L. M. Garcia-Segura (2005). "Glia-neuron crosstalk in the neuroprotective mechanisms of sex steroid hormones." Brain Res Brain Res Rev **48**(2): 273-286.

Garcia-Segura, L. M., J. A. Chowen and F. Naftolin (1996). "Endocrine glia: roles of glial cells in the brain actions of steroid and thyroid hormones and in the regulation of hormone secretion." Front Neuroendocrinol **17**(2): 180-211.

Garrett, J. E. and C. L. Wellman (2009). "Chronic stress effects on dendritic morphology in medial prefrontal cortex: sex differences and estrogen dependence." Neuroscience **162**(1): 195-207.

Genovesio, A., S. P. Wise and R. E. Passingham (2014). "Prefrontal-parietal function: from foraging to foresight." Trends Cogn Sci **18**(2): 72-81.

Ghashghaei, H. T. and H. Barbas (2002). "Pathways for emotion: interactions of prefrontal and anterior temporal pathways in the amygdala of the rhesus monkey." Neuroscience **115**(4): 1261-1279.

Ghashghaei, H. T., C. C. Hilgetag and H. Barbas (2007). "Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala." Neuroimage **34**(3): 905-923.

Gianaros, P. J., J. A. Horenstein, S. Cohen, K. A. Matthews, S. M. Brown, J. D. Flory, H. D. Critchley, S. B. Manuck and A. R. Hariri (2007). "Perigenual anterior cingulate

morphology covaries with perceived social standing." Soc Cogn Affect Neurosci **2**(3): 161-173.

Gianaros, P. J., J. R. Jennings, L. K. Sheu, P. J. Greer, L. H. Kuller and K. A. Matthews (2007). "Prospective reports of chronic life stress predict decreased grey matter volume in the hippocampus." Neuroimage **35**(2): 795-803.

Gianaros, P. J. and S. B. Manuck (2010). "Neurobiological pathways linking socioeconomic position and health." Psychosom Med **72**(5): 450-461.

Gibson, E. L. (2006). "Emotional influences on food choice: sensory, physiological and psychological pathways." Physiol Behav **89**(1): 53-61.

Gibson, K. R. (1991). Myelination and behavioral development: a comparative perspective on questions of neotony, altriciality, and intelligence. Brain maturation and cognitive development: comparative and cross-cultural perspectives. . K. P. Gibson, AC. Hawthorne, NY, Aldine de Gruyter**:** 29-63.

Giedd, J. N. (2004). "Structural magnetic resonance imaging of the adolescent brain." Ann N Y Acad Sci **1021**: 77-85.

Giedd, J. N., J. Blumenthal, N. O. Jeffries, F. X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A. C. Evans and J. L. Rapoport (1999). "Brain development during childhood and adolescence: a longitudinal MRI study." Nat Neurosci **2**(10): 861-863.

Giedd, J. N. and J. L. Rapoport (2010). "Structural MRI of pediatric brain development: what have we learned and where are we going?" Neuron **67**(5): 728-734.

Glowa, J. R. and P. W. Gold (1991). "Corticotropin releasing hormone produces profound anorexigenic effects in the rhesus monkey." Neuropeptides **18**(1): 55-61.

Goelman, G., N. Gordon and O. Bonne (2014). "Maximizing negative correlations in resting-state functional connectivity MRI by time-lag." PLoS One **9**(11): e111554.

Gogtay, N., J. N. Giedd, L. Lusk, K. M. Hayashi, D. Greenstein, A. C. Vaituzis, T. F. Nugent, 3rd, D. H. Herman, L. S. Clasen, A. W. Toga, J. L. Rapoport and P. M. Thompson (2004). "Dynamic mapping of human cortical development during childhood through early adulthood." Proc Natl Acad Sci U S A **101**(21): 8174-8179.

Gogtay, N. and P. M. Thompson (2010). "Mapping gray matter development: implications for typical development and vulnerability to psychopathology." Brain Cogn **72**(1): 6-15.

Goldstein, L. A., E. M. Kurz and D. R. Sengelaub (1990). "Androgen regulation of dendritic growth and retraction in the development of a sexually dimorphic spinal nucleus." J Neurosci **10**(3): 935-946.

Golkar, A., E. Johansson, M. Kasahara, W. Osika, A. Perski and I. Savic (2014). "The influence of work-related chronic stress on the regulation of emotion and on functional connectivity in the brain." PLoS One **9**(9): e104550.

Golub, M., D. Styne, M. Wheeler, C. Keen, A. Hendrickx, F. Moran and M. Gershwin (1997). "Growth retardation in premenarchial female rhesus monkeys during chronic administration of GnRH agonist (leuprolide acetate)." Journal of Medical Primatology **26**(5): 248 - 256.

Gorgolewski, K., C. D. Burns, C. Madison, D. Clark, Y. O. Halchenko, M. L. Waskom and S. S. Ghosh (2011). "Nipype: a flexible, lightweight and extensible neuroimaging data processing framework in python." Front Neuroinform **5**: 13.

Grabenhorst, F. and E. T. Rolls (2008). "Selective attention to affective value alters how the brain processes taste stimuli." Eur J Neurosci **27**(3): 723-729.

Grabenhorst, F., E. T. Rolls and A. Bilderbeck (2008). "How cognition modulates affective responses to taste and flavor: top-down influences on the orbitofrontal and pregenual cingulate cortices." Cereb Cortex **18**(7): 1549-1559.

Graber, J. A. and J. Brooks-Gunn (1996). "Expectations for and precursors to leaving home in young women." New Dir Child Dev(71): 21-38.

Graham, L. C., J. M. Harder, I. Soto, W. N. de Vries, S. W. John and G. R. Howell (2016). "Chronic consumption of a western diet induces robust glial activation in aging mice and in a mouse model of Alzheimer's disease." Sci Rep **6**: 21568.

Grand, A. P., K. M. McCormick, D. Maestripieri and M. M. Sanchez (2005). Effects of infant maltreatment on emotional and HPA axis reactivity in juvenile rhesus macaques. . Society for Neuroscience. Washington DC.

Grant, K. A., C. A. Shively, M. A. Nader, R. L. Ehrenkaufer, S. W. Line, T. E. Morton, H. D. Gage and R. H. Mach (1998). "Effect of social status on striatal dopamine D2 receptor binding characteristics in cynomolgus monkeys assessed with positron emission tomography." Synapse **29**(1): 80-83.

Graves, F. C. and K. Wallen (2006). "Androgen-induced yawning in rhesus monkey females is reversed with a nonsteroidal anti-androgen." Horm Behav **49**(2): 233-236.

Gregor, M. F. and G. S. Hotamisligil (2011). "Inflammatory mechanisms in obesity." Annu Rev Immunol **29**: 415-445.

Gregus, A., A. J. Wintink, A. C. Davis and L. E. Kalynchuk (2005). "Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats." Behav Brain Res **156**(1): 105-114.

Grimm, O., M. J. Jacob, N. B. Kroemer, L. Krebs, S. Vollstadt-Klein, A. Kobiella, U. Wolfensteller and M. N. Smolka (2012). "The personality trait self-directedness predicts the amygdala's reaction to appetizing cues in fMRI." Appetite **58**(3): 1023-1029.

Gundlah, C., S. G. Kohama, S. J. Mirkes, V. T. Garyfallou, H. F. Urbanski and C. L. Bethea (2000). "Distribution of estrogen receptor beta (ERbeta) mRNA in hypothalamus, midbrain and temporal lobe of spayed macaque: continued expression with hormone replacement." Brain Res Mol Brain Res **76**(2): 191-204.

Gunstad, J., R. H. Paul, R. A. Cohen, D. F. Tate, M. B. Spitznagel, S. Grieve and E. Gordon (2008). "Relationship between body mass index and brain volume in healthy adults." Int J Neurosci **118**(11): 1582-1593.

Gust, D. A., T. P. Gordon, M. E. Wilson, A. Ahmed-Ansari, A. R. Brodie and H. M. McClure (1991). "Formation of a new social group of unfamiliar female rhesus monkeys affects the immune and pituitary adrenocortical systems." Brain Behav Immun **5**(3): 296-307.

Haber, S. N. and B. Knutson (2010). "The reward circuit: linking primate anatomy and human imaging." Neuropsychopharmacology **35**(1): 4-26.

Hackman, D. A. and M. J. Farah (2009). "Socioeconomic status and the developing brain." Trends Cogn Sci **13**(2): 65-73.

Haider, L., C. Simeonidou, G. Steinberger, S. Hametner, N. Grigoriadis, G. Deretzi, G. G. Kovacs, A. Kutzelnigg, H. Lassmann and J. M. Frischer (2014). "Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron." J Neurol Neurosurg Psychiatry **85**(12): 1386-1395.

Hains, A. B., M. A. Vu, P. K. Maciejewski, C. H. van Dyck, M. Gottron and A. F. Arnsten (2009). "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 **106**(42): 17957-17962.

Hajnal, A., G. P. Smith and R. Norgren (2004). "Oral sucrose stimulation increases accumbens dopamine in the rat." Am J Physiol Regul Integr Comp Physiol **286**(1): R31-37.

Hanson, J. L., B. M. Nacewicz, M. J. Sutterer, A. A. Cayo, S. M. Schaefer, K. D. Rudolph, E. A. Shirtcliff, S. D. Pollak and R. J. Davidson (2015). "Behavioral problems after early life stress: contributions of the hippocampus and amygdala." Biol Psychiatry **77**(4): 314-323.

Hawrylycz, M. J., E. S. Lein, A. L. Guillozet-Bongaarts, E. H. Shen, L. Ng, J. A. Miller, L. N. van de Lagemaat, K. A. Smith, A. Ebbert, Z. L. Riley, C. Abajian, C. F. Beckmann, A. Bernard, D. Bertagnolli, A. F. Boe, P. M. Cartagena, M. M. Chakravarty, M. Chapin, J. Chong, R. A. Dalley, B. D. Daly, C. Dang, S. Datta, N. Dee, T. A. Dolbeare, V. Faber, D. Feng, D. R. Fowler, J. Goldy, B. W. Gregor, Z. Haradon, D. R. Haynor, J. G.

Hohmann, S. Horvath, R. E. Howard, A. Jeromin, J. M. Jochim, M. Kinnunen, C. Lau, E. T. Lazarz, C. Lee, T. A. Lemon, L. Li, Y. Li, J. A. Morris, C. C. Overly, P. D. Parker, S. E. Parry, M. Reding, J. J. Royall, J. Schulkin, P. A. Sequeira, C. R. Slaughterbeck, S. C. Smith, A. J. Sodt, S. M. Sunkin, B. E. Swanson, M. P. Vawter, D. Williams, P. Wohnoutka, H. R. Zielke, D. H. Geschwind, P. R. Hof, S. M. Smith, C. Koch, S. G. Grant and A. R. Jones (2012). "An anatomically comprehensive atlas of the adult human brain transcriptome." Nature **489**(7416): 391-399.

Henderson, Y. O., G. P. Smith and M. B. Parent (2013). "Hippocampal neurons inhibit meal onset." Hippocampus **23**(1): 100-107.

Herman, J. (2013). "Neural Control of Chronic Stress Adaptation." Frontiers in Behavioral Neuroscience **7**.

Herman, J. P., J. M. McKlveen, M. B. Solomon, E. Carvalho-Netto and B. Myers (2012). "Neural regulation of the stress response: glucocorticoid feedback mechanisms." Braz J Med Biol Res **45**(4): 292-298.

Herod, S. M., A. M. Dettmer, M. A. Novak, J. S. Meyer and J. L. Cameron (2011). "Sensitivity to stress-induced reproductive dysfunction is associated with a selective but not a generalized increase in activity of the adrenal axis." Am J Physiol Endocrinol Metab **300**(1): E28-36.

Herting, M. M., P. Gautam, J. M. Spielberg, R. E. Dahl and E. R. Sowell (2015). "A longitudinal study: changes in cortical thickness and surface area during pubertal maturation." PLoS One **10**(3): e0119774.

Hertzman, C. (1999). "The biological embedding of early experience and its effects on health in adulthood." Ann N Y Acad Sci **896**: 85-95.

Hertzman, C. and M. Wiens (1996). "Child development and long-term outcomes: a population health perspective and summary of successful interventions." Soc Sci Med **43**(7): 1083-1095.

Hill, J. O. (2006). "Understanding and addressing the epidemic of obesity: an energy balance perspective." Endocr Rev **27**(7): 750-761.

Hillman, J. B., L. D. Dorn and H. Bin (2010). "Association of anxiety and depressive symptoms and adiposity among adolescent females, using dual energy X-ray absorptiometry." Clin Pediatr (Phila) **49**(7): 671-677.

Hinde, R. A. and Y. Spencer-Booth (1971). "Effects of brief separation from mother on rhesus monkeys." Science **173**(3992): 111-118.

Hirahara, Y., K. Matsuda, W. Gao, D. N. Arvanitis, M. Kawata and J. M. Boggs (2009). "The localization and non-genomic function of the membrane-associated estrogen receptor in oligodendrocytes." Glia **57**(2): 153-165.

Hogenkamp, P. S., W. Zhou, L. S. Dahlberg, J. Stark, A. L. Larsen, G. Olivo, L. Wiemerslage, E. M. Larsson, M. Sundbom, C. Benedict and H. B. Schioth (2016). "Higher resting-state activity in reward-related brain circuits in obese versus normal-weight females independent of food intake." Int J Obes (Lond).

Hoistad, M. and H. Barbas (2008). "Sequence of information processing for emotions through pathways linking temporal and insular cortices with the amygdala." Neuroimage **40**(3): 1016-1033.

Holder, M. K. and J. D. Blaustein (2013). "Puberty and adolescence as a time of vulnerability to stressors that alter neurobehavioral processes." Front Neuroendocrinol.

Horstmann, A., F. P. Busse, D. Mathar, K. Muller, J. Lepsien, H. Schlogl, S. Kabisch, J. Kratzsch, J. Neumann, M. Stumvoll, A. Villringer and B. Pleger (2011). "Obesity-Related Differences between Women and Men in Brain Structure and Goal-Directed Behavior." Front Hum Neurosci **5**: 58.

Howell, B. R., J. Godfrey, D. A. Gutman, V. Michopoulos, X. Zhang, G. Nair, X. Hu, M. E. Wilson and M. M. Sanchez (2014). "Social subordination stress and serotonin transporter polymorphisms: associations with brain white matter tract integrity and behavior in juvenile female macaques." Cereb Cortex **24**(12): 3334-3349.

Howell, B. R., A. P. Grand, K. M. McCormack, Y. Shi, J. L. LaPrarie, D. Maestripieri, M. A. Styner and M. M. Sanchez (2014). "Early adverse experience increases emotional reactivity in juvenile rhesus macaques: relation to amygdala volume." Dev Psychobiol **56**(8): 1735-1746.

Hryhorczuk, C., S. Sharma and S. E. Fulton (2013). "Metabolic disturbances connecting obesity and depression." Front Neurosci **7**: 177.

Hutchison, R. M., T. Womelsdorf, J. S. Gati, S. Everling and R. S. Menon (2013). "Resting-state networks show dynamic functional connectivity in awake humans and anesthetized macaques." Hum Brain Mapp **34**(9): 2154-2177.

Huttenlocher, P. R. and A. S. Dabholkar (1997). "Regional differences in synaptogenesis in human cerebral cortex." J Comp Neurol **387**(2): 167-178.

Inder, T. E. and P. S. Huppi (2000). "In vivo studies of brain development by magnetic resonance techniques." Ment Retard Dev Disabil Res Rev **6**(1): 59-67.

Iyer, S. P., I. Shafran, D. Grayson, K. Gates, J. T. Nigg and D. A. Fair (2013). "Inferring functional connectivity in MRI using Bayesian network structure learning with a modified PC algorithm." Neuroimage **75**: 165-175.

Jabes, A., P. B. Lavenex, D. G. Amaral and P. Lavenex (2011). "Postnatal development of the hippocampal formation: a stereological study in macaque monkeys." J Comp Neurol **519**(6): 1051-1070.

Jacka, F. N., N. Cherbuin, K. J. Anstey, P. Sachdev and P. Butterworth (2015). "Western diet is associated with a smaller hippocampus: a longitudinal investigation." BMC Med **13**: 215.

Jackowski, A., T. D. Perera, C. G. Abdallah, G. Garrido, C. Y. Tang, J. Martinez, S. J. Mathew, J. M. Gorman, L. A. Rosenblum, E. L. Smith, A. J. Dwork, D. C. Shungu, A. Kaffman, J. Gelernter, J. D. Coplan and J. Kaufman (2011). "Early-life stress, corpus callosum development, hippocampal volumetrics, and anxious behavior in male nonhuman primates." Psychiatry Res **192**(1): 37-44.

Jackson, M. E. and B. Moghaddam (2006). "Distinct patterns of plasticity in prefrontal cortex neurons that encode slow and fast responses to stress." Eur J Neurosci **24**(6): 1702-1710.

Janowitz, D., K. Wittfeld, J. Terock, H. J. Freyberger, K. Hegenscheid, H. Volzke, M. Habes, N. Hosten, N. Friedrich, M. Nauck, G. Domanska and H. J. Grabe (2015). "Association between waist circumference and gray matter volume in 2344 individuals from two adult community-based samples." Neuroimage **122**: 149-157.

Jarrell, H., J. B. Hoffman, J. R. Kaplan, S. Berga, B. Kinkead and M. E. Wilson (2008). "Polymorphisms in the serotonin reuptake transporter gene modify the consequences of social status on metabolic health in female rhesus monkeys." Physiology & behavior **93**: 807-819.

Jayaraman, A., D. Lent-Schochet and C. J. Pike (2014). "Diet-induced obesity and low testosterone increase neuroinflammation and impair neural function." J Neuroinflammation **11**: 162.

Jernigan, T. L. and P. Tallal (1990). "Late childhood changes in brain morphology observable with MRI." Dev Med Child Neurol **32**(5): 379-385.

Joels, M., H. Karst, D. Alfarez, V. M. Heine, Y. Qin, E. van Riel, M. Verkuyl, P. J. Lucassen and H. J. Krugers (2004). "Effects of chronic stress on structure and cell function in rat hippocampus and hypothalamus." Stress **7**(4): 221-231.

Joels, M., H. Karst, H. J. Krugers and P. J. Lucassen (2007). "Chronic stress: implications for neuronal morphology, function and neurogenesis." Front Neuroendocrinol **28**(2-3): 72-96.

Johnson, E. O., T. C. Kamilaris, G. P. Chrousos and P. W. Gold (1992). "Mechanisms of stress: a dynamic overview of hormonal and behavioral homeostasis." Neurosci Biobehav Rev **16**(2): 115-130.

Johnson, P. M. and P. J. Kenny (2010). "Dopamine D2 receptors in addiction-like reward dysfunction and compulsive eating in obese rats." Nat Neurosci **13**(5): 635-641.

Jokinen, J., A. L. Nordstrom and P. Nordstrom (2008). "ROC analysis of dexamethasone suppression test threshold in suicide prediction after attempted suicide." J Affect Disord **106**(1-2): 145-152.

Jung-Testas, I., M. Renoir, H. Bugnard, G. L. Greene and E. E. Baulieu (1992). "Demonstration of steroid hormone receptors and steroid action in primary cultures of rat glial cells." J Steroid Biochem Mol Biol **41**(3-8): 621-631.

Kalin, N. H. and S. E. Shelton (1989). "Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation." Science (New York, N.Y.) **243**: 1718-1721.

Kalin, N. H. and S. E. Shelton (1998). "Ontogeny and stability of separation and threat-induced defensive behaviors in rhesus monkeys during the first year of life." Am J Primatol **44**(2): 125-135.

Kalin, N. H. and S. E. Shelton (2003). "Nonhuman primate models to study anxiety, emotion regulation, and psychopathology." Ann N Y Acad Sci **1008**: 189-200.

Kalin, N. H., S. E. Shelton and R. J. Davidson (2007). "Role of the primate orbitofrontal cortex in mediating anxious temperament." Biol Psychiatry **62**(10): 1134-1139.

Kalin, N. H., S. E. Shelton, A. S. Fox, T. R. Oakes and R. J. Davidson (2005). "Brain regions associated with the expression and contextual regulation of anxiety in primates." Biol Psychiatry **58**(10): 796-804.

Kalynchuk, L. E., A. Gregus, D. Boudreau and T. S. Perrot-Sinal (2004). "Corticosterone increases depression-like behavior, with some effects on predator odor-induced defensive behavior, in male and female rats." Behav Neurosci **118**(6): 1365-1377.

Kang, E. B., J. H. Koo, Y. C. Jang, C. H. Yang, Y. Lee, L. M. Cosio-Lima and J. Y. Cho (2016). "Neuroprotective Effects of Endurance Exercise Against High-Fat Diet-Induced Hippocampal Neuroinflammation." J Neuroendocrinol **28**(5).

Kanoski, S. E. and T. L. Davidson (2011). "Western diet consumption and cognitive impairment: links to hippocampal dysfunction and obesity." Physiol Behav **103**(1): 59-68.

Kaplan, J. R. (2008). "Origins and health consequences of stress-induced ovarian dysfunction." Interdiscip Top Gerontol **36**: 162-185.

Kaplan, J. R., M. R. Adams, T. B. Clarkson, S. B. Manuck, C. A. Shively and J. K. Williams (1996). "Psychosocial factors, sex differences, and atherosclerosis: lessons from animal models." Psychosom Med **58**(6): 598-611.

Kaplan, J. R., H. Chen, S. E. Appt, C. J. Lees, A. A. Franke, S. L. Berga, M. E. Wilson, S. B. Manuck and T. B. Clarkson (2010). "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 **25**(12): 3083-3094.

Kaplan, J. R. and S. B. Manuck (2004). "Ovarian dysfunction, stress, and disease: a primate continuum." ILAR J **45**(2): 89-115.

Kaplan, J. R., S. B. Manuck, M. B. Fontenot and J. J. Mann (2002). "Central nervous system monoamine correlates of social dominance in cynomolgus monkeys (Macaca fascicularis)." Neuropsychopharmacology **26**(4): 431-443.

Kaplan, J. R., S. B. Manuck and C. Shively (1991). "The effects of fat and cholesterol on social behavior in monkeys." Psychosom Med **53**(6): 634-642.

Kaufman, J. and D. Charney (2001). "Effects of early stress on brain structure and function: implications for understanding the relationship between child maltreatment and depression." Dev Psychopathol **13**(3): 451-471.

Keen-Rhinehart, E., V. Michopoulos, D. J. Toufexis, E. I. Martin, H. Nair, K. J. Ressler, M. Davis, M. J. Owens, C. B. Nemeroff and M. E. Wilson (2009). "Continuous expression of corticotropin-releasing factor in the central nucleus of the amygdala emulates the dysregulation of the stress and reproductive axes." Mol Psychiatry **14**(1): 37-50.

Keenan, K. P., M. A. Wallig and W. M. Haschek (2013). "Nature via nurture: effect of diet on health, obesity, and safety assessment." Toxicol Pathol **41**(2): 190-209.

Kessler, R. C., S. Avenevoli and K. Ries Merikangas (2001). "Mood disorders in children and adolescents: an epidemiologic perspective." Biol Psychiatry **49**(12): 1002-1014.

Kessler, R. C., P. Berglund, O. Demler, R. Jin, K. R. Merikangas and E. E. Walters (2005). "Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the National Comorbidity Survey Replication." Arch Gen Psychiatry **62**(6): 593-602.

Kim, J. J. and D. M. Diamond (2002). "The stressed hippocampus, synaptic plasticity and lost memories." Nat Rev Neurosci **3**(6): 453-462.

Knickmeyer, R. C., M. Styner, S. J. Short, G. R. Lubach, C. Kang, R. Hamer, C. L. Coe and J. H. Gilmore (2010). "Maturational trajectories of cortical brain development through the pubertal transition: unique species and sex differences in the monkey revealed through structural magnetic resonance imaging." Cerebral cortex (New York, N.Y. : 1991) **20**: 1053-1063.

Knutson, B., J. Taylor, M. Kaufman, R. Peterson and G. Glover (2005). "Distributed neural representation of expected value." J Neurosci **25**(19): 4806-4812.

Kolb, B. and J. Stewart (1991). "Sex-related differences in dendritic branching of cells in the prefrontal cortex of rats." J Neuroendocrinol **3**(1): 95-99.

Kolber, B. J., M. S. Roberts, M. P. Howell, D. F. Wozniak, M. S. Sands and L. J. Muglia (2008). "Central amygdala glucocorticoid receptor action promotes fear-associated CRH activation and conditioning." Proc Natl Acad Sci U S A **105**(33): 12004-12009.

Koob, G. F. and M. Le Moal (2008). "Review. Neurobiological mechanisms for opponent motivational processes in addiction." Philos Trans R Soc Lond B Biol Sci **363**(1507): 3113-3123.

Koob, G. F. and N. D. Volkow (2016). "Neurobiology of addiction: a neurocircuitry analysis." Lancet Psychiatry **3**(8): 760-773.

Koolschijn, P. C., J. S. Peper and E. A. Crone (2014). "The influence of sex steroids on structural brain maturation in adolescence." PLoS One **9**(1): e83929.

Krahn, D. D., B. A. Gosnell and M. J. Majchrzak (1990). "The anorectic effects of CRH and restraint stress decrease with repeated exposures." Biol Psychiatry **27**(10): 1094-1102.

Krmpotich, T. D., J. R. Tregellas, L. L. Thompson, M. T. Banich, A. M. Klenk and J. L. Tanabe (2013). "Resting-state activity in the left executive control network is associated with behavioral approach and is increased in substance dependence." Drug Alcohol Depend **129**(1-2): 1-7.

Kuhnen, C. M. and B. Knutson (2005). "The neural basis of financial risk taking." Neuron **47**(5): 763-770.

Kumaran, D., H. L. Melo and E. Duzel (2012). "The emergence and representation of knowledge about social and nonsocial hierarchies." Neuron **76**(3): 653-666.

Kurth, F., K. Zilles, P. T. Fox, A. R. Laird and S. B. Eickhoff (2010). "A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis." Brain Struct Funct **214**(5-6): 519-534.

la Fleur, S. E., A. J. van Rozen, M. C. Luijendijk, F. Groeneweg and R. A. Adan (2010). "A free-choice high-fat high-sugar diet induces changes in arcuate neuropeptide expression that support hyperphagia." Int J Obes (Lond) **34**(3): 537-546.

LaMantia, A. S. and P. Rakic (1990). "Axon overproduction and elimination in the corpus callosum of the developing rhesus monkey." J Neurosci **10**(7): 2156-2175.

Lavenex, P., P. Banta Lavenex and D. G. Amaral (2007). "Postnatal development of the primate hippocampal formation." Dev Neurosci **29**(1-2): 179-192.

Lazar, S. W., C. E. Kerr, R. H. Wasserman, J. R. Gray, D. N. Greve, M. T. Treadway, M. McGarvey, B. T. Quinn, J. A. Dusek, H. Benson, S. L. Rauch, C. I. Moore and B. Fischl (2005). "Meditation experience is associated with increased cortical thickness." Neuroreport **16**(17): 1893-1897.

LeDoux, J. E. (2000). "Emotion circuits in the brain." Annu Rev Neurosci **23**: 155-184.

Lee, D. (2008). "Game theory and neural basis of social decision making." Nat Neurosci **11**(4): 404-409.

Lee, R., T. D. Geracioti, Jr., J. W. Kasckow and E. F. Coccaro (2005). "Childhood trauma and personality disorder: positive correlation with adult CSF corticotropin-releasing factor concentrations." Am J Psychiatry **162**(5): 995-997.

Legendre, A. and R. B. Harris (2006). "Exaggerated response to mild stress in rats fed high-fat diet." Am J Physiol Regul Integr Comp Physiol **291**(5): R1288-1294.

Lenroot, R. K. and J. N. Giedd (2006). "Brain development in children and adolescents: insights from anatomical magnetic resonance imaging." Neurosci Biobehav Rev **30**(6): 718-729.

Lenroot, R. K., N. Gogtay, D. K. Greenstein, E. M. Wells, G. L. Wallace, L. S. Clasen, J. D. Blumenthal, J. Lerch, A. P. Zijdenbos, A. C. Evans, P. M. Thompson and J. N. Giedd (2007). "Sexual dimorphism of brain developmental trajectories during childhood and adolescence." Neuroimage **36**(4): 1065-1073.

Lewis, J. W. and D. C. Van Essen (2000). "Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey." J Comp Neurol **428**(1): 112-137.

Li, C. X., S. Patel, E. J. Auerbach and X. Zhang (2013). "Dose-dependent effect of isoflurane on regional cerebral blood flow in anesthetized macaque monkeys." Neurosci Lett **541**: 58-62.

Lieberwirth, C., Y. Pan, Y. Liu, Z. Zhang and Z. Wang (2016). "Hippocampal adult neurogenesis: Its regulation and potential role in spatial learning and memory." Brain Res **1644**: 127-140.

Lindburg, D. G. (1973). "Section 1 Grooming Behavior as a Regulator of Social Interactions in Rhesus Monkeys." Behavioral regulators of behavior in primates: 124.

Lips, M. A., M. A. Wijngaarden, J. van der Grond, M. A. van Buchem, G. H. de Groot, S. A. Rombouts, H. Pijl and I. M. Veer (2014). "Resting-state functional connectivity of brain regions involved in cognitive control, motivation, and reward is enhanced in obese females." Am J Clin Nutr **100**(2): 524-531.

Liston, C., B. S. McEwen and B. J. Casey (2009). "Psychosocial stress reversibly disrupts prefrontal processing and attentional control." Proc Natl Acad Sci U S A **106**(3): 912-917.

Liu, X., X. L. Fan, Y. Zhao, G. R. Luo, X. P. Li, R. Li and W. D. Le (2005). "Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal

injury through both estrogen receptor-alpha and estrogen receptor-beta in microglia." J Neurosci Res **81**(5): 653-665.

Lodge, D. J. and A. A. Grace (2005). "Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity." J Pharmacol Exp Ther **314**(1): 201-206.

Loucks, E. B., L. Pilote, J. W. Lynch, H. Richard, N. D. Almeida, E. J. Benjamin and J. M. Murabito (2010). "Life course socioeconomic position is associated with inflammatory markers: the Framingham Offspring Study." Soc Sci Med **71**(1): 187-195.

Lucassen, P. J., V. M. Heine, M. B. Muller, E. M. van der Beek, V. M. Wiegant, E. R. De Kloet, M. Joels, E. Fuchs, D. F. Swaab and B. Czeh (2006). "Stress, depression and hippocampal apoptosis." CNS Neurol Disord Drug Targets **5**(5): 531-546.

Luine, V. (2016). "Estradiol: Mediator of memories, spine density and cognitive resilience to stress in female rodents." J Steroid Biochem Mol Biol **160**: 189-195.

Lupien, S. J., B. S. McEwen, M. R. Gunnar and C. Heim (2009). "Effects of stress throughout the lifespan on the brain, behaviour and cognition." Nat Rev Neurosci **10**(6): 434-445.

Lyons-Ruth, K., P. Pechtel, S. A. Yoon, C. M. Anderson and M. H. Teicher (2016). "Disorganized attachment in infancy predicts greater amygdala volume in adulthood." Behav Brain Res **308**: 83-93.

Ma, N., Y. Liu, N. Li, C. X. Wang, H. Zhang, X. F. Jiang, H. S. Xu, X. M. Fu, X. Hu and D. R. Zhang (2010). "Addiction related alteration in resting-state brain connectivity." Neuroimage **49**(1): 738-744.

Machado, C. J. and J. Bachevalier (2003). "Non-human primate models of childhood psychopathology: the promise and the limitations." Journal of child psychology and psychiatry, and allied disciplines **44**: 64-87.

Machado, C. J. and J. Bachevalier (2006). "The impact of selective amygdala, orbital frontal cortex, or hippocampal formation lesions on established social relationships in rhesus monkeys (Macaca mulatta)." Behav Neurosci **120**(4): 761-786.

Machado, C. J. and J. Bachevalier (2008). "Behavioral and hormonal reactivity to threat: effects of selective amygdala, hippocampal or orbital frontal lesions in monkeys." Psychoneuroendocrinology **33**(7): 926-941.

Machado, C. J., A. M. Whitaker, S. E. Smith, P. H. Patterson and M. D. Bauman (2014). "Maternal Immune Activation in Nonhuman Primates Alters Social Attention in Juvenile Offspring." Biol Psychiatry.

Maestripieri, D., K. McCormack, S. G. Lindell, J. D. Higley and M. M. Sanchez (2006). "Influence of parenting style on the offspring's behaviour and CSF monoamine

metabolite levels in crossfostered and noncrossfostered female rhesus macaques." Behav Brain Res **175**(1): 90-95.

Magarinos, A. M. and B. S. McEwen (1995). "Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors." Neuroscience **69**(1): 89-98.

Magarinos, A. M., M. Orchinik and B. S. McEwen (1998). "Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox." Brain Res **809**(2): 314-318.

Majdandzic, J., S. Amashaufer, A. Hummer, C. Windischberger and C. Lamm (2016). "The selfless mind: How prefrontal involvement in mentalizing with similar and dissimilar others shapes empathy and prosocial behavior." Cognition **157**: 24-38.

Makino, S., K. Hashimoto and P. W. Gold (2002). "Multiple feedback mechanisms activating corticotropin-releasing hormone system in the brain during stress." Pharmacol Biochem Behav **73**(1): 147-158.

Makino, S., M. A. Smith and P. W. Gold (1995). "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 **136**(8): 3299-3309.

Malkova, L., E. Heuer and R. C. Saunders (2006). "Longitudinal magnetic resonance imaging study of rhesus monkey brain development." Eur J Neurosci **24**(11): 3204-3212.

Margulies, D. S., J. L. Vincent, C. Kelly, G. Lohmann, L. Q. Uddin, B. B. Biswal, A. Villringer, F. X. Castellanos, M. P. Milham and M. Petrides (2009). "Precuneus shares intrinsic functional architecture in humans and monkeys." Proc Natl Acad Sci U S A **106**(47): 20069-20074.

Marin-Husstege, M., M. Muggironi, D. Raban, R. P. Skoff and P. Casaccia-Bonnefil (2004). "Oligodendrocyte progenitor proliferation and maturation is differentially regulated by male and female sex steroid hormones." Dev Neurosci **26**(2-4): 245-254.

Markham, J. A., J. R. Morris and J. M. Juraska (2007). "Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood." Neuroscience **144**(3): 961-968.

Markham, J. A., S. E. Mullins and J. I. Koenig (2013). "Periadolescent maturation of the prefrontal cortex is sex-specific and is disrupted by prenatal stress." J Comp Neurol **521**(8): 1828-1843.

Markov, N. T., M. M. Ercsey-Ravasz, A. R. Ribeiro Gomes, C. Lamy, L. Magrou, J. Vezoli, P. Misery, A. Falchier, R. Quilodran, M. A. Gariel, J. Sallet, R. Gamanut, C. Huissoud, S. Clavagnier, P. Giroud, D. Sappey-Marinier, P. Barone, C. Dehay, Z. Toroczkai, K. Knoblauch, D. C. Van Essen and H. Kennedy (2014). "A weighted and

directed interareal connectivity matrix for macaque cerebral cortex." Cereb Cortex **24**(1): 17-36.

Marques-Iturria, I., R. Pueyo, M. Garolera, B. Segura, C. Junque, I. Garcia-Garcia, M. Jose Sender-Palacios, M. Vernet-Vernet, A. Narberhaus, M. Ariza and M. A. Jurado (2013). "Frontal cortical thinning and subcortical volume reductions in early adulthood obesity." Psychiatry Res **214**(2): 109-115.

Marsland, A. L., P. J. Gianaros, D. C. Kuan, L. K. Sheu, K. Krajina and S. B. Manuck (2015). "Brain morphology links systemic inflammation to cognitive function in midlife adults." Brain Behav Immun **48**: 195-204.

Martel, M. M., K. Klump, J. T. Nigg, S. M. Breedlove and C. L. Sisk (2009). "Potential hormonal mechanisms of attention-deficit/hyperactivity disorder and major depressive disorder: a new perspective." Horm Behav **55**(4): 465-479.

Martin, A. A. and T. L. Davidson (2014). "Human cognitive function and the obesogenic environment." Physiol Behav **136**: 185-193.

Masouleh, S. K., K. Arelin, A. Horstmann, L. Lampe, J. A. Kipping, T. Luck, S. G. Riedel-Heller, M. L. Schroeter, M. Stumvoll, A. Villringer and A. V. Witte (2016). "Higher body mass index in older adults is associated with lower gray matter volume: implications for memory performance." Neurobiol Aging **40**: 1-10.

McCarthy, M. M. (2010). "How it's made: organisational effects of hormones on the developing brain." J Neuroendocrinol **22**(7): 736-742.

McEwen, B. S. (1999). "Stress and hippocampal plasticity." Annu Rev Neurosci **22**: 105-122.

McEwen, B. S. (2001). "Invited review: Estrogens effects on the brain: multiple sites and molecular mechanisms." J Appl Physiol **91**(6): 2785-2801.

McEwen, B. S. (2006). "Protective and damaging effects of stress mediators: central role of the brain." Dialogues Clin Neurosci **8**(4): 367-381.

McEwen, B. S. (2012). "Brain on stress: how the social environment gets under the skin." Proc Natl Acad Sci U S A **109 Suppl 2**: 17180-17185.

McEwen, B. S., C. A. Biron, K. W. Brunson, K. Bulloch, W. H. Chambers, F. S. Dhabhar, R. H. Goldfarb, R. P. Kitson, A. H. Miller, R. L. Spencer and J. M. Weiss (1997). "The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions." Brain Res Brain Res Rev **23**(1-2): 79-133.

McEwen, B. S. and T. A. Milner (2007). "Hippocampal formation: shedding light on the influence of sex and stress on the brain." Brain Res Rev **55**(2): 343-355.

McLaren, D. G., K. J. Kosmatka, E. K. Kastman, B. B. Bendlin and S. C. Johnson (2010). "Rhesus macaque brain morphometry: a methodological comparison of voxel-wise approaches." Methods **50**(3): 157-165.

McLaren, D. G., K. J. Kosmatka, T. R. Oakes, C. D. Kroenke, S. G. Kohama, J. A. Matochik, D. K. Ingram and S. C. Johnson (2009). "A population-average MRI-based atlas collection of the rhesus macaque." Neuroimage **45**(1): 52-59.

McLaughlin, K. J., S. E. Baran and C. D. Conrad (2009). "Chronic stress- and sex-specific neuromorphological and functional changes in limbic structures." Mol Neurobiol **40**(2): 166-182.

Mehta, M. A., N. I. Golembo, C. Nosarti, E. Colvert, A. Mota, S. C. Williams, M. Rutter and E. J. Sonuga-Barke (2009). "Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot." J Child Psychol Psychiatry **50**(8): 943-951.

Meloni, E. G., C. L. Reedy, B. M. Cohen and W. A. Carlezon, Jr. (2008). "Activation of raphe efferents to the medial prefrontal cortex by corticotropin-releasing factor: correlation with anxiety-like behavior." Biol Psychiatry **63**(9): 832-839.

Menon, V. and L. Q. Uddin (2010). "Saliency, switching, attention and control: a network model of insula function." Brain Struct Funct **214**(5-6): 655-667.

Mesulam, M. M. and E. J. Mufson (1982). "Insula of the old world monkey. III: Efferent cortical output and comments on function." J Comp Neurol **212**(1): 38-52.

Meunier, M., J. Bachevalier, E. A. Murray, L. Malkova and M. Mishkin (1999). "Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys." Eur J Neurosci **11**(12): 4403-4418.

Michopoulos, V., S. L. Berga, J. R. Kaplan and M. E. Wilson (2009). "Social subordination and polymorphisms in the gene encoding the serotonin transporter enhance estradiol inhibition of luteinizing hormone secretion in female rhesus monkeys." Biology of reproduction **81**: 1154-1163.

Michopoulos, V., M. P. Diaz and M. E. Wilson (2016). "Social change and access to a palatable diet produces differences in reward neurochemistry and appetite in female monkeys." Physiol Behav **162**: 102-111.

Michopoulos, V., M. Higgins, D. Toufexis and M. E. Wilson (2012). "Social subordination produces distinct stress-related phenotypes in female rhesus monkeys." Psychoneuroendocrinology **37**(7): 1071-1085.

Michopoulos, V., K. M. Reding, M. E. Wilson and D. Toufexis (2012). "Social subordination impairs hypothalamic-pituitary-adrenal function in female rhesus monkeys." Horm Behav **62**(4): 389-399.

Michopoulos, V., D. Toufexis and M. E. Wilson (2012). "Social stress interacts with diet history to promote emotional feeding in females." Psychoneuroendocrinology **37**(9): 1479-1490.

Milanski, M., G. Degasperi, A. Coope, J. Morari, R. Denis, D. E. Cintra, D. M. Tsukumo, G. Anhe, M. E. Amaral, H. K. Takahashi, R. Curi, H. C. Oliveira, J. B. Carvalheira, S. Bordin, M. J. Saad and L. A. Velloso (2009). "Saturated fatty acids produce an inflammatory response predominantly through the activation of TLR4 signaling in hypothalamus: implications for the pathogenesis of obesity." J Neurosci **29**(2): 359-370.

Miller, A. A. and S. J. Spencer (2014). "Obesity and neuroinflammation: a pathway to cognitive impairment." Brain Behav Immun **42**: 10-21.

Miller, A. H., V. Maletic and C. L. Raison (2009). "Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression." Biol Psychiatry **65**(9): 732-741.

Miller, G. E. and E. Blackwell (2006). "Turning Up the Heat: Inflammation as a Mechanism Linking Chronic Stress, Depression, and Heart Disease." Current Directions in Psychological Science **15**(6): 269-272.

Mincic, A. M. (2015). "Neuroanatomical correlates of negative emotionality-related traits: A systematic review and meta-analysis." Neuropsychologia **77**: 97-118.

Miranda-Dominguez, O., B. D. Mills, D. Grayson, A. Woodall, K. A. Grant, C. D. Kroenke and D. A. Fair (2014). "Bridging the gap between the human and macaque connectome: a quantitative comparison of global interspecies structure-function relationships and network topology." J Neurosci **34**(16): 5552-5563.

Mitra, R., S. Jadhav, B. S. McEwen, A. Vyas and S. Chattarji (2005). "Stress duration modulates the spatiotemporal patterns of spine formation in the basolateral amygdala." Proc Natl Acad Sci U S A **102**(26): 9371-9376.

Mitra, R. and R. M. Sapolsky (2008). "Acute corticosterone treatment is sufficient to induce anxiety and amygdaloid dendritic hypertrophy." Proc Natl Acad Sci U S A **105**(14): 5573-5578.

Monje, M. L., H. Toda and T. D. Palmer (2003). "Inflammatory blockade restores adult hippocampal neurogenesis." Science **302**(5651): 1760-1765.

Mor, G., J. Nilsen, T. Horvath, I. Bechmann, S. Brown, L. M. Garcia-Segura and F. Naftolin (1999). "Estrogen and microglia: A regulatory system that affects the brain." J Neurobiol **40**(4): 484-496.

Mora, F., G. Segovia, A. Del Arco, M. de Blas and P. Garrido (2012). "Stress, neurotransmitters, corticosterone and body-brain integration." Brain Res **1476**: 71-85.

Moraes, J. C., A. Coope, J. Morari, D. E. Cintra, E. A. Roman, J. R. Pauli, T. Romanatto, J. B. Carvalheira, A. L. Oliveira, M. J. Saad and L. A. Velloso (2009). "High-fat diet induces apoptosis of hypothalamic neurons." PLoS One **4**(4): e5045.

Morgan, D., K. A. Grant, H. D. Gage, R. H. Mach, J. R. Kaplan, O. Prioleau, S. H. Nader, N. Buchheimer, R. L. Ehrenkaufer and M. A. Nader (2002). "Social dominance in monkeys: dopamine D2 receptors and cocaine self-administration." Nat Neurosci **5**(2): 169-174.

Morris, G., M. Berk, K. Walder and M. Maes (2015). "Central pathways causing fatigue in neuro-inflammatory and autoimmune illnesses." BMC Med **13**: 28.

Morris, M. J., J. E. Beilharz, J. Maniam, A. C. Reichelt and R. F. Westbrook (2015). "Why is obesity such a problem in the 21st century? The intersection of palatable food, cues and reward pathways, stress, and cognition." Neurosci Biobehav Rev **58**: 36-45.

Mufson, E. J. and M. M. Mesulam (1982). "Insula of the old world monkey. II: Afferent cortical input and comments on the claustrum." J Comp Neurol **212**(1): 23-37.

Murphy, K., R. M. Birn, D. A. Handwerker, T. B. Jones and P. A. Bandettini (2009). "The impact of global signal regression on resting state correlations: are anti-correlated networks introduced?" Neuroimage **44**(3): 893-905.

Must, A., J. Spadano, E. H. Coakley, A. E. Field, G. Colditz and W. H. Dietz (1999). "The disease burden associated with overweight and obesity." JAMA **282**(16): 1523-1529.

Myers, B., J. M. McKlveen and J. P. Herman (2012). "Neural Regulation of the Stress Response: The Many Faces of Feedback." Cell Mol Neurobiol.

Myles, I. A. (2014). "Fast food fever: reviewing the impacts of the Western diet on immunity." Nutr J **13**: 61.

Nader, M. A., S. H. Nader, P. W. Czoty, N. V. Riddick, H. D. Gage, R. W. Gould, B. L. Blaylock, J. R. Kaplan, P. K. Garg, H. M. Davies, D. Morton, S. Garg and B. A. Reboussin (2012). "Social dominance in female monkeys: dopamine receptor function and cocaine reinforcement." Biol Psychiatry **72**(5): 414-421.

Nelson, E. E., E. Leibenluft, E. B. McClure and D. S. Pine (2005). "The social re-orientation of adolescence: a neuroscience perspective on the process and its relation to psychopathology." Psychol Med **35**(2): 163-174.

Neumann, C., J. Duboscq, C. Dubuc, A. Ginting, A. M. Irwan, M. Agil, A. Widdig and A. Engelhardt (2011). "Assessing dominance hierarchies: validation and advantages of progressive evaluation with Elo-rating." Animal Behaviour **82**(4): 911-921.

Ng, M., T. Fleming, M. Robinson, B. Thomson, N. Graetz, C. Margono, E. C. Mullany, S. Biryukov, C. Abbafati, S. F. Abera, J. P. Abraham, N. M. Abu-Rmeileh, T. Achoki, F.

S. AlBuhairan, Z. A. Alemu, R. Alfonso, M. K. Ali, R. Ali, N. A. Guzman, W. Ammar, P. Anwari, A. Banerjee, S. Barquera, S. Basu, D. A. Bennett, Z. Bhutta, J. Blore, N. Cabral, I. C. Nonato, J. C. Chang, R. Chowdhury, K. J. Courville, M. H. Criqui, D. K. Cundiff, K. C. Dabhadkar, L. Dandona, A. Davis, A. Dayama, S. D. Dharmaratne, E. L. Ding, A. M. Durrani, A. Esteghamati, F. Farzadfar, D. F. Fay, V. L. Feigin, A. Flaxman, M. H. Forouzanfar, A. Goto, M. A. Green, R. Gupta, N. Hafezi-Nejad, G. J. Hankey, H. C. Harewood, R. Havmoeller, S. Hay, L. Hernandez, A. Husseini, B. T. Idrisov, N. Ikeda, F. Islami, E. Jahangir, S. K. Jassal, S. H. Jee, M. Jeffreys, J. B. Jonas, E. K. Kabagambe, S. E. Khalifa, A. P. Kengne, Y. S. Khader, Y. H. Khang, D. Kim, R. W. Kimokoti, J. M. Kinge, Y. Kokubo, S. Kosen, G. Kwan, T. Lai, M. Leinsalu, Y. Li, X. Liang, S. Liu, G. Logroscino, P. A. Lotufo, Y. Lu, J. Ma, N. K. Mainoo, G. A. Mensah, T. R. Merriman, A. H. Mokdad, J. Moschandreas, M. Naghavi, A. Naheed, D. Nand, K. M. Narayan, E. L. Nelson, M. L. Neuhouser, M. I. Nisar, T. Ohkubo, S. O. Oti, A. Pedroza, D. Prabhakaran, N. Roy, U. Sampson, H. Seo, S. G. Sepanlou, K. Shibuya, R. Shiri, I. Shiue, G. M. Singh, J. A. Singh, V. Skirbekk, N. J. Stapelberg, L. Sturua, B. L. Sykes, M. Tobias, B. X. Tran, L. Trasande, H. Toyoshima, S. van de Vijver, T. J. Vasankari, J. L. Veerman, G. Velasquez-Melendez, V. V. Vlassov, S. E. Vollset, T. Vos, C. Wang, X. Wang, E. Weiderpass, A. Werdecker, J. L. Wright, Y. C. Yang, H. Yatsuya, J. Yoon, S. J. Yoon, Y. Zhao, M. Zhou, S. Zhu, A. D. Lopez, C. J. Murray and E. Gakidou (2014). "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 **384**(9945): 766-781.

Nishizuka, M. and Y. Arai (1981). "Organizational action of estrogen on synaptic pattern in the amygdala: implications for sexual differentiation of the brain." Brain Res **213**(2): 422-426.

Noonan, M. P., J. Sallet, R. B. Mars, F. X. Neubert, J. X. O'Reilly, J. L. Andersson, A. S. Mitchell, A. H. Bell, K. L. Miller and M. F. Rushworth (2014). "A neural circuit covarying with social hierarchy in macaques." PLoS Biol **12**(9): e1001940.

Norjavaara, E., C. Ankarberg and K. Albertsson-Wikland (1996). "Diurnal rhythm of 17 beta-estradiol secretion throughout pubertal development in healthy girls: evaluation by a sensitive radioimmunoassay." J Clin Endocrinol Metab **81**(11): 4095-4102.

Nowakowski, R. S. and P. Rakic (1981). "The site of origin and route and rate of migration of neurons to the hippocampal region of the rhesus monkey." J Comp Neurol **196**(1): 129-154.

Nummenmaa, L., J. Hirvonen, J. C. Hannukainen, H. Immonen, M. M. Lindroos, P. Salminen and P. Nuutila (2012). "Dorsal striatum and its limbic connectivity mediate abnormal anticipatory reward processing in obesity." PLoS One **7**(2): e31089.

Nunez, J. L., D. M. Lauschke and J. M. Juraska (2001). "Cell death in the development of the posterior cortex in male and female rats." J Comp Neurol **436**(1): 32-41.

Nuruddin, S., A. Krogenaes, O. B. Brynildsrud, S. Verhaegen, N. P. Evans, J. E. Robinson, I. R. Haraldsen and E. Ropstad (2013). "Peri-pubertal gonadotropin-releasing hormone agonist treatment affects sex biased gene expression of amygdala in sheep." Psychoneuroendocrinology **38**(12): 3115-3127.

Nuruddin, S., S. Wojniusz, E. Ropstad, A. Krogenaes, N. P. Evans, J. E. Robinson, A. K. Solbakk, M. Amiry-Moghaddam, I. R. Haraldsen and S. Sex On Brain European Research Group (2013). "Peri-pubertal gonadotropin-releasing hormone analog treatment affects hippocampus gene expression without changing spatial orientation in young sheep." Behav Brain Res **242**: 9-16.

O'Doherty, J., J. Winston, H. Critchley, D. Perrett, D. M. Burt and R. J. Dolan (2003). "Beauty in a smile: the role of medial orbitofrontal cortex in facial attractiveness." Neuropsychologia **41**(2): 147-155.

Oler, J. A., A. S. Fox, S. E. Shelton, J. Rogers, T. D. Dyer, R. J. Davidson, W. Shelledy, T. R. Oakes, J. Blangero and N. H. Kalin (2010). "Amygdalar and hippocampal substrates of anxious temperament differ in their heritability." Nature **466**(7308): 864-868.

Oliver, G., J. Wardle and E. L. Gibson (2000). "Stress and food choice: a laboratory study." Psychosom Med **62**(6): 853-865.

Onyike, C. U., R. M. Crum, H. B. Lee, C. G. Lyketsos and W. W. Eaton (2003). "Is obesity associated with major depression? Results from the Third National Health and Nutrition Examination Survey." Am J Epidemiol **158**(12): 1139-1147.

Osterlund, M. K., J. A. Gustafsson, E. Keller and Y. L. Hurd (2000). "Estrogen receptor beta (ERbeta) messenger ribonucleic acid (mRNA) expression within the human forebrain: distinct distribution pattern to ERalpha mRNA." J Clin Endocrinol Metab **85**(10): 3840-3846.

Osterlund, M. K., E. Keller and Y. L. Hurd (2000). "The human forebrain has discrete estrogen receptor alpha messenger RNA expression: high levels in the amygdaloid complex." Neuroscience **95**(2): 333-342.

Owens, M. J. and C. B. Nemeroff (1991). "Physiology and pharmacology of corticotropin-releasing factor." Pharmacol Rev **43**(4): 425-473.

Pace, T. W., F. Hu and A. H. Miller (2007). "Cytokine-effects on glucocorticoid receptor function: relevance to glucocorticoid resistance and the pathophysiology and treatment of major depression." Brain Behav Immun **21**(1): 9-19.

Paiardini, M., J. Hoffman, B. Cervasi, A. M. Ortiz, F. Stroud, G. Silvestri and M. E. Wilson (2009). "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 **23**(2): 286-293.

Pannacciulli, N., A. Del Parigi, K. Chen, D. S. Le, E. M. Reiman and P. A. Tataranni (2006). "Brain abnormalities in human obesity: a voxel-based morphometric study." Neuroimage **31**(4): 1419-1425.

Paolicelli, R. C., G. Bolasco, F. Pagani, L. Maggi, M. Scianni, P. Panzanelli, M. Giustetto, T. A. Ferreira, E. Guiducci, L. Dumas, D. Ragozzino and C. T. Gross (2011). "Synaptic pruning by microglia is necessary for normal brain development." Science **333**(6048): 1456-1458.

Paolini, B. M., P. J. Laurienti, J. Norris and W. J. Rejeski (2014). "Meal replacement: calming the hot-state brain network of appetite." Front Psychol **5**: 249.

Parker, K. J., A. F. Schatzberg and D. M. Lyons (2003). "Neuroendocrine aspects of hypercortisolism in major depression." Horm Behav **43**(1): 60-66.

Pasquali, R., B. Ambrosi, D. Armanini, F. Cavagnini, E. D. Uberti, G. Del Rio, G. de Pergola, M. Maccario, F. Mantero, M. Marugo, C. M. Rotella and R. Vettor (2002). "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 **87**(1): 166-175.

Patel, R., S. Moore, D. K. Crawford, G. Hannsun, M. V. Sasidhar, K. Tan, D. Molaie and S. K. Tiwari-Woodruff (2013). "Attenuation of corpus callosum axon myelination and remyelination in the absence of circulating sex hormones." Brain Pathol **23**(4): 462-475.

Patterson, M. L. (2008). "Back to Social Behavior: Mining the Mundane." Basic and Applied Social Psychology **30**(2): 93-101.

Pau, C. Y., K. Y. Pau and H. G. Spies (1998). "Putative estrogen receptor beta and alpha mRNA expression in male and female rhesus macaques." Mol Cell Endocrinol **146**(1-2): 59-68.

Paxinos, G., X. Huang and A. W. Toga (2000). "The rhesus monkey brain in sterotaxic coordinates." San Diego: Academic Press.

Payne, C., C. J. Machado, N. G. Bliwise and J. Bachevalier (2010). "Maturation of the hippocampal formation and amygdala in Macaca mulatta: a volumetric magnetic resonance imaging study." Hippocampus **20**(8): 922-935.

Pazol, K., J. R. Kaplan, D. Abbott, S. E. Appt and M. E. Wilson (2004). "Practical measurement of total and bioavailable estradiol in female macaques." Clin Chim Acta **340**(1-2): 117-126.

Pelphrey, K. A., R. J. Viola and G. McCarthy (2004). "When strangers pass: processing of mutual and averted social gaze in the superior temporal sulcus." Psychol Sci **15**(9): 598-603.

Perez, S., T. J. Sendera, J. H. Kordower and E. J. Mufson (2004). "Estrogen receptor alpha containing neurons in the monkey forebrain: lack of association with calcium binding proteins and choline acetyltransferase." Brain Res **1019**(1-2): 55-63.

Petrides, M. (2005). "Lateral prefrontal cortex: architectonic and functional organization." Philos Trans R Soc Lond B Biol Sci **360**(1456): 781-795.

Petrides, M. and D. N. Pandya (2007). "Efferent association pathways from the rostral prefrontal cortex in the macaque monkey." J Neurosci **27**(43): 11573-11586.

Petrides, M., F. Tomaiuolo, E. H. Yeterian and D. N. Pandya (2012). "The prefrontal cortex: comparative architectonic organization in the human and the macaque monkey brains." Cortex **48**(1): 46-57.

Pfaff, D. W., N. Vasudevan, H. K. Kia, Y. S. Zhu, J. Chan, J. Garey, M. Morgan and S. Ogawa (2000). "Estrogens, brain and behavior: studies in fundamental neurobiology and observations related to women's health." J Steroid Biochem Mol Biol **74**(5): 365-373.

Pohl, C. R., C. M. deRidder and T. M. Plant (1995). "Gonadal and nongonadal mechanisms contribute to the prepubertal hiatus in gonadotropin secretion in the female rhesus monkey (Macaca mulatta)." J Clin Endocrinol Metab **80**(7): 2094-2101.

Pollitt, R. A., J. S. Kaufman, K. M. Rose, A. V. Diez-Roux, D. Zeng and G. Heiss (2008). "Cumulative life course and adult socioeconomic status and markers of inflammation in adulthood." J Epidemiol Community Health **62**(6): 484-491.

Pope, N. S., T. P. Gordon and M. E. Wilson (1986). "Age, social rank and lactational status influence ovulatory patterns in seasonally breeding rhesus monkeys." Biol Reprod **35**(2): 353-359.

Power, J. D., K. A. Barnes, A. Z. Snyder, B. L. Schlaggar and S. E. Petersen (2012). "Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion." Neuroimage **59**(3): 2142-2154.

Power, J. D., A. Mitra, T. O. Laumann, A. Z. Snyder, B. L. Schlaggar and S. E. Petersen (2014). "Methods to detect, characterize, and remove motion artifact in resting state fMRI." Neuroimage **84**: 320-341.

Price, J., F. Russchen and D. Amaral (1987). "The limbic region.II: The amygdaloid complex." Bjorklund A, Hokfelt T, and Swanson LW (eds.) Handbook of Chemical Neuroanatomy, vol. 5, Integrated Systems of the CNS, part I. Amsterdam: Elsevier Science.

Pruessner, J. C., K. Dedovic, M. Pruessner, C. Lord, C. Buss, L. Collins, A. Dagher and S. J. Lupien (2010). "Stress regulation in the central nervous system: evidence from structural and functional neuroimaging studies in human populations - 2008 Curt Richter Award Winner." Psychoneuroendocrinology **35**(1): 179-191.

Quan, N. and W. A. Banks (2007). "Brain-immune communication pathways." Brain Behav Immun **21**(6): 727-735.

Quirk, G. J. and J. S. Beer (2006). "Prefrontal involvement in the regulation of emotion: convergence of rat and human studies." Curr Opin Neurobiol **16**(6): 723-727.

Raadsheer, F. C., W. J. Hoogendijk, F. C. Stam, F. J. Tilders and D. F. Swaab (1994). "Increased numbers of corticotropin-releasing hormone expressing neurons in the hypothalamic paraventricular nucleus of depressed patients." Neuroendocrinology **60**(4): 436-444.

Radley, J. J., R. M. Anderson, B. A. Hamilton, J. A. Alcock and S. A. Romig-Martin (2013). "Chronic stress-induced alterations of dendritic spine subtypes predict functional decrements in an hypothalamo-pituitary-adrenal-inhibitory prefrontal circuit." J Neurosci **33**(36): 14379-14391.

Raison, C. L. and A. H. Miller (2003). "When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders." Am J Psychiatry **160**(9): 1554-1565.

Raji, C. A., A. J. Ho, N. N. Parikshak, J. T. Becker, O. L. Lopez, L. H. Kuller, X. Hua, A. D. Leow, A. W. Toga and P. M. Thompson (2010). "Brain structure and obesity." Hum Brain Mapp **31**(3): 353-364.

Rajkowska, G. (2000). "Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells." Biol Psychiatry **48**(8): 766-777.

Rapisarda, J. J., K. S. Bergman, R. A. Steiner and D. L. Foster (1983). "Response to estradiol inhibition of tonic luteinizing hormone secretion decreases during the final stage of puberty in the rhesus monkey." Endocrinology **112**(4): 1172-1179.

Reader, B. F., B. L. Jarrett, D. B. McKim, E. S. Wohleb, J. P. Godbout and J. F. Sheridan (2015). "Peripheral and central effects of repeated social defeat stress: monocyte trafficking, microglial activation, and anxiety." Neuroscience **289**: 429-442.

Reardon, L. E., E. W. Leen-Feldner and C. Hayward (2009). "A critical review of the empirical literature on the relation between anxiety and puberty." Clin Psychol Rev **29**(1): 1-23.

Reding, K., V. Michopoulos, K. Wallen, M. Sanchez, M. E. Wilson and D. Toufexis (2012). "Social status modifies estradiol activation of sociosexual behavior in female rhesus monkeys." Horm Behav **62**(5): 612-620.

Register, T. C., C. A. Shively and C. E. Lewis (1998). "Expression of estrogen receptor alpha and beta transcripts in female monkey hippocampus and hypothalamus." Brain Res **788**(1-2): 320-322.

Rettberg, J. R., J. Yao and R. D. Brinton (2014). "Estrogen: a master regulator of bioenergetic systems in the brain and body." Front Neuroendocrinol **35**(1): 8-30.

Reynolds, S. M. and D. S. Zahm (2005). "Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala." J Neurosci **25**(50): 11757-11767.

Richwine, A. F., A. O. Parkin, J. B. Buchanan, J. Chen, J. A. Markham, J. M. Juraska and R. W. Johnson (2008). "Architectural changes to CA1 pyramidal neurons in adult and aged mice after peripheral immune stimulation." Psychoneuroendocrinology **33**(10): 1369-1377.

Rilling, J., D. Gutman, T. Zeh, G. Pagnoni, G. Berns and C. Kilts (2002). "A neural basis for social cooperation." Neuron **35**(2): 395-405.

Rilling, J. K., A. G. Sanfey, J. A. Aronson, L. E. Nystrom and J. D. Cohen (2004). "Opposing BOLD responses to reciprocated and unreciprocated altruism in putative reward pathways." Neuroreport **15**(16): 2539-2543.

Robinson, A. J. and O. Pascalis (2004). "Development of flexible visual recognition memory in human infants." Dev Sci **7**(5): 527-533.

Rolls, E. T. (2008). "Functions of the orbitofrontal and pregenual cingulate cortex in taste, olfaction, appetite and emotion." Acta Physiol Hung **95**(2): 131-164.

Rolls, E. T. (2011). "Taste, olfactory and food texture reward processing in the brain and obesity." Int J Obes (Lond) **35**(4): 550-561.

Rolls, E. T. and C. McCabe (2007). "Enhanced affective brain representations of chocolate in cravers vs. non-cravers." Eur J Neurosci **26**(4): 1067-1076.

Rosene, D. and G. Van Hoesen (1987). The Hippocampal Formation of the Primate Brain. Cerebral Cortex. E. Jones and A. Peters, Springer US. **6:** 345-456.

Rosenkranz, J. A., E. R. Venheim and M. Padival (2010). "Chronic stress causes amygdala hyperexcitability in rodents." Biol Psychiatry **67**(12): 1128-1136.

Rosin, C., S. Colombo, A. A. Calver, T. E. Bates and S. D. Skaper (2005). "Dopamine D2 and D3 receptor agonists limit oligodendrocyte injury caused by glutamate oxidative stress and oxygen/glucose deprivation." Glia **52**(4): 336-343.

Roy, A. K., Z. Shehzad, D. S. Margulies, A. M. Kelly, L. Q. Uddin, K. Gotimer, B. B. Biswal, F. X. Castellanos and M. P. Milham (2009). "Functional connectivity of the human amygdala using resting state fMRI." Neuroimage **45**(2): 614-626.

Roy, B. N., R. L. Reid and D. A. Van Vugt (1999). "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." Endocrinology **140**(5): 2191-2198.

Rubinow, M. J., L. L. Drogos and J. M. Juraska (2009). "Age-related dendritic hypertrophy and sexual dimorphism in rat basolateral amygdala." Neurobiol Aging **30**(1): 137-146.

Sade, D. S. (1967). "Determinants of dominance in a group of free-ranging rhesus monkeys." Social communication among primates: 99-114.

Sallem, K. and N. Logothetis (2006). "A combined MRI and histology atl;as of the rhesus monkey brain in stereotaxiccoordinates." Boston: Academic Press, Elsevier Ltd.

Sallet, J., R. B. Mars, M. P. Noonan, J. L. Andersson, J. X. O'Reilly, S. Jbabdi, P. L. Croxson, M. Jenkinson, K. L. Miller and M. F. S. Rushworth (2011). "Social Network Size Affects Neural Circuits in Macaques." Science **334**: 697-700.

Sanchez, M. M., E. F. Hearn, D. Do, J. K. Rilling and J. G. Herndon (1998). "Differential rearing affects corpus callosum size and cognitive function of rhesus monkeys." Brain Res **812**(1-2): 38-49.

Sanchez, M. M., C. O. Ladd and P. M. Plotsky (2001). "Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models." Dev Psychopathol **13**(3): 419-449.

Sanchez, M. M., L. J. Young, P. M. Plotsky and T. R. Insel (2000). "Distribution of corticosteroid receptors in the rhesus brain: relative absence of glucocorticoid receptors in the hippocampal formation." J Neurosci **20**(12): 4657-4668.

Sapolsky, R. M. (2005). "The influence of social hierarchy on primate health." Science (New York, N.Y.) **308**: 648-652.

Sapolsky, R. M., L. M. Romero and A. U. Munck (2000). "How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions." Endocr Rev **21**(1): 55-89.

Saygin, Z. M., D. E. Osher, K. Koldewyn, R. E. Martin, A. Finn, R. Saxe, J. D. Gabrieli and M. Sheridan (2015). "Structural connectivity of the developing human amygdala." PLoS One **10**(4): e0125170.

Schino, G., A. Troisi, G. Perretta and V. Monaco (1991). "Measuring anxiety in nonhuman primates: effect of lorazepam on macaque scratching." Pharmacol Biochem Behav **38**(4): 889-891.

Schulz, K. M., H. A. Molenda-Figueira and C. L. Sisk (2009). "Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence." Horm Behav **55**(5): 597-604.

Schwartz, S. M., M. E. Wilson, M. L. Walker and D. C. Collins (1985). "Social and growth correlates of the onset of puberty in female rhesus monkeys." Nutrition and Behavior **2**: 225 - 232.

Scott, J. A., D. Grayson, E. Fletcher, A. Lee, M. D. Bauman, C. M. Schumann, M. H. Buonocore and D. G. Amaral (2015). "Longitudinal analysis of the developing rhesus monkey brain using magnetic resonance imaging: birth to adulthood." Brain Struct Funct.

Seeley, W. W., V. Menon, A. F. Schatzberg, J. Keller, G. H. Glover, H. Kenna, A. L. Reiss and M. D. Greicius (2007). "Dissociable intrinsic connectivity networks for salience processing and executive control." J Neurosci **27**(9): 2349-2356.

Seeman, M. V. (1997). "Psychopathology in women and men: focus on female hormones." Am J Psychiatry **154**(12): 1641-1647.

Semmler, A., T. Okulla, M. Sastre, L. Dumitrescu-Ozimek and M. T. Heneka (2005). "Systemic inflammation induces apoptosis with variable vulnerability of different brain regions." J Chem Neuroanat **30**(2-3): 144-157.

Seress, L. (1992). "Morphological variability and developmental aspects of monkey and human granule cells: differences between the rodent and primate dentate gyrus." Epilepsy Res Suppl **7**: 3-28.

Setiawan, E., A. A. Wilson, R. Mizrahi, P. M. Rusjan, L. Miler, G. Rajkowska, I. Suridjan, J. L. Kennedy, P. V. Rekkas, S. Houle and J. H. Meyer (2015). "Role of translocator protein density, a marker of neuroinflammation, in the brain during major depressive episodes." JAMA Psychiatry **72**(3): 268-275.

Sex On Brain European Research Group, S., S. Nuruddin, M. Bruchhage, E. Ropstad, A. Krogenaes, N. P. Evans, J. E. Robinson, T. Endestad, L. T. Westlye, C. Madison and I. R. Haraldsen (2013). "Effects of peripubertal gonadotropin-releasing hormone agonist on brain development in sheep--a magnetic resonance imaging study." Psychoneuroendocrinology **38**(10): 1994-2002.

Shade, R. E., J. R. Blair-West, K. D. Carey, L. J. Madden, R. S. Weisinger, J. E. Rivier, W. W. Vale and D. A. Denton (2002). "Ingestive responses to administration of stress hormones in baboons." Am J Physiol Regul Integr Comp Physiol **282**(1): R10-18.

Shansky, R. M., C. Hamo, P. R. Hof, W. Lou, B. S. McEwen and J. H. Morrison (2010). "Estrogen promotes stress sensitivity in a prefrontal cortex-amygdala pathway." Cereb Cortex **20**(11): 2560-2567.

Shansky, R. M. and J. H. Morrison (2009). "Stress-induced dendritic remodeling in the medial prefrontal cortex: effects of circuit, hormones and rest." Brain Res **1293**: 108-113.

Shaw, P., N. J. Kabani, J. P. Lerch, K. Eckstrand, R. Lenroot, N. Gogtay, D. Greenstein, L. Clasen, A. Evans, J. L. Rapoport, J. N. Giedd and S. P. Wise (2008).

"Neurodevelopmental trajectories of the human cerebral cortex." J Neurosci **28**(14): 3586-3594.

Shepard, J. D., K. W. Barron and D. A. Myers (2000). "Corticosterone delivery to the amygdala increases corticotropin-releasing factor mRNA in the central amygdaloid nucleus and anxiety-like behavior." Brain Res **861**(2): 288-295.

Shively, C. and J. Kaplan (1984). "Effects of social factors on adrenal weight and related physiology of Macaca fascicularis." Physiol Behav **33**(5): 777-782.

Shively, C. A. (1998). "Social subordination stress, behavior, and central monoaminergic function in female cynomolgus monkeys." Biol Psychiatry **44**(9): 882-891.

Shively, C. A., D. P. Friedman, H. D. Gage, M. C. Bounds, C. Brown-Proctor, J. B. Blair, J. A. Henderson, M. A. Smith and N. Buchheimer (2006). "Behavioral depression and positron emission tomography-determined serotonin 1A receptor binding potential in cynomolgus monkeys." Arch Gen Psychiatry **63**(4): 396-403.

Shively, C. A., K. Laber-Laird and R. F. Anton (1997). "Behavior and physiology of social stress and depression in female cynomolgus monkeys." Biological Psychiatry **41**(8): 871-882.

Shively, C. A., T. C. Register, D. P. Friedman, T. M. Morgan, J. Thompson and T. Lanier (2005). "Social stress-associated depression in adult female cynomolgus monkeys (Macaca fascicularis)." Biol Psychol **69**(1): 67-84.

Shively, C. A. and S. L. Willard (2012). "Behavioral and neurobiological characteristics of social stress versus depression in nonhuman primates." Exp Neurol **233**(1): 87-94.

Short, S. J., G. R. Lubach, A. I. Karasin, C. W. Olsen, M. Styner, R. C. Knickmeyer, J. H. Gilmore and C. L. Coe (2010). "Maternal influenza infection during pregnancy impacts postnatal brain development in the rhesus monkey." Biological psychiatry **67**: 965-973.

Shott, M. E., M. A. Cornier, V. A. Mittal, T. L. Pryor, J. M. Orr, M. S. Brown and G. K. Frank (2015). "Orbitofrontal cortex volume and brain reward response in obesity." Int J Obes (Lond) **39**(2): 214-221.

Shughrue, P. J., M. V. Lane and I. Merchenthaler (1997). "Comparative distribution of estrogen receptor-alpha and -beta mRNA in the rat central nervous system." J Comp Neurol **388**(4): 507-525.

Shughrue, P. J. and I. Merchenthaler (2000). "Evidence for novel estrogen binding sites in the rat hippocampus." Neuroscience **99**(4): 605-612.

Shughrue, P. J., P. J. Scrimo and I. Merchenthaler (1998). "Evidence for the colocalization of estrogen receptor-beta mRNA and estrogen receptor-alpha immunoreactivity in neurons of the rat forebrain." Endocrinology **139**(12): 5267-5270.

Sierra, A., A. Gottfried-Blackmore, T. A. Milner, B. S. McEwen and K. Bulloch (2008). "Steroid hormone receptor expression and function in microglia." Glia **56**(6): 659-674.

Silberg, J., A. Pickles, M. Rutter, J. Hewitt, E. Simonoff, H. Maes, R. Carbonneau, L. Murrelle, D. Foley and L. Eaves (1999). "The influence of genetic factors and life stress on depression among adolescent girls." Arch Gen Psychiatry **56**(3): 225-232.

Silk, J. B. (2002). "Practice random acts of aggression and senseless acts of intimidation: The logic of status contests in social groups." Evolutionary Anthropology: Issues, News, and Reviews **11**(6): 221-225.

Silverman, M. N. and E. M. Sternberg (2012). "Glucocorticoid regulation of inflammation and its functional correlates: from HPA axis to glucocorticoid receptor dysfunction." Ann N Y Acad Sci **1261**: 55-63.

Sisk, C. L. and J. L. Zehr (2005). "Pubertal hormones organize the adolescent brain and behavior." Frontiers in neuroendocrinology **26**: 163-174.

Smith, S. M., M. Jenkinson, M. W. Woolrich, C. F. Beckmann, T. E. Behrens, H. Johansen-Berg, P. R. Bannister, M. De Luca, I. Drobnjak, D. E. Flitney, R. K. Niazy, J. Saunders, J. Vickers, Y. Zhang, N. De Stefano, J. M. Brady and P. M. Matthews (2004). "Advances in functional and structural MR image analysis and implementation as FSL." Neuroimage **23 Suppl 1**: S208-219.

Snyder-Mackler, N., J. N. Kohn, L. B. Barreiro, Z. P. Johnson, M. E. Wilson and J. Tung (2016). "Social status drives social relationships in groups of unrelated female rhesus macaques." Anim Behav **111**: 307-317.

Soares, J. M., A. Sampaio, L. M. Ferreira, N. C. Santos, P. Marques, F. Marques, J. A. Palha, J. J. Cerqueira and N. Sousa (2013). "Stress Impact on Resting State Brain Networks." PLoS One **8**(6): e66500.

Sofroniew, M. V. and H. V. Vinters (2010). "Astrocytes: biology and pathology." Acta Neuropathol **119**(1): 7-35.

Sohrabji, F. and D. K. Lewis (2006). "Estrogen-BDNF interactions: implications for neurodegenerative diseases." Front Neuroendocrinol **27**(4): 404-414.

Solomon, M. B., A. R. Furay, K. Jones, A. E. Packard, B. A. Packard, A. C. Wulsin and J. P. Herman (2012). "Deletion of forebrain glucocorticoid receptors impairs neuroendocrine stress responses and induces depression-like behavior in males but not females." Neuroscience **203**: 135-143.

Sovago, J., B. Makkai, B. Gulyas and H. Hall (2005). "Autoradiographic mapping of dopamine-D2/D3 receptor stimulated [35S]GTPgammaS binding in the human brain." Eur J Neurosci **22**(1): 65-71.

Spanagel, R. and F. Weiss (1999). "The dopamine hypothesis of reward: past and current status." Trends Neurosci **22**(11): 521-527.

Spencer-Booth, Y. (1968). "The behaviour of group companions towards rhesus monkey infants." Animal Behaviour **16**(4): 541-557.

Spielberg, J. M., G. A. Miller, A. S. Engels, J. D. Herrington, B. P. Sutton, M. T. Banich and W. Heller (2011). "Trait approach and avoidance motivation: lateralized neural activity associated with executive function." Neuroimage **54**(1): 661-670.

Spinelli, S., S. Chefer, S. J. Suomi, J. D. Higley, C. S. Barr and E. Stein (2009). "Early-life stress induces long-term morphologic changes in primate brain." Archives of general psychiatry **66**: 658-665.

Steinberg, L. (2005). "Cognitive and affective development in adolescence." Trends Cogn Sci **9**(2): 69-74.

Steiner, J., H. Bielau, R. Brisch, P. Danos, O. Ullrich, C. Mawrin, H. G. Bernstein and B. Bogerts (2008). "Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide." J Psychiatr Res **42**(2): 151-157.

Steiner, M., E. Dunn and L. Born (2003). "Hormones and mood: from menarche to menopause and beyond." J Affect Disord **74**(1): 67-83.

Stetler, C. and G. E. Miller (2011). "Depression and hypothalamic-pituitary-adrenal activation: a quantitative summary of four decades of research." Psychosom Med **73**(2): 114-126.

Stoppe, G. and M. Doren (2002). "Critical appraisal of effects of estrogen replacement therapy on symptoms of depressed mood." Arch Womens Ment Health **5**(2): 39-47.

Styner, M., R. Knickmeyer, S. Joshi, C. Coe, S. J. Short and J. Gilmore (2007). "Automatic brain segmentation in rhesus monkeys." 65122L-65122L.

Sutherland, M. T., M. J. McHugh, V. Pariyadath and E. A. Stein (2012). "Resting state functional connectivity in addiction: Lessons learned and a road ahead." Neuroimage **62**(4): 2281-2295.

Swerdlow, N. R., P. L. Hartman and P. P. Auerbach (1997). "Changes in sensorimotor inhibition across the menstrual cycle: implications for neuropsychiatric disorders." Biol Psychiatry **41**(4): 452-460.

Takahashi, N., A. B. Tonchev, K. Koike, K. Murakami, K. Yamada, T. Yamashima and M. Inoue (2004). "Expression of estrogen receptor-beta in the postischemic monkey hippocampus." Neurosci Lett **369**(1): 9-13.

Tang, C. Y. and R. Ramani (2016). "fMRI and Anesthesia." Int Anesthesiol Clin **54**(1): 129-142.

Tang, Y. Y., B. K. Holzel and M. I. Posner (2015). "The neuroscience of mindfulness meditation." Nat Rev Neurosci **16**(4): 213-225.

Teicher, M. H., C. M. Anderson and A. Polcari (2012). "Childhood maltreatment is associated with reduced volume in the hippocampal subfields CA3, dentate gyrus, and subiculum." Proc Natl Acad Sci U S A **109**(9): E563-572.

Tomasi, D. and N. D. Volkow (2013). "Striatocortical pathway dysfunction in addiction and obesity: differences and similarities." Crit Rev Biochem Mol Biol **48**(1): 1-19.

Tottenham, N., T. A. Hare and B. J. Casey (2009). A developmental perspective on human amygdala function. The Human Amygdala. E. Phelps and P. Whalen. New York, Guilford Press**:** 107-117

Tottenham, N., T. A. Hare, B. T. Quinn, T. W. McCarry, M. Nurse, T. Gilhooly, A. Millner, A. Galvan, M. C. Davidson, I. M. Eigsti, K. M. Thomas, P. J. Freed, E. S. Booma, M. R. Gunnar, M. Altemus, J. Aronson and B. J. Casey (2010). "Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation." Dev Sci **13**(1): 46-61.

Tottenham, N. and M. a. Sheridan (2009). "A review of adversity, the amygdala and the hippocampus: a consideration of developmental timing." Frontiers in human neuroscience **3**: 68.

Troisi, A. (2001). "Gender differences in vulnerability to social stress: a Darwinian perspective." Physiol Behav **73**(3): 443-449.

Troisi, A. (2002). "Displacement activities as a behavioral measure of stress in nonhuman primates and human subjects." Stress **5**(1): 47-54.

Troisi, A., G. Schino, M. D'Antoni, N. Pandolfi, F. Aureli and F. R. D'Amato (1991). "Scratching as a behavioral index of anxiety in macaque mothers." Behav Neural Biol **56**(3): 307-313.

Tryon, M. S., C. S. Carter, R. Decant and K. D. Laugero (2013). "Chronic stress exposure may affect the brain's response to high calorie food cues and predispose to obesogenic eating habits." Physiol Behav **120**: 233-242.

Tsai, A. G., D. F. Williamson and H. A. Glick (2011). "Direct medical cost of overweight and obesity in the USA: a quantitative systematic review." Obes Rev **12**(1): 50-61.

Tung, J., L. B. Barreiro, Z. P. Johnson, K. D. Hansen, V. Michopoulos, D. Toufexis, K. Michelini, M. E. Wilson and Y. Gilad (2012). "Social environment is associated with gene regulatory variation in the rhesus macaque immune system." Proc Natl Acad Sci U S A **109**(17): 6490-6495.

Tustison, N. J. and B. B. Avants (2013). "Explicit B-spline regularization in diffeomorphic image registration." Front Neuroinform **7**: 39.

Tustison, N. J., B. B. Avants, P. A. Cook, Y. Zheng, A. Egan, P. A. Yushkevich and J. C. Gee (2010). "N4ITK: improved N3 bias correction." IEEE Trans Med Imaging **29**(6): 1310-1320.

Uchida, R. R., C. M. Del-Ben, G. F. Busatto, F. L. Duran, F. S. Guimaraes, J. A. Crippa, D. Araujo, A. C. Santos and F. G. Graeff (2008). "Regional gray matter abnormalities in panic disorder: a voxel-based morphometry study." Psychiatry Res **163**(1): 21-29.

Uchoa, E. T., G. Aguilera, J. P. Herman, J. L. Fiedler, T. Deak and M. B. de Sousa (2014). "Novel aspects of glucocorticoid actions." J Neuroendocrinol **26**(9): 557-572.

Uematsu, A., M. Matsui, C. Tanaka, T. Takahashi, K. Noguchi, M. Suzuki and H. Nishijo (2012). "Developmental trajectories of amygdala and hippocampus from infancy to early adulthood in healthy individuals." PLoS One **7**(10): e46970.

Ulfig, N., M. Setzer and J. Bohl (2003). "Ontogeny of the human amygdala." Ann N Y Acad Sci **985**: 22-33.

Ullewar, M. P. and S. N. Umathe (2016). "Gonadotropin-releasing hormone agonist prevents l-arginine induced immune dysfunction independent of gonadal steroids: Relates with a decline in elevated thymus and brain nitric oxide levels." Nitric Oxide **57**: 40-47.

Ulrich-Lai, Y. M., S. Fulton, M. Wilson, G. Petrovich and L. Rinaman (2015). "Stress exposure, food intake and emotional state." Stress **18**(4): 381-399.

Ulrich-Lai, Y. M. and J. P. Herman (2009). "Neural regulation of endocrine and autonomic stress responses." Nat Rev Neurosci **10**(6): 397-409.

Uphouse, L., A. Selvamani, C. Lincoln, L. Morales and D. Comeaux (2005). "Mild restraint reduces the time hormonally primed rats spend with sexually active males." Behav Brain Res **157**(2): 343-350.

Vaillancourt, C., M. Cyr, J. Rochford, P. Boksa and T. Di Paolo (2002). "Effects of ovariectomy and estradiol on acoustic startle responses in rats." Pharmacol Biochem Behav **74**(1): 103-109.

Valentino, R. J., S. L. Foote and M. E. Page (1993). "The locus coeruleus as a site for integrating corticotropin-releasing factor and noradrenergic mediation of stress responses." Ann N Y Acad Sci **697**: 173-188.

van Harmelen, A. L., M. J. van Tol, N. J. van der Wee, D. J. Veltman, A. Aleman, P. Spinhoven, M. A. van Buchem, F. G. Zitman, B. W. Penninx and B. M. Elzinga (2010). "Reduced medial prefrontal cortex volume in adults reporting childhood emotional maltreatment." Biol Psychiatry **68**(9): 832-838.

Vasconcelos, A. R., J. V. Cabral-Costa, C. H. Mazucanti, C. Scavone and E. M. Kawamoto (2016). "The Role of Steroid Hormones in the Modulation of Neuroinflammation by Dietary Interventions." Front Endocrinol (Lausanne) **7**: 9.

Veit, R., S. Kullmann, M. Heni, J. Machann, H. U. Haring, A. Fritsche and H. Preissl (2014). "Reduced cortical thickness associated with visceral fat and BMI." Neuroimage Clin **6**: 307-311.

Veldhuis, J. D., J. N. Roemmich and A. D. Rogol (2000). "Gender and sexual maturation-dependent contrasts in the neuroregulation of growth hormone secretion in prepubertal and late adolescent males and females--a general clinical research center-based study." J Clin Endocrinol Metab **85**(7): 2385-2394.

Verdi, J. M. and A. T. Campagnoni (1990). "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 **265**(33): 20314-20320.

Videbech, P. and B. Ravnkilde (2004). "Hippocampal volume and depression: a meta-analysis of MRI studies." Am J Psychiatry **161**(11): 1957-1966.

Vincent, J. L., G. H. Patel, M. D. Fox, a. Z. Snyder, J. T. Baker, D. C. Van Essen, J. M. Zempel, L. H. Snyder, M. Corbetta and M. E. Raichle (2007). "Intrinsic functional architecture in the anaesthetized monkey brain." Nature **447**: 83-86.

Vinuesa, A., C. Pomilio, M. Menafra, M. M. Bonaventura, L. Garay, M. F. Mercogliano, R. Schillaci, V. Lux Lantos, F. Brites, J. Beauquis and F. Saravia (2016). "Juvenile exposure to a high fat diet promotes behavioral and limbic alterations in the absence of obesity." Psychoneuroendocrinology **72**: 22-33.

Visscher, T. L. and J. C. Seidell (2001). "The public health impact of obesity." Annu Rev Public Health **22**: 355-375.

Volkow, N. D., D. Tomasi, G. J. Wang, J. S. Fowler, F. Telang, R. Z. Goldstein, N. Alia-Klein and C. Wong (2011). "Reduced metabolism in brain "control networks" following cocaine-cues exposure in female cocaine abusers." PLoS One **6**(2): e16573.

Volkow, N. D., G. J. Wang, J. S. Fowler, D. Tomasi and R. Baler (2012). "Food and drug reward: overlapping circuits in human obesity and addiction." Curr Top Behav Neurosci **11**: 1-24.

Volkow, N. D., G. J. Wang, F. Telang, J. S. Fowler, P. K. Thanos, J. Logan, D. Alexoff, Y. S. Ding, C. Wong, Y. Ma and K. Pradhan (2008). "Low dopamine striatal D2 receptors are associated with prefrontal metabolism in obese subjects: possible contributing factors." Neuroimage **42**(4): 1537-1543.

Volpe, J. J. (2000). "Overview: normal and abnormal human brain development." Ment Retard Dev Disabil Res Rev **6**(1): 1-5.

Vyas, A., S. Bernal and S. Chattarji (2003). "Effects of chronic stress on dendritic arborization in the central and extended amygdala." Brain Res **965**(1-2): 290-294.

Vyas, A., S. Jadhav and S. Chattarji (2006). "Prolonged behavioral stress enhances synaptic connectivity in the basolateral amygdala." Neuroscience **143**(2): 387-393.

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

Walker, M. L., T. P. Gordon and M. E. Wilson (1982). "Reproductive performance in capture-acclimated female rhesus monkeys (Macaca mulatta)." J Med Primatol **11**(5): 291-302.

Wallen, K. (1990). "Desire and ability: hormones and the regulation of female sexual behavior." Neurosci Biobehav Rev **14**(2): 233-241.

Walther, K., A. C. Birdsill, E. L. Glisky and L. Ryan (2010). "Structural brain differences and cognitive functioning related to body mass index in older females." Hum Brain Mapp **31**(7): 1052-1064.

Wanat, M. J., F. W. Hopf, G. D. Stuber, P. E. Phillips and A. Bonci (2008). "Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih." J Physiol **586**(8): 2157-2170.

Wang, A. C., Y. Hara, W. G. Janssen, P. R. Rapp and J. H. Morrison (2010). "Synaptic estrogen receptor-alpha levels in prefrontal cortex in female rhesus monkeys and their correlation with cognitive performance." J Neurosci **30**(38): 12770-12776.

Wang, J., C. Vachet, A. Rumple, S. Gouttard, C. Ouziel, E. Perrot, G. Du, X. Huang, G. Gerig and M. Styner (2014). "Multi-atlas segmentation of subcortical brain structures via the AutoSeg software pipeline." Front Neuroinform **8**: 7.

Wang, Q., E. W. Verweij, H. J. Krugers, M. Joels, D. F. Swaab and P. J. Lucassen (2014). "Distribution of the glucocorticoid receptor in the human amygdala; changes in mood disorder patients." Brain Struct Funct **219**(5): 1615-1626.

Wang, Z., J. Fan, J. Wang, Y. Li, L. Xiao, D. Duan and Q. Wang (2016). "Protective effect of lycopene on high-fat diet-induced cognitive impairment in rats." Neurosci Lett **627**: 185-191.

Ward, M. A., C. M. Carlsson, M. A. Trivedi, M. A. Sager and S. C. Johnson (2005). "The effect of body mass index on global brain volume in middle-aged adults: a cross sectional study." BMC Neurol **5**: 23.

Wardle, J., J. Waller and M. J. Jarvis (2002). "Sex differences in the association of socioeconomic status with obesity." Am J Public Health **92**(8): 1299-1304.

Warne, J. P. (2009). "Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity." Mol Cell Endocrinol **300**(1-2): 137-146.

Watanabe, Y., E. Gould and B. S. McEwen (1992). "Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons." Brain Res **588**(2): 341-345.

Watson, W. H., J. Cai and D. P. Jones (2000). "Diet and apoptosis." Annu Rev Nutr **20**: 485-505.

Weems, C. F., B. G. Scott, J. D. Russell, A. L. Reiss and V. G. Carrion (2013). "Developmental variation in amygdala volumes among children with posttraumatic stress." Dev Neuropsychol **38**(7): 481-495.

Weissman, J. F., L. A. Pratt, E. A. Miller and J. D. Parker (2015). "Serious Psychological Distress Among Adults: United States, 2009-2013." NCHS Data Brief(203): 1-8.

Widya, R. L., A. de Roos, S. Trompet, A. J. de Craen, R. G. Westendorp, J. W. Smit, M. A. van Buchem, J. van der Grond and P. S. Group (2011). "Increased amygdalar and hippocampal volumes in elderly obese individuals with or at risk of cardiovascular disease." Am J Clin Nutr **93**(6): 1190-1195.

Williams, K. W. and J. K. Elmquist (2012). "From neuroanatomy to behavior: central integration of peripheral signals regulating feeding behavior." Nat Neurosci **15**(10): 1350-1355.

Williamson, D. E., K. Coleman, S. A. Bacanu, B. J. Devlin, J. Rogers, N. D. Ryan and J. L. Cameron (2003). "Heritability of fearful-anxious endophenotypes in infant rhesus macaques: a preliminary report." Biol Psychiatry **53**(4): 284-291.

Wilson, A. C., S. V. Meethal, R. L. Bowen and C. S. Atwood (2007). "Leuprolide acetate: a drug of diverse clinical applications." Expert Opin Investig Drugs **16**(11): 1851-1863.

Wilson, M. E., S. Bounar, J. Godfrey, V. Michopoulos, M. Higgins and M. Sanchez (2013). "Social and emotional predictors of the tempo of puberty in female rhesus monkeys." Psychoneuroendocrinology **38**(1): 67-83.

Wilson, M. E., J. Fisher and K. Chikazawa (2004). "Estradiol negative feedback regulates nocturnal luteinizing hormone and follicle-stimulating hormone secretion in prepubertal female rhesus monkeys." J Clin Endocrinol Metab **89**(8): 3973-3978.

Wilson, M. E., J. Fisher, A. Fischer, V. Lee, R. B. Harris and T. J. Bartness (2008). "Quantifying food intake in socially housed monkeys: social status effects on caloric consumption." Physiol Behav **94**(4): 586-594.

Wilson, M. E., T. P. Gordon and D. C. Collins (1986). "Ontogeny of luteinizing hormone secretion and first ovulation in seasonal breeding rhesus monkeys." Endocrinology **118**(1): 293-301.

Wilson, M. E. and B. Kinkead (2008). "Gene-environment interactions, not neonatal growth hormone deficiency, time puberty in female rhesus monkeys." Biol Reprod **78**(4): 736-743.

Wilson, M. E., K. Pazol, A. Legendre, J. Fisher and K. Chikazawa (2005). "Gonadal steroid modulation of the limbic - hypothalamic - pituitary - adrenal (LHPA) axis is influenced by social status in female rhesus monkeys." Endocrine **26**(2).

Wilson, M. E., S. M. Schwartz, M. L. Walker and T. P. Gordon (1984). "Oestradiol and somatomedin-C influence body weight patterns in premenarchial rhesus monkeys housed outdoors." The Journal of endocrinology **102**(3): 311-317.

Withrow, D. and D. A. Alter (2011). "The economic burden of obesity worldwide: a systematic review of the direct costs of obesity." Obes Rev **12**(2): 131-141.

Wojniusz, S., C. Vogele, E. Ropstad, N. Evans, J. Robinson, S. Sutterlin, H. W. Erhard, A. K. Solbakk, T. Endestad, D. E. Olberg and I. R. Haraldsen (2011). "Prepubertal gonadotropin-releasing hormone analog leads to exaggerated behavioral and emotional sex differences in sheep." Horm Behav **59**(1): 22-27.

Woolley, C. S., E. Gould, M. Frankfurt and B. S. McEwen (1990). "Naturally occurring fluctuation in dendritic spine density on adult hippocampal pyramidal neurons." J Neurosci **10**(12): 4035-4039.

Woolley, C. S. and B. S. McEwen (1992). "Estradiol mediates fluctuation in hippocampal synapse density during the estrous cycle in the adult rat." J Neurosci **12**(7): 2549-2554.

Woolley, C. S. and B. S. McEwen (1993). "Roles of estradiol and progesterone in regulation of hippocampal dendritic spine density during the estrous cycle in the rat." J Comp Neurol **336**(2): 293-306.

Woolrich, M. W., S. Jbabdi, B. Patenaude, M. Chappell, S. Makni, T. Behrens, C. Beckmann, M. Jenkinson and S. M. Smith (2009). "Bayesian analysis of neuroimaging data in FSL." Neuroimage **45**(1 Suppl): S173-186.

Wu, W. F., X. J. Tan, Y. B. Dai, V. Krishnan, M. Warner and J. A. Gustafsson (2013). "Targeting estrogen receptor beta in microglia and T cells to treat experimental autoimmune encephalomyelitis." Proc Natl Acad Sci U S A **110**(9): 3543-3548.

Yacubian, J., J. Glascher, K. Schroeder, T. Sommer, D. F. Braus and C. Buchel (2006). "Dissociable systems for gain- and loss-related value predictions and errors of prediction in the human brain." J Neurosci **26**(37): 9530-9537.

Yeterian, E. H., D. N. Pandya, F. Tomaiuolo and M. Petrides (2012). "The cortical connectivity of the prefrontal cortex in the monkey brain." Cortex **48**(1): 58-81.

Zadran, S., Q. Qin, X. Bi, H. Zadran, Y. Kim, M. R. Foy, R. Thompson and M. Baudry (2009). "17-Beta-estradiol increases neuronal excitability through MAP kinase-induced calpain activation." Proc Natl Acad Sci U S A **106**(51): 21936-21941.

Zakrzewska, K. E., I. Cusin, A. Stricker-Krongrad, O. Boss, D. Ricquier, B. Jeanrenaud and F. Rohner-Jeanrenaud (1999). "Induction of obesity and hyperleptinemia by central glucocorticoid infusion in the rat." Diabetes **48**(2): 365-370.

Zehr, J. L., P. E. Van Meter and K. Wallen (2005). "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 **72**(5): 1087-1094.

Zender, R. and E. Olshansky (2009). "Women's mental health: depression and anxiety." Nurs Clin North Am **44**(3): 355-364.

Zhou, J., H. Zhang, R. S. Cohen and S. C. Pandey (2005). "Effects of estrogen treatment on expression of brain-derived neurotrophic factor and cAMP response element-binding protein expression and phosphorylation in rat amygdaloid and hippocampal structures." Neuroendocrinology **81**(5): 294-310.

Zink, C. F., Y. Tong, Q. Chen, D. S. Bassett, J. L. Stein and A. Meyer-Lindenberg (2008). "Know your place: neural processing of social hierarchy in humans." Neuron **58**(2): 273-283.