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Jiaqi Grace Shen April. 9, 2021

Effects of Selective Neonatal Amygdala Lesions on Flexible Response Selection in Adult Rhesus Macaques

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Abstract

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Recent developmental studies in monkeys show neonatal-onset lesions of the amygdala alter food choice guided by reward value but not choice guided by reward contingency. To build on this study, the same animals with neonatal lesions of the amygdala were tested to assess their Food/Non-food selection strategy with or without satiation. The behavioral tasks measure the animals' selection of Food and Non-food items (Experiment 1), and their ability to switch selection of preferred food towards other preferred food after satiation using the Primary Reinforcer Devaluation paradigm (Experiment 2). We hypothesized that adult monkeys with neonatal neurotoxic amygdala lesions will show an increased preference of Non-food items over regular food items as compared to control animals that will disregard these Non-food items and intact satiation response. The findings showed that neurotoxic neonatal amygdala lesions did not affect food selection in adult rhesus macaques and spared the ability to flexibly switch their selection strategy after satiation. Thus, both early-onset and adult-onset amygdala lesions had no effects on the abilities to select palatable foods over inedible foods and to modulate food selection after satiation. These results are at odd with previous reports indicating that the amygdala lesioned animals had a tendency to select more inedible objects and showed heightened meat preference. Future studies are needed to explore other neural structures related to food preferences and modulation of food intake disruption that are observed in Autism Spectrum Disorders as well as the role of amygdala in emotional appraisal in patients with bulimia and binge eating disorders.

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Effects of Selective Neonatal Amygdala Lesions on Flexible Response Selection in Adult Rhesus Macaques

Jiaqi Grace Shen

Introduction

In recent years, there have been an increasing number of patients with symptoms and diagnosis of eating disorders. According to National Association of Anorexia Nervosa and Associated Disorders (ANDA), at least 30 million people across all ages and genders suffer from eating disorders in the U.S. Eating disorders also have the highest mortality rate of any mental illnesses (Sullivan, 1995). Recent research have shown that major brain system dysfunctions are involved in several eating disorders, including anorexia nervosa, bulimia nervosa, binge eating disorder, and obesity. Observed hypersensitivity to rewarding cues in amygdala, anterior insula, prefrontal cortex, and orbitofrontal cortex indicated the importance of food-related neural response in these brain regions. Patients with binge eating disorder show impaired motivation and reward processing (Balodis et al., 2015). There are also significant neural changes in relation to the severity of eating disorders. Research shows volume reduction and aberrant responses in the insula, amygdala, and occipital cortex in acute stage of illness with binge eating disorder and bulimia (Donnelly et al., 2018). Moreover, bulimic patients differed in reward sensitivity, showed greater arousal in anterior cingulate cortex and insula activation to visual food stimuli (Schienle et al., 2008). Therefore, understanding the neurological source of these disorders is critical for their prevention and treatment.

Brain regions implicated in the modulation of food intake

Several brain regions are critical for modulation of food intake. The hypothalamus is the primary structure implicated in food stimuli regulation. Neurons within the arcuate nucleus of the hypothalamus are responsible for regulating caloric intake, hunger response, glucose metabolism, and energy expenditure by detecting the nutrient status and signals from peripheral hormones (Timper and Brüning, 2017). Yet, other brain areas, such as the insula, orbital frontal cortex, and amygdala, also play a critical role in the modulation of taste, food preference, and aversion. The insular cortex (as well as the insula and insular lobe) is a portion of the cerebral cortex folded deep within the lateral sulcus (the fissure separating the temporal lobe from the parietal and frontal lobes) within each hemisphere of the mammalian brain. In humans, the insula appears to be activated during a wide array of events, including pain, love, emotion, craving, addiction, the enjoyment of music, and taste (Craig, 2009). The orbital frontal cortex is a part of the frontal cortex that sits just above the orbits and has extensive connections with sensory areas as well as limbic system structures involved in memory and emotion regulation, such as the amygdala. Although the specific role of the orbital frontal cortex in food regulation is still debated, studies in monkeys have found that the orbital frontal cortex is critical to modulate decision-making and particularly to modulate food choice selection (Stalnaker et al., 2015). Previous research using adolescent rhesus macaques showed that orbital frontal ablation was correlated with a high number of oral tendencies due to an increased selection of Non-food items (Butter et al., 1969). Finally, the amygdala which has extensive connections with the hypothalamus, insular, and orbital frontal cortex, has also been linked to the modulation of rewards and punishments given its critical role in emotion regulation. The remaining of the paper

will focus on the amygdala and its role in food preference and the modulation of food intake associated with the reward value of food.

Amygdala neuroanatomical and connectional system

The primate amygdala, located in the anterior portion of the medial temporal lobe (see Figure. 1), comprises a set of thirteen interconnected nuclei with different connectional features (for reviews see Amaral et al., 1992; Schumann et al., 2016; Bachevalier, 2019). Convergent sensory information from unimodal as well as polysensory areas of the neocortex forms a strong contingent of sensory inputs to the lateral nucleus, which then projects to the basal nucleus and back upon the sensory cortical areas, providing a way by which affective states could influence sensory inputs at a very early stage of their processing. The basal nucleus also serves as an interface between sensory-specific cortical inputs, and the central nucleus, which relays this information to the brainstem and hypothalamus. These two neural centers are concerned with different aspects of emotional responses, including their behavioral and autonomic manifestations, respectively. Via this pathway, sensory stimuli could influence and activate emotional reactions (Amaral et al., 1992; Kling & Brothers, 1992). Sensory inputs from the basal and accessory basal nuclei reach widespread areas of the ventral striatum, which allows affective states to gain access to cortical and subcortical elements of the motor system and thus modulates behavioral responses, such as facial and vocal expressions, body postures and motions (Everitt & Robbins, 1992; Gothard, 2014). In addition, the basal nucleus of the amygdala has dense interconnections with the orbital region of the prefrontal cortex. Through this pathway, the orbital frontal cortex receives information about the emotional and affective content of sensory stimuli, and sends to the amygdala information about the social content of a situation. Thus, the

connections between the amygdala and orbital frontal cortex may permit the modulation and self-regulation of emotional behavior in relation to rapid changes in food reward value of stimuli as well of social situation or context (Bachevalier & Loveland, 2006; Barbas, 1995; Emery & Amaral, 1999). The amygdala is also strongly interconnected with the insular cortex, a site where bodily sensations, autonomic control, and afferents from brain regions implicated in emotion processing. Both structures are essentially involved in multisensory and affective processing, as well as social functions like empathy (for review see Gogolla, 2017).

Finally, the amygdala significantly interacts with the hippocampal formation, predominantly via the entorhinal cortex, though direct connections also exist (Amaral et al., 1992; Saunders and Rosene 1988; Saunders et al., 1988). This anatomical link between the amygdala and the hippocampus may allow access to and modulation of stored information in cortical areas, such as past experience with affective stimuli (Amaral et al., 1992; Saunders and Rosene, 1988; Saunders et al., 1988). This general anatomical organization of the non-human primate amygdala can also be found in humans, with the most prominent change being the allometric size of the lateral nucleus, which increases from non-human primates to humans (Braak and Braak, 1983; Gloor & Guberman, 1997; Stephan et al., 1987). Presumably, this expansion results from the increase and specialization of the cerebral cortex in primate evolution, reaching its greatest complexity in humans. Although much less is known of the extent of interconnections of the human amygdala with the rest of the brain, there are no reasons to believe that the connectional pattern of the human amygdala will be drastically different from that of other non-human primates; though the connections with certain cortical regions could be more extensive in humans than in non-human primates. Thus, in humans and non-human primates, the amygdala stands in a strategic position to integrate exteroceptive and interoceptive signals,

modulate sensory and autonomic processing, and act upon stored representations of emotional aspects of sensory information.

Role of the amygdala in food intake modulation

Understanding the functions of the amygdala is critical for studying a wide range of clinical disorders. Recent studies have shown that the central amygdala projections to the insular cortex plays an important role in the pathogenesis of obesity and binge-eating disorder. Using fasted mice, Stern and colleagues induced conditioned overconsumption to define the neural circuits that can induce food consumption and satiation response even in the absence of hunger. They found a top-down control of feeding to conditioned overconsumption by the modulation of the central amygdala from the insular cortex (Stern et al., 2020). Research also indicates that in order to promote overconsumption, nitric oxide synthase-1 neurons in the insular cortex project to the central amygdala. This process is able to suppress homeostatic satiety. Livneh and colleagues uncovered a specific pathway from hunger-related hypothalamic neurons to insular cortex using cellular-resolution imaging, circuit mapping, and pathway-specific manipulations of insular cortex in behaving mice across hunger and satiety. They showed that reciprocal connections between the insular cortex and the amygdala also allow signaling of taste and cue-food association (Livneh et al., 2017).

Furthermore, the amygdala is important for associating visual stimuli with the incentive value of food reinforcers. Adult monkeys with total bilateral amygdaloid ablation showed the typical amygdaloid syndrome of hypoemotionality, meat eating despite of unpalatable taste, coprophagia, and excessive exploration with/without orality (Aggleton & Passingham, 1981; Murray et al., 1996). The syndrome also includes hypermetamorphosis, hypersexuality, social

behavior breakdown and reduced neophobia (Aggleton & Passingham, 1982) as well as altered avoidance of unsavory foods and inedible objects that normal animals find of little interest (Murray et al., 1996; Stefanacci et al., 2003). In addition, Baylis and Gaffan (1991) showed that bilateral amygdala ablations resulted in abnormal choices between apple, lemon, olive, and meat by selecting meat or olive more often than normal animals. One important issue with these earlier studies is that the amygdala lesions were performed by aspiration or radiofrequency and thus included not only the amygdala nuclei but also damage to adjacent cortical areas as well as fibers from these cortical areas coursing around and within the amygdala. Such that the behavioral symptoms reported with these types of lesions could not be attributed uniquely to the ablation of the amygdala but also to additional unintended adjacent damage. Therefore, a more recent study (Machado & Bachevalier, 2007), re-evaluated the effects of damage to the amygdala on food intake regulation using neurotoxic lesions that were more restricted to the amygdala nuclei, avoiding damage to adjacent cortical areas and fibers-in-passage. Adult monkeys were tested for Food/Non-food preference prior to receiving bilateral neurotoxic amygdala lesions and were re-tested after recovering from their surgery. The neurotoxic lesions of the amygdala did not alter animals' preferences for palatable foods or raw meat, although they did yield a slight increase in preference for inedible non-foods when comparing animal's choice selection prior and after surgery. In addition, the same animals had normal ability to shift their food selection after satiation with their preferred food when the view of food items predicted the food value, that is animals selected less of their preferred food and more of other food items (Machado & Bachevalier, 2007; Experiment 1). However, the same animals showed abnormal food selection after satiation when objects predicted the food rewards, that is animals continued to select the objects associated with the sated food (Machado & Bachevalier, 2007; Experiment 2).

In addition to reward processing, the amygdala is involved in assessing not only the hedonic value of food but also other affective stimuli such as social stimuli. In a functional MRI studies, Sato and colleagues scanned participants while viewing subliminally and supraliminally presented food images. The results suggested that the amygdala activation was indirectly modulated by non-food-related factors such as social relationships (Sato et al., 2019). Lastly, the amygdala plays an essential role in detecting danger and preventing harm from potential stimuli including predators, unsafe objects, unpalatable foods, and dangerous conspecifics (Machado & Bachevalier, 2006; Machado & Bachevalier, 2007). Study using selective amygdala lesions showed that amygdala lesion is directly responsible for adult rhesus macaques' ability to identify reinforcing stimuli; operated animals were shown to have a weakened fear response to threatening stimuli, more rapid extinction behavior, lower level of avoidance and depression, and slower acquisition (Weiskrantz, 1956; Antoniadis et al., 2007). Thus, it is clear that the amygdala appears to be a critical structure in the service of flexible response selection towards foods. Yet, little is known on when and how the amygdala contributes to flexible response selection during development. Knowledge on this topic could be invaluable given that several developmental neuropsychiatric disorders are linked to changes in food preference and food consumption as well as to pathological changes in the amygdala. For example, Schreck et al. (2004) indicated that children with Autism Spectrum Disorders (ASD) have more feeding problems, including food refusal, idiosyncratic meal time behavior, and acceptance of a limited variety and texture of food items than typically developing children. Many also have strong preferences for a narrow selection of foods. Some even feel compelled to have certain foods in the same place on the plate or to use the same plate at each meal (Raiten & Massaro, 1986; Kimberly & Williams, 2006).

Furthermore, the amygdala is known to be altered in many cases with ASD (Schumman & Amaral, 2006). Given that food preference (Birch, 1999) as well as the amygdala (Payne et al., 2010; Chareyron et al., 2012) develop early in infancy, it is likely that the amygdala may play a critical role in the development of food preference and food intake modulation in human infants as well.

Role of the amygdala in the development of food intake modulation

Recent developmental studies in monkeys from our laboratory have shown that, like adult-onset lesions, neonatal-onset lesions of the amygdala alter food choice guided by reward value (e.g. switching selection strategies when the rewarded value of an object has been altered by food devaluation; Kazama and Bachevalier, 2013), but not choice guided by reward contingency (i.e. how rewarding an object is when it has been associated with pleasant food; Kazama and Bachevalier, 2012, 2013). To build on these earlier findings, in the present study, we used the same animals with neonatal lesions of the amygdala as they reached adulthood to assess their Food/Non-food selection strategy with or without satiation, using the same behavioral tasks that have been designed to study these functions in adult monkeys (Machado and Bachevalier, 2007). The only difference in the testing procedure was that animals that had received their amygdala lesions were tested prior and after their lesions, whereas animals receiving their lesions in the two weeks after their birth were tested only several years after their surgery. Because food preference develops in early infancy and the amygdala is functional early in infancy, we hypothesize that adult monkeys with neonatal neurotoxic amygdala lesions, like those with adult-onset amygdala lesions (Machado & Bachevalier, 2007), will show an increased preference of Non-food items over regular food items as compared to control animals that will

disregard these Non-food items. In addition, using the Reinforcer Devaluation paradigm during which the reinforcement value of each animal's highest-preferred food was decreased by selective satiation prior to Food/Non-food testing, we hypothesize that adult monkeys with neonatal amygdala lesions will not alter satiation response (e.g. avoiding food items that have been devalued), which will be consistent with findings in adult macaques with adult-onset amygdala lesions.

Methods

Subjects

The subjects consisted of twelve adult rhesus macaques (Macaca mulatta), 6 males and 6 females, weighed between 4.5-8 kg and aged between 4-5 years at the start of this study. The animals were acquired from MD Anderson Cancer Center Science Park (Bastrop, TX) between 1 to 4 days after birth and raised at the primate nursery at MD Anderson Cancer Center in Houston, Texas. The following procedures were approved by the Animal Care and Use Committees of the University of Texas Health Science Center at Houston and of Emory University.

Upon their arrival in the primate nursery, the animals were housed individually in small wire-cages located in temperature controlled rooms under 12-hour-long light/night cycle. Individual cages were staked together under an incubator and provided visual, tactile, and auditory inputs between infants in the adjacent cages. Starting from their first weeks of life, the animals were exposed to intensive human contact, had daily social interactions with peers, and received cognitive testing. These rearing procedures were designed by Sackett and colleagues (2002) in order to achieve optimal animals' normal growth and species-specific social skill

development. During the first three months of age, the animals were hand-fed with infant Similac formula (SMA with Iron, Abbot Laboratories) and banana-flavored pellets supplement (PJ Noyes, Cleveland, OH) was added starting at two months. From 3-12 months, their diet consisted of daily fresh fruit and primate chow (PMI Nutrition International, Lab Diet 5037). Water was available ad libitum starting at three month of age. Between day 8-12, animals in Group Neo-A (N = 6, 3 males, 3 females) received neurotoxic amygdala lesions and animals in Group Neo-C (N = 6, 3 males, 3 females) received sham operations and served as controls.

Neuroimaging

As detailed in previous reports (Nemanic et al., 2002, 2004; Machado &Bachevalier, 2006), pre-surgical Magnetic Resonance Imaging (MRI) scans were used to locate the brain structures and calculate the coordinates targeting the amygdala before the surgery. Subjects were sedated under isoflurane gas (1-2%, v/v, to effect). Intravenous 0.45% NaCl drop solution was given to maintain hydration. The animal's head was secured in a non-ferromagnetic stereotaxic apparatus. To relieve pain caused by pressure from head-constraint, subjects' ears and eyes were treated with Emla cream and to prevent ocular dryness, ophthalmic ointment was applied to the eyes. Once transported into the scanner, the animals were placed on a warm pad and wrapped in warm blankets to maintain normal body temperature. Vital signs (heart rate, respiration, blood pressure, body temperature, and expired CO2) were monitored throughout the procedures. MRI scans were acquired with a GE Signa 1.5 Tesla Echo Speed scanner and a 7.5cm circular surface head coil (GE Medical System. Milwaukee, WI). Two MR sequences were obtained: (a) three dimensional, T1-weighted, fast spoiled gradient (FSPGR)-echo sequence, 1-mm thick images and (b) three interleaved fluid attenuated inversion recovery (FLAIR, 3mm thick each offset by

1mm) scans, both taken in the coronal plan. The same two MR sequences were taken again 6-8 days post-surgery. The presurgical T1 images were used to select the injection sites and calculate their stereotaxic coordinates within the amygdala, whereas the post-surgical FLAIR images were used to identify areas of hypersignals indicating edema resulting from cell loss after the ibotenic acid injections and to estimate location and extent of the amygdala lesions.

Surgery

Following the pre-surgical imaging session, the animals were kept under deep anesthesia and brought to the surgical suite. A heating pad was placed under the animal to prevent hypothermia, and vital signs (heart and respiration rates, expired CO2, and temperature) were monitored until the monkey fully recovered from anesthesia. Aseptic techniques were used throughout the surgery. Once the scalp was shaved and the skin disinfected with Nolvasan, a long lasting local anesthetic (Marcaine 25%, 1.5 ml) was injected along the midline incision. Using an electric drill, small craniotomies were made bilaterally on the bone above the amygdala. Bone wax (Ethicon, Inc., Somerville, NJ; 2.5 g size) was used to prevent excessive bone bleeding, and small slits were made in the dura to expose the brain. Each animal's presurgical T1-weighted MR images was evaluated to determine the number of injection sites and positions in the anterior/posterior, medial/lateral and dorsal/ventral planes. For each amygdala, a total of 0.2–0.6 µl (rate: 0.4 µl/minute) Ibotenic acid (Biosearch Technologies, Novato, CA, 10 mg/mL in phosphate buffered saline, pH 7.4) was injected in 4-6 sites (2mm apart in all three planes) through a 10-µl Hamilton syringe. Injections were made simultaneously in both amygdalae. After each injection, a 3-min delay ensued to permit diffusion of the neurotoxin and minimize its spread along the needle track during retraction of the needles.

After the injections, the wound was closed in anatomical layers. The animals were removed from anesthesia and were monitored at the surgical site until they could breathe on their own. The animal was then moved back to the nursery and placed in an oxygen-ventilated incubator until the second day. In order to prevent infection and reduce edema, 25 mg/kg cephazolin was given for 7 days after surgery and 0.4mg/kg dexamethasone sodium phosphate was administered in the initial 12 hours before surgery and for 3-4 days post-surgery, respectively. For pain relief after surgery, 10 mg/kg acetaminophen was given four times a day for three days.

Sham operations in Group Neo-C

The sham-operated animals serve as a control group and receive the same procedures and treatment as those with the neonatal lesions. The only exception is that for all Neo-C animals, except Neo-C1, we performed bilateral craniotomies and dura slits on the animals' cranium but did not penetrate with needles. Neo-C1 was used as an unoperated control.

MRI - based and histological lesion evaluation

We used pre- and post-surgical FLAIR images to detect the presence of hypersignals indicative of edema in brain areas 6-8 days after surgery. This provided an estimate of the location and extent of the amygdala lesions for each Neo-A animal. As the animals reached adulthood and completed all experiments, they were euthanized and their brain processed for histological evaluation. Brains were removed and taken through a series of ascending sucrose solutions in 0.1M phosphate buffer at 4°C, then frozen in dry ice and sliced at 50 μm on a freezing microtome with a freezing stage (Model 860; American Optical Corp., Lorton, VA). A

series of sections at 50 μm intervals was processed with a Nissl stain to visualize cell bodies and a second series of sections at 250 μm intervals was processed with a Gallyas silver stain to visual fiber tracts (Gallyas, 1979). After each histological slice throughout the amygdala was matched to a series of drawings of coronal histological sections from a normal adult rhesus monkey brain at 1 mm interval. The extent of cell loss and gliosis was visually identified on each section and plotted onto the corresponding drawings of the brain of the normal adult monkey. The surface area (in pixels) of cell loss to both the amygdala and adjacent areas (ento- and perirhinal cortices, hippocampus) was measured using ImageJ software (http://rsb.info .nih.gov/ij/). The total volume of damage for each structure was calculated from the measured surface areas in each hemisphere (Gundersen & Jensen, 1987), and expressed as a percentage of the normal volume for that structure, which was previously estimated from the normal adult rhesus monkey brain (detailed in Nemanic, Alvarado, Price, Jackson, & Bachevalier, 2002).

Behavioral paradigm apparatus

We conducted behavioral testing in a sound-shielded room using a Wisconsin General Testing Apparatus (WGTA, see Figure 2). A white-noise generator was also used to mask ambient noises. A tray with three wells spaced 10 cm apart was positioned in front of the animals. Only the two lateral food wells were used to display the Food/Non-food items.

Experiment 1: Food/ Non-food Preference

Subjects' post-surgery preference for Food and Non-food items was measured over a four-day period (Murray et al., 1996). The food selection consisted of six options: banana-flavored pellet (P. J. Noyes, Inc, Lancaster, NH, 1 g size), red M&M candy (Mars Candies,

Hackettstown, NJ), fresh carrot, unsalted peanut, raisin, and meat-flavored dog food beef. Monkeys are vegetarians and do not usually consume raw meat. However, previous data indicates that adult monkeys with amygdala lesions tend to abnormally select and eat raw meat (Weiskrantz, 1956; Butter et al., 1969; Ursin et al., 1969; Aggleton & Passingham, 1981, 1982; Baylis & Gaffan, 1991; Murray et al., 1996; Stefanacci et al., 2003). The Non-food items included rubber band, paper ball, yellow yarn, and cork stopper. All Food and Non-food items (including Meat) were cut into the same small size.

All food items were presented in 14 different random orders. On each trial, the subject was given a pair of two items: two different Food items, two different Non-food items, or one Food and one Non-food items. All animals were given a 15 s to pick one, both, or neither (recorded as "Balk") item. All subjects received every listed pairing twice over a four day period (once in each of the two left-right food wells). For this experiment, we calculated animal's food preference by noting for each trial, which of the two items on the tray was selected first. All experiments were conducted between 12 PM to 2 PM (e.g. 20 h after the last feed and prior to the daily feeding) in order to control for animals' motivation related to feeding schedule.

Experiment 2: Primary Reinforcer Devaluation

Reinforcer Devaluation procedure (adapted from Thornton et al. 1998) was used to assess animals' ability to refrain from picking a devalued food. The animals were presented with 30 trials probe test. Following four day of post-surgery Food/Non-food preference test (see above), the experimenter conducted a devaluation test session consisting of 2 parts. In the first part, we devaluated the preferred food item for each animal by placing 200g of the animals favorite food into the feeding box attached to the animal's cage in its living quarters. The animals were able to

eat to their content for 30 mins. An additional 100g of the same food was added to the feeding box every 15 min until the animals reached the point of satiation defined as over five consecutive minutes without any further food ingestion. For this phase, we recorded the total amount of food consumed and the total time it took for each animal to reach satiation. Immediately after satiation, the animal was brought to the WGTA where the second phase was given. This second phase consisted of 30 trials in which the animals were provided with a choice between one piece of the devalued food along with either their Second preferred food item, another Non-preferred food item or a Non-food item. Although the devalued food was included in all 30 trials, there were only 6 trials including the Second preferred food item, 12 trials including other Non-preferred food items, and 12 trials including the Non-food items. We recorded the animals' selection of the first item selected: either the Devalued food item, the Second preferred food item, the Non-preferred food item, or the Non-food item.

Data analysis

For the Food/Non-food preference, we used the number of time animals' selected a Food or Non-food item for each of the 4 testing days as well as the total number of selected items across the 4 testing days. The total number of "Balks" was also used and analyzed in the same way. General Linear Model ANOVAS were used with Groups (Neo-C and Neo-A) as the between factors and Days (4 testing days) as the within repeated measures factors. If sphericity could not be assumed, a Huynh-Feldt correction was used to adjust degree of freedom. To compare the significant main effects of groups, posthoc t-tests were used. The total number of food types selected across the four days between the two groups was also analyzed with t-tests.

For reinforcer devaluation scores, because the number of trial types was not equal for each item paired with the devalued food item, in order to compare across food type selection for each group, we calculated the percentage Devalued food/ Second preferred food/ Non-preferred food/ Non-food selected across the 30 trials by dividing the number of Food or Non-food items selected by the Total number of time this item was presented over the 30 trials and multiplying by 100. For example, if 8 Non-food items were selected, we divided the total of 8 items by 12 (the total number these items were presented) and multiplied by 100 (i.e. 66.7%). To assess the effect of food devaluation, we first compared the percentage of the 1st preferred food selection and 2nd preferred food selection before devaluation and after devaluation using General Linear Model ANOVAS with Groups (Neo-C vs Neo-A) and Devaluation status (before vs after satiation as the between subject factors and Preferred food type (1st vs. 2nd preferred food items) as the within repeated factor. In addition, the percentages of 2nd preferred food items, non-preferred food items, non-food items were compared using General Linear Model ANOVAS with Groups (Neo-C and Neo-A) as the between factors and food type (3 food types) as the within repeated measure factors to measure the effects of devaluation on discrimination of Food and Non-food items. To compare the significant main factor effects or their interactions, we calculated post hoc comparisons using t-tests Bonferroni corrected.

In order to account for individual variation between lesioned animals, we correlated the volume of amygdala lesion with animal's scores in the two tasks. We calculated the correlation between the extent of lesion and behavioral scores from percentage of preferred food Pearson product moment correlation matrices.

Finally, although neonatal groups included both males and females, sex comparisons were not included due to the small sample size (3 males and 3 females in each group).

For the comparisons between the effects of neonatal amygdala lesions to those obtained previously on animals with adult-onset amygdala lesions, we used the scores of each animals published in Machado and Bachevalier (2007) and three way ANOVA with repeated measure comparing group, timing of the lesions, and food types. We compared each food type between the neonatal and adult groups using t-test to detect the effects of timing of the lesions.

Results

Histological verification of lesions

As described earlier (Payne & Bachevalier, 2019), the extent of bilateral amygdala damage in all cases averaged 43.4%, but extended across the entire amygdala (see Table 1). Four cases (Neo-Aibo-2, -3, -4, and -6) incurred substantial and symmetrical lesions, and included the dorsolateral portion of the amygdala (i.e., lateral, basolateral, basal accessory, and central nuclei) while sparing the most ventromedial portion. In one cases (Neo-Aibo-1), the amygdala damage was substantial and asymmetrical (left greater than the right) but included the dorsolateral nuclei in each side. Finally, case Neo-Aibo-5 had the smallest asymmetrical damage to the amygdala, including the most central portion of the amygdala nuclei on the left (i.e., basal and accessory basal nuclei) and the dorsolateral portion on the right (lateral and central nuclei). The extent of unintended damage to the adjacent cortical areas and the anterior portion of the hippocampus were negligible for all cases. Figure 3 shows histological stains for cell body through the extent of the amygdala in the case with the least amygdala damage (Neo-Aibo-5) and the most amygdala damage (Neo-Aibo-2; Figure 4-A), and fibers of passage through the largest slice of the amygdala for Neo-C-2 (Figure 4-B) and Neo-Aibo-2 (Figure 4-C). Note the sparing of fibers in the areas within the amygdala was where the neurotoxin was injected.

Effects of Neonatal amygdala lesions on food preference and devaluation

Experiment 1:

The total number of Food items, Non-food items, Meat selected and Balks emitted by animals in Groups Neo-A and Neo-C for the four days of testing as well as the total number of each item category across the 4 days are illustrated in Figure 5.

The Total Food items selected across the four days did not differ between group [t (10) = -0.70, p = 0.500]. The repeated measure Group × Day ANOVA indicated no significant main effect of Group [F (1, 10) = 0.414, p = 0.534] and no interaction between Group and Day [F (3, 30) = 0.247, p = 0.863], but the Day factor almost reached significance [F (3, 30) = 2.863, p = 0.053], indicating that preference for food items slightly increased across the four days (see Figure 5A).

Although animals in Group Neo-A selected slightly more Non-food items (X ± SEM: 38.83 ± 3.56) than Group Neo-C (23.17 ± 9.08), the group difference did not reach significance [t (10) = 1.607, p = 0.139]. The repeated measures Group × Day ANOVAs revealed no significant Group effect [F (1, 10) = 1.848, p = 0.204] and no interaction [F (3, 30) = 0.14, p = 0.81], but a significant Day effect [F (3, 30) = 3.336, p = 0.033]. As shown in Figure 5B, both groups decreased their selection of Non-food items from Day 1 to Day 4. Planned comparisons between each day, using t-test with Bonferroni correction indicated that the consumption of Non-food items decreased significantly from Day 2 to Day 3 [F (1, 10) = 8.696, p = 0.015] and from Day 2 and Day 4 [F (1, 10) = 11. 364, p = 0.007], and failed short of significance from Day 1 and Day 4 [F (1, 10) = 3.894, p = 0.077].

The Total Meat items selected did not reach significance [t (10) = 0.188, p = 0.855]. The repeated measure Group × Day ANOVA indicated no main effect of Group [F (1, 10) = 0.092, p

= 0.768] and no interaction between Group and Day [F (3, 30) = 0.557, p = 0.647], and the Day factor failed short of significance [F (3, 30) = 2.537, p = 0.075].

The Total Balks emitted across the 4 days did not differ between the 2 groups [t (10) = -1.165, p = 0.271]. The repeated measure Group × Day ANOVA indicated no main effect of Group [F (1, 10) = 1.444, p = 0.257] and of Day [F (3, 30) = 1.207, p = 0.324], and no interaction between Group and Day [F (3, 30) = 0.139, p = 0.994].

Experiment 2:

During the satiation procedures, the two groups did not significantly differ in the time animals took to reach the satiation criterion [t (10) = 0.743, p = 0.475] as well as in total amount of preferred food consumed [t (10) = 0.636, p = 0.658].

Satiation effect was determined by calculating a difference Score for each animal = [Percent of 1st preferred food taken prior to devaluation – Percent of 1st preferred food taken after Devaluation]. The result shows positive scores for both Group Neo-C (X ± SEM: 80.83 ± 3.91) and Group Neo-A (X ± SEM: 82.67 ± 7.38) indicating that all subjects were able to switch their selection strategy by consuming less preferred food after satiation [Group effect: t (10) = -0.371, p = 0.718].

We also compared the amount of the 1st and 2nd preferred food items selected prior to satiation with the amount of the same preferred food items selected after satiation using a three way ANOVA (Group X before-after satiation X 1st and 2nd preferred food items, with repeated measure for the food types). As shown in Figure 6, although the overall pattern of response selection did not differ between the two groups [Group effect: F (1, 20) = 0.595, p = 0.449; Group X Satiation status: F (1, 20) = 1.14, p = 0.298; Group X Satiation Status X Preferred food

type: F (1, 20) = 0.571, p = 0.459], for both groups the specific preferred food item selected changed from prior satiation to after satiation. That is, before satiation, animals selected more of their 1st preferred food than their 2nd preferred food, whereas after being sated with their 1st preferred food, they switch their food selection and selected more of the 2nd preferred food than the sated 1st preferred food [Satiation Status X Food type interaction: F (1, 20) = 89.789, p = 0.000]. Thus, as shown in Figure 6 both Groups Neo-C and Neo-A selected less of the 1st preferred food after satiation than before satiation [t (10) = -20.66, p < 0.00001 and t (10) = -12.53, p = 0.00003, respectively]. Interestingly, both Groups Neo-C and Neo-A selected more of their 2nd preferred food after satiation than before satiation with 1st preferred food [t (10) = 3.53, p = 0.008, and t (10) = 3.05, p = 0.014, respectively].

We finally determined whether, after satiation with their preferred food item, the animals could still show good discrimination between the Second preferred food, Non-preferred food, and Non-food or whether the satiation still have altered the discrimination between the 3 types of items. A repeated measure ANOVA (Group × Food type) indicated no main effect of Group [F (2, 20) = 0.705, p = 0.506], and the interaction between Group and Food type did not reach significance [F (2, 20) = 0.705, p = 0.506]. However, the Food type factor did reach significance [F (1, 20) = 17.192, p = 0.000], indicating that all animals could still discriminate the different Food/Non-food items after the satiation.

Correlations between extent of amygdala lesions and behavioral scores in the Neo-A group

The extent of lesion and preferred food choice in Experiment 1 was analyzed with Pearson product moment correlation matrices. The results show no significant correlation

between extent of amygdala lesions and percent Food selected ($r = -0.363$, $p > 0.05$), percent Non-food selected ($r = 0.206$, $p > 0.05$), and percent Meat selected ($r = -0.850$, $p > 0.05$).

Effects of Adult amygdala lesions on food preference and devaluation

Experiment 1:

The total number of Food items, Non-food items, Meat and Balks selected by animals in Groups Ad-A and Ad-C for the four days of testing as well as the total number of each item category across the 4 days are illustrated in Figure 7.

The Total Food items selected across the four days did not differ between groups [$t(16) = 1.667$, $p = 0.115$]. The repeated measure Group × Day ANOVA indicated no main effect of Group [$F(1, 16) = 2.839$, $p = 0.111$] and of Day [$F(3, 48) = 1.17$, $p = 0.331$], and no interactions between Group and Day [$F(3, 48) = 1.327$, $p = 0.279$].

The Total Non-food items selected across the 4 days did not differ between groups [$t(16) = 0.092$, $p = 0.928$]. The repeated measures Group × Day ANOVAs revealed no significant Group effects [$F(1, 16) = 0.015$, $p = 0.904$] and Day effect [$F(3, 48) = 0.257$, $p = 0.856$], and no interaction [$F(3, 48) = 1.746$, $p = 0.17$].

The Total Meat selected across the 4 days did not differ between groups [$t(16) = -0.812$, $p = 0.429$]. The repeated measure Group × Day ANOVA indicated no main effect of Group [$F(1, 10) = 0.659$, $p = 0.429$] and of Day factor [$F(3, 48) = 0.193$, $p = 0.9$] and no interaction between Group and Day [$F(3, 48) = 0.502$, $p = 0.682$].

The Total Balks emitted across the 4 days did not differ between the 2 groups [$t(16) = -1.023$, $p = 0.322$]. The repeated measure Group × Day ANOVA indicated no main effect of

Group [F (1, 16) = 1.10, p = 0.31] and of Day [F (3, 48) = 0.263, p = 0.852], and no interaction between Group and Day [F (3, 48) = 0.165, p = 0.919].

Experiment 2:

During the satiation procedures, the two groups did not significantly differ in the time it took to reach the satiation criterion [t (16) = -0.571, p = 0.576], as well as in total amount of preferred food consumed [t (16) = -0.777, p = 0.449].

Satiation effect was determined by calculating a difference Score for each animal = [Percent of 1st preferred food taken prior to devaluation – Percent of 1st preferred food taken after Devaluation]. The result shows positive scores for both Group Neo-C (X ± SEM: 67.53 ± 5) and Group Neo-A (X ± SEM: 68.52 ± 6.31) indicating that all subjects were able to switch the selection strategy by consuming less preferred food after satiation [Group effect: t (16) = -0.122, p = 0.904].

After satiation, we also compared the amount of the 1st preferred food item and 2nd preferred food item selected prior to satiation with the amount of the same two preferred food items selected after satiation using a three way ANOVA (Group X before-after satiation X 1st and 2nd preferred food items with repeated measure for the food types). As shown in Figure 8, although the overall pattern of response selection did not differ between the two groups [Group effect: F (1, 32) = 0.91, p = 0.765; Group X Satiation status: F (1, 36) = 0.063, p = 0.803; Group X Satiation Status X Preferred food type: F (1, 32) = 0.279, p = 0.601], for both groups the 1st preferred food item selected decreased from prior satiation to after satiation. That is, before satiation, animals selected more of their 1st preferred food than their 2nd preferred food, whereas after being sated with their 1st preferred food, they switch their food selection and selected more

of the 2nd preferred food than the sated 1st preferred food [Satiation Status X Food type interaction: $F(1, 32) = 276.579$, $p = 0.000$]. Thus, as shown in Figure 8 both Groups Neo-C and Neo-A selected less of the 1st preferred food after satiation than before satiation [$t(16) = -13.499$, $p < 0.00001$ and $t(16) = -10.86$, $p < 0.00001$, respectively]. Interestingly, both Groups Neo-C and Neo-A selected more of their 2nd preferred food after satiation than before satiation with 1st referred food [$t(16) = 2.83$, $p = 0.011$, and $t(16) = 2.434$, $p = 0.020$, respectively].

We finally determined whether, after satiation with their preferred food item, the animals could still show good discrimination between the Second preferred food, Non-preferred food, and Non-food or whether the satiation still have altered the discrimination between the 3 other types of food items. A repeated measure ANOVA (Group × Food type) indicated no main effect of Group [$F(1, 16) = 3.722$, $p = 0.072$], and the interaction between Group and Food type did not reach significance [$F(2, 32) = 0.307$, $p = 0.738$]. However, the Food type factor did reach significance [$F(2, 32) = 19.347$, $p = 0.000$], indicating that all animals could still discriminate the different food items after the satiation.

Correlations between adult amygdala lesions and behavioral scores

As reported by Machado and Bachevalier (2007), there were no significant correlations between the extent of amygdala lesions in adult animals and food preference (Experiment 1) and devaluation (Experiment 2) scores.

Comparisons between the effects of neonatal amygdala lesions and adult-onset amygdala lesions on food preference

Figure 9 illustrates the Food preference scores in animals with neonatal amygdala lesions (Neo-A) and those with adult-onset amygdala lesions(Ad-A) and their age-matched sham-operated controls (Neo-C and Ad-C) for each Food items and Balks. The data were analyzed using a three way ANOVA with Groups (A vs C) X Timing of lesions (infant vs adult) X Food types (Food vs Non-food vs Meat) with repeated measures for the last factor. Neither the Group effect nor the Timing of lesion effect reached significance [F (1, 26) = 2.604, p = 0.119 and F (1, 26) = 0.000, p = 0.99, respectively], but the effect of Food types was significant [$F_{Huynh\text{-}Feldt}$ (2, 46.3) = 679.45, p = 0.000]. In addition, the interactions between Group X Food Type and between Group X Time at lesion did not reach significance [$F_{Huynh\text{-}Feldt}$ (2, 46.3) = 1,124, p = 0.33 and F (1, 26) = 0.07, p = 0.79, respectively]. However, the interaction between Food types and Time at lesion reached significance [$F_{Huynh\text{-}Feldt}$ (2, 46.3) = 7.335, p = 0.002] as well as the triple interaction (Groups X Time at Lesion X Food Type) [$F_{Huynh\text{-}Feldt}$ (2, 46.3) = 3.217, p = 0.048]. As shown in Figure 9, this triple interaction indicated that, although both neonatal and adult-onset lesions resulted in similar amount of Food selected and Balk emitted, animals in both Groups Neo-C and Neo-A selected less Non-food items than animals in Group Ad-C and Ad-A [Control: t (58) = -4.817, p = 0.00; Lesions: t (58) = -2.223, p = 0.030]. Animals in Neo-A and Neo-C also selected more Meat than animals in Group Ad-A, but the difference reached significance only between Neo-A and Ad-A [t (58) = 3.9, p = 0.000].

Discussion

The present study assessed the effects of neonatal amygdala lesions in adult monkeys on their preferred selection of Food, Non-food and Meat items (Experiment 1) as well as on the shifts in their selection of Food and Non-food items after being sated with their preferred food (Experiment 2). Finally, the study also compared the effects of neonatal amygdala lesions with those of amygdala lesions performed in adulthood from an earlier report (Machado and Bachevalier, 2007) to assess any potential sparing of functions associated with early brain damage. For the food preference test (Experiment 1), our findings demonstrated that, as compared to control animals, animals with selective lesions of amygdala performed either in infancy or in adulthood did not affect the normal abilities to select Food over Non-food items and did not significantly increase animals' preference to select Meat. However, both neonatal control animals and animals with neonatal amygdala lesions selected slightly less Non-food items than adult controls and adult lesioned animals (see Figure 9B), but more Meat items than adult lesioned animals, although this increased in Meat selection reached significance only for the neonatal amygdala lesions but not their controls (see Figure 9C). Furthermore, both the neonatal and adult-onset amygdala lesions had no impact on selection of Food and Non-food items after satiation (Experiment 2). First, like their respective controls, both animals with early or late amygdala lesions took the same amount of time to reach satiation and consumed similar amount of their preferred food. Second, like their controls, both animals with neonatal or adult amygdala lesions were able to switch their selection strategies after satiation by selecting less of the sated food but more of their next preferred food and were able to show normal discrimination between Food and Non-food items. The results finally showed that the extent of amygdala

damage did not correlate with any of the behavioral scores for both animals with the neonatal lesions and those with the adult lesions.

The effects of amygdala lesions on food preference

This is the first study to show that neurotoxic neonatal amygdala lesions did not affect food selection in adult rhesus macaques. Overall, the data indicated that across the four days of testing both Groups Neo-C and Neo-A slightly increased their selection of Food items while decreasing their selection of Non-food items. Thus, animals in Group Neo-C and Neo-A selected more Food items (Mean: 54% and 51%, respectively) than Non-food items (Mean: 12% and 21%, respectively). This pattern of results parallels that of animals that had received their lesions in adulthood (Groups Ad-C and Ad-A: 47% and 51% of Food items, and 24% and 25% of Non-food items). It is important to note here that, although the lack of change on post-surgery palatable food selection after adult-onset amygdala lesions is similar to that reported in the earlier report with the same animals (Machado and Bachevalier, 2007), yet the lack of change of Non-food selection after adult-onset amygdala lesions is at odd with the increased of Non-food selection reported by Machado and Bachevalier (2007). After further investigation of the data analysis from this previous report, it appears that the authors included the Meat items within the category of Non-food items, whereas here we analyzed these two categories of item separately. In addition, they showed that the increased selection of Non-food items was between their pre-surgery scores and post-surgery scores and not a significant group difference in their post-surgery scores.

To sum up, when only post-surgery scores are analyzed, both neonatal and adult-onset amygdala lesions did not alter the selection of palatable and unpalatable food items. Both of

these findings differed from those reported in previous studies using adult-onset amygdala lesions. First, contrary to the current findings, Izquierdo and Murray (2007) reported that bilateral neurotoxic amygdala lesions in adult monkeys resulted in decreased preference for three foods that were highly preferred by control animals (fruit snacks, raisins and M&M candies). In addition, two previous reports indicated that neurotoxic amygdala lesions in adult monkeys heightened selection of inedible items and the tendency to explore these items with their mouths (Murray et al., 1996; Stefanacci et al., 2003). Although all studies had used neurotoxic amygdala damage, the different outcomes between these earlier studies and the current one in Non-food selection is most likely related to the extent of the amygdala damage as well as damage to cortical areas surrounding the amygdala given that aspiration lesions of the amygdala in adult monkeys, which include cortical tissues on the parahippocampal gyrus (perirhinal and entorhinal cortex) as well as damaging fibers in passage, are known to result in heighten selection of Non-food and intense fidgeting with the items (Weiskrantz, 1956; Aggleton & Passingham, 1981, 1982; Baylis & Gaffan, 1991) which is one of the whole marks of the Klüver-Bucy syndrome (Klüver & Bucy, 1939). Indeed, inspection of the amygdala lesions for the 5 monkeys in the Murray et al. study (1996) indicated that, in all five cases, the amygdala damage was extensive (range, 85.2–100%) and included some of the fibers coursing on the lateral border of the amygdala as well as the entorhinal and perirhinal cortex, whereas in all six neonatal cases the amygdala damage varied from 23.8 to 55.1% without encroachment to the lateral fibers and adjacent cortical areas and in all nine adult cases the amygdala damage varied from 37.0 to 90% with no additional damage to the ento- and perirhinal cortex.

In addition, the abnormal selection of Meat item was not observed after either the neonatal or adult-onset lesions as compared to their controls. In fact, the selection of Meat item

was extremely low with only 8 Meat items for Group Neo-A as compared to 7 Meat items for Group Neo-C over a total of 36 Meat items presented and with only 3 Meat items for Group Ad-A and 5 Meat items for Group Ad-C. The lack of heightened Meat selection after both neonatal or adult amygdala lesions in the current study also differs from the clear selection of Meat or meat-flavored food seen after radio-frequency (Aggleton & Passingham, 1981), aspiration (Ursin et al., 1969; Baylis & Gaffan, 1991) or even neurotoxic (Murray et al., 1996) amygdala lesions. It has been shown that even neurotoxic lesion with ibotenic acid could result in demyelination of fibers of passage when injected centrally (Murray et al., 1996), whereas fiber bundles are protected from the inflammatory response (Coffey et al., 1988). Thus, since Aggleton & Passingham (1981) reported that heightened Meat ingestion followed complete amygdala lesion, but not subtotal damage, it is likely that the subtotal amygdala lesions and the lack of encroachment to the adjacent cortical areas of the neonatal and adult amygdala damage in the present study could be responsible for these different results.

Overall, we did not find profound differences between the neonatal and adult amygdala lesions in Food, Non-Food, and Meat selection, yet there were at least two minor differences between the neonatal and adult amygdala lesions. First, animals in both Groups Neo-C and Neo-A selected less Non-food items (Means: 23.17 and 38.83, respectively) as compared to Groups Ad-C (Mean: 43.89) and Ad-A (Mean: 44.22) (See Figure 9B). Since this reduction in preference for Non-food items was present for both the neonatal control and neonatal amygdala groups, this effect could not have resulted from a sparing of function due to the early timing of the amygdala lesions. Rather, this reduction in the selection of the Non-food items could have resulted from extensive experience the animals received with palatable Food and Non-food prior to the preference testing. For example, after receiving their neonatal lesions between 8-10 days of age,

animals with the neonatal lesions received 4-5 years of experience with palatable food and inedible food items/objects in their home cage or during cognitive testing after their surgery and prior to being presented with the food preference task. By contrast, animals with adult-onset lesions were tested 1-2 months after recovering from their surgical procedure and in between had not received any cognitive tests involving food rewards and objects of different material, such as plastic, wood, and rubber. Thus, animals with neonatal amygdala lesions may have had extensive experience with Non-food items or objects that could have slightly reduced their tendency to select inedible items during the task. The different outcomes after neonatal and adult-onset lesions could also be related to the different rearing conditions the animals received when they were infants. Neonatal-onset lesioned animals were reared by human caregivers and social interactions with peers when moved into larger enclosures at one year of age. They were also allowed permanent social contacts with peers (Kazama and Bachevalier, 2014). Whereas adult-onset lesioned animals were raised in social groups at the Bastrop Primate Research Center (Bastrop, TX).

Second, both animals with neonatal amygdala lesions selected more Meat (Mean: 8.33) than animals with adult-onset amygdala lesions (Mean: 2.89) and this slight increase in Meat selection was also seen in the neonatal control group (Mean: 7.13 Meat) compared to the adult control animals (Mean: 5.33 Meat) though the group difference between the control groups did not reach significance (see Figure 9C). It is unlikely that this effect of the neonatal amygdala lesions resulted from differences in the extent of damage to the amygdala since all neonatal cases had less extensive damage to the amygdala (average: 43%) than the nine adult cases (68%). An alternative explanation could relate to the timing of the lesions. There are extensive literature indicating that neonatal brain lesions to a specific structure enhance neuroplasticity and result in

either spared or more severely affected functions (Cramer et al., 2011; Meyers et al., 2019; Goldman, 1974; Kolb et al., 2016). Research on developmental postnatal neuroplasticity also suggests an onset of critical point in the amygdala with perineuronal nets density that decreases between three months to adulthood, indicating increased neuroplasticity during neonatal stages (Mcgillis, 2018). Thus, increased neuroplasticity after neonatal amygdala lesions could have resulted in an increased tendency to select more Meat items as compared to the adult amygdala lesions. Finally, the slight enhanced selection of Meat items after both neonatal control and amygdala lesions could simply be ascribed to the use of meat-flavored dog food for the neonatal groups instead of raw meat for the adult groups. It is possible that the raw meat could have been more aversive to the monkeys than meat-flavored dog food.

Effects of neonatal and adult-onset amygdala lesions on Primary Reinforcer Devaluation

Both neonatal and adult-onset amygdala lesions spared the animals' ability to switch their food selection strategy after satiation with their preferred food, suggesting that the amygdala is not involved in modulation of flexible response selection after devaluation of primary food reinforcers. This finding is consistent with previous research that emphasized the important involvement of orbital prefrontal cortex, but not the amygdala, in the modulation of flexible response selection after satiation (Machado and Bachevalier, 2007). It is also interesting to note that, although neonatal amygdala damage spared the ability to switch selection of food after satiation with a preferred food, it severely impaired the animals' ability to flexibly shift object choices away from those objects associated with devalued food rewards (Kazama and Bachevalier, 2014). These different outcomes of both the neonatal and adult amygdala lesions indicate that, although the orbital frontal cortex alone (without the participation of the amygdala)

could support flexible modulation of response selection directed towards primary reinforcers (reward value of food), the interactions between the amygdala and the orbitofrontal cortex are necessary to support flexible modulation of response selection directed to secondary reinforcers (reward value of objects) as demonstrated earlier (Baxter et al., 2000).

Summary

The results indicate that early-onset and adult-onset amygdala lesion spared the abilities to select palatable foods over inedible foods and to modulate food selection after satiation. Although the neonatal amygdala did not alter food preference and primary reinforcer devaluation, this does not imply this null effect was due to experimental procedural error or mistakes in the way the experiments were carried over. The same animals with neonatal amygdala lesions were impaired in several other tasks, including the Fear Potentiated task (Kazama et al., 2012) during which they took longer to develop a strong fear response to the negative stimuli, the Secondary Reinforcer Devaluation task (Kazama and Bachevalier, 2013) for which they showed an inability to flexibly switch their choice strategy when secondary reinforcers are used, as well as the Human Intruder task (Raper et al., 2013) during which lack strong modulation of their emotional responses to social threats.

As regard to food preference and the amygdala, it is unlikely that disruption of the developmental processes of amygdala may be the source of the food preference disruption and intake reported in neuropsychiatric disorders, such as Autism Spectrum Disorders (ASD). Specifically, children with ASD have idiosyncratic meal time, acceptance of limited food, strong preference and selection of foods and also show atypical neuropathological changes in the amygdala (Bauman and Kemper, 1994; Aylward et al., 1999; Schumann et al., 2014), yet the

present results suggest that these food related changes in ASD cannot be directly linked with disruption of early development of amygdala, which developed early in human and non-human primate infants (Schreck et al., 2004; Payne et al., 2010; Chareyron et al., 2012). Further research is therefore warranted to explore the source of food preferences and modulation of food intake disruption in ASD. Second, the results also suggest that the amygdala is not involved in flexibly modulate goal-directed responses after food satiation, at least when primary food reinforcers are assessed and thus may have a relative minor role in clinical eating disorders including obesity and binge eating. Previous fMRI research examining neural activity relating to different levels of severity in bulimia nervosa and binge eating disorder showed aberrant response in amygdala as well as insula, middle frontal gyrus and occipital cortex in human subjects (Donnelly et al., 2018). Although our results suggested that amygdala is not directly involved in satiation responses, further research should investigate whether the amygdala could be involved in emotional appraisal and processing of food reward that could lead to inflexible responses towards food selection seen in patients with bulimia and binge eating disorders.

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Figures

Figure 1. Illustration of the non-human primate amygdala with the major nuclei labeled in the image, including central (CE), medial (M), basal (B), and lateral (L) nuclei, which are the largest nuclei. This illustration shows three different coronal views of the amygdala from the most posterior (left), middle (center) and anterior (right) levels.

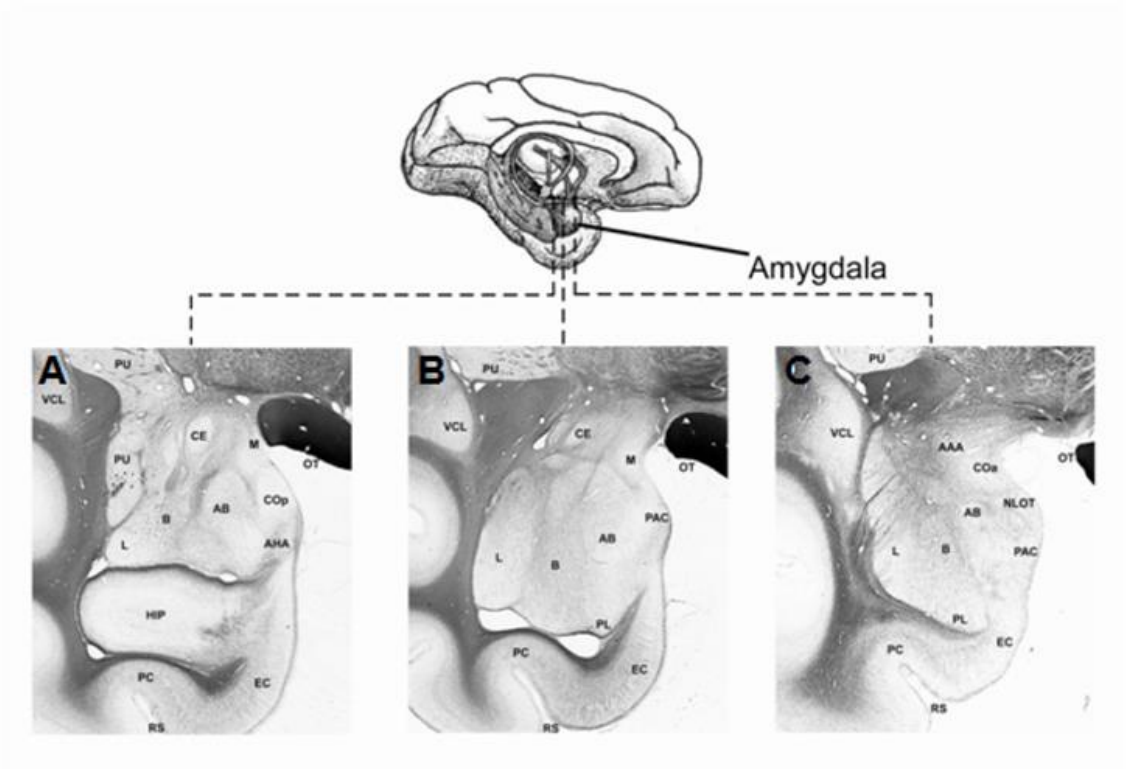


Figure 2. Drawing of a Wisconsin General Testing Apparatus. In A, the opaque screen is lowered to obstruct the animal's view of the stimulus tray onto which food rewards are placed into the left and right small food by the experimenter. When the opaque screen is raised and the one-way vision screen is lowered, the animal can select the food while the view of the experimenter is obstructed. Photo in B illustrates, the position of the animal and experimenter and the animal displacing an object to retrieve a food reward.

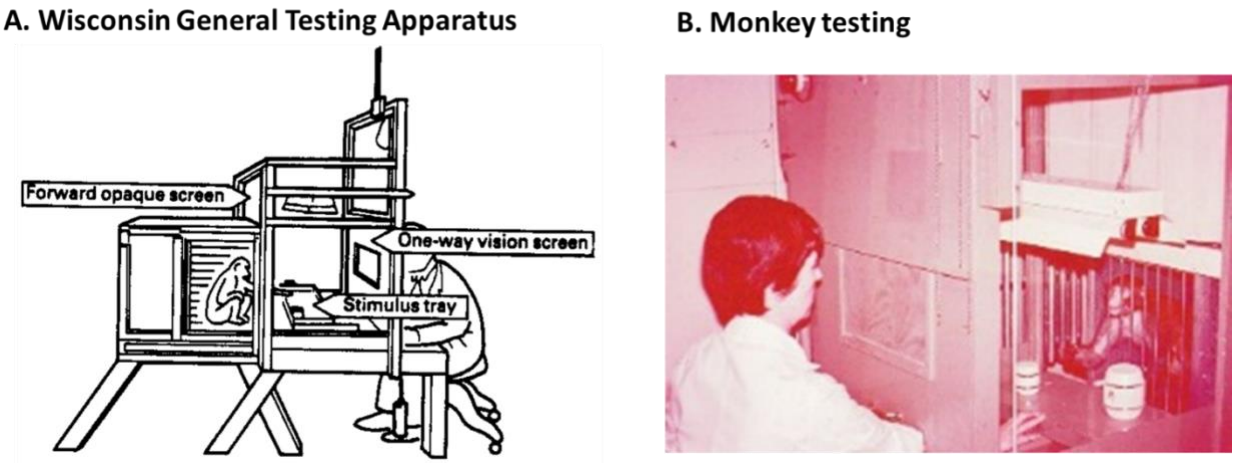


Figure 3. Amygdala lesion extent: Least amygdala damage. Photomicrographs of 50 μm coronal sections of the left and right amygdalae stained for cell bodies via Nissl depicting the animal with the least amygdala damage (left: 17.6%; right: 30.0%). Black arrows highlight the edges of damage. From Payne and Bachevalier, 2019; Figure 1.

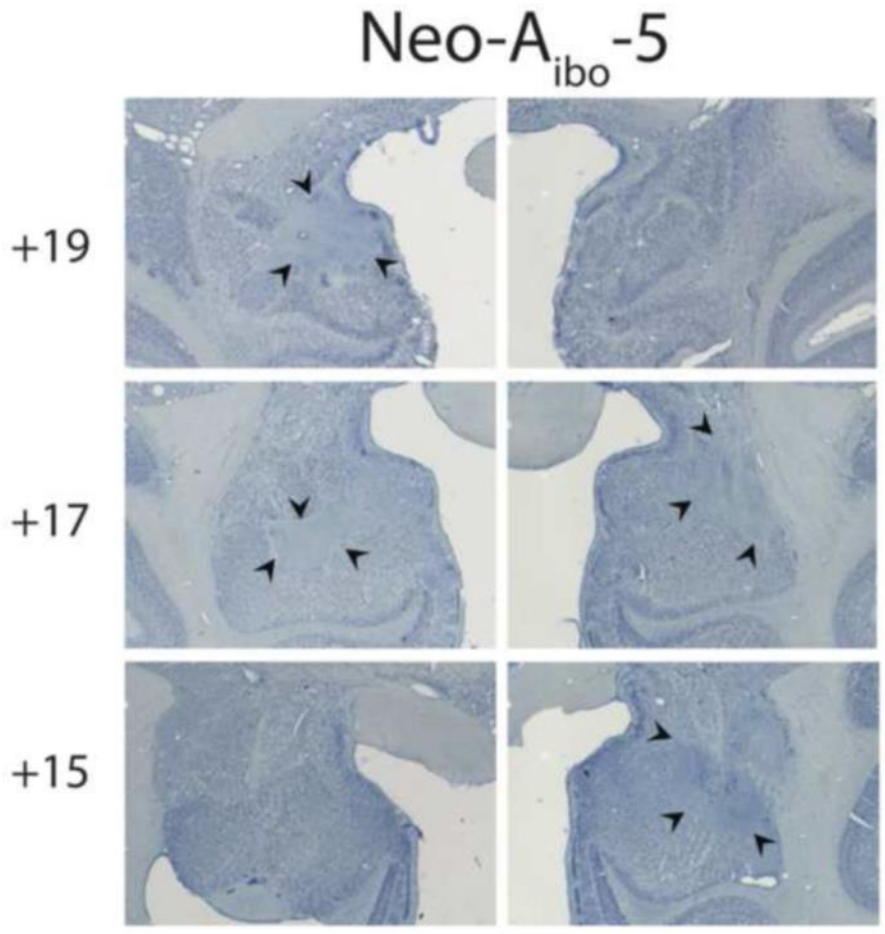


Figure 4. Amygdala lesion extent: Greatest amygdala damage. Photomicrographs of 50 μm coronal sections of the left and right amygdala stained for cell bodies via Nissl (A) and fiber tracts via silver (B, C). Panel A depicts cell loss in the animal with the greatest amygdala damage (left: 45.2%; right: 65.0%). Panel B shows the fibers of passage through the largest section of the amygdala in a Neo-C animal. Panel C shows that the neurotoxic lesions spared the fibers of passage within the amygdala. Black arrows highlight the edges of damage. See the online article for the color version of this figure. From Payne and Bachevalier, 2019; Figure 2.

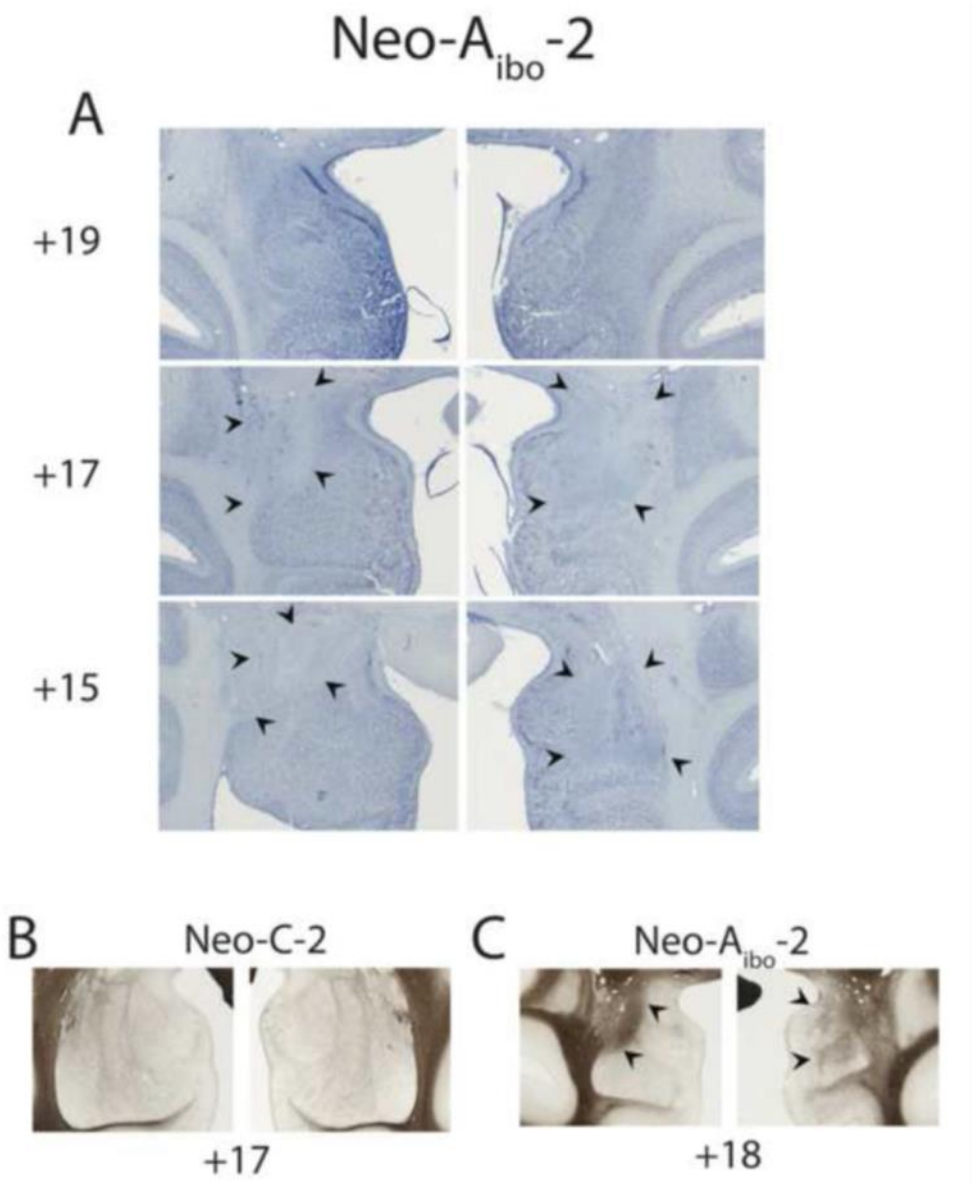


Figure 5. Preference test. Mean number of Total Food (A), Total Non-food (B), and Total Meat (C) selected and Total Balks (D) emitted for animals with neonatal amygdala lesions (Neo-A, blue bars) and controls (Neo-C, orange bars). Vertical bars indicate standard errors of the mean.

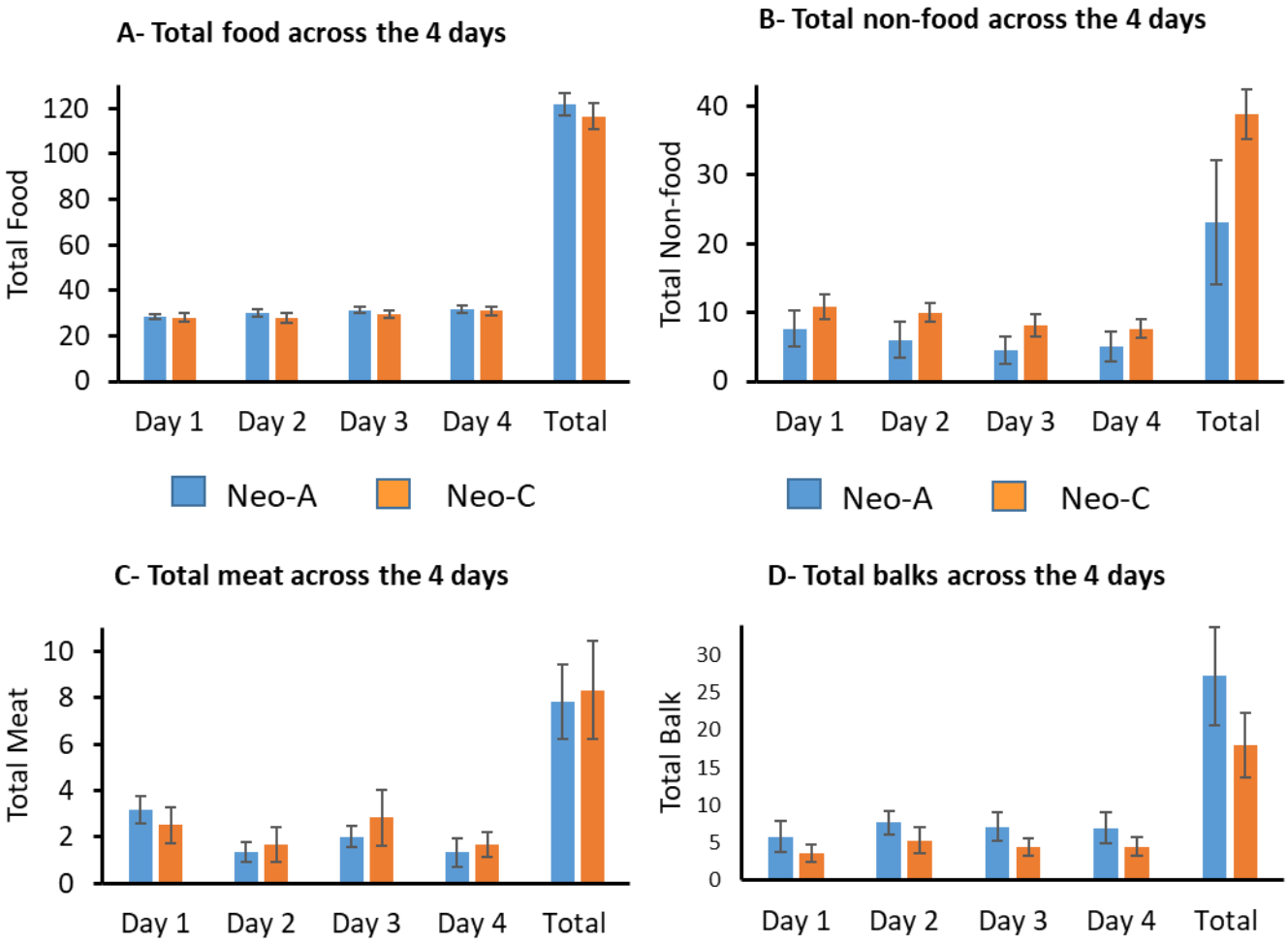


Figure 6. Devaluation test. Percent 1st preferred food items (Purple bars) and 2nd preferred food items (Green bars) selected before satiation (solid bars) and after satiation (Hatched bars) for Group Neo-C (left side) and Group Neo-A (right side). * P < 0.05

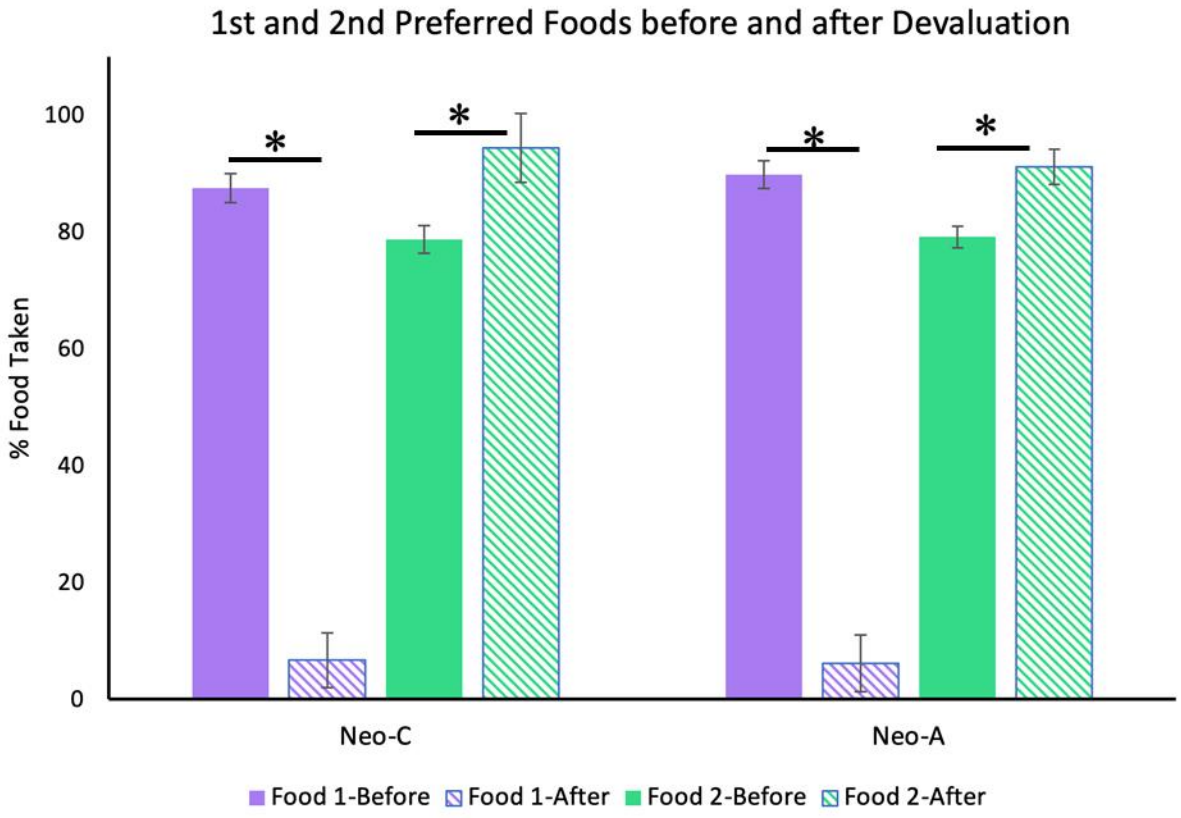


Figure 7. Preference test. Food, Non-food, Meat and Bulk item preference. Mean number of Food (A), Non-food (B), Meat (C), and Balk (D) selected by each adult monkey experimental group with amygdala lesion and control (blue and orange bars respectively). Vertical bars indicate standard errors of the mean.

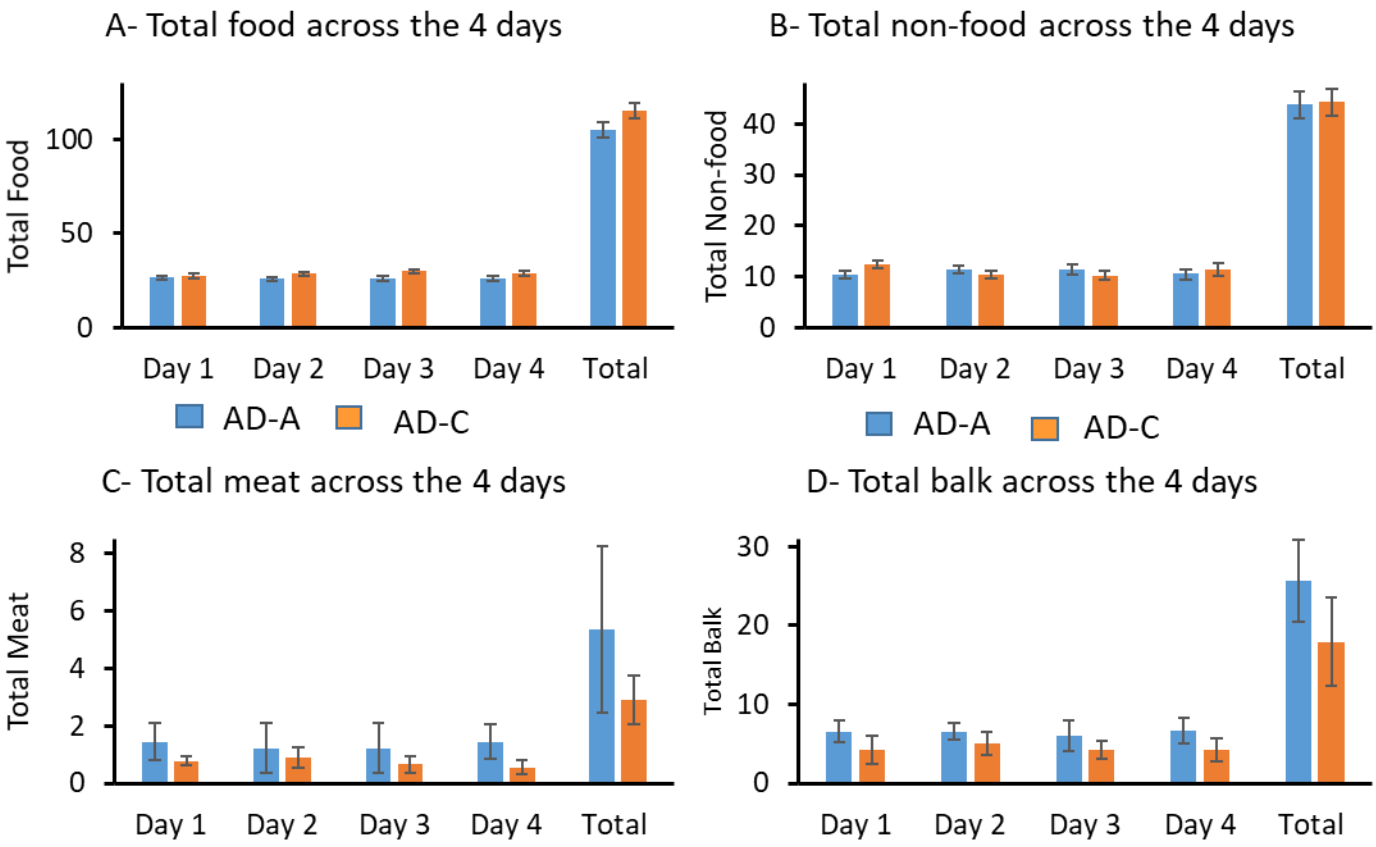


Figure 8. Devaluation test. Percent 1st preferred food items (Purple bars) and 2nd preferred food items (Green bars) before satiation (solid bars) and after satiation (Hatched bars) for Group Ad-C (left side) and Group Ad-A (right side). Vertical bars indicate standard errors of the mean. * P < 0.05

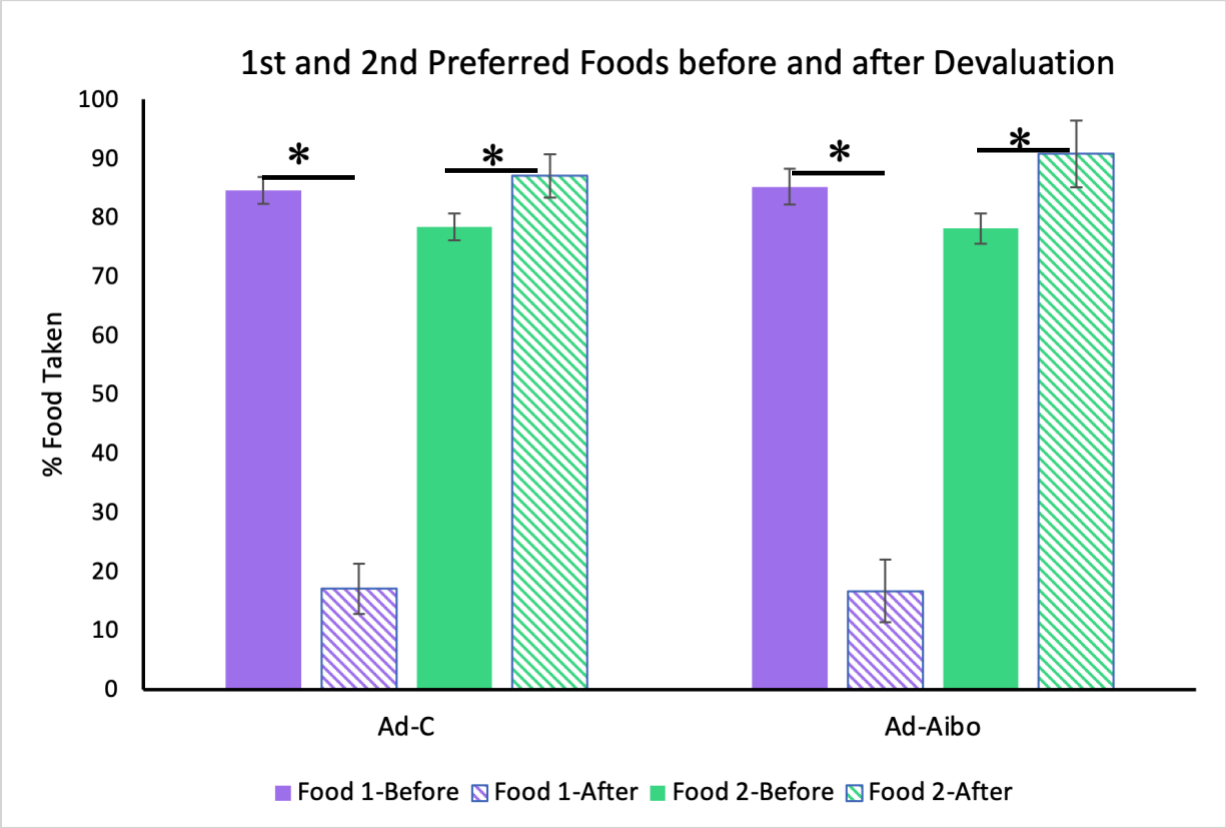
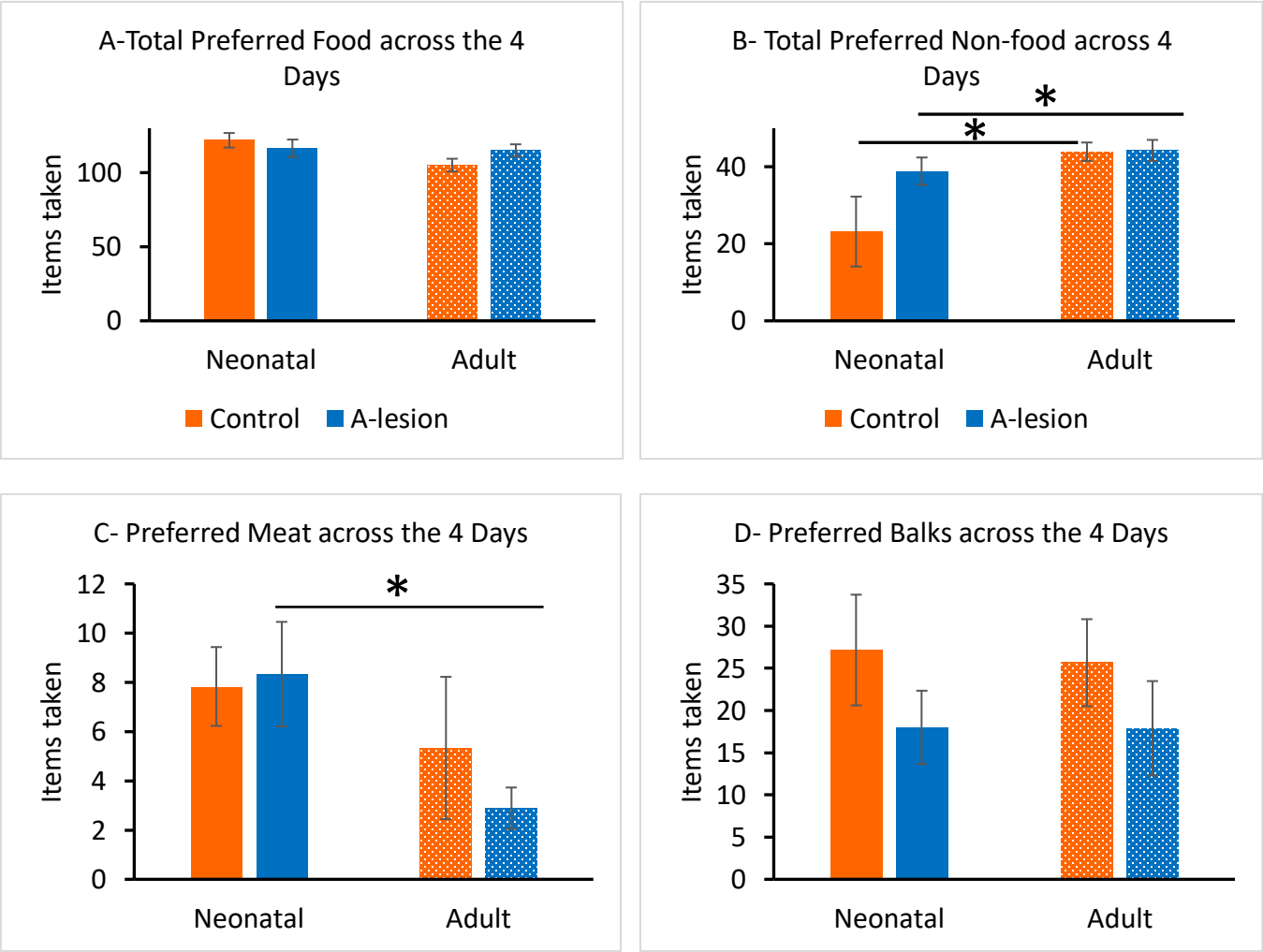


Figure 9. Comparison between neonatal lesions and adult-onset lesions. Mean number of Total Food (A), Total Non-food (B), Total Meat (C), and Total Balk (D) selected by monkeys with neonatal lesions (Neo-C, solid orange and Neo-A, solid blue) and by monkeys with adult-onset lesions (Ad-C, hatched orange and Ad-A, hatched blue). Vertical bars indicate standard errors of the mean. * $P < 0.05$



Tables

Table 1

Percentage of Cell Loss in Amygdala as Measured on Postmortem Histological Slices

	L%	R%	X%	W%
Neo-Aibo-1	61.2	35.0	48.1	21.4
Neo-Aibo-2	45.2	65.0	55.1	29.4
Neo-Aibo-3	40.3	40.0	40.1	16.1
Neo-Aibo-4	42.9	51.4	47.2	22.1
Neo-Aibo-5	17.6	30.0	23.8	5.3
Neo-Aibo-6	46.8	45.9	46.4	21.5
Mean	42.3	44.6	43.4	19.3

Note. Mean = average damage per group; L% = percent damage in the left hemisphere; R% = percent damage in the right hemisphere; X% = average damage in both hemispheres; W% = weighted average damage to both hemispheres (W% = (L% X R%)/100; Hodos & Bobko, 1984). From Payne and Bachevalier, 2019; Table 1).