Investigating Neural Mechanisms of Altered Social Motivation Following Brief Social Satiation & Deprivation in Rats

Michelle G. Soriano
Department of Neuroscience and Psychology
Lake Forest College
Lake Forest, Illinois 60045

Social behaviors are an important marker for normal brain development in children and young adults. Several psychiatric disorders are characterized by their negative effects on social behaviors, such as autism spectrum disorder and schizophrenia. Social interactions are inherently rewarding, and any changes to social motivation may reflect alterations in how the brain encodes the value of social interactions. Although no statistically significant effects were found, female rats in the social deprivation condition showed a trend toward increased investigation and orientation towards a social stimulus, suggesting a potential increase in social drive following isolation. ZIF analysis indicated a negative correlation between ZIF expression in the basolateral amygdala (BLA) and nose-to-nose sniffing, suggesting that BLA activation may reflect stress and reduce prosocial behavior. Sex-specific effects and individual variability were observed, with females showing lower ZIF expression in the CG1 region compared to males.

1. INTRODUCTION

1.1 A Homeostatic Framework For Studying Social Behaviors

Social interactions include any form of communication and/or interaction between two or more individuals of the same species (Chen & Hong, 2018; Sato et al., 2023). While the terms "social behaviors" and "social interactions" are frequently used interchangeably throughout the literature (Chen & Hong, 2018; Lee et al., 2004; Modi & Sahin, 2019; Sato et al., 2023; Shankar, 2023; van Kerkhof et al., 2014; Wu & Hong, 2022), this thesis will make a distinction between the terms for the sake of clarity. The term "social behavior" will refer to the observable actions of an individual towards conspecifics, while "social interactions" will refer to the mutual exchange of social cues. Making this minor distinction between the terms is intended to highlight the feedback loop between an individual's unique neural mechanisms and their social environment. The goal of this thesis is to explore how the nervous system adapts to the social environment and drives social engagement as a dynamic physiological state.The introduction will focus on exploring the neural substrates of social cognition and their adaptations to social need fulfillment. By reviewing the current literature on what is known regarding the physiological basis for social homeostasis, gaps in knowledge can be further explored and addressed.

1.2 Social Behaviors are Evolutionarily Conserved Mechanisms of Survival

Social interactions are vital for survival and reproductive success across species, making social behaviors an essential driver of evolutionary fitness (Sato et al., 2023). Various versions of social behaviors have been observed across species, with even the simplest species demonstrating a range of affiliative behaviors, such as the sharing of resources (Benabentos et al., 2009). For example, Dictyostelium discoideum, unicellular organisms known as "social amoebas", have been observed to aggregate upon starvation and decide to sacrifice themselves to support the survival of the others, if necessary (Benabentos et al., 2009). Despite knowing some of the other amoebas will "cheat" and refuse to sacrifice themselves, these social amoebas accept the potential risk and choose social cooperation above their self-interests (Benabentos et al., 2009). This example demonstrates how social behaviors have been observed in organisms without nervous systems, suggesting that they have been selected for in evolutionary history from a common ancestor due to their contributions to survival (Chen & Hong, 2018; Sato et al., 2023). These simple organisms employ an evolutionary strategy that considers the reciprocal nature of social interactions, where an individual's actions may not always elicit the desired reaction.

This concept of reciprocity within social interactions becomes more complex in species with nervous systems, as they enable more sophisticated forms of communication. Male Drosophila melanogaster, better known as fruit-flies, are known to perform various social behaviors to demonstrate their fitness as a potential mate for their preferred female. This includes singing to her by vibrating his wings, dancing in circles around her, and tapping her with his forelegs to activate mechanosensitive pheromone receptors (Sokolowski, 2010). If the female decides he is worthy, she will show she is receptive by presenting him with her vaginal plate, otherwise she kicks him away and leaves (Sokolowski, 2010). This example not only demonstrates that male fruit-flies are experts at handling social rejection, but it also illustrates the idea that as the nervous system becomes more complex, so too do social interactions. These fruit-flies, even with their simple nervous systems, remain capable of rich social interactions which utilize an amalgamation of chemosensory, mechanosensory, visual, and auditory cues from conspecifics (Benabentos et al., 2009). The sensory cues are reciprocal and dynamic, forming a feedback loop that gives the individual milliseconds to decide upon their next behavior and predict the response from their interaction partner (Chen & Hong, 2018).

Social behaviors become increasingly context dependent as the goals of the organism evolve past fulfilling their base survival and sexual needs (Sato et al., 2023; Wu & Hong, 2022). In mammals, there is a marked difference in the complexity of social behaviors compared to those of the social amoeba or fruit fly examples previously mentioned. It is hypothesized that the evolutionary origins of mammalian social behaviors are an adaptation of maternal behavior, with much of the same circuitry involved (Modi & Sahin, 2019). This is most evident in altricial species, such as humans, which are born with underdeveloped brains and are helpless without their caregivers to provide food, warmth, and protection (Sato et al., 2023; Wu & Hong, 2022). Mammals can be dependent on their caregivers for years at a time, thus a social bond between progeny and caregiver is required for survival across species. This social bond involves circuits that produce rewarding sensations to the caregiver and neuromodulators that promote feelings of attachment to make the bond lasting. The neural contributors to their sociality have thus evolved to enable the formation and maintenance of lasting social bonds (Sato et al., 2023; Wu & Hong, 2022).

1.3 The Social Environment Affects Brain Development

Beyond maternal social behaviors, the behaviors of the helpless infant are motivated by an evolutionary drive to form a social bond to their caregiver (Ferrara et al., 2023). Their social behaviors are centered on getting their caregiver to meet their needs, such as crying for their mother's attention. The neural circuits involved in promoting this social bond will continue developing throughout the lifespan and produce social behaviors gaining in complexity (Ferrara et al., 2023). The emerging social behavior changes that occur throughout development are dynamically influenced by the social environment of the developing individual (Figure 1). As the individual's social circle expands beyond their caretakers, such as entering school and meeting other developing minds, their brain will begin to develop essential circuits between the prefrontal cortex and the amygdala (Matthew & Tye, 2019).

Dopamine, the neurotransmitter of reward and motivation, modulates this circuit between the developing medial prefrontal cortex and the amygdala throughout adolescence, heightening social awareness and sensitivity to social reward and rejection (Matthew & Tye, 2019). The expanding social environment, coupled with the individual's growing independence from their caregiver introduces the importance of social inclusion versus social exclusion. Sensitivity to social rejection during adolescence is a mechanism of evolution, whereby the growing individual must seek out their new social contacts to ensure their survival (Matthew & Tye, 2019). If the developing individual can learn how to respond to the social behaviors of their peers, they will continue developing the social skills

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*This author wrote this paper as a senior thesis under the direction of Dr. J Amiel Rosenkranz necessary for the inherently dynamic nature of social interactions (Ferrara et al., 2023). For social animals, reacting to the social cues of others is instinctual, however, selecting the appropriate response is a learned skill (Eslinger et al., 2021). This skill of processing social information and using it to determine the best course of action is known as social cognition.

1.4 Social Cognition

Social cognition refers to the ability to decode the intentions of others from their social behaviors and then decide on the socially appropriate actions in response (Arioli et al., 2018). It reflects the integration of a wide variety of cognitive processes related to salience, reward-seeking, motivation, self-monitoring, and empathy (Bicks et al., 2015). Social cognition becomes refined throughout development as the individual learns how their behaviors influence others. Failure to develop the necessary skillset for engaging with others can reflect dysfunctional or underdeveloped circuits (Ferrara et al., 2023; Lee et al., 2004). Distinct facets of social cognition include social motivation, knowledge of one's own internal state, and knowledge of social cue interpretation (Bicks et al., 2015).

Significant deficits to any one of these facets of social cognition is often enough to warrant a psychiatric evaluation. Social dysfunctions are a fundamental dimension of many psychiatric disorders, which is why the Research Domain Criteria initiative put forward by the National Institute of Mental Health has asked researchers to focus their studies on social deficits (National Institute of Mental Health, 2024). The initiative is aimed at encouraging researchers to study mental health and psycoathology in the context of neurobehavioral functioning. They emphasize the need for researchers to ground their studies in understanding the neurobiology and physiological impact of psychiatric disorders because while the mechanisms remain poorly understood, so do the interventions to alleviate them. Additionally, social cognition is deeply intertwined with the ability to plan, remain focused, and to problem solve(Arioli et al., 2018). Neuroimaging studies have suggested that the prefrontal cortex and the amygdala both play a central role in facilitating the adaptive problem solving that takes place during social interactions (Lee et al., 2004).

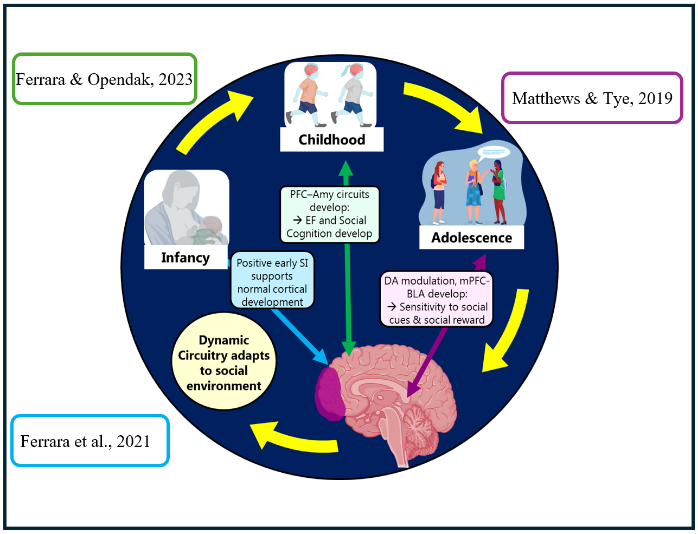

Figure 1. Social Interactions are guided by and influenced by brain development.
The social environment forms a dynamic feedback loop with the nervous system, where healthy social interactions support typical prefrontal cortical-amygdala circuit development (Ferrara et al., 2021; Ferrara & Opendak, 2023). This circuit becomes refined with social experience, adapting to the more complex interactions in adolescence and becoming more sensitive to social cues with increased dopamine modulation driving the pursuit of social rewards (Matthew & Tye, 2019).

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1.5 Social Homeostasis: Loneliness is a Social Pain

Homeostasis refers to physiological processes that maintain stable states through compensatory mechanisms to meet physiological needs (Matthews & Tye, 2019). The state which functions as the "set point" is the state of homeostatic balance. Any deviations from homeostatic balance recruit coordinated responses to elicit interactions with the environment until the deviation is corrected (Matthews & Tye, 2019).

Deviations from homeostatic balance can also evoke motivated behaviors in response to unmet physiological needs that challenge the organism's survival. Natural behaviors, such as drinking or feeding, are initiated by motivated "drive" states which are regulated by neural circuits shaped by selective pressure (Betley et al., 2013). These motivated behaviors are negative "drive" states, where aversive conditions such as overheating, hunger, or thirst are actively avoided for the physical discomfort they cause (Matthews & Tye, 2019). Neural control of homeostatic balance requires flexible coordination of circuit components for these complex survival-oriented behaviors to be evoked (Betley et al., 2013; Matthews & Tye, 2019). Homeostasis has been classically understood as systems used to maintain thermoregulation, energy levels, and osmoregulation. One of the major goals of neurobiology has been to understand how neural adaptations are recruited and how they direct behavior. Social homeostasis has been proposed as a neuroscience model for understanding the neural adaptations that occur as adaptive functions to regulate behaviors in response to social interactions (Matthews & Tye, 2019).

Social homeostasis is the ability of individuals to compare the perceived quantity and quality of their social contacts and compare it to their established set-point. This comparison will adjust the amount of effort the individual is willing to expend to seek social engagement until they have reached their optimal set point (Lee et al., 2021). This optimal set point for social homeostasis is subjective and unique to the individual's perception. Within this model, loneliness functions as an aversive signal designed to promote motivated behaviors to seek social engagement (Matthews & Tye, 2019). Loneliness is defined as subjectively perceived social isolation, and it is unique to the individual (Hawkley, 2022). Functional MRI studies have noted that individual differences in perceived isolation predicted the brain's response to social information, showing more activation of the visual cortex when presented with unpleasant social images (Hawkley, 2022).

Much like hunger pains or extreme thirst, loneliness is the brain's painful reminder that social needs have not been met. The mechanism that drives social engagement is known as social motivation.

1.6 Social Motivation: Social Dysfunctions are Circuit Dysfunctions

Sensory perception systems play a vital role in social cognition. Social information is processed first through the dominant sensory modality of the organism. In humans, social information is processed first through visual perception. Recognition of a conspecific as familiar or unfamiliar is obtained through visual perception of their facial identity and facial expressions are used to infer their intentions (Babinet et al., 2021). In rodents, olfaction is the dominant sensory modality for processing social information. Rodents use their sense of smell to obtain social information, making olfactory cues the rat equivalent of social cues (Modi & Sahin, 2019).

Social sensory information is given greater attention than non-social stimuli in both humans and rodents, and this process requires a brain-wide effort to coordinate across multiple circuits and the recruitment of neuromodulators (Modi & Sahin, 2019). The prefrontal cortex plays a major role in attending to incoming social information, and one of the contributors to the heightened sensitivity and selective attention towards social stimuli is the amygdala.

1.6.1 The Almond at the Center of it All: The Amygdala

The amygdala is an almond-shaped cluster of nuclei located in the anterior part of the medial temporal region composed of grey matter (Watson et al., 2010). It is located anterior to the hippocampus and plays an essential role in emotional and affective processing (Watson et al., 2010). The circuitry of the amygdala has been well-conserved across evolution, with mammals and non-mammals alike possessing an amygdala that performs analogous functions across species (see Figure 3) (Janak & Tye, 2015). Most research dedicated to the amygdala has focused on its role in fear and stress, however, the true role of the amygdala extends far beyond this simple characterization. While the amygdala does play an important role in the detection of threats, its true purpose is to promote recognition of information from the environment critical for survival (Labuschagne et al., 2024). This includes enhancing the salience of emotionally relevant stimuli (Janak & Tye, 2015). In simple terms, it helps the brain prioritize emotionally important stimuli by modulating attention towards it, influencing the memories that form around it, and affecting the behavioral responses evoked by the stimuli.

This places the amygdala at the core of the brain's emotion processing network (Balderston et al., 2015). The amygdala serves as a gateway in processing sensory information, sending and receiving projections across multiple brain regions to integrate sensory and cognitive inputs (Modi & Sahin, 2019; Watson et al., 2010). Several psychiatric disorders are affected by amygdala dysfunction, such as autism spectrum disorder, anxiety, and addiction (Huang et al., 2022). Neuroendocrine factors and neurotransmitters modulate the activity of the amygdala, influencing the neuronal activation patterns to evoke the socially appropriate response (Janak & Tye, 2015). Of these neurotransmitters, dopamine (DA) and oxytocin play an essential role in the amygdala's influence over establishing salience to social stimuli. The ventral tegmental area (VTA) is located in the midbrain and composed of ~60% dopaminergic (DA neurons) neurons, making this area an important source of DA in the mesocorticolimbic dopamine system, which our little amygdala is smack dab in the middle of (Cai & Tong, 2022).

The DA provided by the VTA is hypothesized to mediate the rewarding sensations associated with social interactions and social motivation overall (Modi & Sahin, 2019). The amygdala sends inputs back to the VTA, as well as the nucleus accumbens (NAc), another important dopamine heavy region that mediates reward in the striatum. By influencing the dopaminergic tone at multiple levels, the amygdala drives the DA needed to reinforce the valence of social stimuli (Modi & Sahin, 2019). At the same time this is occurring, oxytocin, a neuropeptide that influences social bond formation, is also increasing the salience of the social signals (Chu et al., 2012; Modi & Sahin, 2019; Sharp, 2017). When oxytocin is co-expressed with dopamine in medium spiny neurons found in the NAc, this enhances the rewarding sensations of social bonds (Modi & Sahin, 2019). The amygdala can be divided by the functional roles of its subnuclei, and the one most central to sociality is the basolateral amygdala. The basolateral amygdala receives projections from the VTA, is modulated by oxytocin, and it maintains reciprocal connections with the NAc and prefrontal cortex (Chu et al., 2012; Modi & Sahin, 2019; Sharp, 2017). This places the basolateral amygdala as an essential component of social motivation and social awareness.

1.6.2 The Basolateral Amygdala: Linking Sensory Input to Emotional Meaning

The basolateral amygdala (BLA) contributes to social perception by imparting emotional valence to potentially rewarding stimuli (Watson et al., 2010). This means that the BLA helps decide what the brain considers important enough to attend to, by weighing potential risks and rewards that the stimuli poses. Because of this, the BLA is central to decision-making, social perception, and emotional responses (Chang et al., 2015). The BLA receives input from sensory cortices and helps evaluate external cues for emotional or social significance, such as facial expressions or tone of voice. Meta-analysis of functional MRI studies has demonstrated that emotion-evoking stimuli, such as negative facial emotions (grimacing, frowning), altered the connectivity between the BLA and prefrontal cortex (Labuschagne et al., 2024). The impact of altered signaling in the BLA is often hyper-excitability, as the BLA requires tonic inhibition, meaning stable and constant inhibitory signaling for the stable regulation of emotional processing (Sharp, 2017). The tonic inhibition of the BLA is maintained by the glutamatergic (excitatory) cortical inputs it receives, which sounds counter intuitive to need excitation to downregulate the already hyperexcitable BLA. The inhibitory interneurons in the BLA, which mediate connections between other neurons, are activated when they receive excitatory cortical inputs, essentially turning the inhibition 'on' (Sharp, 2017). This is known as feed-forward inhibition and it is what controls the BLA's inherent excitability, as it ensures the BLA's principal glutamatergic neurons maintain more stable firing rates (Janak & Tye, 2015; Sharp, 2017). When this feedback inhibition is disrupted, the BLA's hyperexcitability is associated with disrupted motivation, anxiety, and emotional dysregulation (Janak & Tye, 2015; Sharp, 2017).

Along with these excitatory projections from multiple subcortical regions, membrane receptors throughout the BLA also work to modulate the activity of the BLA's GABAergic (inhibitory) interneurons (Janak & Tye, 2015; Sharp, 2017). Dopamine receptors expressed by BLA GABAergic interneurons inhibit GABA release when activated by projections from the VTA (Janak & Tye, 2015; Sharp, 2017). The inhibition of GABA release by the VTA's projections suppresses the feed-forward inhibition of the BLA's principal neurons by GABA interneurons and increases overall BLA activity (Sharp, 2017). The BLA is part of a frontotemporal system that innervates several regions across the corticolimbic system, including the anterior cingulate cortex found in the medial prefrontal cortex (Huang et al., 2022).

1.6.3 Prefrontal Cortex

Along with the amygdala, another telencephalic powerhouse key to orchestrating higher order brain functions is the prefrontal cortex (PFC). The PFC refers to the region of the frontal cortex located anterior to the premotor cortex and supplementary motor area (Grossmann, 2013; Hathaway & Newton, 2025). The PFC came to the forefront of biomedical researchers' attention in 1848 when Phineas Gage miraculously survived having an iron tamping rod pierce through his frontal lobe (Harlow, 1868). Before his injury, Gage was reportedly a calm and dependable man who drastically changed after his accident, becoming short-tempered and disorganized (Harlow, 1868). His famous brain injury has been cited in every neuroscience and psychology textbook since then for revealing the PFC's functional connectivity as the neural substrate for personality and cognition (Anastasiades & Carter, 2021; Grossmann, 2013). Numerous neuropsychiatric disorders have been associated with neuromodulator dysregulations which impact the circuit level functions of the PFC (Gamo & Arnsten, 2011; Kas et al., 2014). These disorders include obsessive-compulsive disorder, attention deficit hyperactivity disorder, depression, schizophrenia, bipolar disorder, and autism spectrum disorder (Gamo & Arnsten, 2011). These disorders highlight the centrality of PFC functionality for its role in exerting cognitive control over thoughts and behaviors. Without the inhibitory control of the PFC to focus attention to relevant targets and filter out irrelevant stimuli, social and cognitive deficits can emerge (Chini & Hanganu-Opatz, 2021). Integration of external cues with internal emotional states underlies the PFC's ability to enable executive functions such as planning, attention, and decision-making to take place (Reppucci & Petrovich, 2016). Since Phineus Gage's accident, other lesion studies have demonstrated that functionally distinct subcomponents of the PFC work together to rapidly generate meaningful interpretations of incoming information (Kas et al., 2014; Szczepanski & Knight, 2014). Cytoarchitectonics, the study of the structural arrangement of cells in neural tissues, has been utilized to map out these divisions by their specific connectivity and contributions to functionality (Anastasiades & Carter, 2021; Kiwitz et al., 2020; Van De Werd et al., 2010). The cytoarchitectonic features of the PFC suggest that after it receives sensory input from sensory cortices, long-range afferents to its subdivisions from the ventral striatum, hypothalamus, and amygdala are integrated (Anastasiades & Carter, 2021; Kiwitz et al., 2020; Van De Werd et al., 2010). These connections have been implicated in priming the PFC for complex social cognitive processes by coordinating reciprocal loops with sites of sensory perception, motivation, and emotional valence (Kas et al., 2014; Modi & Sahin, 2019). The PFC subregion responsible for the integration of emotional and social information is the medial prefrontal cortex (mPFC) (Grossmann, 2013).

1.6.4 Medial Prefrontal Cortex

Located on the medial surface of the frontal lobe, the mPFC is composed of a complex network of interconnected regions thought to enable different forms of processing related to the expression of social behavior (Modi & Sahin, 2019). The afferent projections to the mPFC induce local circuit activity which facilitate higher sensory processing in response to social stimuli by the PFC (Chen et al., 2024). This increased sensory processing allows for heightened awareness of the environment and selective attention to potential threats (Capuzzo & Floresco, 2020). The mPFC is known as a key structure in the social circuit, where it mediates memory retrieval, cognitive flexibility, and inhibitory control to anticipate consequences (Chen et al., 2024). The mPFC is comprised of three main subregions in rats: the cingulate cortex the prelimbic cortex and the infralimbic cortex. The cingulate cortex (CG1) is involved in a wide range of functions, such as the regulation of emotion, decision-making, executive control, and prosocial behaviors (Simon IV & Rich, 2024). It is the rat equivalent of the anterior cingulate cortex (ACC) in humans, aiding in social processing which involves the intake of social cues to provide context and enable meaningful interpretations of them (Chen et al., 2024; Simon IV & Rich, 2024). The CG1 coordinates attentional processing to make judgements on social behavior, described as cost-benefit calculation that considers the entire context of a situation (Guo et al., 2019; Kitagawa et al., 2024). Several studies on observational fear have considered the CG1 as an empathy- promoting subregion, and the prelimbic cortex is more associated with fear expression (Chen et al., 2024). The connections it has to the hypothalamic-pituitary-adrenal (HPA) axis link its activation to stress and some studies have noted that prelimbic activity can reflect social motivation to dominance related decisions (Grossmann, 2013). For the infralimbic cortex, it is often considered to have a somewhat opposing role when compared the prelimbic, where the infralimbic cortex is associated in social buffering and stress recovery (Nett & LaLumiere, 2021). The infralimbic cortex (IL) is implicated in reward-seeking behaviors, but it can also regulate extinction, as some studies have observed the IL influence the inhibition of cocaine seeking (Nett & LaLumiere, 2021). The human equivalent of the infralimbic cortex is the ventromedial PFC, which mediates behavioral inhibition (Nett & LaLumiere, 2021).

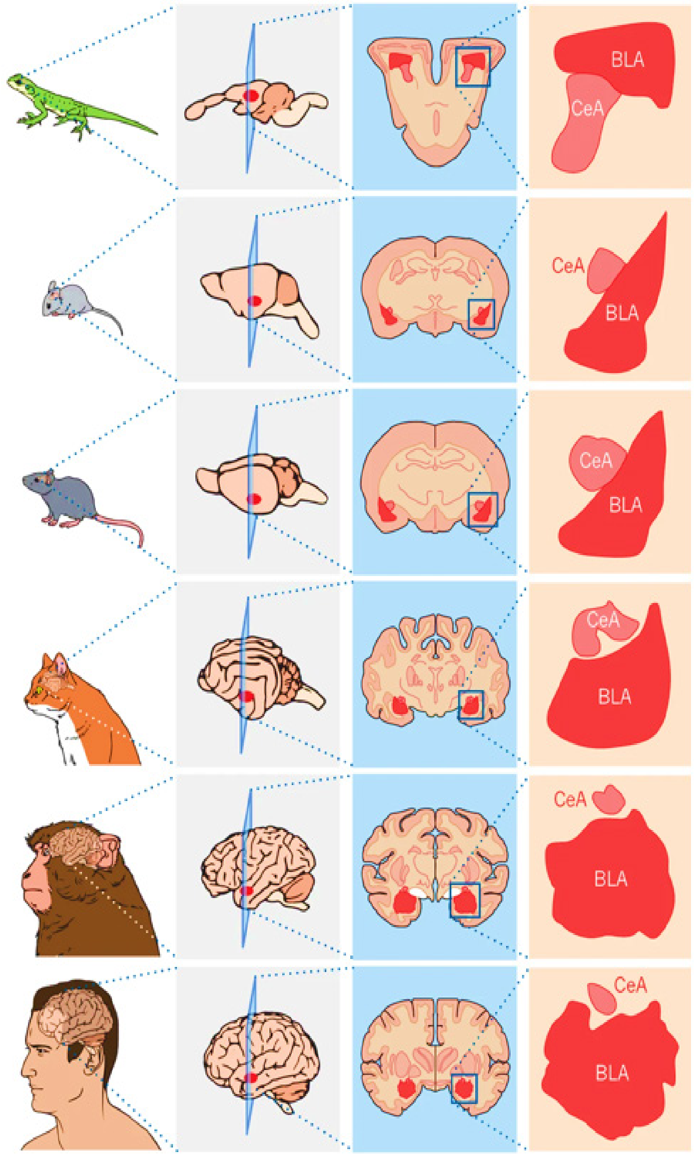


Figure 2. The Amygdala in All of Us

Amygdalar nuclei and its basic circuit connections and functions are conserved across species, the images shown are coronal sections from the brains of a lizard, mouse, rat, cat, monkey and human. This image is included to showcase how there is an amygdala in all of us, emphasizing the conserved nature of its contributions to sociality and survival. Image from Janak & Tye, 2015.

1.7 Social Deprivation vs Social Isolation: Historical Context, Ethical Delineation

In 1958, Harlow and Zimmerman infamously raised baby Rhesus monkeys in isolation chambers with either a "cloth mother" that provided comfort but no food, or a "wire mother" that provided food but no comfort. This study deprived the Rhesus monkeys of social touch, and the intense distress and permanent behavior deficits demonstrate the powerful role of social contact in shaping behavior and brain development. Their study established the importance of social and emotional bonds, as the monkeys would choose the comfort of touch over food. While their study is often cited as a foundational work, it is important to note that their study is now widely condemned for its extreme and unnecessary cruelty. It is now well established in the literature that long term social isolation produces detrimental effects on behavior and brain function, causing anxiety, depression-like symptoms and even self-harming. In contrast, the present study does not employ isolation, is not interested in replicating cruel studies, and did not revisit the questions we already have the answers to: long term social isolation is detrimental. Importantly, no rats were harmed in this study and they were housed in the same housing room as their former cage mates, leaving them with olfactory, auditory, and visual contact with conspecifics. Brief social deprivation (< 24 hours) was used as one of the conditions to mimic ethologically relevant reductions in social interaction to study how these brief periods of reduced social contact may shift motivational states and increase social drive.

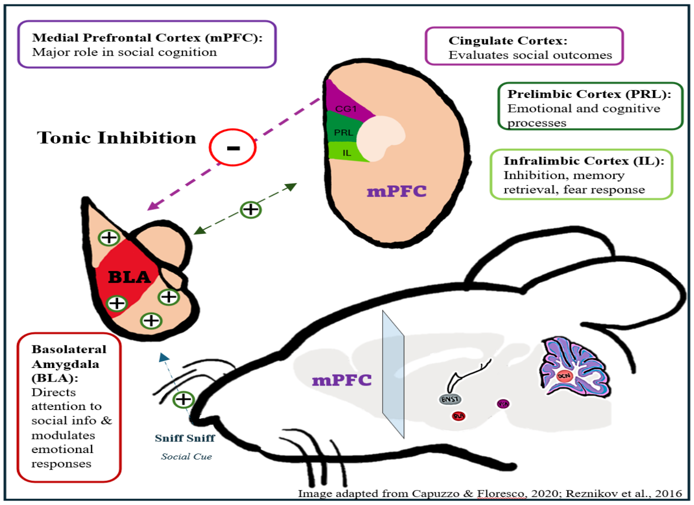


Image adapted from Capuzzo & Floresco, 2020; Reznikov et al., 2016

Figure 3: Prefrontal Cortex-Amygdala Circuit
Involved in social cognition, emotional regulation, and decision-making.

1.7.1 Hypothesis

We hypothesized that (1) depriving rats of fulfilling their need for social interaction would increase ACC-BLA activity social drive, (2) sating those social needs would lower ACC-BLA activity and social drive, and (3) male and female neural activity would differ and manifest in distinct behavioral phenotypes.

2. MATERIALS AND METHODS

2.1 Sprague-Dawley rat husbandry and acclimation to handling

All experiments were approved by the Institutional Animal Care and Use Committee at Rosalind Franklin University of Medicine and Science. Subjects were young adult male and female Sprague–Dawley rats purchased from Envigo (male n = 29, female n = 33; Indianapolis, IN, USA). Rats were housed in the Rosalind Franklin University Biological Resource Facility in a climate-controlled animal room (2 per cage before experimental housing manipulations, separated by sex; ± 2 ◦C; 60% humidity) under a 12 h light/dark cycle (lights off at 8AM) and fed standard rat chow and water ad libitum. Male rats arrived at the animal facility at postnatal day (PND) 70-75 and females at PND 65-75 and acclimated for at least 7 days prior to experimental procedures, this included 48 hours to adjust to the stress of shipping and at least 3 days to acclimate to human handling by the researchers. At the time of all experiments without survival surgery, males were between the ages of PND 77-82 and females were between PND 73-83; rats with surgical manipulations were allowed a minimum of four weeks to recover and tested between PND 122-132.

2.2 Social Conditions

To study how brief changes to the social environment can influence social behaviors, rats were randomly assigned to one of three conditions: social deprivation, social satiation, or control housing. Rats placed in the brief social deprivation group were separated from their cagemate and single-housed without enrichment for at least 24 – 48 hours before conducting behavioral assays. Rats in the social satiation group were housed in groups of 4 rats per cage overnight. Because all rats arrived at the animal facility in 2 rats per cage housing, this was used as control housing and no changes were made to the control group's housing.

2.3 Behavior Assays

2.3.1 Experimental Timeline

Before testing began, rats were weighed and marked with identifying tail marks and fur dye using animal safe dye. On experimental day 1, rats were allowed to acclimatize to the middle portion of the social preference chamber for 15 minutes in pairs. On experimental day 2, they were placed into the experimental housing conditions to which they were randomly assigned. On experimental day 3, rats were tested individually in a social preference test, with ten minutes to explore the empty apparatus and an additional 10 minutes to explore after adding the novel stimuli. On experimental day 4, rats were placed into the middle chamber of the social preference apparatus in pairs for a social interaction session with their original cagemate for 10 minutes. Rats were perfused 40-60 minutes after ending the social interaction session. Rats were tested across a period of less than one week between 8am and 6pm and weighed before each testing session.

2.3.2 Acclimation to three-chamber social preference apparatus

Rats were acclimated to the three-chamber apparatus used for the social preference tests in pairs with their original cagemate. The middle chamber of the apparatus was used as a miniature open field and rats were placed for 15 minutes one day before housing manipulations.

2.3.3 Social Preference Test

To assess how the experimental manipulations affected the social preferences of rats, they were placed individually into a three-chamber social preference apparatus on experimental day three for a social preference test. Before testing, rats were removed from their experimental housing and placed into transport cages before being brought into the testing room. Their transport cage was placed on a table near the testing apparatus before retrieving the novel rat. Novel rats were the same sex as the test rat and were alternated to avoid overtiring them. Novel rats were also placed into transport cages and brought into the testing room before being placed on the same table as the test rat. During the first part of the testing, the experimental rat was allowed to freely explore the entire apparatus with two empty novel stimuli cages placed in the corners of opposing chambers. This allowed them to acclimate to the chamber before the novel stimuli was added. After ten minutes, the researcher confined the rat to the middle chamber of the apparatus by gently placing wall inserts on either side of it. Then a novel rat of the same sex was placed into one of the cages and a novel object (brightly colored toy) was placed into the opposite cage before removing the wall inserts and allowing the experimental rat to freely explore the apparatus with the added novel stimuli for an additional 10 minutes. After the second part of testing was completed, the experimental rat was removed from the chamber and placed back into a transport cage before being returned to experimental housing. The novel rat was then removed and returned to its original housing.

2.3.4 Social Interaction Assay

The middle chamber of a three-chamber social preference apparatus was used as a modified open field by adding wall inserts to limit rats to only the middle chamber. This was done to limit the need for repeated acclimations to different chambers. On experimental day 4, rats were placed in pairs into the middle chamber of a three-chamber social preference apparatus for 10 minutes under dim white light (approximately 15 lx). Video was captured (DBK 27AUR0135, The Imaging Source, Charlotte, NC, USA) and acquired with ANY-maze software (Stoelting, Inc., Wood Dale, IL, USA), although the software was not used for scoring. They were tested with their former cage mates, in the case of those that were in the social deprivation condition (1 rat/cage). The animals in the social satiation condition (4 rats/cage) were tested with their original cagemates. Controls (2 rats/cage) were tested with their cage mates.

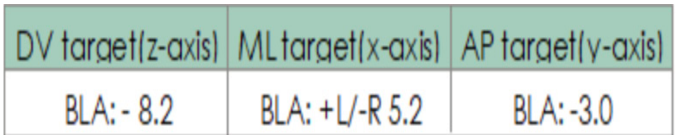
Animals were perfused within 40-60 minutes of the end of the assay. Because olfaction is the primary social cue in rats, analysis of sniffing behavior provides a behaviorally relevant measure of social engagement.

2.3.5 Supervised Machine Learning Behavior Classification

Each social interaction assay was recorded, and the videos were manually labeled in SLEAP (Social LEAP Estimates Animal Poses). SLEAP is an open source deep-learning based framework for multi-animal pose tracking (Pereira et al., 2022). SLEAP uses deep neural networks that require training and proofreading. Because of this, the types of videos used to train it affect its ability to accurately learn how to estimate the poses of each animal without mixing them up. As some of the social interaction assays have excessive glare from the animals urinating in the chamber, not all of the videos have accurate pose estimations yet. Training the models is an ongoing effort, and so the data analyzed in this portion will only include the videos that have been reliably tracked. After training SLEAP, pose estimations were obtained and fed into another software, SIMBA. SimBA is an open-source software that uses supervised machine learning algorithms to perform supervised behavioral classification (Goodwin et al., 2024). Sniffing bouts were calculated using pose estimation and spatial metrics extracted from SimBA and SLEAP. A sniffing bout was defined as the Euclidean distance between a rat's nose and conspecific's body remaining within ~100 mm (converted from pixels) for a duration of at least 3 seconds. The total number of sniffing bouts was calculated, as well as the total time sniffing, and the average duration of a sniffing bout was calculated (total time sniffing / total number of sniff bouts). Sniffing events were categorized into three interaction types: nose-body, nose-anogenital, and nose-nose. Due to limitations in some video quality, such as overexposure, or grainy quality, certain interactions have yet to be analyzed accurately. Training the model to work with videos where the animals urinate in the chamber has been an ongoing challenge, as the glare confuses the algorithms into thinking the bright spot is a white rat. The videos with unreliable tracking will not be involved in the following analysis.

2.4 Retrograde Tracer Surgery

In a separate set of experiments, retrograde virus was intracranially injected into the basolateral amygdala (BLA). Rats were first anesthetized with 4% isoflurane and oxygen until deeply anesthetized and maintained at 1.5–2.5% for the remainder of the surgery. Lidocaine was applied to their ears using a cotton swab to prevent ear pain from the ear bars, and an eye moistening solution was applied to their eyes after they were anesthetized. Rats received a 0.2 mL injection of lidocaine to the scalp parallel to the body to prevent additional discomfort. A 10 µL Hamilton syringe with a 33-gauge needle was mounted onto a stereotaxic infusion. All groups received alternating unilateral infusions from bregma, with the following stereotaxic coordinates used for the retrograde injections to target the BLA: dorsal/ventral = -8.2, medio/lateral = +/- 5.2, and anterior/posterior = -3.0. The syringe was left in place for an additional 5 min following virus infusion to allow for diffusion. They were given meloxicam before waking to ensure no additional discomfort, and they received daily meloxicam following 3 days after the procedure.



DV target(z-axis)	ML target(x-axis)	AP target(y-axis)
BLA: - 8.2	BLA: +L/-R 5.2	BLA: -3.0

Table 1. BLA Retrograde Injection Stereotaxis Coordinates
Each rat received an injection to either left or right BLA (n = 12).

2.5 Microscopy

Amygdala regions were identified based on the rat brain atlas (Watson, 2007). Basolateral amygdala regions were captured on a Nikon Eclipse E600 microscope (Melville, NY, United States) using a 10× objective lens. Each animal had two slides, one for Zif268 staining and another for pPDH staining. Each slide had three cortex sections and three amygdala sections. Each cortex had three regions of interest captured bilaterally for a total of six images per cortex section (6 CG1, 6 PRL, 6 IL per slide). Each amygdala section had bilateral squares captured (3 left BLA, 3 right BLA per slide). Images were taken using a 4x objective lens to ensure proper mapping for each region of interest back to the rat brain atlas. Images were then exported as TIFF files, and particles were quantified using ImageJ software (NIH, Bethesda, MD, United States).

2.6 Sacrifice of Rats using Timed Transcardial Perfusions

Rats were deeply anesthetized via inhalation of isoflurane vapor in a closed chamber until they were fully unresponsive to paw pinch and eye blink reflex check. Once fully anesthetized, a thoracotomy was performed immediately to expose the heart and the diaphragm was incised to prevent the animal from regaining consciousness. Brains were extracted, post-fixed in 10% formalin for 24 hours, and stored in PBS. Brains were sliced coronally at 40 µm using a vibratome (Leica CM3050 S), and cryoprotected in a freezing medium until further processing.

2.7 Immunohistochemistry

Immunohistochemistry was used to measure the activation and inactivation of the basolateral amygdala (bilateral), cingulate cortex, prelimbic cortex, and infralimbic cortex of each rat. Out of the 5 cohorts, 3 of them were stained with phosphorylated pyruvate dehydrogenase, a novel antibody staining method used as a proxy of cellular inactivation. This included the cohort that had the survival surgery, as well as one additional male and female cohort. All 5 cohorts were stained with immediate early gene zif268 as a proxy of cellular activity. These antibodies were stained on separate slides with ALEXA FLUOR 488 goat anti-rabbit and coverslipped with DAPI counterstain.

2.7.1 pPDH staining for neural inactivation

Brains were sliced on a vibratome in 40 µm sections and mounted onto gelatinized slides. Slides are washed in phosphate-buffered saline (PBS) to remove debris and prepare the tissue for staining. Slides were incubated in a 1% hydrogen peroxide solution (PBS + 0.1% H_2O_2) used to quench endogenous peroxidase activity that could cause nonspecific staining. Slides were permeabilized (PBS + 0.3% Triton X100). and incubated in a blocking solution for 1 hour (PBS + 5% normal goat serum) before incubating with the phosphorylated pyruvate dehydrogenase (pPDH) antibody at 1:250 dilution to target the protein of interest for 24 hours at 4°C. Slices were then incubated in a secondary solution (1:500, Alexa Fluor 488) for 2 h, rinsed with wash buffer, and cover slipped with a DAPI counterstain.

2.7.2 ZIF staining for neural activation

Brains were sliced on a vibratome in 40 µm sections and mounted onto gelatinized slides. Slides are washed in phosphate-buffered saline (PBS) to remove debris and prepare the tissue for staining. Slides were incubated for 15 minutes in a 1% hydrogen peroxide solution (PBS + 0.1% H_2O_2) used to quench endogenous peroxidase activity that could cause nonspecific staining. Slides were washed in wash buffer (PBS + 0.1% Tweenx20) and permeabilized (PBS + 0.8% Triton X100) for 15 minutes. Slides were then incubated in blocking solution for 30 minutes (PBS + 5% normal goat serum), and then EGR1 (zif268) antibody (1:500 dilution) for 48 hours at 4°C. Slides were then incubated in a secondary solution (1:500, Alexa Fluor 488) for 2 h, rinsed with wash buffer, and cover slipped with a DAPI counterstain.

2.8 Quantification and Statistical Analysis

2.8.1 Quantification of ZIF268

For quantification of EGR1 (ZIF268), images were analyzed by ImageJ. The Paxinos and Watson rat brain atlas was referenced to delineate individual brain regions by drawing Regions of Interest (ROI) using the ROI Manager function by ImageJ. ROIs were saved as .zip files for batch analysis. Image brightness and contrast were normalized across all images within each experiment, using a single parameter across all control and experimental groups. Cell counting of DAPI labeled nuclei (DAPI$^+$) was performed using the particle analysis function in Image. Cell counting was performed on positive staining cells (ZIF$^+$) using custom Macro scripts modified from the methods of Ferrara et al (2019). The script looped through each image, applying background subtraction and difference of Gaussian filtering before converting the image to binary and applying the watershed function to separate overlapping particles. The script then located the appropriate ROI.ZIP file for each image and ran the “Analyze Particles” function within each region of interest using a circularity between 0.65 and 1.00 to ensure specificity to ZIF$^+$ labeled cells. ZIF expression was quantified as a proportion of total cell count (ZIF$^+$/DAPI$^+$).

2.8.2 Quantification of pPDH

For quantification of pPDH, images were analyzed by using the same methods for delineating brain regions mentioned in the ZIF quantification with the addition of adding a square of the fiber tracts adjacent to the BLA or cortical layers I/II for the mPFC to the Roi Manager to use for background correction. Custom Macro scripts were generated using parameters modified from the methods section of (Yang et al., 2024). Image brightness and contrast were normalized across all images within each experiment, using a single parameter across all control and experimental groups. Cell counting of DAPI labeled nuclei (DAPI+) was performed using the particle analysis function in ImageJ. Custom Macro scripts were used to measure the mean intensity of positive cells (PPDH+) to account for the more diffuse distribution of cytoplasmic and neurite staining. The script looped through each image and applied the standardized contrast adjustments before locating the appropriate ROI.ZIP file for each image and applying the “Measure” function to quantify the mean intensity for each region of interest and the background square. Final intensity values were calculated by subtracting background intensity from ROI intensity.

2.8.3 Quantification of Retrograde Virus-Labeled Neurons

For quantification of retrograde virus-labeled neurons, images were analyzed by using the same methods for delineating brain regions mentioned in the ZIF quantification. Brightness and contrast were adjusting using consistent parameters however some images were adjusted if the retrograde labeled neurons were too bright to separate from each other. Injection site accuracy was assessed using the “Integrated Density” function to measure labeling density. Cell counting of DAPI labeled nuclei was performed using the particle analysis function in ImageJ. Cell counting was performed on positive staining cells (RETRO+) using custom Macro scripts modified from methods of Ferrara et al., 2019. Scripts looped through each image and applied the subtract background function and difference of Gaussian filtering before making them binary and applying the watershed function to separate overlapping particles. The script then located the appropriate ROI.ZIP file for each image and ran the “Analyze Particles” function within each region of interest using a circularity between 0.4 and 1.00.

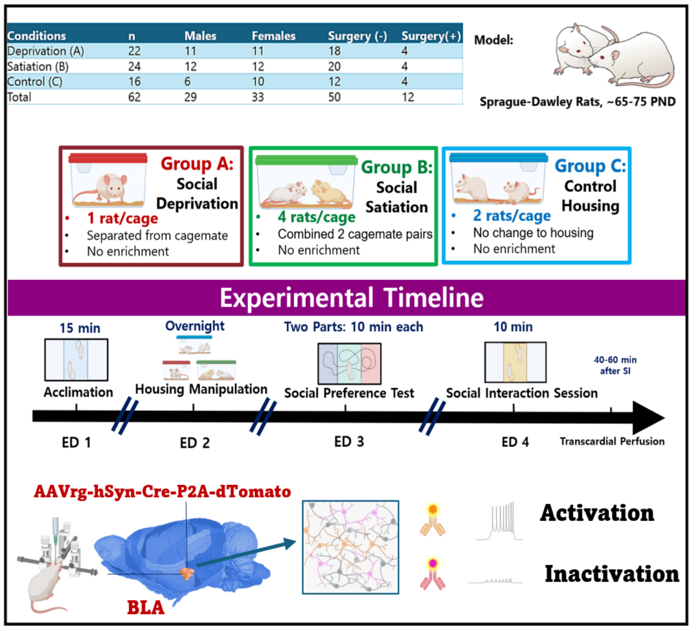
2.8.4 Statistical analysis

All statistical analyses were performed using Python (v3.12.9). Jupyter Lab and Spyder were used as integrated development environments for organizing data, performing analyses, and generating visualizations. For comparisons between time spent with the social stimuli versus the nonsocial stimuli, paired samples t-tests were used on the raw scored collected from Any-maze. For between-group comparisons, raw scores collected from ANY-maze, SIMBA, and ImageJ were transformed as needed and analyzed using one-way ANOVA. Two-way ANOVA was used to assess interactions between sex and experimental condition. Post hoc comparisons were corrected for multiple testing using Sidak’s correction. Multivariate linear regression models were performed using ordinary least squares (OLS) to explore relationships between variables. Assumptions of normality and homogeneity of variance were assessed using the Omnibus, Shapiro-Wilk, and Levene’s tests along with visual inspection using Q-Q plots of residuals. When assumptions were violated, non-parametric tests were used: Kruskal-Wallis and Mann-Whitney U for comparisons between groups and aligned rank transform (ART) ANOVA for interaction effects. Post hoc comparisons for non-parametric analyses were performed using Dunn’s test and ART-corrected post hoc. Exploratory analysis, such as dimensionality reduction techniques and unsupervised machine learning techniques were used to explore the dataset and understand its structure before performing statistical analysis. Custom Python and Macro scripts were developed to conduct these analyses, and original code is available upon request.

3. Results

3.1.0 Social Preference Test Measures

To assess the effect of the conditions on recognition of social cues, social motivation, and social preference, animals were individually tested in a dark room using a three-chambered social preference task. Prior to testing, each animal was placed into the opaque black plexiglass social preference apparatus for a 10 minute acclimation period. This allowed them to freely explore all three chambers of the apparatus in the presence of empty stimuli cages located on either end. This period allowed animals to acclimate to the testing environment and minimize any potential stress induced from handling or change of scenery. Following acclimation, a novel object and a novel age- and sex-matched rat were placed into each cage. Interactions with the social stimulus (novel rat) and non-social stimulus (novel object) were recorded and quantified across five primary behavioral measures: orienting towards the social stimulus, total investigation time, total number of investigation bouts, and average investigation bout duration. To control for any potential confounds due to stress and anxiety, the following additional measures were included: time spent freezing, time spent in the middle chamber relative to the left or right arms, overall locomotion, and weight changes. These metrics were included to aid in interpretation of findings and to ensure that any significant behavioral differences between conditions reflected social preference specifically, rather than general stress responses.



Conditions	n	Males	Females	Surgery (-)	Surgery(+)
Deprivation (A)	22	11	11	18	4
Satiation (B)	24	12	12	20	4
Control (C)	16	6	10	12	4
Total	62	29	33	50	12

Figure 4. Experimental Design

The sample included males and females (n = 62) across three conditions: social deprivation, social satiation, and a control condition. The experimental timeline for spanned a period of four days for each cohort (typically 12 rats per cohort) for young adult Sprague Dawley rats (PND 65-75). One cohort was used for intracranial injections to the basolateral amygdala for retrograde tracing.

Each of the behavioral measures from the social preference test was designed to compare interactions with the social stimulus (T_S) against interactions with the non-social stimulus (T_{NS}). A relative preference ratio (R_{SP}) was calculated, $R_{SP} = T_{S} / T_{NS}$, and used to assess the relative magnitude of social preference while preserving the original units of measurement. This ratio used interactions with the non-social stimulus as the animal's baseline novelty preference. Values range from 0 to infinity and were used to compare the relative magnitude of social preference, with higher values indicating a greater preference for the social stimulus.

Behavioral tracking software was used to quantify the amount of time spent in each of the designated investigation zones placed two inches around each cage. An investigation bout begins when the animal's head is inside the investigation zone and it is oriented towards the zone and ends when its head is no longer inside the zone, or when it stops orienting towards the zone. The number of investigation bouts is equal to the number of bouts that began. The total time spent investigating each of the zones was used for analysis to compare differences in the duration of time in seconds that were spent with the social stimulus compared to the non-social stimulus. The average duration of investigation bouts was calculated by dividing the total time spent investigating (seconds) by the total number of bouts of investigation to the zone. The total amount of time the animal was oriented towards each of the investigation zones was also analyzed. Orienting begins when the is oriented towards the investigation zone while its head is outside of the zone. Orientation is determined by the angle (60°) between the animal's vector (determined by a point at the base of the neck) and a zone vector (determined by multiple points on the borders of the zone).

For overall stress measures, the total number of freezing bouts while investigating the zones were analyzed. Freezing is reported as a count of the start of each bout, and each time the animal starts to freeze the count is increased by one. For this measure, manual verification of each recorded video was necessary and videos were retracked when necessary to ensue that the video tracking software was not mislabeling freezing behavior. After verifying the true freezing count, the number reported by the software was adjusted. There was not freezing behavior observed by every animal, so analysis was limited to binary classifications of whether or not the animal froze (0: did not freeze, 1: did freeze) and the total percentage of animals that froze by condition was documented.

Outlier detection using Interquartile Range (IQR) with a standard multiplier of 1.5 was initially performed on the R_{SP} values, however after noticing that 3-8 animals were being flagged per measure, a social preference index was included as an additional validity check to avoid compromising the true variability within the dataset. One subject was dropped from the social preference analysis due to repeated escape attempts that forced the assay to end early. The social preference indexes for each measure, $I_{SP} = (T_S - T_{NS}) / (T_S + T_{NS})$, were compared between groups by dividing the difference between the social and non-social stimuli and dividing by the total time spent at both cages. The index ranges from -1 to 1, with scores closer to -1 suggesting preference for the non-social stimulus, scores closer to 1 suggesting preference for the social stimulus, and scores of 0 indicating there is no preference for either the non-social or social stimulus. Unlike the preference ratio, the preference index assesses both the magnitude and directionality of preference. Both I_{SP}, and R_{SP} were used to interpret the effect of social condition on social engagement, social interest, social interaction, and overall social preference. To ensure that natural variability is not lost to strict outlier thresholds given the sample size, the IQR multiplier was increased to a value of 2.2 to leave the natural, meaningful differences intact and only dropping extreme outliers. Outlier detection was performed by condition within each individual measure.

3.2.0 Stress Response Results

To determine whether the social conditions induced a stress response, the average time spent freezing near the social and non-social stimulus, and the time spent in the middle chamber compared to the left and right chambers was assessed. Because the novel stimuli were in the left and right chambers, time spent in the periphery can be classified as an investigatory behavior. Time spent in the middle chamber can be interpreted as avoidance, anxiety, or a lack of motivation to investigate either stimulus. The means and standard deviations of average investigation bout to each chamber are presented in Table 2.

A two-way ANOVA was performed to determine if sex (male, female), condition (deprivation, satiation, control), and interactions between sex and condition affected the average time spent in the middle chamber. Sex did not have a significant effect, F(1, 55) = 1.14, p = .29, $\eta p^2 = .02$, nor did condition, F(2, 55) = .83, p = .44, $\eta p^2 = .029$, and there was no significant interactions between sex and condition on the average duration of time spent in the middle chamber, F(2, 55) = .677, p = .512, $\eta p^2 = .024$ (Table 3).

Table 2

Descriptive Table of Average Investigation Bout to the Each Chamber

			Chamber					
			Left		Middle		Right	
Sex	Condition	N	M	SD	M	SD	M	SD
Female	Deprivation	10	3.89	1.51	2.28	0.57	2.78	1.21
	Satiation	12	3.74	0.96	2.24	0.58	3.00	0.95
	Control	10	4.54	1.36	2.99	1.89	3.08	0.74
Male	Deprivation	11	2.93	0.37	2.48	0.87	2.84	0.83
	Satiation	12	3.44	0.83	3.13	2.35	5.03	6.28
	Control	6	3.68	1.30	2.85	0.93	2.50	0.53

Table 3

Two-Way ANOVA Summary: Average Investigation Bout in Middle Chamber

Source	Sum of Squares	df	MS	F	p	$\eta^2 p$
Condition	3.37	2	1.69	0.83	0.441	0.029
Sex	2.31	1	2.31	1.14	0.291	0.02
Condition * Sex	2.75	2	1.37	0.68	0.512	0.024
Residual	111.67	55	2.03			

A repeated-measures ANOVA was conducted to determine whether condition (deprivation, satiation, control) had a significant effect on the average investigation bouts to each chamber of the apparatus (left, middle, right). The means and standard deviations are presented in Table 2. The repeated-measures ANOVA revealed a significant main effect of chamber, F(2, 116) = 5.81, p = .004, $\eta^2 p = .091$, indicating that rats spent different amounts of time across chambers. The Greenhouse-Geisser correction was applied due to violation of sphericity (W = .552, p < .001). There was no significant main effect of condition, F(2, 58) = 0.99, p = .377, $\eta^2 p = .033$, and no significant interaction between condition and chamber, F(4, 116) = 1.55, p = .192, $\eta^2 p = .051$ (Table 4). Post-hoc pairwise comparisons with Holm correction showed that rats spent significantly more time in the left chamber (M = 3.68, SD = 1.15) compared to the middle chamber (M = 2.65, SD = 1.41), p <.001, and more time in the right chamber (M = 3.30, SD = 2.93) compared to the middle chamber, p = .043, regardless of condition. No significant difference was found between the left and right chambers, p = .336. See Figure 5 for violin plots of average investigation bout distributions and representative heatmaps for each condition.

Table 4

Repeated-Measures ANOVA Summary: Time Spent in Each Chamber by Condition

Source	Sum of Squares	df1	df2	MS	F	p-unc	p-GG-corr	np2	eps	sphericity	W-spher	p-spher
Condition	12.056	2	58	6.028	0.992	0.377	NaN	0.033	NaN	NaN	NaN	NaN
Chamber	33.439	2	116	16.72	5.813	0.004	0.011	0.091	0.691	FALSE	0.552	0
Irteraction	17.856	4	116	4.464	1.552	0.192	NaN	0.051	NaN	NaN	NaN	NaN

Note. Greenhouse-Geisser correction applied to Chamber due to violation of sphericity.

Next, the number of freezing bouts near the social and non-social stimulus were used as a measure for stress induced by the novel stimuli. As there were animals that did not freeze at both stimulus cages and some that did not freeze at all, freezing near the cages was converted into a binary (1: froze, 2: did not freeze) for each stimulus. The percentage of rats freezing near the novel stimuli cages is shown in Table 5 by sex and condition.

Table 5

Descriptive Table of Freezing Proportions Near Novel Stimuli

			Location					
			Social			Non-Social		
Sex	Condition	n	Yes Freeze	No Freeze	% froze	Yes Freeze	No Freeze	% froze
Female	Deprivation	10	7	3	70	8	2	80
	Satiation	12	10	2	83.3	8	4	66.7
	Control	10	9	1	90	8	2	80
Male	Deprivation	11	9	2	81.8	5	6	45.5
	Satiation	12	9	3	75	8	4	66.7
	Control	6	5	1	83.3	4	2	66.7

A chi-square test of independence showed that there was no significant association between condition and freezing near the social stimulus, X^2 (2, N = 61) = .676, p = .713, nor the non-social stimulus, X^2 (2, N = 61) = .712, p = .700. See Figure 6 for bar graphs of freezing behaviors near the novel stimulus cages.

3.3.0 Average Investigation Bout

The duration of the average investigation bout was used as a baseline measure for the animal's broad preference, as it combined two of the other core measures (total time investigating the zone / total number of investigations to the zone). For the preference ratio, this value indicates the proportional magnitude of preference, measured in seconds, for the social stimulus relative to the non-social stimulus. For the preference index, the average duration of bouts to both the social and non-social investigation zones are considered to assess the effect on preference towards both the social and non-social stimulus.

Two animals were removed from the preference ratio and preference index measures after being flagged as outliers by IQR. Normality of the preference ratio for total time investigating was assessed using the Shapiro-Wilk and Omnibus tests, both of which indicated significant deviations from a normality across conditions. This was further confirmed by Q-Q plot inspection and the Jarque-Bera test which showed there was skewness and high kurtosis. Because of this, non-parametric measures were used for the preference ratio for violating assumptions.

To test whether interactions between sex and condition affected the preference ratio average investigation bout, an Aligned Rank Transform (ART) ANOVA was performed. The ART ANOVA suggested that there was not a significant interaction between sex and condition on the preference ratio for average investigation bout duration, F(2, 54) =2.31, p = .109, ηp^2 =.079. The results also indicated that there was not a significant effect of condition, F(2, 54) = 2.23, p =.118, ηp^2 =.076, or of sex, F(1, 54) = 1.31, p = .256, ηp^2 = .024.

The preference ratio values were then split by sex to determine whether individual sex differences would emerge when analyzing the effect of condition on average investigation bout duration. A Kruskal-Wallis H test was performed on the preference ratios of the males to determine whether condition had a significant effect average investigation bout duration to the social stimulus. Condition did not have a significant effect on the preference ratios of the males, H(2) =1.644, p = .440, ηp^2 = .059. Next, the preference ratios of the females were assessed using the Kruskal-Wallis H test, with no significant effect of condition found on average investigation bout duration, H(2) = 4.220, p = .121, ηp^2= .141.

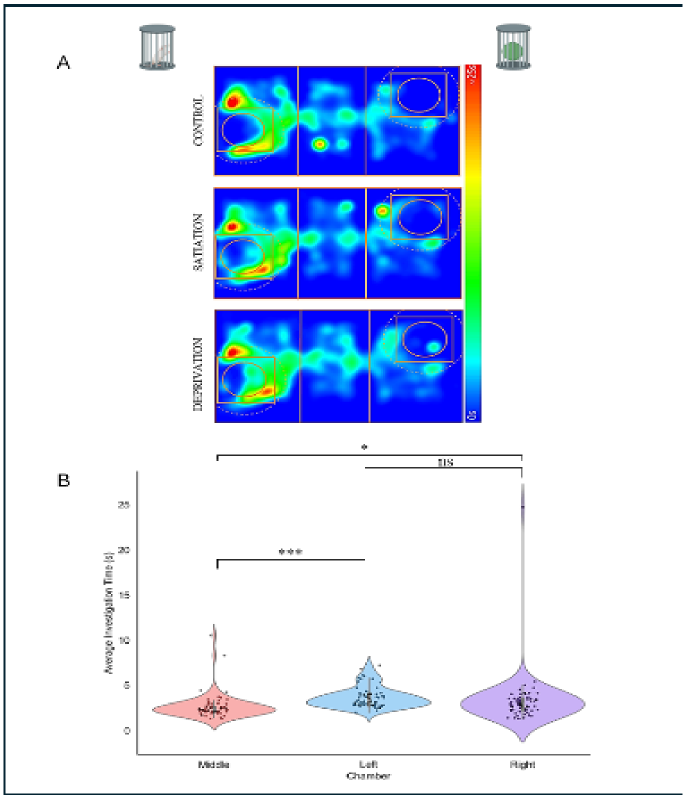


Figure 5. Chamber Preference During Social Preference Test

A. Representative heatmaps of time spent in each chamber with the social stimuli in the left chamber and the non-social stimuli in the right (n = 61). B. Violin plots of average investigation bout distribution for each chamber indicating preference for peripheral chambers regardless of condition. Median, interquartile range, and 95% confidence intervals are shown within each plot. ***p < .001, **p < .01, *p < .05.

Next, the social preference index for average investigation bout duration was examined. A two-way ANOVA was performed using the preference index to investigate whether social condition (deprivation, satiation, control), sex (male, female) or condition and sex interactions had a significant effect on the average investigation bout duration. The results indicated a trend towards a significant effect of condition, F(2, 54) = 2.71, p = .076, ηp^2 =.091, on the preference index score of average investigation bout duration, such that the animals in the social deprivation condition (M =.421, SE = .039), spent a greater amount of time with the social stimulus compared to the animals in the social satiation condition (M = .284, SE = .043), p =.072. The results also indicated that there was not a significant effect of sex, F(1, 54) = .704, p = .405, ηp^2 = .013, on preference index scores of average investigation bout duration. There were no significant interactions between sex and condition, F(2, 54) = .1.02, p = .369, ηp^2 =.036, on preference index scores of average investigation bout duration.

Given the trend towards a significant interaction of condition on the preference index scores of average investigation bout, a one-way ANOVA within each sex was conducted to test for the possibility of sex-specific behavioral responses. A one-way ANOVA of the males showed no significant effects of condition on the preference index, F(2, 26) = .415, p = .664, ηp^2 =.031, indicating that the average investigation bout was not impacted by the social conditions of the males.

A one-way ANOVA of the females indicated that condition did have a significant effect on the preference index of females, F(2, 28) = 3.389, p = .048, ηp^2 = .194. Post-hoc using Tukey’s HSD showed a trend towards a significant difference in preference index scores between the females in the social deprivation condition (M = .422, SE = .057) and the females in the social satiation condition (M = .217, SE = .031), p = .065. This result indicates two findings: (1) the females in the social deprivation condition had longer investigation bouts to the social stimulus, and (2) the females in the social satiation condition spent more time on average investigating the non-social stimulus compared to the deprivation females.

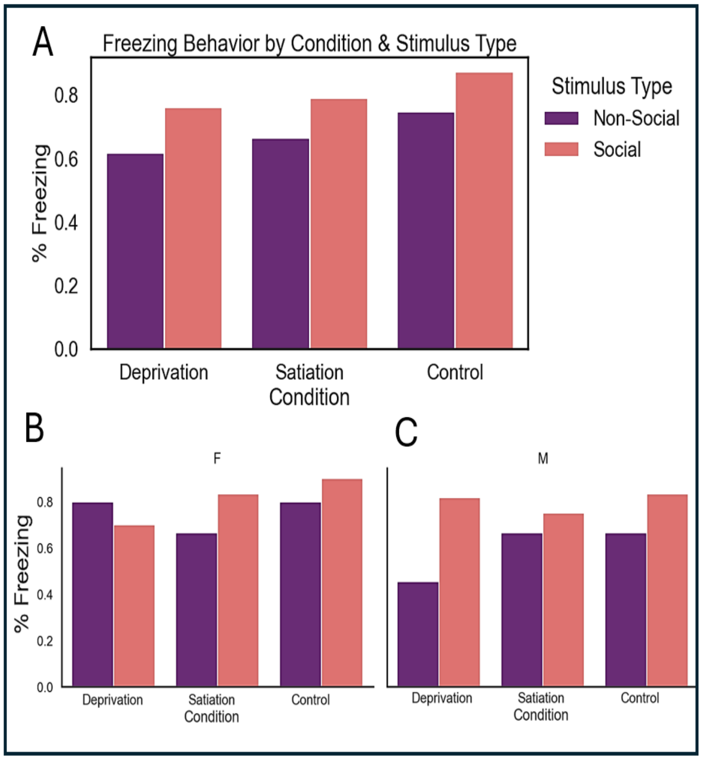


Figure 6. Freezing Behavior By Condition, Sex, and Stimulus Type
A. Bar graph showing the proportion of rats by condition that froze at either the social or non-social stimulus (n = 61). B. Proportion of female rats that froze at either the social or non-social stimulus (n = 32). C. Proportion of male rats that froze at either the social or non-social stimulus (n = 29).

3.4.0 Total Number of Investigation Bouts

To assess differences in social preference, the total number of investigations to both the social and non-social stimulus cages were assessed. Behavioral tracking software was used to quantify the amount of time spent in each of the designated investigation zones placed two inches around each cage. An investigation bout begins when the animal’s head is inside the investigation zone and it is oriented towards the zone and ends when its head is no longer inside the zone, or when it stops orienting towards the zone. The number of investigation bouts is equal to the number of bouts that began.

A two-way ANOVA was first conducted to determine whether the total number of investigation bouts varied based on the conditions manipulating opportunities for social need fulfillment (social deprivation, social satiation, and control housing), sex (male, female), or sex and condition interactions had a significant effect on the total number of investigation to the social stimulus. The main effect of condition was not significant, F(2,56) = .022, p = .979, ηp^2 =.001 however there was a trend towards a main effect of sex, F(1, 56) = 3.309, p = .069, ηp^2 = .056. The preference ratio did not show a significant interaction of sex and condition on the total number of investigation to the social stimulus, F(2, 56) = 1.993, p = .146.

A two-way ANOVA was also performed on the preference index, and there was a significant main effect of sex on the number of investigations to the social stimuli, F(1,56) = 5.48, p =.024, ηp^2 = .089, such that males (M =.383, SE =.043) investigated the social stimulus a greater number of times than the females (M = .256, SE = .034) The social preference index did not have significant of effect of condition, F(2, 56) = .103, p = .901, ηp^2 = .004, nor were there significant interactions of sex and condition, F(2, 56) = .914, p = .407, ηp^2 = .032.

Next, a one-way ANOVA was conducted to determine whether the total number of investigation bouts varied based on the conditions manipulating opportunities for social need fulfillment (social deprivation, social satiation, and control housing). The results showed no significant differences between the social conditions, for either the preference ratio, F(2, 59) = 0.001, p = .999, ηp^2 = .001 or the social preference index, F(2, 59) = .012, p = 0.989, indicating no effect of condition on the number of investigations to the social stimulus.

3.5.0 Total Time Investigating

To assess differences in social preference, the time spent investigating both the social and non-social stimulus cages was analyzed. Normality of the preference ratio for total time investigating was assessed using the Shapiro-Wilk and Omnibus tests, both of which indicated deviations from a normal distribution across conditions. This was further confirmed by Q-Q plot inspection and the Jarque-Bera test which showed there was skewness and high kurtosis. Because of this, the Kruskal-Wallis H test was conducted to assess the effect of condition on total time investigating the social stimulus. The Kruskal-Wallis H test indicated condition had no significant effect on preference ratios of total time spent investigating the social stimulus, H(2) = 1.05, p = .59. To test whether interactions between sex and condition affected the preference ratio for total investigation time, an aligned ranks transformation (ART) ANOVA was performed. The ART ANOVA suggested that there was no significant interaction between sex and condition on the preference ratio, F(2,56) =1.471, p = .238, ηp^2=0.05.

The preference ratio values were then split by sex to determine whether individual sex differences would emerge when analyzing the effect of condition on time spent investigating the social stimulus. A Kruskal-Wallis H test was performed on the preference ratios of the males to determine whether condition had a significant effect on total time spent investigating the social stimulus. Condition did not have a significant effect on the preference ratios of the males, H(2) = 1.58, p = .455, ηp^2 = .054. Next, the preference ratios of the females were assessed using the Kruskal Wallis H test, with no significant effect of condition found, H(2) = 3.436, p =.179, ηp^2=.111.

The social preference index was also slightly skewed, and an omnibus test indicated that it did not have a normal distribution, however the kurtosis was only slightly elevated, and the skew was moderate. The residuals were inspected using Q-Q plots and found to only have slight deviations from normality. Given that ANOVA is generally robust to moderate violations of its assumptions, it was determined that the analysis would proceed with parametric testing (Caldwell et al., 2020).

A two-way ANOVA was performed using the preference index to assess whether social condition (deprivation, satiation, control), sex (male, female), or sex and condition interactions had a significant effect on the total time spent investigating the social stimulus. There was a main effect of sex on total time spent investigating, F(1, 50) = 4.78, p = .03, ηp² =.08, such that males spent (M = .68, SE =.04) more time investigating the social stimulus compared to females (M = .52, SE = .04) regardless of condition. Condition also had a significant effect on total time investigating, F(2, 50) = 4.20, p = .02 ηp² = .143, such that deprivation males (M=.64, SE=.03) spent significantly more time with the social stimulus than the satiation females (M =.39, SE=.06), p=.031. The satiation females spent significantly less time with the social stimulus than the female controls (M=.66, SE= .04), p =.039, and more than the male controls as well (M=.68, SE = .05), p=.044. The interaction of sex and condition did not show significant effects, F(2, 50) = 1.51, p = .231, ηp² = .056.

Given the significance of sex on the total investigation time, a one-way ANOVA within each sex was conducted to test for the possibility of sex-specific behavioral responses. A one-way ANOVA using the preference index of the males to examine the effect of condition on total investigation time suggested that condition did not significantly affect total investigation time, F(2, 26) = .267, p =.768, ηp² = .02. The one-way ANOVA performed on the preference index of the females suggested that condition did have a significant effect on total investigation time, F(2, 24) = 6.66, p = .005, ηp² = .357.

3.6.0 Total Time Orienting

Orienting is considered a considered a measure of vigilance and sociability. This measures the amount of time each animal spent orienting towards the social and non-social stimulus. Total time orienting was examined to determine if the conditions manipulating the opportunities to have social needs met had an impact on vigilance.

IQR outlier detection flagged four animals as outliers when assessing the preference index, all from the social deprivation condition. The threshold was increased to a multiplier of 2.5 to ensure that the outlier check wasn't too sensitive, however the same four animals were still flagged. In fact, the multiplier increased to 2.9 and the animals were still flagged, only when the multiplier was set to a value of 3 were there no outliers detected. This validated that they were truly outliers, and they were not included in the total time orienting analysis (2 male, 2 female). The IQR outlier detection was run separately on the preference ratio, as it uses a different metric for computing the values and may detect different outliers when assessing magnitude in seconds, and this flagged one animal as an outlier (deprivation condition, female, also flagged in the preference index outlier detection).

Normality of the preference ratio for total time orienting towards the social stimulus was assessed using the Shapiro-Wilk and Omnibus tests, both of which indicated significant deviations from a normal distribution across conditions. This was further confirmed by Q-Q plot inspection and the Jarque-Bera test which showed there was skewness and high kurtosis at the tails. Because of this, the non-parametric tests were used on the preference ratio for total time orienting towards the social stimulus.

An ART ANOVA was performed to determine if the preference ratio for total time spent orienting towards the social stimulus varied by sex (male, female), social condition (deprivation, satiation, control), and the interaction between sex and social condition. The results suggested that there was not a significant effect of social condition on orienting towards the social stimulus, F(2, 54) = 2.30, p = .109, ηp² = .079, nor a significant effect of sex on the preference ratio for orienting F(1, 54) =.083, p =.775, ηp² = .022. There was not a significant interaction of social condition and sex on the preference ratio of time spent orienting towards the social stimulus, F(2, 54) = 1.23, p = .299, ηp² = .044.

The preference ratio values were then split by sex to determine whether individual sex differences would emerge when analyzing the effect of condition on average investigation bout duration. A Kruskal-Wallis H test was used to assess whether condition significantly affected male preference ratios for total time orienting towards the social stimulus. The results indicated that there was no significant effect of condition, H(2) = .402, p = .818, ηp² = .014, on the preference ratio for time spent orienting towards the social stimulus. Next, a Kruskal-Wallis H test was used on the females to determine whether social condition influenced the preference ratio for orienting towards the social stimulus. The results did indicate that there was a slight trend towards a significant effect of condition on the female's preference ratio for total time orienting towards the social stimulus, H(2) = 4.948, p = .084, ηp² = .165.

Next, a two-way ANOVA was conducted on the preference index to determine whether sex (male, female), condition (deprivation, satiation, control) and interactions between sex and condition had a significant effect on the preference index scores for total time orienting towards either the social or non-social stimulus. The results indicated a trend towards a significant effect of condition, F(2, 51) = 2.89, p = .064, ηp² = .098, such that the social deprivation condition (M = .382, SE = .026) spent more time oriented towards the social stimulus compared to the social satiation (M = .25, SE = .045) condition, p = .054. There was not a significant effect of sex, F(1, 51) = 2.65, p = .11, ηp² = .049, nor a significant interaction of sex and condition on the preference index for total time orienting, F(2, 51) =.699, p = .501, ηp² = .027. To further explore if the trend towards a significant effect of condition, the preference index scores were then split by sex to assess whether sex-dependent differences in orientation preference would emerge. A one-way ANOVA on the preference index of the males was conducted to determine if condition impacted male orientation preference, and the results suggested that there was not a significant effect of condition on male orientation preference F(2, 24) = .889, p = .424, ηp² = .069. A one-way ANOVA on the preference index of the females to assess the effect of condition showed a slight trend towards a significant effect of condition, F(2, 27) = 2.83, p = .07, ηp² = .173. This indicated that the females in the deprivation (M=.35, SE = .042) condition showed a slight preference towards the social stimulus compared to the females in the social satiation (M =.183, SE = .05), p = .082.

4.1 Social Interaction Measures

To assess whether social engagement was affected by the social conditions, the animals were placed in pairs into the middle chamber of the social preference apparatus. They were tested with their former cage mates, in the case of those that were in the social deprivation condition. The animals in the social satiation condition, which had four animals in one cage, were tested with their original cagemates. Controls were tested with their cage mates. The middle chamber of the social preference apparatus served as a modified open field, with wall inserts added to ensure the animals remained in the middle chamber for the duration of the assay. The social interaction assay was 10 minutes long and the animals were perfused within 40-60 minutes of the end of the assay.

Because olfaction is the primary social cue in rats, analysis of sniffing behavior provides a behaviorally relevant measure of social engagement. Sniffing bouts were calculated using pose estimation and spatial metrics extracted from SimBA and SLEAP, as described in the methods section. A sniffing bout was defined as the Euclidean distance between a rat's nose and conspecific's body remaining within ~100 mm (converted from pixels) for a duration of at least 3 seconds. The total number of sniffing bouts was calculated, as well as the total time sniffing, and the average duration of a sniffing bout was calculated (total time sniffing / total number of sniff bouts). Sniffing events were categorized into three interaction types: nose-body, nose-anogenital, and nose-nose.

Due to limitations in some video quality, such as overexposure, or grainy quality, certain interactions have yet to be analyzed accurately. Training the model to work with videos were the animals urinate in the chamber has been an ongoing challenge, as the glare confuses the algorithms into thinking the bright spot is a white rat. The videos with unreliable tracking will not be involved in the following analysis.

4.2.0 Nose-anogenital Results

Nose-anogenital sniffing is a way for rats to gain more social information from each other, so we wanted to investigate whether there would be a significant effect of the social conditions on the total time spent sniffing. A two-way ANOVA was performed assessing the affect of social condition and sex and their interactions on the log transformed total time sniffing in the nose-anogenital category. The result indicated that there was not a significant effect of condition on nose-anogenital sniffing, F(2,39) = 1.27, p = .293, ηp^2 = .922, nor a significant effect of sex F(1, 39) = 1.65, p = .206, ηp^2 = .089. Ther was not a significant interaction of sex and condition on nose-anogenital sniffing, F(2, 39) = .78, p = .47, ηp^2 = .038. A Pearson correlation revealed a strong, statistically significant positive relationship between average nose-body and nose-nose sniffing bout durations (r = 0.751, p < 0.001), suggesting consistent patterns of social engagement across sniffing targets. There was a negative correlation between BLA zif/DAPI ratio and the average duration of nose-to-nose sniff bouts, which approached statistical significance (Pearson r = -0.288, p = 0.058). This trend suggests that increased BLA activation may be associated with reduced close social contact duration.

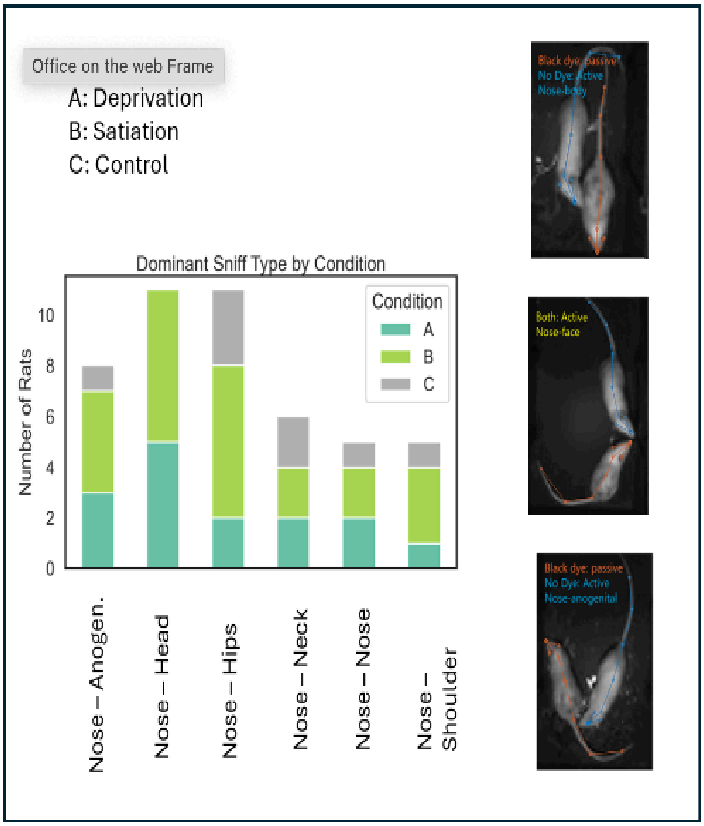


Figure 7: Nose-X
Nose-X distribution by number of rats per group.

5.1.0 BLA ZIF Expression

The total count of ZIF expressed was converted to a proportional value comparing the count of cells expressing ZIF to the total cell count in the region of interest (ZIF_p = (ZIF^{+} / DAPI$^{+)}$ * 100). The values were then log transformed after they failed to meet the assumptions necessary to perform parametric testing.

Two-way ANOVA was conducted to determine the effect of condition (deprivation, satiation, control), sex (male, female), and interactions between sex and condition on the ZIF expression in the BLA. The results suggested that condition did not significantly impact ZIF expression in the BLA, F(2, 54) = .054, p = .947, ηp^2 = .002, and neither did sex F(1, 54) = .139, p = .710, ηp^2 = .002. The results did not indicate a significant interaction of sex and condition influencing ZIF expression in the BLA, F(2, 54) = .835, p = .439, ηp^2 = .029.

The groups were then split by sex to investigate whether the effects of condition would emerge in a sex specific manner. A one-way ANOVA was conducted on the males to determine whether condition would impact the amount of ZIF expression in the BLA and the results suggested there was no significant effect of condition, F(2, 24) =.708, p = .502, ηp^2 = .056. A one-way ANOVA on the females also indicated that condition did not have a significant effect of ZIF expressed in the BLA F(2, 30) = .395, p = .677,ηp^2=.025.

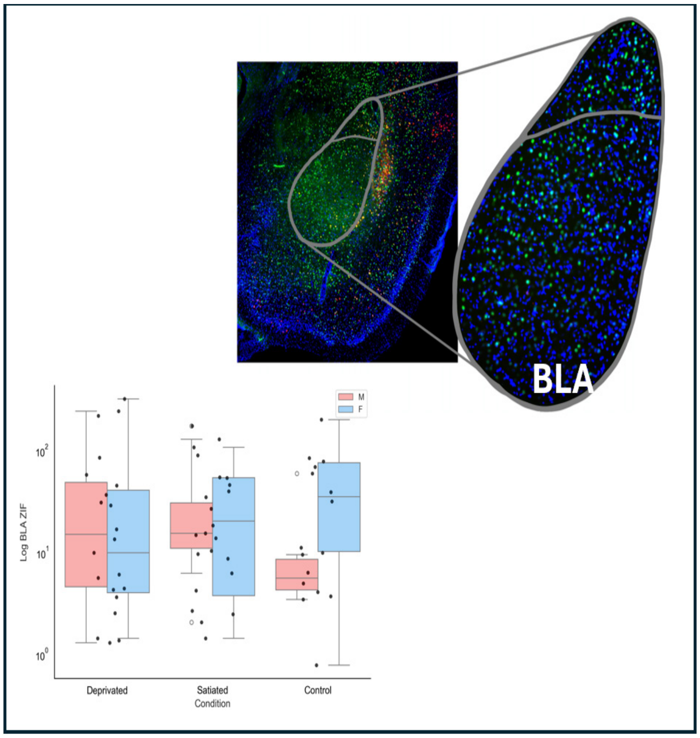


Figure 8: Basolateral-amygdala ZIF counts. The basolateral amygdala is pictured above, and the graph indicates the distribution of the data set.

5.2.1 CG1 ZIF Expression

The values were then log transformed with a small constant to avoid a log of zero (log10 + 1e-5) to reduce the impact of outliers and preserve the sample size. The values were back transformed into their original ZIF+ / DAPI+ for interpretation and visualization.

A two-way ANOVA was conducted to assess the effect of condition (deprivation, satiation, control), sex (male, female), and their interactions on ZIF expression in the cingulate cortex (CG1). The results indicated that there was not a main effect of sex on CG1 ZIF expression, F(1,47) = .9511, p = .334, ηp^2 = .019, nor of condition F(2, 47) = 1.04, p =.359, ηp^2 = .043. However, the interaction between sex and condition had approached significance, F(2, 47) = 3.144, p = .052,

ηp^2 = .118. Pairwise comparisons indicated that the female control group (M = .23, SE = .04) had a higher ratio of cells expressing ZIF in the cingulate cortex than the control males (M = .13, SE = .12) after back transforming the values from log. The Sidak adjusted p value for this comparison was p = .072, while the uncorrected p value was p = .025.

After this, they were split by sex to investigate whether there where any sex specific effects of the conditions that were missed on the two-way ANOVA. A one-way ANOVA was performed on the males suggested that there was not a significant effect of condition, F(2, 26) = 1.48, p = .253, ηp^2 =.10, on expression of ZIF in the cingulate cortex. A one-way ANOVA performed on the females also indicated that condition did not have a significant effect on ZIF expression in the cingulate cortex, F(2, 29) = .45, p =.642, ηp^2 = .031.

5.2.2 Prelimbic Cortex ZIF Expression

A two-way ANOVA was conducted to determine the effects of sex (male, female) on condition (deprivation, satiation, control) on the log transformed ZI+/DAPI+ expression in the prelimbic cortex (PRL).

The results showed no significant effect of sex on the proportion of cells expressing ZIF in the PRL, F(1, 45) = .065, p = .800, ηp^2 = .001, nor a main effect of condition, F(2, 45) = .174, ηp^2 = .075. The results also indicated that there was not a significant interaction between sex and condition on PRL ZIF expression, F(2, 45) = .90, p = .413, ηp^2 = .039.

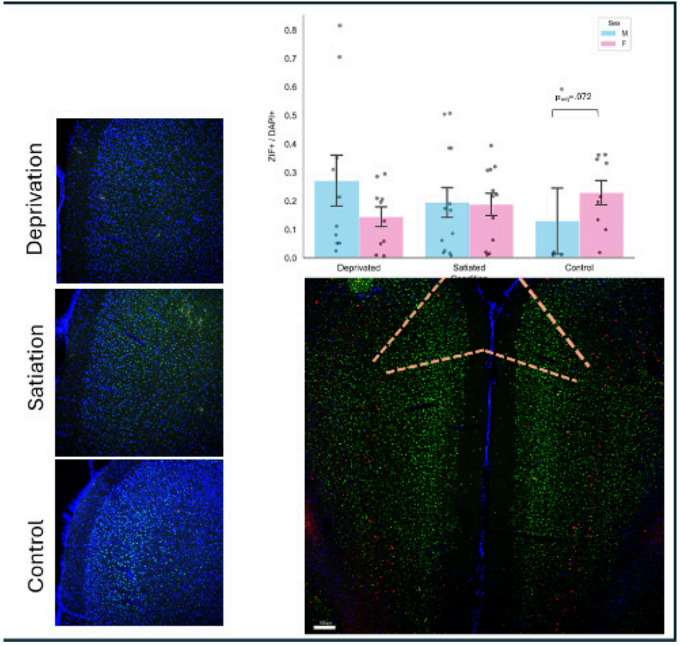


Figure 9: Cingulate Cortex ZIF. The results indicated that females express less of the CG1 ZIF compared to the males, however no other result were significant.

5.2.2 Prelimbic Cortex ZIF Expression

A two-way ANOVA was conducted to determine the effects of sex (male, female) on condition (deprivation, satiation, control) on the log transformed ZI+/DAPI+ expression in the prelimbic cortex (PRL).

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5.2.3 Retrograde Tracer Results

One cohort from the study underwent survival surgeries to determine if correlations could be made between circuit level activity in the BLA and social motivation, sociality, neural activation and inactivation patterns. A cohort of 18 animals were tested together, with 6 of them serving as non-surgery controls. The animals (n = 12 M/F) received bilateral intracranial injections of retrograde tracers to the BLA and followed the same behavioral assays as previous cohorts. Given the smaller sample, this analysis was mostly exploratory.

A two-way ANOVA was conducted on the subset of rats with retrograde injections to determine if condition (deprivation, satiation, control), sex (male, female), or interactions between sex and condition had a significant effect on the ZIF+/DAPI+ proportion of expression in the cingulate cortex. There was a main effect of sex on the ZIF+/DAPI+ expression in the cingulate cortex, F(1, 5) = 9.95, p = .025, ηp^2 = .067, such that females (M =29.60, SE = 3.85) has significantly lower expression than the males (M = 78.23, SE = 13.41). There was not a significant effect of condition, F(2, 5) = .219, p = .561, ηp^2 = .206, and there was not a significant interaction of sex and condition, F(2, 5) =1.61, p = .289, ηp^2 = .391.

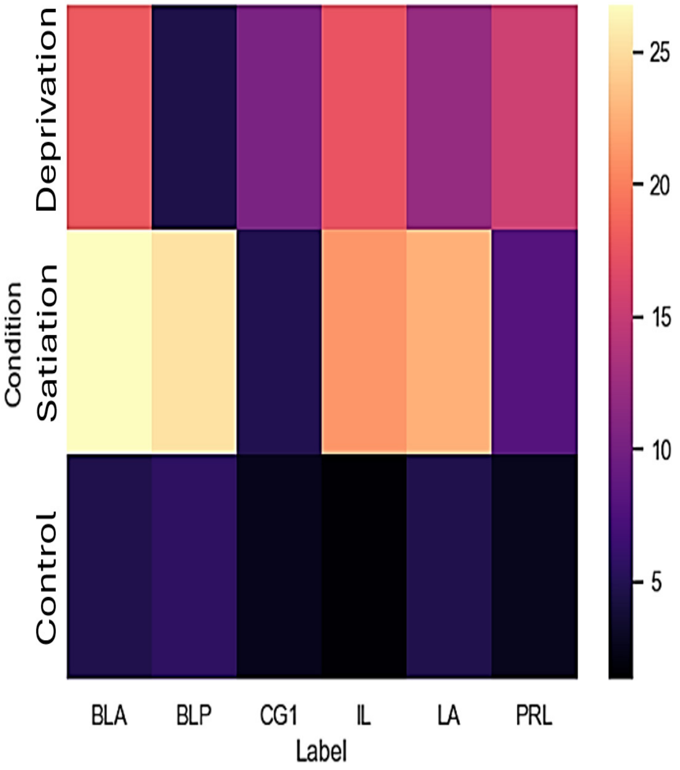

Figure 10. Heatmap of Retrograde Virus Count by Region of Interest and Condition
Heatmap indicating expression of the retrograde tracer by count.

Discussion

This study aimed to assess how short-term manipulation to social experiences impact social motivation, social preference, and neural activation in rats. Female rats in the deprivation condition showed a trend toward increased time investigating and orienting towards the social stimulus. This suggests a potential increase in social drive following isolation. This could point to a slight trend in heightened vigilance following deprivation, which is consistent with prior studies that have linked social isolation to increased social approach behavior.

The data from the social interaction test did not show significant differences in nose-anogenital sniffing time, however there was a positive correlation between nose-body and nose-nose sniffing, which could support the reliability of the social behavior metrics. However, the pose estimations are still a work in progress that will continue on as the rest of the data is analyzed. Due to tracking limitations in a subset of the videos, some data were excluded, and this likely reduced power to detect meaning effects in the social interaction measures.

The basolateral amygdala is a key structure for evaluating the emotional salience of social cues and assigning affective valence to sensory input (Janak & Tye, 2015). Previous work has shown that hyperexcitability of BLA principal neurons is associated with anxiety-like states, in part due to reduced GABAergic inhibition within local circuits (Sharp, 2017). The present study found that increased ZIF activity in the BLA was negatively correlated with nose-to-nose sniffing. This suggests that BLA activation may reflect an anxiety or stressed state that reduced motivation towards the prosocial investigatory sniffy behavior. Several of the results had sex specific effects that showed differences in male and female exploratory behavior, as well as a few that approached significance. One of the most interesting results indicated that female CG! ZIF expression was significantly lower in the females than in the male of the cohort that had the survival surgery. This could indicate that an inhibitory component from CG1 activation is regulating the activity of the BLA for females and possibly impacting their responses to the conditions. It could also indicate that the males were more stressed out, and that their hyperexcitable BLA simply required more tonic inhibition to return to homeostasis. Exploratory analysis using principle component analysis on the amount of retrograde-labeled neurons indicated that when the dimensionality of the dataset is reduced to two principle components, the major source of variance for the social satiation group is all comes from the medial prefrontal cortex elements, suggesting that even when they are reduced, the satiation group is still distinct as a cluster.

There were also a few indications that the effects of the conditions may not be sensitive enough to view both sexes as a whole. Within group variability may have also been affecting the statistical power of the results, as there were several indications suggesting that the male and female controls to which every test was compared to were too different from each other to use as a reliable control group.

The results from the social preference test could be indicative of so effect of condition, however, it is likely that further analysis addressing the major sources of variability, such as identification of a behavioral phenotypes in the dataset, such as the responder status that was explored. This seemed to reveal the presence of natural differences unique to each rat, and this indicated that personal responses play a more important role in the effect that the social condition is inducing. Studies looking at fMRI in humans have indicated that there is increased amygdala-ACC signaling in response to social stimuli, which will be explored further as the data set is more thoroughly analyzed.

Consistent with human neuroimaging studies indicating increased amygdala–vACC and amygdala–mPFC connectivity in response to social exclusion (Lass-Hennemann et al., 2023), brief social deprivation in the current rodent model may recruit similar limbic–prefrontal circuitry to enhance social motivation. The ventral anterior cingulate cortex (vACC) and medial prefrontal cortex (mPFC) have been implicated in emotional processing, social reward, and adaptive stress coping, with sex-specific modulation observed in both rodents and humans.

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