AN AMYGDALAR – INSULAR – PREFRONTAL CIRCUIT MEDIATING SOCIAL AFFECTIVE BEHAVIOR

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An Amygdalar - Insular - Prefrontal Circuit Mediating Social Affective Behavior

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Abstract: The perception of others as safe or threatening informs how we respond to others in a social setting. These social affective behaviors require the detection of sensory stimuli and the appraisal of others' affective states to orchestrate adaptive behavioral responses. This process is also informed by one's own internal state and environment. The neural circuitry underlying this behavior consists of a wide network of brain regions that communicate to execute social behaviors. However, the neural mechanisms mediating social affective behavior require further investigation. Therefore, the objective of this dissertation is to add detail to our understanding of the specific brain circuits involved in social affective behavior. The insula is a key node within this circuitry, necessary for approach and avoidance behaviors in a social affective preference (SAP) test where adult rats prefer interactions with stressed juveniles but avoid interactions with stressed adults. Here, I investigated the roles of a basolateral amygdala projections to the insula and insular projections to the PL in SAP testing and present evidence indicating the necessity of both these tracts to social affective behaviors. The results described here along with the reviewed literature support a potential amygdalar-insular-prefrontal circuit responsible for detecting social valence, integrating external stimuli with internal states, and selecting and executing context-appropriate social affective behaviors.

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ANOVA	Analysis of Variance
ASD	Autism Spectrum Disorder
BLA	Basolateral Amygdala
BNST	Bed Nucleus of the Stria Terminalis
BOLD	Blood Oxygen Level Dependent
CeA	Central Amygdala
CNO	Clozapine-N-Oxide
CRF	Corticotropin-Releasing Factor
CSB	Cognitive Social Brain
dmPFC	Dorsomedial Prefrontal Cortex
fMRI	Functional Magnetic Resonance Imaging
IL	Infralimbic Cortex
I.P.	Intraperitoneal
LA	Lateral Amygdala
MCH	Melanin-Concentrating Hormone
MD	Mediodorsal Thalamus
mPFC	Medial Prefrontal Cortex
NAc	Nucleus Accumbens
PAG	Periaqueductal Gray
PFC	Prefrontal Cortex
PL	Prelimbic Cortex

PV	Parvalbumin
ROI	Region of Interest
SAP	Social Affective Preference
SBN	Social Brain Network
SDMN	Social Decision-Making Network
SOM	Somatostatin
USV	Ultrasonic Vocalization
vHPC	Ventral Hippocampus
vmPFC	Ventromedial Prefrontal Cortex

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Chapter 1: An introduction to social affective behavior and its neural correlates

1.1 Social Affective Behavior

Social behaviors are an essential component of life; necessary for the survival, proliferation, and enrichment of a species (Rilling and Sanfey, 2011). These behaviors are diverse and range from innate and habitual to complex and goal-directed (Wei et al., 2021). Innate social behaviors include defensive responses to predatory cues such as odors or vocalizations (Blanchard et al., 1990; Dielenberg and McGregor, 2001), or parental behaviors towards offspring (Kohl and Dulac, 2018). Affiliative behaviors, which are characterized by actions promoting social bonds and cooperation, represent more adaptive and flexible social behaviors. This includes behaviors such as grooming, vocalizations, and physical contact, which strengthen bonds between individuals (Lim and Young, 2006; Stoesz et al., 2013; Walker and McGlone, 2013). Conversely, agonistic behavior involves competitive or conflict-driven interactions that range from overt aggression to social dominance (Kudryavtseva, 2000). These behaviors provide the framework for hierarchies within social groups (Qu et al., 2017). Cooperative behaviors, which are essential for tasks like foraging and raising offspring, provide another example of social behavior's importance to both the success of individuals and the greater community (Schuster, 2002; Jiang et al., 2021). Reproductive behavior is especially important to species survival and proliferation and encompasses inherently social aspects like courtship rituals, mate selection, and copulation (Anholt et al., 2020). Finally, altruistic behaviors, where individuals sacrifice their own well-being for the benefit of others, further exemplify the complexity and adaptability of social interactions (Wrighten and

Hall, 2016). These diverse types of social behavior shape the dynamics and longevity of populations and influence species evolution over time.

Successful coordination of behavior amongst social groups depends on a number of factors. First, the behaviors of individuals reflect their own internal states and motivations. Second, other group members observe those behaviors. Third, to interpret the behavior of others, the observer must also incorporate information about the interaction context and their own internal states. Understanding what the expressions of others mean and using that information to orchestrate social responses is crucial to many aspects of social life (Spunt and Adolphs, 2019). To understand how internal states relate to behaviors, the circumplex model of affect describes moment-to-moment changes in affective state as a product of the interaction between arousal and valence. Emotional experiences, then, fall along a continuum of pleasure-to-displeasure and activation-to-deactivation (Posner et al., 2005). Affective states can be thought of as the foundation for behaviors such as facial expressions, vocalizations, or odors that others may use to make inferences about the affective states (or emotions) of others (Sterley and Bains, 2021). When an observer detects these behaviors in others he or she may make predictions about the nature of the other's affective state. Often, the expression of affect, or *social affect*, elicits behaviors in the observer which are shaped by 1) the prediction about the other's affect, 2) the observer's own affective state, and 3) the specific interaction context. Here, I will define the overt behaviors that are elicited upon exposure to conspecifics with different affective states as *social affective behaviors.*

In humans, social affective behaviors involve complex processes such as theory of mind and perspective-taking, both of which contribute to empathy, a key facet of social motivation (Preckel et al., 2018; Weisz and Zaki, 2018). Social affective behaviors in rodents include emotion contagion, social approach and avoidance, social buffering and consoling (Meyza et al., 2017; Keysers et al., 2022). Going further, perturbations to internal states including hunger, stress, sickness, and sleep are also known to influence social affective behaviors(Pettijohn et al., 2012; Beattie et al., 2015; Beery and Kaufer, 2015; Eisenberger et al., 2017). Given the importance of social affective behaviors to the wellness and proliferation of social species, understanding the underlying neurobiology is a fundamental goal.

The flexibility of social affective behavior is the product of the coordinated activity of complex neural circuits within the brain that allow for rapid responses to changing environmental and social contexts. Importantly, dysfunction of this neural circuitry contributes to disordered cognitive, emotional, and social processes (Fernández et al., 2018; Wang et al., 2023). Social impairments arising from neural dysfunction are characteristic symptoms of neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (Brüne, 2005; Baslet et al., 2009; Lai et al., 2014; Fernández et al., 2018). Researchers have dedicated years to exploring the mechanisms underlying social behaviors in an effort to understand how these behaviors manifest across species. In doing so, researchers also aim to gain insight into how circuit disruption contributes to

the social deficits commonly present in patients with ASD or schizophrenia to begin developing targeted interventions aimed at alleviating these symptoms.

1.2 Network models of social affective behavior

In humans, much of what is known about the circuits governing social behaviors comes from fMRI studies examining BOLD activation across brain regions during a variety of social tasks (Insel and Fernald, 2004; Masten et al., 2011). While these studies have informed our fundamental understanding of the brain regions and functional connectivity orchestrating social behavior, limitations in cognitive neuroscience techniques prevent investigation of the specific circuits and cellular and molecular processes underlying this behavior. Many anatomical substrates of social behavior are conserved across species, including rodents which display behavioral correlates of affective social behavior seen in humans (Bartal et al., 2011), making them a suitable model for translational social affective research. For example, “observer” rats will exhibit stress-related behavior such as social avoidance when housed with a rat that previously underwent social defeat stress (Carnevali et al., 2017), indicating emotional contagion. Rats presented with a novel tone in presence of a rat previously conditioned to be fearful of that tone will show increased freezing to that tone the following day, indicating social transfer of fear (Bruchey et al., 2010). Broadly, a large body of work using rodents reveals the capacity for social transmission of information in a number of tasks (Monfils and Agee, 2019). This evidence of social affective behavior in rodents in conjunction with technological

advancements in molecular, cellular, and viral genetic techniques has paved the way for uncovering the neural mechanisms of social affective behaviors (Roth, 2016; Haggerty et al., 2020; Roth and Ding, 2020).

The explosion in research dedicated to identifying neural correlates of social behavior has led to the proposal of multiple models of the "social brain" (Prounis and Ophir, 2020a), including the Social Decision Making Network (SDMN) (O'Connell and Hofmann, 2011a) and the Cognitive Social Brain (CSB) (Prounis and Ophir, 2020b). Prior to the establishment of the SDMN, Newman (1999) proposed the Social Brain Network (SBN), consisting of limbic forebrain and midbrain structures. While investigating the role of the medial amygdala in rodent sexual behavior, Newman recognized that this region acted as a node in a larger network responsible for executing a number of social behaviors, including male reproductive behaviors. A region was included in the SBN if it met the following three criteria: it was reciprocally connected to other regions in the network, it contained sex steroid hormone receptors, and it was identified as an important regulator of more than one social behavior. Newman established that social behaviors are not the product of any one region or circuit but the result of a pattern of activation in a network of regions (Newman, 1999). While this description represented a major advance in how the neural substrates of social behavior are understood, the functionality of the SBN was limited to the regulation and execution of these behaviors and did not account for the evaluation of various external stimuli. This limitation led to the proposal of the SDMN by O'Connell and Hofmann (2011). Using comparative biology, they

proposed a network consisting of the mesolimbic reward system and the SBN that is conserved across species (O'Connell and Hofmann, 2011a). The mesolimbic reward system has been shown to mediate attention, detect salient stimuli, and drive motivated behaviors (Alcaro et al., 2007; Wickens et al., 2007) while the SBN is hypothesized to organize multiple social behaviors (Newman, 1999). Additionally, these two networks share common structures in the lateral septum and bed nucleus of the stria terminalis (BNST). The classification of this integrated SDMN provided an anatomical and evolutionary foundation for studying the neural basis of social decision-making across species. The SDMN serves as a framework for investigating both the motivation behind and execution of social behaviors at the circuit level.

A strength of the SDMN is its use of comparative anatomy to propose a network of conserved regions involved in social decision-making and to demonstrate how these structures have evolved over time. Many less-complex organisms lack neocortical structures that are conserved between rodents and humans, leading to the omission of these structures in the SDMN. These neocortical regions contribute to sensory integration and goal-directed behaviors in rodents (Gogolla et al., 2014; Howland et al., 2022) and higher-order social cognition in humans and non-human primates (Adolphs, 2001; Amodio and Frith, 2006).

In their recent review, Prounis and Ophir (2020) work to bridge the gap between the SDMN and another view of the social brain that they term the Cognitive Social Brain (CSB) (Prounis and Ophir, 2020b). The CSB was initially

proposed by Brothers (1990) and was defined primarily through the study of social behaviors in non-human primates (Brothers, 1990). By this account, the social brain consists of a network of regions that include the basolateral amygdala (BLA) and more cortical structures like the insula, the anterior cingulate cortex (ACC), and the medial prefrontal cortex (mPFC). The CSB orchestrates appropriate social behavior through the accurate perception and evaluation of affective information conveyed by social targets (Brothers, 1990). Taken together, the SDMN is positioned to integrate elementary components of social motivation (safety, danger, reproductive potential, etc.) with the proximate mediators of behavioral response (approach, avoidance), while the CSB is positioned to shape these behaviors with perception, expectation, memory, and cognitive emotional components that give social interactions their typical complexity.

The establishment of these network models has provided a framework for studying the neural circuitry underlying social behavior. Knowing the anatomical and regional connectivity broadly dictating various social functions allows for more targeted investigation of specific brain regions and circuits. In the following sections, I will highlight three regions of note within this joint SDMN/CSB network and their roles in various non-social and social behaviors. The following chapters will detail experiments investigating the circuitry between these regions in social affective behavior.

1.3 Basolateral amygdala in valence detection and social motivation

The BLA has received a considerable amount of attention due its role in a number of functions including fear memory formation and expression (Chen et al., 2019; Maren et al., 1996), reward behavior (Murray, 2007), threat detection (Amir et al., 2019; Sepahvand et al., 2023), and social behavior (Bickart et al., 2014; Gangopadhyay et al., 2021). In the following sections, I will detail the BLA neuroanatomy that allows for this multifunctionality, as well as discuss its role in valence detection, which contributes to each of the functions mentioned above.

1.3.1 Anatomical connectivity of the BLA

The BLA is a cortically-derived nucleus in the heterogeneous amygdalar complex located within the medial temporal lobe (Swanson and Petrovich, 1998). BLA connections with both limbic and cortical structures inform its function (Davis and Shi, 2000). Thalamic input allows for the relay of sensory information (LeDoux et al., 1990), while connections with the striatum, specifically the nucleus accumbens (NAc), mediate reward-related behaviors (Ambroggi et al., 2008). Further reciprocal connections are shared with the hippocampus, sensory, insular, and prefrontal cortices (Allen et al., 1991; McDonald, 1998; Sah et al., 2003; Hoover and Vertes, 2007). Importantly, this region is also heavily interconnected with other amygdala subnuclei, including the central amygdala (CeA) (Swanson and Petrovich, 1998). BLA anatomy is conserved between humans and rodents (O'Connell and Hofmann, 2011a) and its intricate connectivity allows it to serve as a hub for integrating sensory stimuli, recognizing

affective information, and influencing various aspects of motivated behaviors (Smith and Torregrossa, 2021).

1.3.2 BLA as valence detector

A prominent view of BLA function is its role as a valence detector (Kyriazi et al., 2018; O'Neill et al., 2018; Zhang and Li, 2018; Pignatelli and Beyeler, 2019). Given its thalamic and sensory afferents and striatal and cortical efferents, the BLA is well situated to assess the valence of stimuli in an environment to inform subsequent behavior. In humans, amygdala activity increases in response to both positive and negative stimuli compared to neutral stimuli (Garavan et al., 2001; Anders et al., 2008). Olfactory cues ranging from pleasant-to-unpleasant are encoded within the amygdala (Jin et al., 2015) and the intensity of these cues alters activity (Winston et al., 2005). Additionally, appetitive and aversive tastes activate the amygdala (O'Doherty et al., 2001) and spatially distributed, valence-specific modulation informs one's choice in food (Tiedemann et al., 2020). Amygdala activity also increases in response to emotionally valenced faces (i.e. happy or fearful) (Morris et al., 1996). Interestingly, the perceived direction of another's facial expression also modulates amygdala activity, with angry faces targeted toward an observer eliciting a greater amygdalar response than angry faces targeted toward a bystander (Sato et al., 2004). The amygdala's ability to differentiate between opposingly valenced expressions also contributes to one's judgment of another's trustworthiness (Santos et al., 2016). Together, these findings suggest that amygdala activation is correlated with both the valence and

intensity of stimuli, potentially contributing to its role in affective processing that influences behavioral outcomes and aspects of social cognition in humans.

A number of non-human primate studies have also contributed to BLA valence encoding literature. During an operant conditioning task in which macaques learned to push or pull a joystick to avoid an aversive or accept an appetitive outcome, respectively, single unit recordings of BLA neurons revealed neurons that preferentially responded to the valence and intensity of a stimulus. Additionally, the behavioral response scaled with stimulus intensity, with macaques responding more quickly to stronger stimuli (Iwaoki and Nakamura, 2022). In terms of social cognition, activity in the amygdala increases in response to threatening vs. appeasing facial expressions (Hoffman et al., 2007). The BLA also tracks the value of given rewards in a modified dictator game, influencing social choice (Grabenhorst et al., 2019). These studies provide further evidence that the amygdala is active in response to valenced stimuli.

The rodent literature on valence processing in the BLA is extensive and intersectional, investigating functionality at the cellular-, regional-, and circuit-level (O'Neill et al., 2018; Pignatelli and Beyeler, 2019). At the cellular level, genetically-defined subsets of BLA neurons selectively respond to unconditioned, innate positive or negative stimuli (i,.e. foot shocks vs. opposite sex conspecific exposure, sucrose vs. quinine, etc) (Gore et al., 2015; Kim et al., 2016; Zhang and Li, 2018). Specific genetically-defined excitatory populations of these neurons are controlled by a local inhibitory circuit such that only one population may be excited at a given time. In this way, BLA neurons that preferentially

respond to a positively-valenced stimulus can be selectively excited to drive appetitive behaviors or inhibited when a negatively-valenced stimulus is presented and an aversive or avoidant outcome is necessary (Kim et al., 2016). These findings indicate that valence-responsive BLA neurons can be defined by their projection targets. These projection-defined neurons are responsible for learning to associate cues with positive or negative outcomes (Sangha et al., 2013; Beyeler et al., 2018), further indicating this region's role in assigning valence to stimuli and informing behavioral outcomes. At the regional level, BLA lesions impair the acquisition of a conditioned stimulus in fear conditioning (Maren et al., 1996). On the other hand, while BLA lesions do not prevent appetitive Pavlovian conditioning (Parkinson et al., 2000), photostimulation of this region during an appetitive conditioning task intensified responding to the CS compared to control conditions, suggesting that BLA activation can enhance the appetitive qualities of a stimulus (Servonnet et al., 2020). At the circuit level, BLA neuronal populations display distinct input-output circuits depending on their valence-specific behaviors (Zhang et al., 2021) and photostimulation of projections to the NAc and CeA facilitate positive and negative reinforcement, respectively (Beyeler et al., 2016, 2018). In sum, rodent studies have revealed both genetically and anatomically-defined populations of valence detecting neurons in the BLA that drive behaviors.

1.3.3 Social motivation in the BLA

A lack of motivation for social engagement is a major symptom of disorders such as ASD, with amygdalar dysfunction commonly linked to this

behavioral deficit (Baron-Cohen et al., 2000; Zalla and Sperduti, 2013). A number of studies reveal the BLA's essential role in social behaviors. Amygdala lesions in human patients impairs the recognition of emotions (Adolphs et al., 2002) and is necessary for social experiential learning, with patients suffering from bilateral BLA damage unable to adjust behaviors when interacting with trustworthy vs. untrustworthy partners (Santos et al., 2016; Rosenberger et al., 2019). In primates, BLA neurons signal social preferences in a modified dictator game (Chang et al., 2015) and simulate decision-making processes of partners via social learning (Grabenhorst et al., 2019). Social behavior is also bidirectionally modulated by the primate amygdala, with activation or inhibition of the BLA suppressing or increasing social behavior, respectively (Wellman et al., 2016). Similarly, decreasing GABA function, and thereby increasing excitatory transmission, in rodent BLA decreases social behavior (Paine et al., 2017). Social behavior in rodents is also modulated via BLA efferents. Activation of either BLA-mPFC or BLA-ventral hippocampus (vHPC) projections reduces social interaction while inhibition facilitates interaction (Felix-Ortiz and Tye, 2014; Felix-Ortiz et al., 2016). Chapter 2 details further work investigating this region's role in social behavior, specifically linking its projections to the insula and valence-detecting properties to social affective preference behavior. In sum, the amygdala is likely involved in recognizing or responding to the valence of various social and nonsocial stimuli, potentially assigning affective significance to these stimuli that help to inform subsequent behavior.

1.4 Insula as a site of interoception, sensory integration and social affective processing

Social affective behavior requires the integration of internal and external signals to achieve an appropriate behavioral response. These signals are constantly changing as new non-social or social stimuli emerge in an environment or as internal motivations shift according to adjustments in bodily states. As a site of multisensory input, the insula is well-situated to monitor internal states and track changes in environmental stimuli.

1.4.1 Insula neuroanatomy

The insula is a cortical region that exhibits a complex neuroanatomy across species, located beneath the lateral sulcus in humans and primates and spanning the lateral surface of each hemisphere in rodents (Gogolla, 2017). Generally, despite some differences in scale and organization, human and rodent insula share connectivity with analogous regions (O'Connell and Hofmann, 2011a; Rogers-Carter and Christianson, 2019). The insula receives input from a number of cortical regions, including the somatosensory and prefrontal cortices (Shi and Cassell, 1998), supporting the integration of sensory information. It also exhibits interhemispheric connectivity, which could be critical to social affective processing (Glangetas et al., 2022). Subcortical projections include the thalamus, basal ganglia, and hypothalamus (Allen et al., 1991), which allow for the regulation of autonomic processes. Emotion- and memory-related functions in this region are sustained via connections with the limbic system (Allen et al.,

1991; Shi and Cassell, 1998), including the amygdala and hippocampus. The anatomical organization of the insula informs its role in sensory processing and interoception, two functions that contribute to social affective behavior.

1.4.2 The interoceptive insula

Interoception refers to the awareness of one's internal sensations, including cardiovascular signals, hunger, temperature, pain, and respiration ((Bud) Craig, 2003; Chen et al., 2021b). Homeostatic regulation is critical as high interoceptive awareness contributes to anxiety disorders while low awareness contributes to depression and schizophrenia (Paulus and Stein, 2010). As detailed above, insula anatomy subserves its interoceptive properties so functional deficits may contribute to these neuropsychiatric disorders.

In humans, insula activity is linked to the regulation of a number of autonomic functions, including heart rate (Chouchou et al., 2019; Keller et al., 2020), blood pressure (Sanchez-Larsen et al., 2023) and respiration (Keller et al., 2020). Deficits in attention to visceral interoceptive information is linked to decreased activity in bilateral insula and is correlated to increased depressive symptoms in patients with Major Depressive Disorder (Avery et al., 2014). Nociceptive and cortical inputs to the insula also contribute to its role in pain perception, linking pain sensation to emotional responses while also contributing to one's reported empathy for pain in others (Mutschler et al., 2011). In this way, interoceptive awareness in the insula is inherently linked to socio-emotional behavior.

Rodent insula function is also integral to bodily awareness and homeostasis. Visceral sensations from the body are processed within the insula, with bilateral lesions resulting in significantly weakened visceral sensitivity following chronic restraint stress (Yi et al., 2014). Innate compensatory responses to signals of internal discomfort, such as “lying on belly” during gastrointestinal malaise, are also mediated by the insula (Aguilar-Rivera et al., 2020). Insula inactivation blunts symptoms of malaise induced by acute lithium administration and reduces drug-seeking behavior in rats given amphetamines (Contreras et al., 2007). Representations of interoceptive states in the insula are also essential to balancing responses to fear-inducing stimuli and guiding behavior. Insula reactivity to a fear conditioned stimulus results from the integration of the predictive value of the cue with bodily signals arising during the acute freezing response (Klein et al., 2021). In mice, *in vivo* electrophysiological recordings within the posterior insula revealed that tachycardia increases activity within this region at both the cellular and population-level, while inhibition reduces the anxiogenic effects observed during tachycardia (Hsueh et al., 2023). Optogenetic activation of the posterior insula and its projections to the CeA triggers defensive behaviors and increases respiratory rate in mice, revealing a top-down mechanism for control of anxiety and aversive states (Gehrlach et al., 2019a). These findings further point to the interconnectedness of interoception, exteroception, and overt behavior.

1.4.3 Exteroceptive sensory integration in the insula

Internal state signals and external stimuli are inextricably linked. This is because internal states are constantly informed and updated by incoming external cues. A key function of the insula is its ability to integrate these interoceptive signals as described above with relevant external stimuli.

Human studies have revealed the insula as a multimodal sensory processor. Intracerebral insula stimulation evokes somatosensory, auditory, olfactory and gustatory responses (Mazzola et al., 2006). In an fMRI study, anterior insula responded most strongly to the onset of nociceptive stimulation while posterior insula preferentially responded to sustained non-nociceptive input, indicating that the insula is specialized to respond to various somatosensory inputs along its rostral-caudal gradient (Hu et al., 2015). The insula also monitors changes in temperature (Craig et al., 2000), encodes and integrates representations of taste and smell (Small, 2010; Mazzola et al., 2017; Avery et al., 2020), responds to positively or negatively valenced auditory cues (Zhang et al., 2019), evaluates emotions of facial expressions (Motomura et al., 2019), and processes both visual and vestibular stimuli (Frank et al., 2014).

Rodent insula is similarly situated to integrate multisensory inputs. Mice with neuropathic pain show increases in synaptic plasticity in the insula, indicating this region's essential role in processing nociceptive input and modulating pain response (Qiu et al., 2013). The insula is also essential to taste representation (Yiannakas and Rosenblum, 2017; Chen et al., 2021a) and smell, with gustatory and olfactory chemosignals converging in rodent anterior insula

(Shipley and Geinisman, 1984; Mizoguchi et al., 2016). Lesions of the insula prevent the association of taste and smell in rats, further implicating this region in the integration of these two modalities (Sakai and Imada, 2003). There is also evidence for audio-tactile integration within this region with auditory stimuli activating the insula and concurrent administration of varying tones with air puffs enhancing this activation (Gogolla et al., 2014). Perhaps the most convincing evidence for multimodal sensory integration in the insula is the presence of distinct insular fields that respond to auditory, somatosensory, or multisensory cues, with responses in the multisensory field displaying non-linear summation of these two modalities. This suggests that specific interactions between auditory- and somatosensory-responsive cells are contributing to activity within this multisensory field and combining multisensory information (Rodgers et al., 2008). In sum, the insula processes features from a number of sensory modalities, integrating these external signals and updating internal states accordingly to respond to changes in environmental and social contexts.

1.4.4 Social affective processing in the insula

Human neuroimaging studies have identified the insula as one of a few structures where activity is correlated with empathic processes. Insular resection in humans contributes to deficits in emotion recognition of facial expressions and a reduction in perspective-taking of others (Boucher et al., 2015). Functional specialization across the rostral-caudal gradient of the insula may also contribute to its ability to link sensation with emotion perception. Anterior insula is activated in response to pain experienced by oneself and pain experienced by a loved one

while posterior insula is only responsive to one's own pain (Singer et al., 2004). Similarly, posterior insula responds to the sensory aspects of affective auditory tones while the anterior insula responds to the emotional contents of these cues (Zhang et al., 2019). Communication between anterior and posterior subregions of the insula are also essential to the processing of affective tactile stimuli, with functional connectivity increasing between posterior and anterior insula during pleasant stroking (Davidovic et al., 2019).

In rodents, the insula is crucial for conveying affective states via facial expressions, a critical component of social interactions and another example of interoception intersecting with affective behaviors (Dolensek et al., 2020). Anterior insula is implicated in a number of social behaviors: lesions prevent social recognition memory formation (Min et al., 2023), inhibition reduces targeted helping behavior (Cox et al., 2022a), activation restores rescue behavior following deficits induced by heroin self-administration (Tomek et al., 2020), and inactivation reverses hypernociception experienced by observer mice who's cagemates had undergone sciatic nerve constriction (Zaniboni et al., 2018). Posterior insula is also essential to approach and avoidance behavior in specific social contexts (Rogers-Carter et al., 2018b) which will be reviewed in more detail below. Chapters 2 and 3 will discuss experiments aimed at investigating insula circuitry with the BLA and the prelimbic cortex (PL), respectively. These findings, along with the existing literature reviewed above, contribute to a growing view of the insula as a site of convergence for external and internal information,

assignment of affective significance, and modulation of behavioral outputs based on representations of one's environment.

1.5 Executive control and social cognition in the medial prefrontal cortex

As discussed in section 1.1 above, social behaviors can be habitual or goal-directed, with structures such as the medial amygdala or striatum potentially driving more habitual social behaviors and cortical regions like the mPFC mediating goal-directed behaviors. Goal-directed behaviors require an individual to evaluate environmental and internal stimuli to execute an optimal action in situations where automatic responses may not be most beneficial (Ceceli and Tricomi, 2018). Executive control mechanisms allow for behavioral flexibility and modulation of more automatic processes via top-down signaling. Deficits in executive control contribute to biases toward habit-based decisions in many neuropsychiatric disorders, including ASD and social anxiety disorder (Alvares et al., 2016). Due to its subcortical and cortical connections, the mPFC is a crucial node involved in executive control and guiding goal-directed decision-making.

1.5.1 mPFC neuroanatomy across species

The mPFC is a complex structure and researchers have long debated the anatomical and functional homology of the PFC across species, with some discussion over what potential PFC substrates in rodents directly correlate to non-human primates or humans (Preuss, 1995; Uylings et al., 2003) and whether these substrates are functionally similar. Though cognitive complexity differs between species, a substantial body of evidence has indicated rodents share

anatomically and functionally homologous PFC subregions with non-human primates and humans(Uylings et al., 2003; Bicks et al., 2015).

Human PFC is often divided into a number of subregions, including the dorsolateral and orbitofrontal PFC and ACC. Rather than provide a comprehensive anatomical review of each of these subregions here, I will briefly focus on medial PFC, which can be further subdivided into a dorsal and ventral portion that are functionally distinct but extensively interconnected (Bzdok et al., 2013). Broadly, the dorsomedial portion of the PFC (dmPFC) shares connections with the inferior frontal gyrus, temporo-parietal junction, and middle temporal gyrus. These connections with high-order association and multimodal cortical areas contribute to the dmPFC's role in more cognitive functions, including attentional processing, perspective-taking and moral judgement (Bzdok et al., 2013; Isoda and Noritake, 2013). On the other hand, the ventromedial portion of human PFC (vmPFC) is preferentially connected with more limbic and reward-related medial brain structures, including the ventral striatum and hippocampus. Subsequently, the vmPFC is associated with emotion regulation, value-based decision making, and the processing of self-relevant information (Bzdok et al., 2013; Hiser and Koenigs, 2018).

As discussed above, the existence of a rodent mPFC homologous to that in humans and non-human primates has been contentious. What is generally agreed upon is that rodent mPFC consists of multiple subregions, each with its own distinct connectivity profile and functional specialization (Laubach et al., 2018). The mPFC in rodents can be categorized into a dmPFC and vmPFC

based on connectivity patterns that are broadly homologous to the anatomical connections discussed in human and non-human primates above (Oh et al., 2014). Similar to humans and non-human primates, these broad subdivisions also delineate a cognitive vs. affective dorsal-ventral functional axis that is informed by differences in neuroanatomy. The dmPFC consists of the secondary motor cortex, the ACC, and the dorsal portion of the PL. Rodent dmPFC shares reciprocal connections with higher order sensory cortices, association areas, and motor systems that include the spinal cord and brainstem nuclei (Hoover and Vertes, 2007; Zingg et al., 2014; Hintiryan et al., 2016; Kamigaki, 2019), allowing for the processing and integration of external information and control over motor output. The vmPFC, which includes the ventral portion of the PL and the infralimbic cortex (IL), is less connected with sensory cortices, instead sharing denser reciprocal connections with limbic structures, including the hippocampal formation and amygdala (Vertes, 2004; Gabbott et al., 2005; Hoover and Vertes, 2007). The vmPFC is also the primary target of the agranular insula (Mathiasen et al., 2023), further positioning this portion of the mPFC to evaluate visceral and affective information and guide motivated behavior. Regarding cortico-striatal connectivity, the dmPFC sends inputs to the dorsal striatum, supporting its role in outcome-dependent goal-directed behavior, while the vmPFC projects to the NAc, with the PL portion targeting the core and the IL portion targeting the shell (Berendse et al., 1992). Together, these vmPFC-striatal circuits support both appetitive goal-directed behavior and habit responding, respectively. In sum, the mPFC’s complex neuroanatomy and subregional specificity contributes to its

functional diversity and involvement in tasks related to working memory (Funahashi, 2017), attention (Rossi et al., 2009), executive control (Kesner and Churchwell, 2011; Kamigaki, 2019), goal-directed behaviors (Pinto and Dan, 2015; Howland et al., 2022), and social behavior and cognition (Bicks et al., 2015; Kietzman and Gourley, 2023).

1.5.2 mPFC as a center for executive control and goal-directed behavior

Executive control refers to a set of processes that enable individuals to plan, initiate, monitor, and adjust their behavior in a goal-directed manner dependent on environmental and internal factors. These mental processes therefore require monitoring changes in sensory information and internal states in order to determine an optimal action while inhibiting multiple alternative outcomes. The PFC is well-positioned to serve this function, computing expected outcomes via bottom-up signals and executing adaptive responses via top-down control (Kamigaki, 2019). This function is evident in the mPFC's activity during various aversive and appetitive tasks. In humans, the mPFC is involved in predicting and evaluating action outcomes (Alexander and Brown, 2011; Forster and Brown, 2011), a task which involves both appraising and interpreting various stimuli in order to direct behavior. In rodents, mPFC neural activity increases in response to cues and contexts that predict aversive or appetitive outcomes (Pratt and Mizumori, 2001; Gentry and Roesch, 2018), while inhibition of this region resulted in increased premature responding in a reaction time task (Risterucci et al., 2003), further implicating this region's role in predicting and executing the most advantageous outcome out of all possible outcomes.

Cognitive flexibility, the ability to switch between tasks or behaviors or shift attention, is essential to executive control and is a core function of the mPFC. In humans, mPFC displays regional specialization depending on the cognitive flexibility required, with antero-dorsal prefrontal cortex involved in attentional set shifting and postero-ventral prefrontal cortex involved in reorganizing stimulus-response associations (Nagahama et al., 2001; Kim et al., 2011). In rodents, mPFC disinhibition led to an impaired ability to track changes in reward outcomes in a reward-discounting choice task (Gruber et al., 2010). Lesions of the mPFC impaired attention in a visual discrimination task while inhibition of the PL also impaired rodents' performance in a task in which the difficulty level was unpredictable, suggesting this region's necessity in tasks requiring more attention (Williams et al., 1999; Kahn et al., 2012). Dopaminergic modulation of the mPFC also mediated attentional processes in rodents in a novel visual working memory task (Chudasama and Robbins, 2004) and ample evidence suggests cholinergic modulation of the mPFC is linked to attention and cue detection (Bloem et al., 2014), indicating a role for neuromodulatory control of cognitive and behavioral flexibility within the mPFC.

Another essential component of executive control mediated by the mPFC is response inhibition, which requires the suppression of certain actions that are inappropriate in a given context in order to execute adaptive goal-directed behaviors. In a Go/No-Go task, human mPFC displayed decreased activity during response inhibition, with greater deactivation corresponding to fewer errors in the response inhibition task (H. Rodrigo et al., 2014). In rodents, layer V

pyramidal neuron excitability within the PL decreased after acquisition of a response inhibition task and this was correlated with successful withholding of an inappropriate response (Hayton et al., 2010), suggesting a PL-specific role for action selection. Successful response inhibition was also associated with increased synaptic plasticity within cortico-striatal PL neurons and increased glutamatergic transmission to ventral striatum (Hayton et al., 2011), providing evidence for a circuit mechanism for top-down impulse control.

Executive control is also essential to the short-term active maintenance of information, known as working memory. Human mPFC activity is linearly related to working memory load and maintenance (Braver et al., 1997; Kamiński et al., 2017). Interestingly, dmPFC is more active during maintenance of emotional content vs visual content while maintenance of both of these requires suppression of vmPFC (Smith et al., 2018), indicating that executive control of working memory also requires communication within the mPFC itself. Inhibition of the mPFC in rodents results in impaired working memory in rats and it appears that aspects of working memory tasks coincide with increases in spiking frequencies and changes in neuronal firing synchrony within the mPFC (Yang et al., 2014). Neuronal ensembles within the mPFC encode various aspects of working memory in a T-maze, including spatial- , outcome-, and choice-related information (Yang and Mailman, 2018). This study conducted by Yang et al. not only identified strategic neuronal encoding during working memory but also revealed that these ensembles are specially tuned to compute and adjust behavioral outcomes. In the following section, I will discuss executive control in

the mPFC in relation to this goal-directed behavior, specifically in the context of social decision-making.

1.5.3 Social cognition and decision-making within the mPFC

Certain social behaviors and interactions require a degree of decision-making that is informed by environmental contexts and internal representations. Goal-directed social behavior aims to achieve social goals, including forming and maintaining relationships, navigating social hierarchies, and interpreting one's affective state and intentions. With its ability to integrate bottom-up exogenous and endogenous information and compute an optimal decision out of many potential outcomes, the mPFC is well-situated to guide social decision-making behavior.

Both the dorsal and ventral portions of the PFC have been linked to various aspects of social cognition and decision making. The dmPFC in particular displays increased activation when forming impressions of an individual (Harris et al., 2007) and when taking on the perspective of another (D'Argembeau et al., 2007), suggesting this region plays a role in interpreting or predicting the mental or emotional state of another. Patients with vmPFC lesions performed worse than healthy controls on theory of mind tasks (Xi et al., 2011), displayed decreased prosocial behavior in various social decision-making tasks(Krajbich et al., 2009), and exhibited social apathy and abnormal social decision making (Barrash et al., 2000). This region is also active in healthy subjects when judging negatively valenced social scenarios, such as cutting a line (Grossman et al., 2010), when generating thoughts containing episodic or social features (Konu et al., 2020),

and when experiencing feelings of social acceptance (Gunther Moor et al., 2010), indicating that this region may inform social decisions based on moral judgements, self-generated thought, and desire to be liked. In primates, the PFC appears to maintain a representation of one's social state that informs social decision-making (Fujii et al., 2009). In sum, the mPFC in human and non-human primates appears to generate representations of other's emotional state or character, promote feelings of social reward, and judge social scenarios, all of which contribute to its ability to inform social decisions and interactions.

An extensive body of work has investigated the mPFC's role in rodent social behavior (for a thorough review that includes cross-species comparison see Kietzman and Gourley, 2023), particularly those behaviors that require a degree of social processing, including social motivation. Broadly, rodent mPFC is necessary for a number of social processes, including social recognition (Morici et al., 2015; Sakamoto and Yashima, 2022), dominance (Dulka et al., 2018; Li et al., 2023), memory(Xing et al., 2021), and affective discrimination (Scheggia et al., 2020). A number of *in vivo* recording experiments have linked mPFC neuronal activity to these various social behaviors: subsets of mPFC neurons are active during social approach to a novel mouse (Lee et al., 2016), non-overlapping mPFC neural populations respond to the onset and offset of behavior in addition to encoding aspects of social salience and novelty (Liang et al., 2018), neurons in the dmPFC of mice encode features of conspecific sex across contexts and this predicts sex preferential social behavior (Kingsbury et al., 2020). There is also evidence for interbrain synchrony within the dmPFC

between mice during various social behaviors, with synchronous cells encoding one's own behavior and the behavior of their partner (Kingsbury et al., 2019). Together, these findings indicate that mPFC activity is correlated with behavioral outputs, changes in social contexts, and various conspecific features and therefore may serve as a locus for social affective behavior in rodents

Within the mPFC, local inhibitory microcircuits exhibit tight control over computational processes, long-range efferents and afferents, and behavioral outputs (Anastasiades and Carter, 2021; Yang et al., 2021), making the balance of excitatory and inhibitory neurotransmission within this region a crucial component of social behavior. For example, elevation of excitatory activation in the mPFC disrupted social interaction, while increasing inhibitory activation rescued this behavior (Yizhar et al., 2011). Accordingly, specific classes of GABAergic interneurons exhibit profound effects on social behavior. Optogenetically increasing the excitability of PV interneurons within the mPFC rescued social deficits in CNTNAP2 knockout mice, which exhibit autism-like phenotypes (Selimbeyoglu et al., 2017). Similarly, PV neurons within the dmPFC are specifically active directly prior to a social bout initiated by a focal mouse and activation of these populations promotes sociability (Bicks et al., 2020). Alternatively, photoinhibition of mPFC somatostatin (SOM) interneurons abolished affective state discrimination in mice (Scheggia et al., 2020), indicating specialized roles for specific classes of interneurons. These cell-type specific functions and microcircuity within the mPFC allow for even finer control of social

behavior, in some cases biasing social decisions based on the social affect of a conspecific.

The mPFC's anatomical connections with other nodes in the SBN also informs its role in social behavior. Projections from the BLA, a structure implicated in a number of anxiety-like behaviors, to the mPFC bidirectionally modulate social interaction in the resident-intruder test, with optogenetic stimulation of this pathway reducing interaction and inhibition facilitating interaction, indicating that this tract may convey internal state information related to anxiety that informs social behavior (Felix-Ortiz et al., 2016). Projections from the mPFC to downstream structures like the hypothalamus, a region essential to aggression, and PAG, a brainstem structure necessary for defensive behaviors, promote social dominance behavior and mediate social avoidance (Franklin et al., 2017; Padilla-Coreano et al., 2022). These circuits provide yet another example of the mPFC's function as an executive controller, modulating social behaviors via top-down signals.

Another important consideration when discussing mPFC social function is its subregional heterogeneity. Though many studies target the mPFC generally, there is sufficient evidence for functional specialization across subregions. For example, PL lesions, but not ACC lesions, impaired social recognition and investigation (Yashima et al., 2023). Broadly, the PL and IL have often been cited as having dissociable, opposing roles in fear expression, extinction, and reward behavior (Gourley and Taylor, 2016). Accordingly, projections from each of these regions to BLA appear to have opposing effects on social preference, with

activation of PL projectors and inhibition of IL projectors abolishing social preference behavior in mice. Further, it seems that PL-BLA neurons encode non-social-specific negative valence that may influence the affective state of the subject, thereby affecting social behavior (Huang et al., 2020). The PL's ability to encode multiple aspects of stimuli and contexts is evident in its projections to the NAc, which encode both social and spatial aspects of an environment. Activation of the PL-NAc pathway decreased social preference and subsets of these NAc-projections neurons were responsive depending on the location of the social stimulus (Murugan et al., 2017). In sum, these findings point to the mPFC's ability to differentially drive social behavior via its varied connectivity across subregions, and its capacity to synthesize information that includes valence and spatial location, and internal state signals. Chapter 4 expands on this literature, detailing a role for insula-PL circuitry in social affective preference behavior.

1.6 Studying social affective behavior: The SAP test

Researchers use a number of behavioral paradigms to study different aspects of social interaction. Some paradigms, like the three-chamber and social preference test, quantify a rodent's preference for social behavior generally and may reflect social motivation (Jabarin et al., 2022). The resident-intruder paradigm and social avoidance test measure a rodent's aggression or avoidance, respectively, of a potentially threatening stimulus animal (Toth and Neumann, 2013). In terms of social affective behavior, a few studies have demonstrated a rodent's capacity for prosocial behaviors toward stressed others (Meyza et al.,

2017), and these provide a good model for empathy-like behaviors in rodents (de Waal and Preston, 2017). Studying social affective behavior, however, provides a unique challenge in that many of the paradigms used require some form of learning or exposure to a conspecific experiencing pain, which may be driven by other motives, including a desire for social contact (Silberberg et al., 2014), or be a product of conditioned behavior (Kim et al., 2019). To minimize potentially tangled motivations when studying social affective behavior, our lab utilizes a Social Affective Preference (SAP) test to quantify the unconditioned behavior of an experimental rat toward conspecifics of varying affective states.

During SAP testing, which is discussed throughout this dissertation and described in detail in the methods sections of Chapters 2 and 3, experimental rats are presented with two conspecifics, one that experiences 2 mild 5s footshocks directly prior to testing, inducing a negative affective state, and one that is naïve to stress and in a neutral affective state. Time spent with each conspecific is recorded over the course of a 5 minute testing session. Preference behavior is modulated by the affective state and age of the conspecifics, among other factors. Experimental adult rats prefer stressed juveniles to naïve juveniles and this preference is flipped for adult conspecifics (Rogers-Carter et al., 2018b). A number of other factors modulate an experimental rat's preference in this paradigm including familiarity and illness (Rogers-Carter et al., 2018a; Rieger et al., 2022c). Multiple neuromodulatory systems, including serotonin, corticotropin-releasing factor (CRF), and oxytocin, modulate this behavior, specifically within the insula (Rogers-Carter et al., 2018b; Rieger et al., 2022a; Ng et al., 2023).

Optogenetic and chemogenetic inhibition of the insula (Rogers-Carter et al., 2018b) and its projections to the NAc (Rogers-Carter et al., 2019) also abolishes the preference behavior observed in SAP testing, positioning the insula as a crucial node within a social affective brain network (Rogers-Carter and Christianson, 2019). This dissertation expands on this circuitry using the SAP test, implicating insula connectivity with both the BLA and PL subregion of the mPFC in this social affective behavior.

1.7 Overview of Dissertation Aims

The overarching aim of this dissertation is to investigate whether the insula's connections with other nodes within the SDMN and CSB are necessary for social affective behavior toward stressed others. This requires cell- and tract-specific experimental manipulations that can only be performed in rodent models at the moment. The BLA and PL were identified as nodes of interest due to their connectivity with the insula (McDonald, 1998; Shi and Cassell, 1998; Hoover and Vertes, 2007; Gehrlach et al., 2020a; Mathiasen et al., 2023) and c-fos activation during social interactions with conspecifics of varying affect (Rogers-Carter et al., 2018b). Therefore, the first goal of this work was to determine whether the BLA and its projections to the insula mediate social affective preference behaviors in male and female rats. The second goal was to focus on insula efferents to the PL and determine whether social affective behavior was dependent on this circuitry.

Chapter 2 will present findings published in Djerdjaj et al, 2022, and Djerdjaj et al, 2023 (Djerdjaj et al., 2022, 2023), detailing the role of the BLA,

insula, and the BLA-insula pathway in social affective behavior in male and female rats. In males, chemogenetic inhibition of both the BLA and its projections to the insula abolished social affective preference for stressed juveniles in the SAP test. In females, pharmacological inactivation of the BLA and the insula also abolished social affective preference behavior typically observed towards stressed juveniles and naïve adults. Interestingly, oxytocin administration into the BLA of females increased social exploration of both naïve juvenile and adult conspecifics while insular oxytocin administration had no effect on this behavior. This revealed a sex-specific effect of oxytocin, as insular oxytocin increased social investigation in male rats (Rogers-Carter et al., 2018b) but had no effect in females.

Chapter 3 will present experimental data investigating the insula-PL tract in social affective behavior. Pharmacological inhibition of the PL via muscimol abolished preference for stressed juveniles. Retrograde tracing confirmed projections to the PL arise most prominently from the anterior insula followed by the posterior insula. A relatively novel anterograde, transsynaptic, chemogenetic viral strategy was implemented to specifically inhibit cells of the PL that receive monosynaptic input from either the anterior or posterior insula during SAP testing. These two tracts were targeted separately due to functional differences evident across insula subregions. Chemogenetic inhibition of PL cells that are postsynaptic to the insula, regardless of whether they originated in anterior or posterior insula, abolished social affective preference behavior toward stressed juveniles and naïve adults. To determine whether these PL populations are

specifically recruited to guide behavior in more complex social contexts, experimental rats underwent opposite sex preference tests. Chemogenetic inhibition of PL cells postsynaptic to insula had no effect on male experimental rats' preference for female conspecifics. Due to the importance of the E/I balance in the mPFC to social behavior, immunohistochemical staining was used to classify PL cells postsynaptic to the extent of the insula as either glutamatergic or GABAergic. This revealed the insula preferentially targets glutamatergic neurons within the PL, with anterior insula inputs representing a significantly larger population of cells.

Overall, these findings reflect the initial overarching aim of this project: expanding on our prior knowledge of the insula's role in social affective behavior, I report evidence for both a BLA-insula and an insula-PL circuit that are necessary for the detection and appraisal of the affective states of others and for the orchestration of appropriate behavioral output. These results lend support to the hypothesis that the insula represents a crucial node within a larger social affective brain network and its connections with limbic and cortical structures is an essential component of rodent social affective decision making.

Chapter 2: Basolateral amygdala and insula circuitry underlie social affective behavior in male and female rats

The contents of this chapter have been published in the following research articles:

Djerdjaj, A., Ng, A. J., Rieger, N. S. & Christianson, J. P. The basolateral amygdala to posterior insular cortex tract is necessary for social interaction with stressed juvenile rats. *Behavioural Brain Research* **435**, 114050 (2022).

Djerdjaj, A. *et al.* Social affective behaviors among female rats involve the basolateral amygdala and insular cortex. *PLOS ONE* **18**, e0281794 (2023).

2.1. Forward

The use of animal models allows us to perform translational research, granting us mechanistic insight into the neural systems underlying behavioral processes such as social affective behavior (Möhrle et al., 2020; Jabarin et al., 2022). The circuitry connecting nodes within both the SDMN and SBN is conserved between rodents and humans and regions in these networks share general functions across species (O'Connell and Hofmann, 2011a; Prounis and Ophir, 2020a), allowing for the use of rats as a model organism for human behavior. As discussed in Chapter 1, the insula, a proposed node within the SDMN (Rogers-Carter and Christianson, 2019), is involved in a number of sensory and behavioral processes, including integration of multimodal sensory information (Benarroch, 2019), salience detection (Uddin, 2015), interoception (Livneh et al., 2020), and social affective behaviors (Singer et al., 2009). It is also bidirectionally connected to the BLA (Shi and Cassell, 1998), a region essential to fear- and reward-related behaviors (Wassum and Izquierdo, 2015; Sun et al., 2020), valence detection (Smith and Torregrossa, 2021), and social behavior (Gangopadhyay et al., 2021). A number of studies in rodents link insula-BLA circuit activity to social affective behaviors, including the encoding of observational pain (Zhang et al., 2022b). These regions also display functional connectivity in human neuroimaging studies when processing emotional faces (Stein et al., 2007; Pohl et al., 2013). While these regions independently have clear roles in recognizing or appraising positive and negative cues and

orchestrating behavioral responses to various stimuli, few studies have tackled the role this pathway specifically plays in social affective behavior. In the following chapter, I present behavioral findings from experiments targeting and inhibiting BLA, insula, and BLA→insula circuitry during SAP testing in rats.

The first portion of this chapter was published in *Behavioral Brain Research* and focuses on the functional role of the BLA and its projections to the insula in male rats during social affective behavior. In 2019, we published a paper detailing the role of the insula to nucleus accumbens (NAc) pathway in social affective behavior in male rats. This paper, which I had worked on as a research technician, had a huge influence on this first project of mine. When conceiving of this initial project, I was interested in continuing to identify insula circuitry underlying this behavior, specifically how afferents to the insula may contribute to or modulate social decision making behavior. The BLA immediately stood out as a region of interest, with its inclusion in the SDMN, its known bidirectional projections to the insula, and its multifunctionality in a variety of valence-related behaviors. Additionally, BLA projections to the nucleus accumbens mediate reward-seeking behavior (Ambroggi et al., 2008), positioning this region as yet another potential node within our lab's growing framework of a social affective brain network. When determining the experimental approach I would take for this study, I considered techniques we utilized in the insula-NAc experiments. Here, we used both chemogenetic and optogenetic methods to

achieve inhibition of NAc-projecting insula terminals but determined that chemogenetic manipulation provided the best method for neuronal inhibition because it allowed for free movement during social interactions and had been used in prior studies investigating related circuitry (Venniro et al., 2017; Jaramillo et al., 2018b). All of this contributed to the conception of this BLA-insula project, which found that this region and pathway is necessary for social approach to stressed juveniles in the SAP paradigm.

When beginning this BLA-insula project, my intention was to use both male and female Sprague-Dawley rats to account for any potential sex differences in the function of this circuitry. Based on prior work from our lab, in the absence of any manipulation, age and affect had similar effects on SAP behavior in males and females (Rogers-Carter et al., 2018b). Familiarity modulated this behavior in a sex-specific way, with females spending significantly more time investigating stressed adult cagemates (Rogers-Carter et al., 2018a). However, we had no evidence that this behavior was insula-dependent in female rats. I first sought to determine insula's social affective function in female rats before beginning to include them in my BLA-insula project. With the help of an undergraduate, Natalie Cortapassi, I conducted a study wherein 6 female rats received insula lesions and 6 received a sham surgery. Three weeks after surgeries, these rats underwent SAP testing with juvenile conspecifics. The results of this study yielded no significant results, with rats displaying no clear preference for either juvenile conspecific in either the sham or lesion group. While we performed surgeries, post-operative care, handling, and SAP testing as

we had in males, there is evidence that sex differences, strain differences, and even vendor differences, exist in stress responses to handling and general husbandry (Weinberg et al., 1978; Bs et al., 2003; Tsuda et al., 2020), pain response post-surgery (DeLeo and Rutkowski, 2000), and general response to anesthesia (Siegal and Dow-Edwards, 2009). Measures of anxiety also vary across sex depending on the test used, though estrous cycle in females does not contribute to this sex difference (Scholl et al., 2019). A recent review article has also stressed considering sex differences in the use of various neuroscience tools, including chemogenetics (Cea Salazar et al., 2024).

Due to our inability to replicate the SAP behavior that we typically observe in males in female rats post-surgery and a lack of knowledge of the insula's role in female SAP behavior, I proceeded to run the BLA-insula studies in male subjects exclusively. The COVID shutdown later put a halt to animal studies for a period of time, by which point I had completed the male studies for the BLA-insula project. I published these findings with the intention of reevaluating how SAP testing was run with females once the lab reopened in order to eventually replicate these findings in female rats.

The second portion of this chapter was published in *PLOS ONE* and focuses on these female SAP studies, specifically the role of the insula and BLA in social affective behavior. For these studies, I implanted indwelling cannula into either the BLA or insula of female rats to reversibly inactivate these regions via mircoinjection of muscimol, a $GABA_A$ agonist, prior to SAP testing. Based on literature detailing potential sex differences in female rat's response to post-

operative care and handling, I extended the amount of recovery time allowed between surgery and testing from 3 weeks to 4 weeks and increased the amount of handling prior to SAP testing from 5 days to 15 days. This approach proved to be effective in replicating SAP behavior in female control rats and allowed for the findings detailed below.

2.2. The basolateral amygdala to posterior insular cortex tract is necessary for social interaction with stressed juvenile rats

2.2.1. Introduction

Situationally appropriate social interactions require that an individual use the information contained in the behaviors and characteristics of social targets to make inferences about the age, sex, motivations and emotions of the other. This process is thought to be the product of neural activity distributed across the central nervous system that detects and evaluates social information and links it to social behaviors. Several conceptions of so-called “social brain networks” exist to capture the elementary neuroanatomical, neurochemical and neurobiological basis for social behavior (also referred to as the Social Behavior Network, Cognitive Social Brain, etc.) (Brothers, 1990; Goodson, 2005; Prounis and Ophir, 2020a; Newman, 1999). Building on the pioneering and influential social brain models of Newman (1999) and Goodson (2005), O’Connell and Hofmann (2011) used a comparative biology approach to describe a Social Decision Making Network (SDMN) which incorporated the social brain models of Newman and Goodson with the mesolimbic reward circuit, expanding the framework for a

wider range of psychological factors (e.g. attention, salience, motivation) to social behavior (O'Connell and Hofmann, 2011a).

One region of interest within this SDMN is the amygdala, a heterogeneous region made up of cortically- and striatally-derived nuclei located within the medial temporal lobe (Swanson and Petrovich, 1998). The basolateral amygdala (BLA), in particular, is a collection of nuclei that has received attention for its role in a number of functions, including fear memory formation and expression (Chen et al., 2019; Maren et al., 1996) and reward behavior (Ambroggi et al., 2008). It receives sensory input from both the thalamus and the cortex and sends projections to a number of subcortical and cortical structures, including the insula (Davis and Shi, 2000). A prominent view about BLA function is that it is a valence encoder (Beyeler et al., 2018; Pignatelli and Beyeler, 2019; Smith and Torregrossa, 2021; Zhang et al., 2021). Subsets of BLA neurons selectively respond to positive or negative valence cues and these seem to be anatomically organized (Sangha et al., 2013; Namburi et al., 2015; Kim et al., 2016; Beyeler et al., 2018; Zhang et al., 2021). For instance, thalamic and cortical inputs relay sensory information to the BLA (LeDoux et al., 1990) which contains projections to the central amygdala and bed nucleus that mediate fear/threat related processes (Hartley et al., 2019) and projections to the nucleus accumbens (NAc) that contribute to reward valuation and appetitive behaviors (Ambroggi et al., 2008).

Regarding social behavior, the BLA is included in the SDMN because of its high expression of social hormone receptors (Chang et al., 2015) and

interconnections with other nodes in the social brain (O'Connell and Hofmann, 2011b). Roles for the BLA in regulating social anxiety (Felix-Ortiz et al., 2016) suggest that the BLA may influence social approach and avoidance behaviors and that anxiogenic circumstances (e.g. stressors) can shift appraisal of social cues to be more negative (Christianson et al., 2010). At the circuit level, BLA inputs to the ventral hippocampus, another region in the SDMN, exhibit bidirectional control over social behavior in rodents: inhibition of BLA terminals in this region increased social interactions in a behavioral paradigm while activation reduced social interactions (Felix-Ortiz and Tye, 2014). In non-human primates, activation of the BLA also suppressed social behavior (Wellman et al., 2016). The BLA's ability to distinguish between valenced stimuli and influence sociability at the circuit-level position this region as key to understanding how emotion shapes social behavior.

The comparative biology approach used to define the SDMN necessarily excluded brain regions, such as neocortex, that do not have obvious homology across taxa. In mammals, neocortical regions contribute to sensory integration (Gogolla et al., 2014) and social cognition (Adolphs, 2001; Amodio and Frith, 2006), and neuroimaging studies suggest that functional connectivity between amygdala and cortical regions is fundamental to socioemotional behaviors (Baas et al., 2008; von dem Hagen et al., 2013; Gorka et al., 2015). Of the cortical structures innervated by the BLA, the insula is of particular interest to the investigation of social decision making. The insular cortex spans the lateral surface of each hemisphere in rodents. Along its rostral-caudal axis, the insula

consists of granular, dysgranular, and agranular subdivisions that differ in cytoarchitecture and projection patterns (Allen et al., 1991; Gogolla, 2017). The insula receives visceral, gustatory, nociceptive, and thermal sensory inputs (Kobayashi, 2011), positioning it as a hub for sensory integration (Rodgers et al., 2008; Gogolla et al., 2014) and salience detection (Menon and Uddin, 2010a). Insula projections are varied but include a number of cortical and subcortical regions important to social behavior including the medial prefrontal cortex (mPFC), the NAc, and the BLA. Similar to the BLA, insula connectivity informs function (Rogers-Carter and Christianson, 2019). For example, projections to the mPFC may be linked to pain perception (Euston et al., 2012), while NAc projections are implicated in reward-seeking behavior (Jaramillo et al., 2018b, 2018a; Gehrlach et al., 2019a). Generally, the anterior portion of the insula is associated with emotional awareness in humans (Lamm and Singer, 2010; Gu et al., 2013a) and appetitive behaviors in rodents (Peng et al., 2015; Pushparaj et al., 2015; Haaranen et al., 2020), while the posterior portion is implicated in interoception in humans (Kuehn et al., 2016) and in aversive state processing (Gehrlach et al., 2019a) and social decision-making in rodents (Rogers-Carter et al., 2018b). Importantly, the posterior insula also receives a dense fiber input from the posterior BLA, positioning it as a site for the integration of external valence information with internal states (Allen et al., 1991; Kobayashi, 2011; Gehrlach et al., 2020a). Human neuroimaging studies strongly associate insula function and connectivity with social affective processes including emotion recognition and empathy (Wicker et al., 2003; Singer et al., 2009).

In a social affective preference (SAP) test, a paradigm in which social emotions shape experimental behaviors, rats prefer interactions with stressed, isosexual juveniles but avoid interactions with stressed, isosexual adults compared to their naïve counterparts. This behavior is dependent on insula activity as treatments that interfere with insula function eliminate or reverse the social preferences typically observed in the SAP test (Rogers-Carter et al., 2018b, 2019; Rieger et al., 2022c, 2022a). The goal of the current study was to investigate the contributions of the BLA and its anatomical projections to the posterior insular cortex in social emotional behavior in the SAP test with juvenile conspecifics. Because the BLA is thought to encode the emotions of others (Phelps and LeDoux, 2005) and the valence of stimuli (Beyeler et al., 2018; Pignatelli and Beyeler, 2019; Smith and Torregrossa, 2021; Zhang et al., 2021) while the insula integrates interoceptive sensory information (Gogolla et al., 2014; Rogers-Carter and Christianson, 2019) and coordinates social approach and avoidance (Rogers-Carter et al., 2018b, 2019; Rieger et al., 2022c, 2022a), we predicted that chemogenetic inhibition of the BLA and BLA-insula neurons would interfere with the social approach to stressed juvenile conspecifics that is typical of rats in the SAP test.

2.2.2. Materials and Methods

2.2.2.1 Subjects

Male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate to the vivarium in the Boston College

Animal Care Facility for at least 7 days before any procedure was carried out. Adult experimental rats arrived weighing 225-250g and juvenile conspecifics arrived at PN21. Experimental rats were housed in pairs while juvenile conspecifics were housed in triads. The vivarium maintained a 12h light/dark cycle and food and water were available *ad libitum*. Behavioral testing was conducted within the first 4 hours of the light cycle. All procedures were approved by the Boston College Institution Animal Care and Use Committee and adhered to the Public Health Service *Guide for the Care and Use of Laboratory Animals.*

2.2.2.2 Chemogenetic Procedures

To inhibit the BLA and the terminals of BLA-insula neurons during SAP tests, we used a chemogenetic approach. Chemogenetics involve the transduction of a viral vector carrying the gene encoding a modified receptor that is coupled to the $G\alpha_i$ G-protein receptor signaling cascade (hM4Di) (Roth, 2016). We used the same parameters reported for chemogenetic control of the insula-NAc tract (Rogers-Carter et al., 2019). The first set of experiments focused on local inhibition of the BLA. Experimental adult male rats underwent surgery under inhaled anesthesia (2-5% v/v isoflurane in O_2) and a virus containing hM4Di under a neuron specific promoter (pAAV5-hSyn-hM4D(Gi)-mCherry; Addgene catalog #50475-AAV5; titer: 9 x 10^{12}GC/mL) was microinjected bilaterally into the BLA of experimental rats (from bregma: A/P -2.5, M/L +/- 5.0, D/V -8.5) at a rate of 100nL/min to a total volume of 500nL per side. Five minutes were allowed for diffusion. Systemic administration of the hM4Di actuator, clozapine-N-oxide

(CNO), should cause hyperpolarization of hM4Di containing neurons in the BLA (Armbruster et al., 2007; Roth, 2016). The second experiment focused on inhibition of BLA terminals in the posterior insular cortex. As above, hM4Di-containing virus was injected bilaterally into the BLA. To direct CNO to the BLA terminals, indwelling cannula (Plastics One) were implanted in the posterior insular cortex (from bregma: A/P -1.8, M/L +/- 6.5, D/V -6.9) and fixed in place with stainless steel screws and acrylic cement. Cannula were fitted with stylets to maintain patency. Subsequent microinjection of CNO to the insula should reduce terminal neurotransmitter release from BLA terminals in the insula, preventing the relay of information from the BLA to insula (Mahler et al., 2014; Rogers-Carter et al., 2019). Importantly, in our prior studies we verified that CNO hyperpolarized hM4Di expressing neurons in whole cell patch clamp recordings and reduced Fos immunoreactivity (Rogers-Carter et al., 2019). A control group entailing rats that have either no virus or a sham virus were not included in this study because in our experience the dose of CNO and route of injection, i.p. or intracerebral microinjection did not influence SAP behavior in rats without hM4Di (Rogers-Carter et al., 2019). After surgery, rats were administered meloxicam (1 mg/kg, Eloxiject; Henry Schein) and the antibiotic penicillin (1 mg/kg, Combi-Pen; Henry Schein). Rats were allowed 2-3 weeks of recovery before undergoing behavior testing as described below. To inhibit the BLA, experimental rats received either an intraperitoneal (I.P.) vehicle injection (DMSO in Saline) or CNO (3 mg/kg; Tocris) 45 minutes prior to SAP testing. This dose is consistent with prior work in which 3 mg/Kg CNO was behaviorally active when injected into subjects

expressing chemogenetic receptors but inert in sham controls(Roth, 2016). To inhibit BLA-insula terminals, either 0.5μL of vehicle (sterile PBS) or CNO (1 μM) was microinjected to the insula 30 minutes prior to testing. We used a CNO dose of 1 μM previously to inhibit insula neuron terminals in the NAc with behavioral effects in hM4Di expressing rats and no effect in sham controls (Rogers-Carter et al., 2019) which is consistent with similar approaches reported by others (Mahler et al., 2014; Stachniak et al., 2014).

2.2.2.3 Social Affective Preference (SAP) test

This procedure allows for the observation of a rodent's behavior when presented with conspecifics of varying social affect. The SAP paradigm takes place in a large plastic arena (50 x 40 x 20 cm; L x W x H) with beta chip bedding and a transparent plastic lid. Juvenile conspecifics were individually placed into clear acrylic chambers (18 x 21 x 10cm; L x W x H) comprised of acrylic rods spaced 1 cm apart to allow for direct interaction. These chambers are placed at opposite ends of the plastic arena during testing. Testing consists of 2 habituation days followed by 2 test days. On day 1, experimental rats were placed into their own plastic arena and allowed 60 minutes of acclimation. On day 2, experimental rats were placed in their arena for 60 min and then underwent 5 minutes of behavior testing where they were presented with two naïve juvenile conspecifics in the acrylic chambers. Days 3 and 4 test the experimental rats' social affective preference after injection of vehicle or CNO, as described above. On day 3, rats were placed in the arena for 60 min and then presented with a pair of unfamiliar

conspecifics. One conspecific received an acute stressor of 2 footshocks (5s, 1 mA, inter-shock interval of 50s); the other conspecific was naïve to any treatment. A trained observer quantified the amount of time the experimental rat spends investigating each conspecific. Social investigation was defined as time spent sniffing or touching the conspecific through the acrylic bars. Testing on day 4 proceeded identically to day 3 with rats receiving the opposite drug treatment from the day prior in a counterbalanced, within-subjects design. Conspecifics were used for no more than 3 days of testing. All tests were recorded in digital video. Recordings were scored by a trained observer blind to the experimental conditions to establish inter-rater reliability.

2.2.2.4 Histology

At the end of behavioral testing, experimental rats were overdosed with tribromoethanol and perfused with cold 0.01 M heparinized PBS followed by 4% paraformaldehyde. Dissected brains were stored in 4% paraformaldehyde for 24 h and then transferred to 30% sucrose for at least 2 days. 40 μm coronal sections were obtained in series on a freezing cryostat (Leica). Insula sections were directly mounted on gelatin-coated slides and stained with cresyl violet for verification of cannula placement. BLA sections were directly mounted onto slides and coverslipped with Vectashield containing DAPI (Vector Laboratories) to visualize mCherry, a genetically encoded fluorescent protein fused to hM4Di, and determine the spread of hM4Di under a fluorescent microscope (Zeiss Axiomanger Z.2). Additional sections containing posterior insular cortex were

used to verify expression of the mCherry fusion protein with immunohistochemistry stain exactly as described in Rogers-Carter et al (2019).

2.2.2.5 Statistical Analysis

Data from rats receiving stereotaxic injections were only included when site-specificity criteria were met. For BLA injections, rats were included if mCherry expression was brightest in the posterior BLA and LA (-2.5mm posterior from bregma or further). The virus frequently spread to the central amygdala (CeA), potentially due to the large injection volume or the interconnectedness of these two regions. Although hM4Di-mCherry expression spread into the CeA in some rats, the CeA does not project to posterior insula (Allen et al., 1991; Shi and Cassell, 1998; Swanson and Petrovich, 1998; Gehrlach et al., 2020a), allowing us to conclude that the behavioral effects of insular CNO were caused by inhibition of axon terminals of neurons with soma in the BLA (Allen et al., 1991; Shi and Cassell, 1998; Swanson and Petrovich, 1998; Gehrlach et al., 2020a). Inclusion criteria for BLA-insula terminal inhibition experiments required evidence of i.) bilateral expression of hM4Di in the BLA, ii.) bilateral cannula placement in the posterior insular cortex and iii.) mCherry expression in the posterior insula confirmed through immunohistochemical stains. Sample sizes were determined by conducting a priori power analyses in G*Power using effect sizes observed

previously (Rogers-Carter, 2018) and N = 12 was determined to be appropriate to achieve power ≥ 0.70. Several cohorts of rats went through these procedures to reach the target sample size. Datasets were tested for normality and sphericity prior to analysis and found to be suitable for t-test and analysis of variance.

Social exploration times were compared using repeated measures two-way Analysis of Variance (ANOVA) with conspecific stress and drug treatment (vehicle or CNO) as within-subjects variables. Main effects and interaction effects were deemed significant at $p<0.05$ and post-hoc analysis consisted of Tukey's multiple comparison test. Preference for the stressed conspecific in each condition was calculated as a percentage of time investigating the stressed conspecifics relative to the total time spent investigating both conspecifics and compared using paired t-tests. Statistical analyses were conducted in Prism 8 (Graphpad Software).

2.2.3. Results

2.2.3.1 BLA is necessary for social investigation of stressed juveniles.

To determine the effect of BLA inhibition on social affective preference, adult male experimental rats received bilateral injections of a viral vector containing an hM4Di receptor into the BLA and later underwent SAP tests with juvenile conspecifics after systemic vehicle or CNO injection (Figure 2.1A). After review of virus placement, 12 rats were found to meet criteria for inclusion based on placement (Figure 2.1B&C). The amount of time the experimental rat spent

investigating the naïve and stressed conspecific (defined as direct physical touch) was analyzed with ANOVA where drug treatment (vehicle vs. CNO) and conspecific affect (naïve vs. stressed) were treated as within-subjects factors. One additional rat was excluded from analyses because of unusually high social investigation in the SAP test (greater than 60s total, identified as an outlier by Grubb's Test) resulting in a final sample size N=11. Experimental rats preferred interaction with stressed juveniles after vehicle injection but appeared to lose this preference after CNO (Figure 2.1D). There was a main effect of social affect ($F(1,10)=19.03$, $p=0.001$) but the drug by affect interaction did not reach significance ($F(1, 10)=2.47$, $p=0.147$). Post-hoc analysis using Tukey's multiple comparison test revealed a significant difference between social investigation of naïve and stressed conspecifics in the vehicle condition ($p=0.047$) but no difference between naïve and stressed in the CNO condition ($p=0.81$) suggesting that the main effect was carried primarily by preference for stressed juveniles in the vehicle condition. The preference for the stressed conspecific was calculated as a percentage of the total time spent investigating both conspecifics (Figure 2.1E). A paired samples t-test revealed a significant preference for the stressed juvenile under vehicle compared to CNO ($t(10)= 2.462$, $p=0.034$). In summary, BLA inhibition by hM4Di interfered with social approach to stressed juveniles.

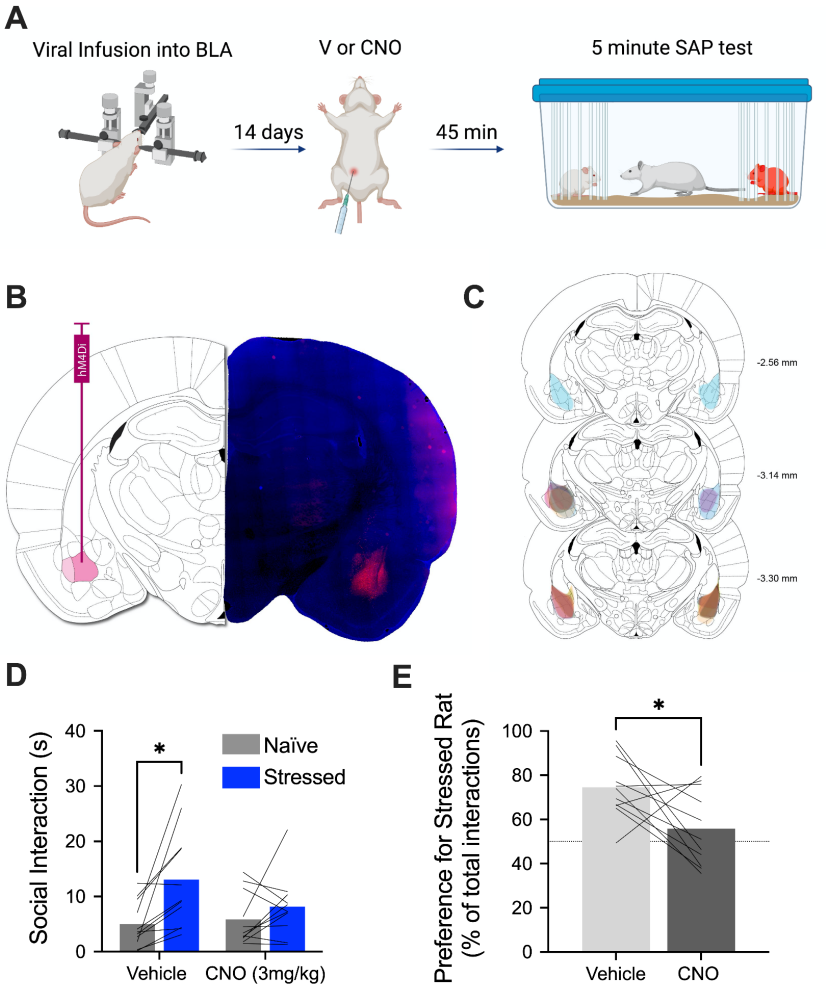


Figure 2.1 Chemogenetic inhibition of the BLA reduced social preference for stressed juveniles. **A.** Schematic diagram of the experimental design. AAV containing the gene coding for hM4Di was bilaterally injected into the BLA and, 2 weeks later, rats received vehicle (V) or CNO injections 45 min before SAP tests with juvenile conspecifics. **B.** Intended target for virus in BLA (left) and representative mCherry expression in the BLA (right, blue= DAPI, red= native mCherry). **C.** Map of viral expression in BLA from experimental rats (N=11, each rat represented by a different color). **D.** Mean (with individual replicates) time spent exploring the naïve and stressed conspecifics during the 5 min. SAP test. After vehicle injections, rats preferred social investigation of stressed juveniles compared to naïve juveniles (p=0.047), which was abolished after CNO treatment (3mg/kg). **E.** Data from **D** presented as a preference for the stressed conspecific as a percentage of total social interaction. Experimental rats preferred interaction with the stressed juvenile under vehicle treatment, which was abolished after CNO treatment (p=0.034). *p<0.05. Diagram in A created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998), use pending permission.

2.2.3.2 BLA inputs to the insular cortex mediate social approach to stressed juveniles.

To determine if insula-projecting BLA neurons are necessary for social approach to stressed juveniles, experimental rats received bilateral injections of a viral vector containing an hM4Di receptor in the BLA and bilateral cannula implants in the posterior insular cortex and later received SAP tests after vehicle or CNO infusion to the insula (Figure 2.2A). After inspection of cannula placement, BLA terminal expression in the posterior insula, and mCherry expression in the BLA, the amount of time the experimental rat spent investigating each conspecific was analyzed in n=14 rats which met the criteria for inclusion (Figure 2.2B&C). After vehicle injections, experimental rats spent more time investigating the stressed juvenile; no preference was evident after CNO injections (Figure 2.2D). A 2-way ANOVA analyzing social interactions across conditions revealed a main effect of social affect, ($F(1,13) = 5.622$, $p=0.034$), and a drug by social affect interaction, ($F(1, 13) = 14.25$, $p=0.0023$). Post-hoc analysis revealed a significant difference between social investigation of naïve and stressed conspecifics in the vehicle condition ($p=0.0057$) and social investigation of naïve conspecifics in the vehicle and CNO conditions ($p<0.05$). The preference for the stressed rat was calculated as described above. A paired t-test revealed a significant difference ($t(13) = 4.909$, $p<0.0003$), with the percent investigation score for the stressed juvenile significantly decreased under CNO (Figure 2.2E). Taken together, these results indicate that chemogenetic inhibition

of BLA terminals in the insula interferes with social approach to stressed juveniles.

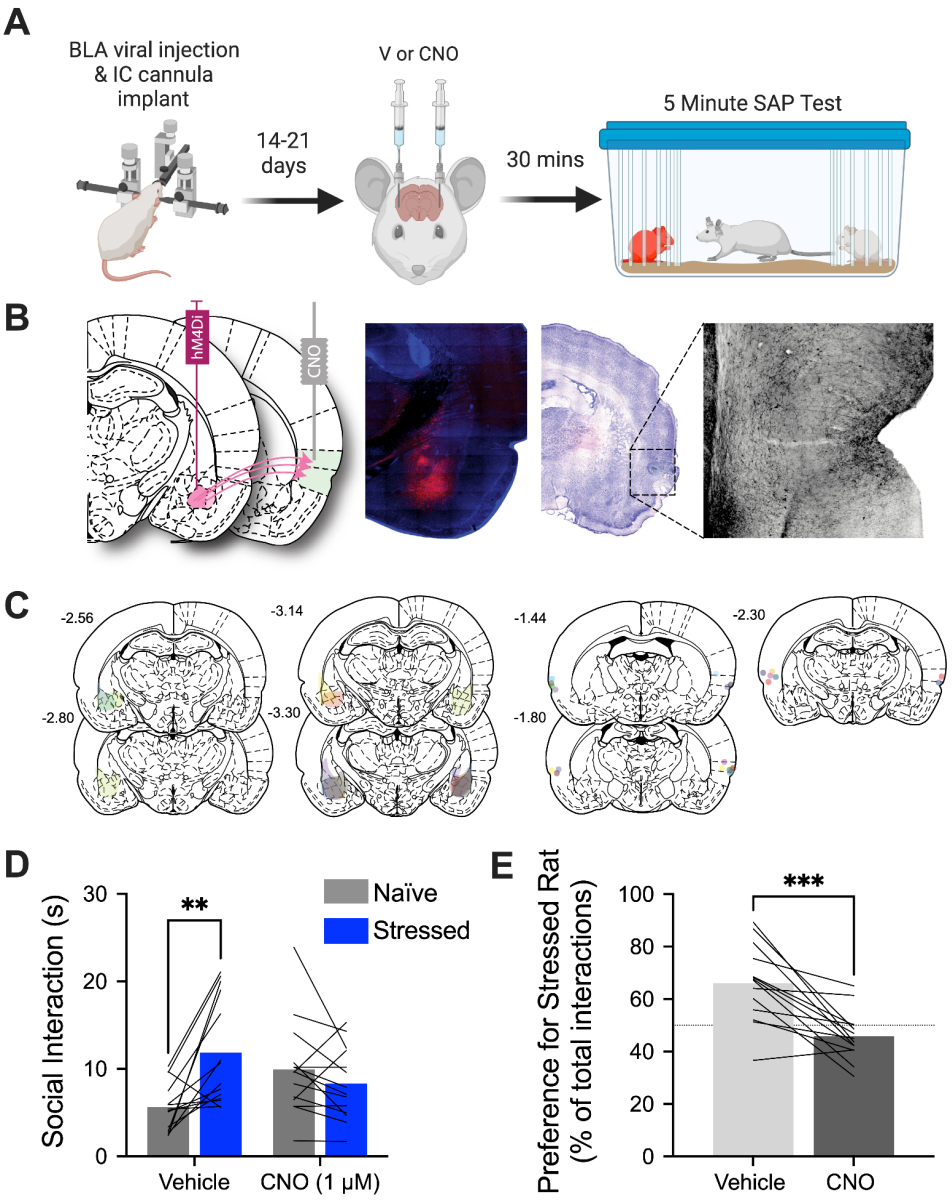


Figure 2.2 Chemogenetic inhibition of BLA to pIC terminals blocked social affective preference for stressed juvenile conspecifics. **A.** Schematic diagram of experimental approach. AAV containing the gene coding for hM4Di was bilaterally injected into the BLA and cannula were implanted into the insula and 2-3 weeks later rats received vehicle (V) or CNO (1μM) infusions 30 min before SAP tests with juvenile conspecifics. **B.** LEFT: Schematic of viral injection into the BLA and cannula implant into posterior insula with (left-to-right): i) representative image of hM4Di-mCherry expression (red = native mCherry, blue = DAPI), ii) cresyl violet image of cannula damage, and iii) mCherry amygdala terminal fiber expression. **C.** Maps of viral expression in BLA (left side) and cannula placements in posterior insular (right side, individual rats are represented by different colors) **D.** Mean (with individual replicates) time spent interacting with the naïve

and stressed juvenile conspecifics during the 5 min SAP tests after vehicle or CNO injection. In the vehicle condition, rats spent more time investigating stressed juvenile conspecifics than naïve juvenile conspecifics (p=0.005). In the CNO condition rats explored the stressed and naïve conspecifics equally. **E.** Data from (D) expressed as the mean (with individual replicates) preference for the stressed conspecific as a percentage of the total social interaction. CNO injections reduced preference for the stressed juvenile (p=0.0003). ** p<0.01, *** p<0.001. Diagram in A was created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998), use pending permission.

2.2.4. Discussion

Seeking to more completely describe the neural basis for this type of social affective behavior, we investigated whether preference for stressed juveniles involved the BLA, a region involved in many forms of social and non-social affective process, and its projections to the posterior insula, a region known to mediate social approach to stressed juveniles. In a social affective preference paradigm, experimental adult rats typically initiate interactions with stressed juveniles but avoid interactions with stressed adults (Rogers-Carter et al., 2018b, 2018a, 2019; Rieger et al., 2022c, 2022a). Chemogenetic inhibition of the BLA and the BLA axon terminals in the insula both reduced time spent investigating stressed juveniles. These findings provide a mechanistic basis for how emotional valence, encoded in the BLA, may reach the insula where it might be integrated with other situational information to determine when to approach or avoid other animals in distress.

The decision to approach or avoid is informed by cues emitted by conspecifics. In rats, affect is conveyed by ultrasonic vocalizations (USVs) (Brudzynski, 2013; Knutson et al., 20021016), chemical alarm signals (Sterley and Bains, 2021) and overt behaviors (such as behavioral immobility). USVs in the 50-Hz range are associated with positive affect and prosociality and elicit tonic decreases in amygdala activity of listeners. We found that in interactions with stressed juveniles, fewer of the prosocial 50Hz USVs were present (Rogers-Carter et al., 2018b). In contrast, USVs in the 22-Hz range signal aversive states and elicit tonic increases in amygdala activity (Parsana et al., 2012; Seffer et al.,

2014). Importantly, rats with BLA lesions do not respond to either type of USV, indicating that this region is critical for recognizing and responding appropriately to vocalizations (Schönfeld et al., 2020). Stressed rats also release chemical alarm signals that are reliably identified by others (Valenta and Rigby, 1968; Kiyokawa et al., 2006). These alarm signals trigger increased *c-fos* expression in a number of brain regions of target rats, including the BLA (Kiyokawa et al., 2005; Kyriazi et al., 2018), positioning it as a potential site for identifying these olfactory signals. *In vivo* recordings and activity-dependent tagging reveal that BLA neurons preferentially encode stimuli that predict either reward or punishment (Beyeler et al., 2016), and unconditioned stimuli that are inherently positive or negative (e.g., sucrose vs. quinine) which is thought to reflect valence-coding within the BLA (Belova et al., 2008; Gore et al., 2015). While it has not been tested directly, it is likely that social affective cues, such as USVs, odors and overt behaviors are assigned valence in the same way within the BLA. In the SAP test, BLA inhibition may have disrupted the encoding, or the assignment of valence, to alarm signals emitted by stressed juveniles rendering the test rat unable to differentiate the affective states of the conspecifics. Rather than reducing overall social behavior, this resulted in experimental rats spending comparable amounts of time investigating each conspecific, likely due to cortical regions lacking the necessary valence information that would be relayed by the BLA to inform appropriate decision-making behavior.

Valence-coding is organized by the anatomical targets of the glutamatergic projection neurons of the BLA. For example, activation of BLA

projections to the NAc drive reward-seeking behaviors (Ambroggi et al., 2008; Stuber et al., 2011). Projections to mPFC bidirectionally modulate social and anxiety-related behaviors with activation *reducing* and inactivation *facilitating* social interaction in a resident-juvenile intruder paradigm (Felix-Ortiz et al., 2016). The insula is one of many targets of these projection neurons. As a site of interoception (Aguilar-Rivera et al., 2020; Rodríguez et al., 2020) and multisensory input (Rodgers et al., 2008), the insula is crucial for the perception of bodily states and integration of socially-salient information, both of which contribute to social decision-making. In this study, terminal inhibition of insula-projecting BLA neurons abolished preference for a stressed juvenile. This suggests a bottom-up process, with the BLA responsible for encoding the valence of social affective cues, and relaying this socially-salient information to the insula for further processing. An important goal of future work will be to establish whether BLA neurons that project to the insula exhibit valence-specificity and if the same neurons that are involved in approach to stressed juveniles are involved when rats avoid conspecifics, for example when the target is a stressed adult, or is sick (Rogers-Carter et al., 2018b; Rieger et al., 2022c).

In any social circumstance, information about the interaction setting (context) and the interactants (self/other) is necessary to decide what behaviors are appropriate. Our studies thus far used a familiar, neutral setting and test rats that are naive to laboratory manipulations. In this preparation, we can make the assumption that the motivation to approach or avoid the conspecifics is a consequence of conspecific features. In addition to the preference to interact with

stressed juveniles, there are several other conspecific features that drive social choice. For example, i.) rats prefer interactions with novel/unfamiliar conspecifics (Smith et al., 2015; Rogers-Carter et al., 2019), ii.) male rats avoid stressed adults (Rogers-Carter et al., 2018b; Rieger et al., 2022a), iii.) female, but not male, rats approach stressed cagemages (Rogers-Carter et al., 2018a), and iv.) adult rats avoid sick adults but not sick juveniles (Rieger et al., 2022c). Moving forward, we must account for contextual and internal factors that are expected to interact with the conspecific factors (e.g., age, sex, familiarity, affect, illness) and alter what social behaviors are appropriate. For example, would rats continue to approach stressed juveniles in a dangerous environment? More specifically, does the BLA relay contextually relevant valence information to the insula that in turn modifies a rat's behavior towards stressed others? Recently, Toyoshima et al. demonstrated that a prior stress experience caused adult rats to prefer interactions with stressed adult conspecifics (Toyoshima et al., 2021); would a rat that was hungry or sick engage in prosocial behaviors with hungry or sick others? In humans, both the amygdala and insula are activated in response to aversive odors or tastes while only the insula responds to observing others' experiences of disgust (Royet et al., 2003; Small et al., 2003; Wicker et al., 2003). Interestingly, glutamatergic inputs from the insula to the BLA encode observational pain in rodents (Zhang et al., 2022b). Could insula to BLA neurons be preferentially recruited for recognizing a conspecific's affective state based on one's own prior experience? Addressing these questions will delineate the systems biology underlying complex social decision-making.

Based on the anatomical connectivity and the growing body of mechanistic studies that show the insula mediates behaviors in context-, internal- and external-state specific ways we suggest that the insula is a site where streams of information relating to the internal and external senses summate for distribution to executive control networks to shape decisions. To test this idea we would need to determine whether insular activity is involved as we vary the nature of the interaction setting and the experience of the test subject in the SAP test. Because some social behaviors, such as social novelty preference, do not require the insula (Rogers-Carter et al., 2019), we hypothesize that the insula becomes important when the relevant contextual, internal and social information is sufficiently complex to need executive cognition to direct behavior. In this framework, removing amygdala input to the insula renders this executive process deficient of information about social valence that may typically trigger prosocial behavior toward stressed others.

Socio-emotional behaviors typical of autism spectrum disorder and schizophrenia (Insel and Fernald, 2004; Brüne, 2005; Baslet et al., 2009; Lai et al., 2014) are attributed, in part, to neurodiversity in the amgydalo-insular circuit (Baron-Cohen et al., 2000; Nagai et al., 2007; Baas et al., 2008; von dem Hagen et al., 2013; Gorka et al., 2015). The work described here provides the first mechanistic support of a critical role for amygdalo-insular communication in socioemotional behavior. A greater understanding of this circuitry and its function within the broader social brain will inform targeted treatments for social affective disorders. Future studies may build on this finding to determine what type of

information is encoded by the insula projecting BLA neurons and to characterize the range of behaviors that this tract supports.

2.3. Social affective behaviors among female rats involve the basolateral amygdala and insular cortex

2.3.1. Introduction

The capacity of one individual to detect and make decisions based on the emotional or arousal state of another is key to understanding both healthy and pathological social interactions. In humans, these processes are thought to be fundamental to empathic cognition. When describing such elementary parts of empathy, de Waal argued, “The lowest common denominator of all empathic processes is that one party is *affected* by another’s emotional or arousal state” (de Waal, 2008). This primal, automatic process is sensory in nature and it, by definition, precedes the higher order manifesta-tions of empathy that are widely studied in humans (Panksepp and Panksepp, 2013) and increasingly observed in laboratory rodents (Bartal et al., 2011; Decety, 2011; Sivaselvachandran et al., 2018; Mony et al., 2018; Hernandez-Lallement et al., 2020). Thus, to detect the emotion of another is the precursor to emotion contagion, emotion recognition, empathic helping, perspective taking, and so on, all of which shape an individual’s reactions to and interactions with others. Abnormalities in these ‘higher’ processes are central to numerous psychiatric conditions including autism spectrum disorder (ASD), Fragile X, depression, psychopathy, schizophrenia, borderline personality disorder and alexithymia (Decety and

Moriguchi, 2007; Thioux and Keysers, 2010; Thoma et al., 2011; Decety et al., 2013; Ripoll et al., 2013). Relevant to the present study, there are marked sex differences in prevalence of social dysfunction in psychopathology (Zlotnick et al., 2001; Bao and Swaab, 2010; Ferri et al., 2018; Gogos et al., 2019) making the translational study into sex differences in the neurobiology of social affective behaviors a high priority.

To investigate the neurobiology of simple empathy-like processes in a translational model, our laboratory developed a social affective preference (SAP) test (Rogers-Carter et al., 2018b, 2019; Djerdjaj et al., 2022; Rieger et al., 2022c). The SAP test allows for the observation of a rat's unconditioned response to a pair of stimuli rats, one of which had been exposed to a stressor and the other naïve to treatment. Interestingly, when given the choice between naïve and stressed juveniles, adult test rats spend more time exploring the stressed juveniles, but this preference was exactly the opposite if the stimuli rats were post pubertal adults. We conducted extensive behavioral ethography of the experimental and conspecific rat behaviors including ultrasonic vocalizations (Rogers-Carter et al., 2018b); and concluded that the approach behavior of the experimental rat is a reliable dependent measure that is influenced by the social affective signals of the conspecifics. Thus, the SAP test reflects something well established with regard to human social behavior: features of the target stimulus, including age, are critical determinants to whether or not an individual will approach, help or avoid another in distress (Staub, 1974).

In our first behavioral studies, we included both male and female rats and the pattern of approach to stressed juveniles and avoidance of stressed adults was present in both sexes within the same testing parameters (Rogers-Carter et al., 2018b, 2018a). However, when we began to investigate neurobiological mechanisms, which entail more invasive and stressful procedures such as cannula implant surgeries and injections, female SAP behavior became more variable while male behavior was robust. This led us to pursue neural mechanisms first in males (reviewed below) while seeking parameters for female SAP tests that would be compatible with modern neuroscience technologies.

Our studies in male rats initially focused on the insula. The insula is central to the prevailing neuroanatomical models of empathy, which have substantial support from human neuroimaging studies (Decety, 2011; Bernhardt and Singer, 2012). Classically identified as a neural locus of somatosensory interoception ((Bud) Craig and D, 2009; Chen et al., 2021b), there has been an explosion of fMRI studies over the last 10+ years implicating insula in an impressive range of cognitive processes (Kurth et al., 2010), including but not limited to emotion recognition, salience detection, pain, drug craving and so on (Gu et al., 2012; Chen et al., 2014a; Naqvi et al., 2014; Boucher et al., 2015; Molnar-Szakacs and Uddin, 2022). It is likely that many of these can be tied to an elementary function of integration and salience detection, a consequence of insula's multisensory inputs and reciprocal connectivity with brain networks serving attention, arousal, action, and emotion (Menon and Uddin, 2010b). Thus, we hypothesized that behavior towards or away from stressed conspecifics in the

SAP test would require the insula. Indeed, interaction with stressed conspecifics increases activity in the insula and pharmacological, optogenetic, and chemogenetic inhibition all abolish preference behaviors (Rogers-Carter et al., 2018b, 2019; Rieger et al., 2021; Djerdjaj et al., 2022; Rieger et al., 2022c). Importantly, oxytocin and corticotropin releasing factor, peptides implicated in social behavior (Rigney et al., 2022) and stress responses (Vasconcelos et al., 2020), respectively, were found to be necessary modulators of insular synaptic physiology and the corresponding receptors necessary for social affective preference behaviors (Rogers-Carter et al., 2018b; Rieger et al., 2021). Subsequently, numerous other reports have shown important roles for the insula in social affective behaviors in males (Gehrlach et al., 2019a; Miura et al., 2020; Zhang et al., 2022b; Cox et al., 2022a).

We interpret the importance of the insula to social affective behaviors as a consequence of its interconnections with the network of highly conserved brain regions that orchestrate social behavior (Rogers-Carter and Christianson, 2019) and our recent work has addressed the necessity of this connectivity. The basolateral amygdala (BLA) is a key component of several network models of social cognition including the Social Decision Making Network (Tremblay et al., 2017) and “social brain” (Adolphs, 2010) and sends a dense fiber input to the posterior insula (Allen et al., 1991; Kobayashi, 2011). BLA neurons are remarkably sensitive to conspecific behavior (Mosher et al., 2014); respond during direct observation of a conspecific receiving shock (Jeon et al., 2010); and are activated upon exposure to a stressed conspecific (Knapska et al., 2006;

Mikosz et al., 2015). We hypothesized that detection or appraisal of social stress stimuli and the integration of this external valence information with internal states would require the BLA and its projections to the insula, respectively. In males, chemogenetic inhibition of the BLA and its projection terminals in the insula did in fact abolish social preference for stressed juveniles (Djerdjaj et al., 2022), providing a mechanistic basis for the detection, appraisal, and behavioral response to emotionally salient stimuli.

Sex differences in the prevalence and manifestation of neuropsychiatric disorders such as ASD and schizophrenia (Mandy et al., 2012; Ochoa et al., 2012; Ferri et al., 2018; Ratto et al., 2018; Gogos et al., 2019; de Giambattista et al., 2021) warrant research into the corresponding neurobiology of socioemotional behavior in females. Underlying differences in functional connectivity during emotion recognition and empathy-related processes are thought to contribute to sex-specific manifestations of psychiatric disorders (Preis et al., 2013; Tavares et al., 2022). For example, amygdala-insula connectivity is increased in human females when viewing the faces of stressed others compared to males(Mather et al., 2010). Sex differences in network function could be attributed to sex differences in the distribution of oxytocin fibers and receptors. Male rats have greater oxytocin binding in the agranular insula, indicating a potential sex-specific role of oxytocin in the insula(Dumais et al., 2013). Less is known about differences in oxytocin receptor expression and function in the BLA, though other amygdala subnuclei, such as the medial and central amygdala, display sex-specific oxytocin binding densities and functions

(Dumais et al., 2013; Dumais and Veenema, 2016; Caldwell, 2018). Insular oxytocin in males increases excitatory synaptic transmission and is necessary for appropriate social affective preference behavior, while its role in female insula remains unknown (Rogers-Carter et al., 2018b).

The goal of the current study was to determine if female rats utilize the same neural systems to navigate social affective decision making that we have discovered in males. As noted above, prior efforts to study these systems in females failed because the behavior phenotype was not robust to surgical procedures and injections. To overcome this we lengthened the time allotted for recovery from surgery to 4 weeks and acclimated the rats to handling and experiment interaction for 15 days prior to SAP tests (Rieger et al., 2022b). Here, we infused muscimol, the $GABA_A$ receptor agonist, to the insula or BLA of female rats for reversible pharmacological inhibition during SAP testing with either juvenile or adult isosexual conspecifics. We also sought to ascertain whether oxytocin affects females similarly to males by infusing oxytocin into the insula or BLA prior to one-on-one social interaction tests.

2.3.2. Materials and Methods

2.3.2.1 Animals

All rats used were female Sprague-Dawleys purchased from Charles River Laboratories (Wilmington, MA). All rats were allowed to acclimate to the vivarium in the Boston College Animal Facility for at least 7 days prior to any procedure being carried out. Experimental subjects arrived weighing 225-250 grams and

were housed in pairs. Juvenile conspecifics arrived at PN21 and adult conspecifics arrived at PN55. Conspecifics were housed in groups of 3. Food and water were available *ad libitum*. Rats were housed on a 12h light/dark cycle with behavioral experiments occurring within the first 4 h of the light cycle. Boston College Institution Animal Care and Use Committee approved all procedures and the Public Health Service *Guide for the Care and Use of Laboratory Animals* was adhered to.

2.3.2.2 Surgical Procedures - Cannula Implantation

To inhibit insula and BLA activity indwelling cannula were implanted bilaterally into either the insula or BLA to allow for direct infusion of muscimol or vehicle into each region. Experimental adult females underwent surgery under inhaled anesthesia (2-5% v/v isoflurane in O_2). Bilateral cannula (Plastics One) were implanted into the posterior insula (from bregma: A/P -1.8, M/L +/-6.5, D/V -6.9) or the BLA (from bregma: A/P -2.8, M/L +/- 4.5, D/V -8.4). Cannula were fixed in place with stainless steel screws and acrylic cement and were fitted with stylets to maintain patency. After surgery, rats were subcutaneously injected with meloxicam (1 mg/kg, Eloxiject; Henry Schein) as well as the antibiotic penicillin (1 mg/kg, Combi-Pen; Henry Schein). 10 mL lactated Ringer's solution was delivered in two doses of 5 mL subcutaneously on the right and left side of the body. Rats were allowed 4 weeks of recovery before undergoing behavioral procedures as described below. 3 weeks prior to behavioral testing, test rats were wrapped in a towel and handled by an experimenter for 1 minute each

weekday for a total of 15 days of handling. This was done to ensure rats were fully habituated to handling prior to drug infusion. To inhibit either the insula or the BLA, 1 hour prior to testing, muscimol (100 ng/side in 0.5mL of saline) was microinjected at a rate of 1μL/min with an additional 1 minute diffusion time (Rieger et al., 2022c). We have used this dose previously (Chen et al., 2016; Rogers-Carter et al., 2018b; Sarlitto et al., 2018).

2.3.2.3 Social Affective Preference (SAP) test

This procedure allows for the quantification of a rodent's behavior when interacting with conspecifics of varying social affect. The SAP paradigm takes place in a plastic arena (76.2cm × 20.3cm × 17.8 cm, Length × Width × Height) with beta chip bedding and a transparent plastic lid. Conspecifics were placed into individual clear acrylic chambers (18 x 21 x 10cm; L x W x H) made up of acrylic rods spaced 1 cm apart that allows for direct interaction. During testing, these chambers containing conspecifics are placed at opposite ends of the arena. Testing consists of 2 habituation days followed by 2 test days. On day 1, experimental rats were placed in the testing room for 1 hr and exposed to the testing arena for 15 minutes before being returned to their home cage. On day 2, experimental rats were placed in the plastic testing arena for 5 minutes of behavior testing where they were presented with two naïve juvenile or adult conspecifics in the acrylic chambers. Days 3 and 5 test the experimental rats' social affective preference after injection of vehicle or muscimol, as described above. Rats were placed in the arena for 1 h and then presented with a pair of

unfamiliar conspecifics. One conspecific received an acute stressor of 2 footshocks immediately preceding placement in its chamber of the testing arena (5s, 1 mA, inter-shock interval of 50s); the other conspecific was naïve to any treatment. Both conspecifics were always novel and unfamiliar to the experimental rat on each day of testing. A trained observer quantified the amount of time the experimental rat spends investigating each conspecific. Social investigation was defined as time spent sniffing or touching the conspecific through the acrylic bars. After tests on day 3, rats were returned to their home cage and left undisturbed for 48 h to ensure muscimol cleared. Testing on day 5 was the same with rats receiving the opposite drug treatment as on day 3 in a counterbalanced, within-subjects design. All tests were recorded in digital video. Recordings were scored by a trained observer blind to the experimental conditions to establish inter-rater reliability.

2.3.2.4 One-on-one social interaction tests

Three days after SAP testing, the same experimental rats underwent one-on-one social exploration tests. A one-on-one social interaction was conducted to test the generality of our findings that insular oxytocin is necessary and sufficient for social affective behavior in males. Previously, we've found that an oxytocin receptor antagonist delivered to the insula of males prevented approach to stressed juveniles and avoidance of stressed adults in the SAP test. Importantly, oxytocin itself delivered to the insula increased interactions with juveniles and reduced interactions with adults in the absence of social stress (Rogers-Carter et

al., 2018a). Therefore, we concluded that oxytocin in the insula was both necessary and sufficient to account for behavior in the SAP test. Here we used the one-on-one social interaction test with oxytocin infusions as a gain-of-function test of the role of the oxytocin system in female social behavior. Each experimental rat was placed into a standard plastic tub cage with beta chip bedding and a wire lid 1 h prior to testing. Testing consisted of a naive juvenile or adult being introduced into the experimental rat's cage for 5 minutes. In our prior work with both males and females we quantified a range of behaviors apparent in the social interaction and SAP contexts and found that only social exploratory behaviors (sniffing, pinning, allogrooming) initiated by the experimental rat were sensitive to conspecific age and stress (Rogers-Carter et al., 2018b, 2018a). These were timed by an observer and used in the primary analysis. Because oxytocin may have affected other aspects of behavior in the current study, we also quantified immobility, self-grooming, rearing, pinning, anogenital sniffing during these tests. Each experimental rat was given tests on consecutive days, once 15 minutes after receiving bilateral infusions of oxytocin (0.5 μL of 500 nM in 0.9% saline vehicle equivalent to 250 pg oxytocin per side) and once after receiving bilateral infusions of the vehicle. This time and dose was selected based on our prior studies (Rogers-Carter et al., 2018b). Drug injections were delivered at a rate of 1μL/min with an additional 1 minute diffusion time and order was counterbalanced (Rogers-Carter et al., 2018b).

2.2.3.5 Cannula placement verification

After behavioral testing was finished, experimental rats were overdosed with tribromoethanol and decapitated. Directly prior to this, a vaginal lavage was taken from each rat to identify the day of estrous. After decapitation, the brains were removed, and flash frozen for slicing. Brains were sectioned at 40 μm using a freezing cryostat (Leica CM1860 UV) and slices were mounted on gelatin-coated slides. A cresyl violet stain was performed for verification of cannula placement under a microscope.

2.2.3.6 Statistical analysis

Social interactions were defined as sniffing or touching of the conspecifics and timed by experimenters blind to treatment. Inter-rater reliability was regularly established. Data from experimental rats were only included if site-specificity criteria were met after verification of cannula placement. For insula cannula, data were included if the lowest point of cannula damage was found in the posterior insula. For BLA injections, data were included if the lowest point of cannula damage was found in the BLA. It is important to note that some rats only had unilateral injections but were included if cannula placement was correct in order to minimize the amount of animals used (Glangetas et al., 2022). Social interaction and preference behaviors were analyzed using a repeated measures Analysis of Variance (ANOVA). Main effects and interaction effects were deemed significant at $p < 0.05$ and followed by Sidak post-hoc tests to maintain experiment-wise type 1 error rate to $\alpha < 0.05$. Preference for the stressed

conspecific in each condition was calculated as a percentage of the total time spent investigating both conspecifics (time interacting with stressed / (time interacting with naïve + time interacting with stressed) x 100) and compared with ANOVA. One-on-one social interaction data was analyzed using paired t-tests. Preference for social interaction under oxytocin was calculated as a percentage of time spent interacting under vehicle and analyzed using one sample t-tests. Statistical analyses were conducted with Prism 9 (Graphpad Software).

2.3.3. Results

2.3.3.1 Insula is necessary for social approach to stressed juveniles and naïve adults in female rats.

To determine the effect of insula inhibition on female social affective preference for juveniles, adult female experimental rats received bilateral cannula implants in the insula and later underwent SAP testing with female juvenile conspecifics after vehicle or muscimol injections (Figure 2.3A). After cannula verifications, 12 rats met the inclusion criteria (Figure 2.3B). The amount of time the experimental rat spent investigating the naïve and stressed conspecific was analyzed with ANOVA where drug treatment (vehicle vs. muscimol) and conspecific affect (naïve vs. stressed) were treated as within-subjects factors. Experimental rats preferred interaction with stressed juveniles after vehicle injection but appeared to lose this preference after muscimol administration (Figure 2.3). There were main effects of both drug treatment ($F(1,11) = 4.90$, $p = 0.049$, $\eta^2 = 6.57$) and social affect ($F(1,11) = 9.33$, $p = 0.011$, $\eta^2 = 16.5$) as well as

a drug by affect interaction ($F(1,11) = 7.42$, $p = 0.020$, $\eta^2 = 6.77$). Post-hoc comparison revealed a significant difference between social investigation of naïve and stressed juveniles in the vehicle condition ($p = 0.0009$) that was not present in the muscimol condition ($p = 0.517$, Figure 2.3C). A separate cohort of adult females with insula cannula were tested with adult conspecifics. After cannula verifications, 9 rats met inclusion criteria (Fig 1B) and were analyzed as above resulting in a significant drug by affect interaction ($F(1,8) = 98.3$, $p < 0.0001$, $\eta^2 = 30.5$). Post-hoc comparisons revealed that experimental rats spent significantly more time investigating naïve adults in the vehicle condition ($p < 0.0001$) and significantly less time investigating naïve adults in the muscimol condition ($p = 0.006$, Figure 2.3D).

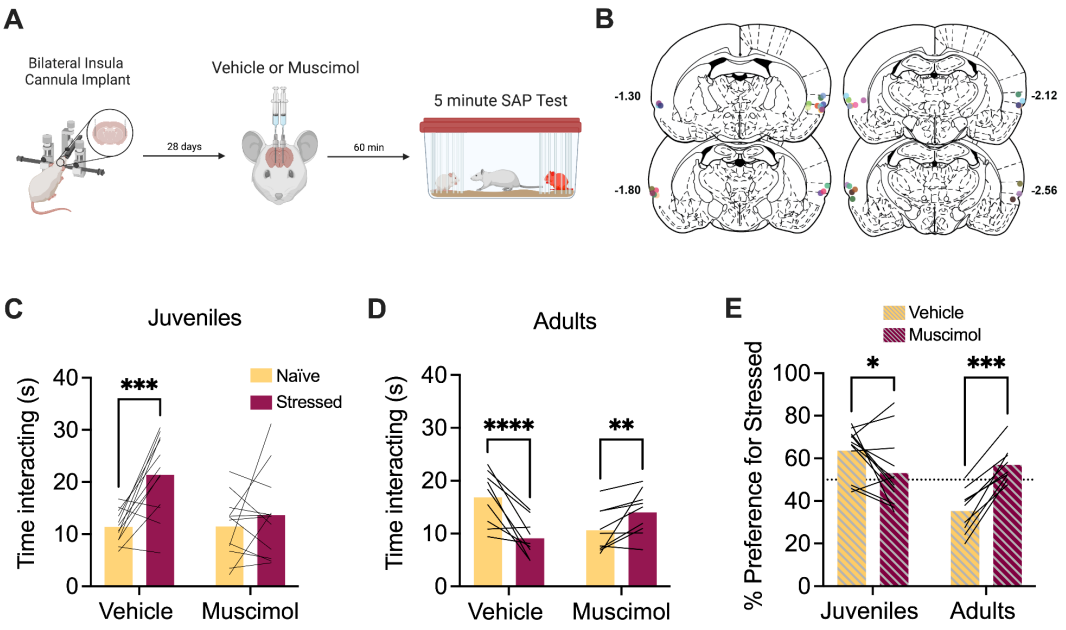


Figure 2.3 Pharmacological inactivation of the insula in female rats interfered with social affective preference behavior.

A. Schematic diagram of experimental design. Bilateral cannula were implanted into the insula of female test rats and, 4 weeks later, rats received saline or muscimol infusions 60 minutes before SAP testing with juvenile or adult conspecifics. **B.** Schematic of cannula implants into the posterior insula. **C.** Mean (with individual replicates) time spent interacting with the naïve or stressed juvenile conspecifics during the 5 min SAP test. After vehicle injections, rats preferred interactions with stressed juveniles compared to naïve juveniles *(*p = 0.0009), which was abolished after muscimol infusion (100 ng per side in 0.5µL saline). **D.** Mean (with individual replicates) time spent interacting with the naïve or stressed adult conspecifics during the 5 min SAP test. After vehicle injections, rats preferred interactions with naïve adults compared to stressed adults ($p < 0.0001$), which was reversed by muscimol infusions ($p = 0.006$). **E.** Data from C and D presented as a mean (with individual replicates) preference for the stressed conspecific as a percentage of total social interaction to allow a comparison of drug effect by age. Experimental rats preferred interactions with stressed juveniles ($p = 0.028$) and naïve adults ($p = 0.0002$) under vehicle treatment, which was abolished following muscimol infusion. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Diagram in A created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998).

The preference for the stressed conspecific in each experiment was calculated as a percentage of the total time spent investigating both conspecifics. A 2-way ANOVA was performed with drug treatment (vehicle vs. muscimol) as a within-subjects variable and age (juvenile vs. adult) as a between-subjects variable. This revealed a main effect of age ($F(1,19) = 7.25$, $p = 0.014$, $\eta^2 = 15.3$) and a drug by age interaction effect ($F(1,19) = 29.7$, $p < 0.0001$, $\eta^2 = 26.5$). Post-hoc comparisons revealed a significant change in preference for stressed juveniles ($p = 0.028$) and naïve adults ($p = 0.002$) when comparing vehicle to muscimol (Figure 2.3E). In summary, insula muscimol infusions in female rats interfered with social preference for stressed juveniles and naïve adults.

2.3.3.2 BLA inhibition abolishes social preference for stressed juveniles and naïve adults in female rats.

To determine the effects of BLA inhibition on social approach to stressed juveniles, bilateral cannula were implanted into the BLA of adult female rats through which muscimol was injected 1 h prior to testing (Figure 2.4A). Twelve rats met inclusion criteria after cannula verifications (Figure 2.4B). It is important to note that, to minimize the number of test subjects used, 3 rats with correct unilateral cannula placements were included. 2 rats were excluded from testing due to clogged/faulty cannula while 10 rats were excluded from analysis due to wrong cannula placement. Similar to above, experimental rats preferred interaction with stressed juveniles after vehicle injection but appeared to lose this preference after muscimol administration (Figure 2.4C). The amount of time

spent investigating the naïve and stressed conspecifics was recorded and a 2-way ANOVA with drug treatment (vehicle vs. muscimol) and social affect (naïve vs. stressed) as within-subjects variables resulted in a significant drug by social affect interaction ($F(1,11) = 7.04$, $p = 0.022$, $\eta^2 = 9.00$). Post-hoc comparisons did not yield any significant differences, although the difference in time spent investigating the stressed vs. the naïve juvenile in the vehicle condition approached significance ($p = 0.054$, Figure 2.4C). Notably, in this experiment 4 of 12 females avoided the stressed juvenile in the vehicle condition which is a slightly larger portion than typically observed but consistent with our prior work where preference fell along a normal distribution with some animals avoiding juveniles(Rogers-Carter et al., 2018b); it is possible that here the inclusion of cannulas and injections moved more subjects to the low end of this distribution. A separate cohort of adult female rats were tested with adult conspecifics and analyzed as above; 9 rats, including 4 with correct unilateral cannula placements, met inclusion criteria after cannula verifications (Figure 2.4B). Three rats were excluded from analysis due to wrong cannula placement. The ANOVA resulted in a significant main effect of social affect ($F(1,8) = 18.95$, $p = 0.0024$, $\eta^2 = 37.0$) and a drug by social affect interaction effect ($F(1,8) = 14.04$, $p = 0.0057$, $\eta^2 = 9.52$). Post-hoc comparisons revealed that experimental rats spent significantly more time investigating naïve adults in the saline condition ($p < 0.0001$) (Figure 2.4D).

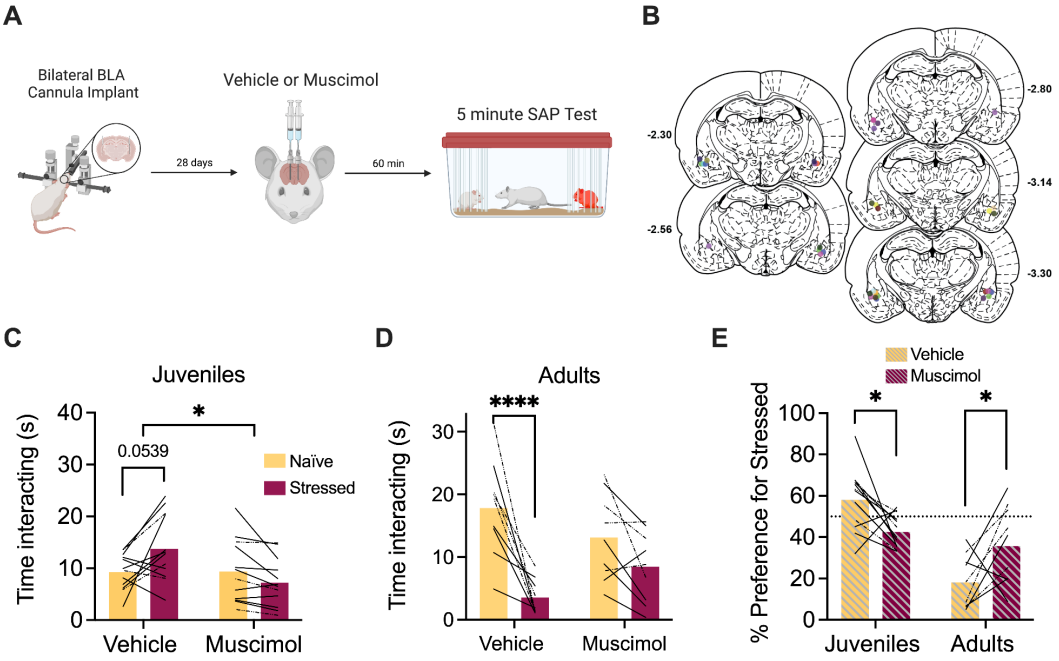


Figure 2.4 Pharmacological inactivation of the BLA in female rats interfered with social affective preference behavior.
A. Schematic diagram of experimental design. Bilateral cannula were implanted into the BLA of female test rats and, 4 weeks later, rats received saline or muscimol infusions 60 minutes before SAP testing with juvenile or adult conspecifics. **B.** Schematic of cannula implants into the BLA. **C.** Mean (with individual replicates) time spent interacting with the naïve or stressed juvenile conspecifics during the 5 min SAP test. Solid lines indicate bilateral BLA injections while dashed lines indicate unilateral injections. After vehicle injections, rats preferred interactions with stressed juveniles compared to naïve juveniles ($p = 0.054$), which was abolished after muscimol infusion (100 ng per side in 0.5µL saline) A social affect x drug interaction effect was observed ($p = 0.022$). **D.** Mean (with individual replicates) time spent interacting with the naïve or stressed adult conspecifics during the 5 min SAP test. After vehicle injections, rats preferred interactions with naïve adults compared to stressed adults ($p < 0.0001$), which was reduced following muscimol infusion. **E.** Data from C and D presented as a preference for the stressed conspecific as a percentage of total social interaction (with individual replicates). Experimental rats preferred interactions with stressed juveniles ($p = 0.038$) and naïve adults ($p = 0.04$) under vehicle treatment, which was abolished following muscimol infusion. *$p < 0.05$, ****$p < 0.0001$. Diagram in A created with BioRender.com. Atlas images recreated from Paxinos and Watson (1998).

The preference for the stressed conspecific was calculated as described above and a 2-way ANOVA with drug treatment (saline vs. muscimol) as a within-subjects variable and age (juvenile vs. adult) as a between-subjects variable resulted in a main effect of age ($F(1,19) = 34.9$, $p < 0.0001$, $\eta^2 = 35.9$) and a drug by age interaction effect ($F(1,19) = 12.9$, $p=0.0019$, $\eta^2 = 18.0$). Post-hoc comparisons revealed a significant change in preference for stressed juveniles ($p = 0.038$) or naïve adults ($p = 0.04$) between vehicle and muscimol (Figure 2.4E). In all, these results indicate that BLA infusions of muscimol impair approach to stressed juveniles and avoidance of stressed adults in female rats.

2.3.3.3 Oxytocin in the BLA, but not the insula, increases social interaction with naïve juvenile and adult conspecifics.

The foregoing results begin to establish that female rats use some of the same brain regions found to be important for social affective behaviors in males. In our first investigations of this neurobiology in male rats, we found that oxytocin was a necessary and sufficient modulator of the insular cortex. Given known sex differences in oxytocin receptor expression(Dumais et al., 2013; Dumais and Veenema, 2016; Smith et al., 2017), we next sought to test whether oxytocin would alter social interactions in females. To determine whether oxytocin infusions to the insula or BLA effect social behavior in female rats, 3 days after SAP testing, 10 rats with bilateral insula cannula and 21 rats with BLA cannula received infusion of either vehicle or oxytocin (500 nM) 15 minutes prior to one-

on-one social interaction tests (Figure 2.5A). Initially, these tests were conducted with juvenile conspecifics (n=10 for insula; n=12 for BLA; see Figures 2.3B and 2.4B for cannula verifications). Because oxytocin in the BLA augmented social interaction with juveniles, we then repeated the experiment for BLA oxytocin infusion with adult conspecifics (n = 9; see Figure 2.4B for cannula verifications). Time spent exploring the naïve conspecific over the course of the 5 min test was recorded. Initially, a 2-way ANOVA with drug treatment (vehicle vs. oxytocin) as a within-subjects variable and age (juvenile versus adult) as a between-subjects variable was performed on the data obtained from rats with BLA cannula, revealing a main effect of drug ($F(1,19) = 12.8$, $p = 0.002$, $\eta^2 = 18.9$) but no significant effect of conspecific age or interactions. Therefore, data from BLA juvenile and adult social interaction tests were pooled together to compare the effect of oxytocin across regions. An ANOVA with drug treatment (vehicle vs. oxytocin) as a within-subjects variable and brain region (insula versus BLA) as a between-subjects variable resulted in main effects of drug ($F(1,29) = 8.29$, $p = 0.007$, $\eta^2 = 0.04$) and brain region ($F(1,29) = 13.9$, $p = 0.0008$, $\eta^2 = 0.25$). Post-hoc comparisons revealed a significant increase in social interaction after oxytocin infusion into the BLA compared to vehicle *(*$p = 0.0008$) with no difference in the insula ($p = 0.72$, Figure 2.5B). Time spent exploring after oxytocin was converted to a percentage of vehicle exploration time and one sample t-tests were conducted comparing the mean percentage of time spent exploring after oxytocin infusion to a theoretical mean of 100%. Social interaction

did not increase after infusion of oxytocin into the insula but did increase after infusion into the BLA (p = 0.001, Fig 3C).

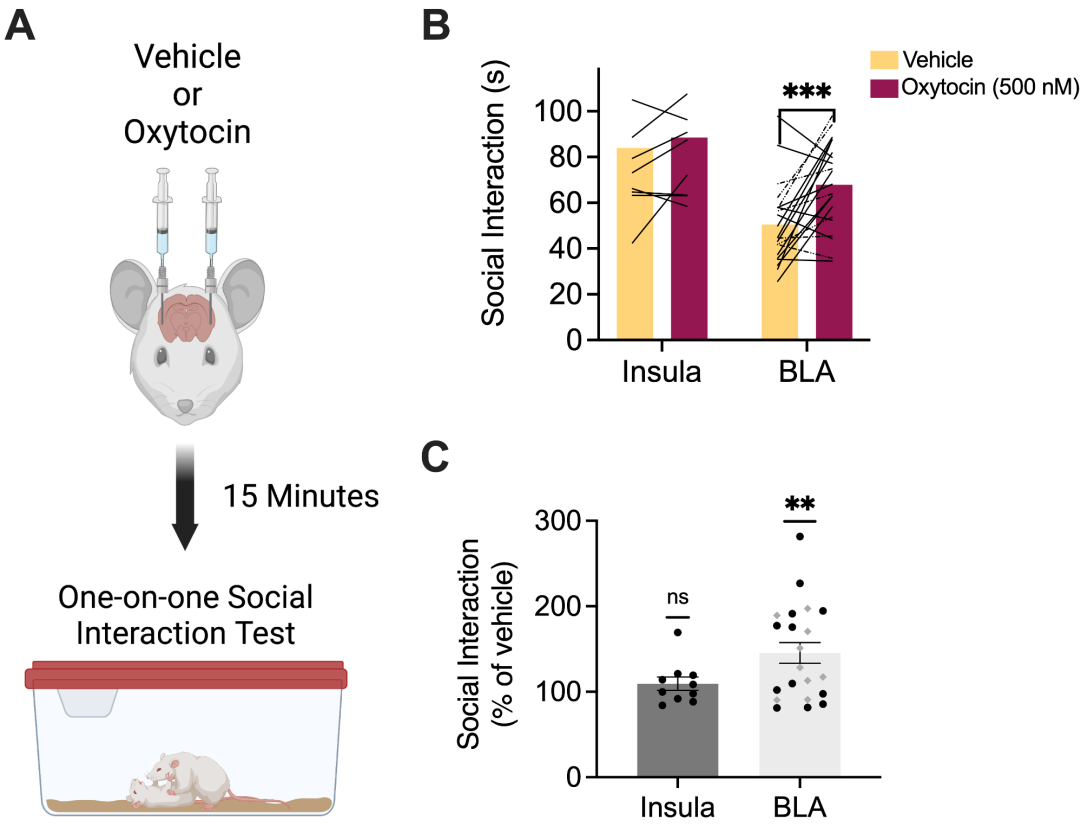


Figure 2.5 Oxytocin infusion into the BLA, but not the insula, of female test rats increased social interaction with naïve juvenile and adult conspecifics.
A. Schematic diagram of experimental design. One week after SAP testing, female test rats with bilateral cannula implants in either the insula or BLA received saline or oxytocin (500 nM) infusions 15 min prior to one-on-one social interaction tests with naïve juvenile or adult conspecifics. **B.** Mean (with individual replicates) time spent interacting with naïve juvenile and adult conspecifics during a 5 min social interaction test after vehicle or oxytocin infusion into the insula or BLA. Solid lines indicate bilateral BLA injections while dashed lines indicate unilateral injections. Oxytocin in the insula had no effect on social interaction with juvenile conspecifics compared to social interaction after vehicle infusions. Oxytocin in the BLA significantly increased social interaction with both juvenile and adult conspecifics (p = 0.0008). **C.** Data from panel B presented as social interaction under oxytocin as a percentage of social interaction under vehicle (with SEM). Oxytocin in the BLA produced a significant increase in social interaction with juveniles (black circles) and adults (gray diamonds) compared to 100% (p = 0.0013). See Supplemental Figures S1

and S2 for additional data analysis. **p < 0.01, ***p < 0.001. Diagram in A created with BioRender.com.

Oxytocin infusions to the BLA had noticeably variable effects which may be attributable to fluctuations in oxytocin receptor expression associated with estrus phase (Dumais et al., 2013). We analyzed the BLA data again with subjects grouped by estrus phase. An ANOVA with drug (vehicle vs. oxytocin) as a within-subjects variable and estrus phase (proestrus vs. diestrus vs. metestrus vs. estrus) as a between-subjects variable revealed a main effect of drug ($F(1,17) = 7.4$, $p = 0.014$, $\eta^2 = 11.8$) but no significant effect of phase or drug by phase interactions. Thus, estrus phase does not appear to mediate the variability in oxytocin efficacy on social interaction when infused to the BLA.

2.3.4. Discussion

We investigated whether posterior insula or BLA inhibition in female rats interfered with preference for stressed juveniles and naïve adults typically observed in a social affective preference test (Rogers-Carter et al., 2018b; Djerdjaj et al., 2022). Akin to similar results in males, muscimol infusions into either the insula or the BLA reduced time spent interacting with stressed juveniles and naïve adults, respectively. In contrast to its prosocial effects in males, infusion of oxytocin into the insula had no effect on social interaction with juvenile conspecifics in females, suggesting a sex-specific role of this neuropeptide in social behavior. Alternatively, oxytocin infusion into the BLA of female rats increased social interaction with both juvenile and adult conspecifics,

establishing a potential prosocial role of BLA oxytocin in females. These results add to our understanding of the neural circuit basis for social emotional behavior in rats.

The internal state of an observer rat and external sensory stimuli emitted from a conspecific inform the observer's decision to approach or avoid another. Interoception and salience detection are mediated in part by the insula (Menon and Uddin, 2010b), an accessory node to the social decision-making network (Rogers-Carter and Christianson, 2019). Anatomical studies incorporating both male and female mice have reported no major sex differences in insula connectivity with other brain regions (Gehrlach et al., 2020a), supporting the idea that similar anatomical structure may give rise to similar functions across sexes. Indeed, we've shown here that posterior insula activity in adult females is necessary for social affective preference for stressed juveniles and naïve adults, like our findings in male rats (Rogers-Carter et al., 2018b). Given its position as an intersection for multisensory information processing, it's likely that the insula guides social affective behavior through its integration of external stimuli and internal states.

A conspecific's affective state is conveyed via external signals that include ultrasonic vocalizations (Brudzynski, 2013; Knutson et al., 20021016), chemosignals (Sterley and Bains, 2021), and overt behaviors which influence an observer's behavior. Vocalizations are especially important to social interaction as they may signal positive or negative affect to observers. In rats, 22-Hz calls are associated with aversive states while 50-Hz calls signal positive states

(Seffer et al., 2014). In males, the BLA responds differentially to each call, increasing activity to 22-Hz calls and decreasing activity to 50-Hz calls (Parsana et al., 2012). While BLA responses to vocalizations in females are unknown, female rodents convey affective states via vocalizations in social situations similar to males (Maggio and Whitney, 1985; Moles and D'amato, 2000; Moles et al., 2007). Chemosignals are also essential to social behavior and BLA inhibition in females abolishes prioritization of social odorants over nonsocial odorants, specifically urine from female conspecifics (Song et al., 2021). Given its known role as a valence detector in males, it's likely that the BLA acts similarly in females, encoding positive or negative affective states of others based on socially derived cues.

Social stimuli can be appraised as either potentially rewarding (*e.g.,* a mate) or hazardous (*e.g.,* a dominant male) which would likely lead to opposing social behaviors, approach or avoidance, respectively. In the SAP test, juvenile stress signals may be perceived as non-threatening and motivate parental stress-buffering approach behaviors, while adult stress signals might be perceived as cues of imminent social danger and motivate defensive or avoidant behaviors. In a prior analysis of Fos in male rats that underwent social interactions with either naïve or stressed juveniles or adults, we observed the correlation between BLA and insular Fos to vary based on the age and stress of the conspecific (Rogers-Carter et al., 2018b) and inhibition of BLA synaptic terminals within the posterior insula interfered with social approach (Djerdjaj et al., 2022) suggesting that this is a functionally important circuit and insula-

projecting amygdala neurons relay information about the emotional value of the social stimuli to the insular cortex.

An inference made in our prior work is that engagement with stressed conspecifics evokes a specific pattern of activity within the insula and the associated social decision-making network that is distinct from the pattern of activation that results from interactions with naive conspecifics. In males, neuromodulators that are well established contributors to social and stress-related behaviors, oxytocin and corticotropin releasing factor, augment insular cortex intrinsic and synaptic excitability and their receptors are necessary for typical social affective preference (Rogers-Carter et al., 2018b; Rieger et al., 2022b). Sex differences in the oxytocin system are common (Rilling et al., 2014; Caldwell, 2018) and there are age (Dumais et al., 2013; Dumais and Veenema, 2016; Smith et al., 2017) and maternal (Marlin et al., 2015) factors that affect cortical oxytocin receptor functions. In Wistar rats, adult males have greater oxytocin receptor binding compared to females (56, but see Ref. 64) providing a possible explanation for a lack of effect of oxytocin infusions on social interaction here. The current data suggest that oxytocin is not important to insula function in female rats, however a limitation of our study is the use of only a single dose of oxytocin. If there are fewer receptors or sex specific receptor pharmacology larger dose schedules should be explored. Interestingly, oxytocin had prosocial effects when administered to the BLA of females. While cell-type distribution of oxytocin receptors within the BLA remains unclear, this region is predominantly composed of glutamatergic neurons (Vereczki et al., 2021). Projection targets of

these glutamatergic neurons help define their valence-encoding properties and drive appetitive or aversive behaviors. It's possible that oxytocin administration to this region increases excitatory transmission via oxytocin receptors on these projection neurons to targets such as the insula, which in turn drives social approach. However, the current methods do not allow us to make the conclusion that oxytocin in the BLA is necessary for social affective preference. A hypothesis to test in future studies would be that oxytocin released during interactions with stressed juveniles augments BLA neurons projecting to the insula providing a mechanism to drive insula activity. Looking beyond the oxytocin system, neuromodulators that define the social decision-making networks, including vasopressin, opioids, and dopamine should be explored in the female brain.

The SAP phenomenon tested here rests on the finding that experimental rats prefer interactions with stressed over naïve juveniles and naïve over stressed adults (Rogers-Carter et al., 2018b). While the exact impetus for this behavior in rodents is unknown, work done in both humans and rodents grants us insight into potential motivations and sex differences for this social decision-making process. Human neuroimaging in parents revealed correlated activity in a network of brain regions that mediate parental impulses and behaviors (Swain, 2011), though women display a greater incentive salience toward infants (Hahn et al., 2013) and exhibit stronger amygdala activation in response to infant vocalizations than men (Sander et al., 2007). In rodents, virgin females are more prone to parental behaviors than virgin males and fluctuations in hormones following gestation increases maternal behaviors in females (Bridges, 1996;

Lonstein and De Vries, 2000). The BLA may be a crucial mediator of this behavior, as lesions impaired maternal behaviors toward pups in virgin female mice (Martel et al., 2008). Taking these findings into consideration, it is likely that prosocial behavior towards stressed juveniles could be motivated by parental responsiveness, and this may be more pronounced in females. On the other hand, adults may avoid stressed adults as a defensive behavior, serving to avoid close contact with aggressive or otherwise harmful others. Consistent with this interpretation, exposure to either direct or socially transmitted stress alters social interactions directed toward stressed adult conspecifics suggesting that internal states interact with the appraisal of social danger (Toyoshima et al., 2021, 2022). However, neither the current data or our prior work with males has uncovered the neural circuits that mediate avoidance of adult conspecifics in the SAP test beyond the insula. Sex differences exist in the circuitry governing social avoidance behaviors and oxytocin acts as a sex-specific modulator of these behaviors (Bangasser and Cuarenta, 2021). Therefore, actions of oxytocin elsewhere in the social decision-making network could contribute to avoidance, providing a starting point to expand the neural circuit mechanism of social affective decision-making.

Chapter 3: An insula-prelimbic circuit mediating social affective behavior

3.1. Introduction

As discussed above, social interactions are crucial to species' survival and success, and how one detects and responds to another's social affective state is critical to a number of social behaviors that include both positive, affiliative behaviors and negative, agonistic behaviors. Several accepted network models underlying social behavior, including the "social brain," (Newman, 1999), the Social Decision Making Network (SDMN) (O'Connell and Hofmann, 2011a), and the Cognitive Social Brain (CSB) (Prounis and Ophir, 2020a) are responsible for this appraisal of social affective cues, integration of external and internal stimuli, and execution of adaptive social responses. Deficits in the connectivity and function of these networks result in abnormal social affective processing and behavior, hallmark symptoms of neuropsychiatric disorders such as autism spectrum disorder (ASD) and schizophrenia (Insel and Fernald, 2004; Brüne, 2005; Baslet et al., 2009; Lai et al., 2014), emphasizing the need for preclinical research uncovering the mechanisms of this network activity.

Up to this point, much has been discussed and reviewed about the insula's role in social affective behavior. In brief, the insula's multisensory input and connections to cortical and subcortical regions position it as a key inflection point for the integration of internal and external signals and coordination of context-appropriate behavioral outputs. We have reported a role for this region in a social affective preference (SAP) test, where factors such as age and stress modulate a test rat's behavioral response to conspecifics (Rogers-Carter et al., 2018b, 2018a; Rieger et al., 2022c, 2022b). Afferents to and efferents from the

insula also mediate this behavior, with inhibition of both the BLA-insula and insula-NAc pathways interfering with social preference for stressed juveniles (Rogers-Carter et al., 2019; Djerdjaj et al., 2022). Importantly, these studies have focused exclusively on posterior insula, which is linked to aversive processes (Gehrlach et al., 2019b).

There is ample evidence for differential functionality along the rostral-caudal gradient of the insula (Centanni et al., 2021). While broadly the insula is essential to sensory integration (Gogolla et al., 2014), interoception ((Bud) Craig and D, 2009; Chen et al., 2021b), empathy-related processes (Gu et al., 2012; Chen et al., 2014b; Boucher et al., 2015), and motivated behaviors (Wager and Barrett, 2017), there is functional specificity within this region in terms of these various operations. The anterior insula is implicated in more affective components of behavior, in both humans (Lamm and Singer, 2010; Gu et al., 2013a), where activity has been linked to the processing of emotional salience (Chen et al., 2014b), and in rodents, where inactivation of this region blunts targeted helping behavior but has no effect on social preference (Cox et al., 2022b). Within this context, this region may receive specific social affective stimuli relayed from regions like the BLA that convey distress in others, potentially contributing to emotion contagion within observers that motivates a behavioral response. This interpretation may suggest that the anterior insula organizes salient affective or socially relevant information and recruits other regions like frontal or motor cortices to execute adaptive responses. (Gu et al., 2013b; Molnar-Szakacs and Uddin, 2022). Anterior insula connectivity supports

this role in social affective behaviors, with strong anatomical connections to cortical regions and the amygdala (Gehrlach et al., 2020b).

As we move down the rostral-caudal axis to the posterior insula, inputs from sensory cortices and thalamic regions increase (Gehrlach et al., 2020b), corresponding with a shift toward somatosensory functions, including touch (Björnsdotter et al., 2009; Limanowski et al., 2020), temperature (Vestergaard et al., 2023), and pain perception (Segerdahl et al., 2015; Kadakia et al., 2022). This functional connectivity contributes to the view of posterior insula as the main interoceptive detector of this region (Kuehn et al., 2016; Aguilar-Rivera et al., 2020). Importantly, these generalized functions attributed to the anterior and posterior portions of the insula are not exclusive to these subregions, and there is evidence for functional overlap between them, with anterior insula implicated in some interoceptive processes (Zaki et al., 2012) and posterior insula involved in social affective behaviors (Rogers-Carter et al., 2018c, 2019; Rieger et al., 2022c, 2022a). The relatively indissociable roles of anterior and posterior insula in interoception, sensory processing, and motivated behaviors is a consequence of the interconnectivity of these subregions.

Internal signals and states are inextricably linked to one's perception of and interaction with his or her environment. In auditory fear conditioning, the insula responds to both the predictiveness of a conditioned stimulus and changes in heart rate that signal fear, resulting in the inhibition of this region facilitating extinction in low fear states but preventing extinction in high fear states (Klein et al., 2021). Considering these findings, Klein and colleagues proposed that the

insula may be an interface for matching interoceptive information with explicit cues that are predictive of threat and that when there is a mismatch, insula activity can calibrate behavior. In a social affective preference test, rats must recognize environmental and conspecific features while maintaining awareness of internal bodily states necessitating a role for the insula in this behavior. However, few studies have sought to determine whether the anterior and posterior insula contribute differentially to this behavior, a discrepancy addressed in the following experimental procedures.

The mPFC, a node within the CSB (Prounis and Ophir, 2020b), shares anatomical connections with the insula (Hoover and Vertes, 2007) and has been implicated in a wide range of cognitive functions, including attentional processes (Rossi et al., 2009; Wolf et al., 2014), working memory (Curtis and D'Esposito, 2003; Funahashi, 2017), goal-directed behavior (Hasselmo, 2005; Ostlund and Balleine, 2005), and decision making (Euston et al., 2012). In rodents, this region is made up of a number of subregions which include the anterior cingulate (ACC), the prelimbic (PL), and infralimbic (IL) cortices. Each of these subdivisions exhibits distinct cytoarchitecture, anatomical connections, and functions (Vertes, 2004; Gabbott et al., 2005; Hoover and Vertes, 2007; Anastasiades and Carter, 2021). For example, more dorsal portions of the mPFC (ACC and dorsal PL) receive cortical input from sensory, motor, and association regions and are associated with motor functions while ventral regions like the ventral PL and IL receive input from limbic structues and the midline thalamus contributing to its role in more motivational and emotional processes (Hoover and

Vertes, 2007). The heterogeneous anatomy of the mPFC gives rise to its complex functional diversity. Ca^{2+} imaging of the mPFC in mice during a sensory discrimination task revealed cell-type differences in task-related responses, with SOM interneurons preferentially responding to motor action while vasoactive intestinal peptide-positive interneurons responded to action outcomes. Additionally, other interneuron types and pyramidal neurons displayed heterogeneous responses to various sensory, motor, and outcome related task information (Pinto and Dan, 2015). These findings support a role for the mPFC in goal-directed behavior. Given that prefrontal dysfunction contributes to a number of neuropsychiatric disorders (Gilbert et al., 2008; Zhou et al., 2015; Ajram et al., 2017), a growing body of research seeks to better understand how this region mediates these cognitive processes.

The mPFC's role in social cognition and subsequent behavior is of particular interest. Social cognition requires the evaluation of others' affective states along with awareness of one's own internal state to make an informed and appropriate decision in a given social context. In humans, mPFC activity has been linked to emotion recognition (Wolf et al., 2014), moral judgment (Young and Koenigs, 2007; Young et al., 2010; Tassy et al., 2012), and perspective-taking (D'Argembeau et al., 2007; Vaccaro et al., 2022), all of which influence social decisions. In a three-agent task in which primates could offer food reward to other group members, neurons within the mPFC track social choice and reward that help to inform a subject's future decisions (Báez-Mendoza et al., 2021). Distinct populations of neurons within primate ACC have also been linked

to predicting another's intentions and formulating one's own choices based on these predictions (Haroush and Williams, 2015). In rodents, an extensive body of work has correlated activity within various subregions and neural populations of the mPFC to social approach (Lee et al., 2016; Liang et al., 2018), affective discrimination (Scheggia et al., 2020), social rank (Li et al., 2022), and the processing of socially relevant olfactory cues (Levy et al., 2019). Anatomical targets of specific subregions of the mPFC may inform this region's role in social behavior. Valence-encoding mPFC neurons that project to the BLA, a region implicated in valence detection, modulate social behavior. Specifically, activation of negatively-valenced BLA-projecting PL neurons abolished social preference (Huang et al., 2020). Interestingly, activation of PL projections to the NAc, a region involved in reward behavior, also interfered with social preference behavior (Murugan et al., 2017). Taken together, it's likely the mPFC tracks changes in social contexts that help inform decisions and orchestrate motivated behavior via top-down executive control.

The anatomical connections between the insula and the mPFC and the role each plays in various aspects of social affective behavior serve as compelling evidence for a potential circuit connecting the two regions that is responsible for processing social affective information and executing adaptive behavioral responses. Based on the findings detailed above, it's possible that the insula integrates social affect with internal states, potentially creating a representation of one's social environment, while the mPFC synthesizes processed multisensory information to inform social decisions and alter activity of

downstream targets. Further supporting the idea of a potential social affective circuit between these two regions, human neuroimaging studies have linked aberrant functional connectivity between insula and mPFC subregions to deficits in threat processing and attention (Klumpp et al., 2012; Qi et al., 2021) that contribute to disorders such as ASD and schizophrenia (Chai et al., 2011; Guo et al., 2019). However, few mechanistic studies have attempted to uncover what type of information is shared via this pathway and how it contributes to social decision-making behavior. A more comprehensive understanding of the structural and functional connectivity between the insula and mPFC is required to inform targeted treatments for social affective disorders.

The heterogeneity of mPFC function requires subregion specificity. The PL receives input from the extent of the insula (Hoover and Vertes, 2007), encodes social novelty (Zhao et al., 2022) among other functions in the social realm, and has projections to downstream regions that, when altered, contribute to anxiety- and autism-related behaviors (Luo et al., 2023). Additionally, a network analysis revealed this region was tightly correlated with insula activity during exposure to naïve or stressed conspecifics (Rogers-Carter et al., 2018c), potentially positioning it as a crucial node necessary for social decision-making.

The goal of the current study was to address this gap in insula-mPFC knowledge and investigate this tract's functional significance to social behavior. Projections to the PL originating from both the anterior and posterior insula were first confirmed using traditional retrograde tracing techniques. The PL's role in SAP testing was then tested via infusion of muscimol, a $GABA_A$ receptor agonist,

into this region. A transsynaptic, anterograde, chemogenetic approach was then taken to silence PL neurons postsynaptic to either anterior or posterior insula inputs (hereafter: PL_{aIC} and PL_{pIC} neurons) during SAP testing. Additionally, immunohistochemistry was utilized to classify these insula-innervated PL populations as glutamatergic or GABAergic. In sum, we report that this insula-PL pathway is necessary for social affective behaviors towards stressed others but not opposite-sex conspecifics, positioning this circuit as critical to social contexts where affective discrimination is required.

3.2. Materials and Methods

3.2.1 Animals

Male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) and allowed to acclimate to the vivarium in the Boston College Animal Care Facility for at least 7 days before any procedure was carried out. Adult experimental rats arrived weighing 225-250g, juvenile conspecifics arrived at PN21, and adult conspecifics arrived at P55. Experimental rats were housed in pairs while juvenile and adult conspecifics were housed in triads. The vivarium maintained a 12h light/dark cycle and food and water were available *ad libitum*. Behavioral testing was conducted within the first 4 hours of the light cycle. All procedures were approved by the Boston College Institution Animal Care and Use Committee and adhered to the Public Health Service *Guide for the Care and Use of Laboratory Animals.*

3.2.2 Surgical procedures - Retrograde tracers

To quantify insula projections to the PL, 100nL of 4% Fluorogold (Fluorochrome), a retrograde tracer, was deposited unilaterally into the PL. Adult male rats underwent surgery under inhaled anesthesia (2-5% v/v isoflurane in O_2). Fluorogold was unilaterally microinjected into the PL (from bregma: A/P +3.2, M/L +/-0.6 D/V -3.5) at a rate of 100nL/min to a total volume of 100nL. Five minutes were allowed for diffusion. After surgery, rats were subcutaneously injected with meloxicam (1 mg/kg, Eloxiject; Henry Schein) as well as the antibiotic penicillin (1 mg/kg, Combi-Pen; Henry Schein). 10 mL lactated Ringer's solution was delivered in two doses of 5 mL subcutaneously on the right and left side of the body. Rats were perfused as described below 10 days after surgery. This amount of time allowed for retrograde expression of Fluorogold in the regions of interest.

3.2.3 Surgical Procedures - Cannula Implantation

To inhibit PL activity, indwelling cannula (Plastics One) were implanted bilaterally into either the PL to allow for direct infusion of muscimol or vehicle. Experimental adult males underwent surgery under inhaled anesthesia (2-5% v/v isoflurane in O_2). Bilateral cannula (Plastics One) were implanted into the PL (from bregma: A/P +3.0, M/L +/-0.5 D/V -3.2). Cannula were fixed in place with stainless steel screws and acrylic cement and were fitted with stylets to maintain patency. After surgery, rats were subcutaneously injected with meloxicam (1 mg/kg, Eloxiject; Henry Schein) as well as the antibiotic penicillin (1 mg/kg, Combi-Pen; Henry Schein). 10 mL lactated Ringer's solution was delivered in two doses of 5 mL

subcutaneously on the right and left side of the body. Rats were allowed 3 weeks of recovery before undergoing behavioral procedures as described below. 1 week prior to behavioral testing, test rats were wrapped in a towel and handled by an experimenter for 1 minute each weekday for a total of 5 days of handling. This was done to ensure rats were fully habituated to handling prior to drug infusion. To inhibit the PL, 1 hour prior to SAP testing, muscimol (100 ng/side in 0.5mL of saline) was microinjected at a rate of 1μL/min with an additional 1 minute diffusion time (Rieger et al., 2022c). We have used this dose previously (Chen et al., 2016; Rogers-Carter et al., 2018b; Sarlitto et al., 2018).

3.2.4 Cannula placement verification

After behavioral testing was finished, experimental rats were overdosed with tribromoethanol and decapitated. After decapitation, the brains were removed and flash frozen for slicing. Brains were sectioned at 40 μm using a freezing cryostat (Leica CM1860 UV) and slices were mounted on gelatin-coated slides. A cresyl violet stain was performed for verification of cannula placement under a microscope.

3.2.5 Surgical procedures - Chemogenetic manipulations

The introduction of viruses for anatomical and mechanistic studies has proven very useful in the last several years and has allowed for increased cell-type or circuit selectivity (Haggerty et al., 2020). One viral technique that targets specific cell populations involves introducing cre recombinase along with a virus that will

cause the expression of a cre-dependent (i.e. “floxed”) reporter. To inhibit PL neurons postsynaptic to insula projections, an anterograde, transsynaptic, cre-dependent, chemogenetic approach was used. Experimental adult male rats underwent surgery under inhaled anesthesia (2-5% v/v isoflurane in O_2). In one set of rats, an anterograde, transsynaptic AAV encoding cre-recombinase (pENN-AAV1-hSyn-Cre-WPRE-hGH; Addgene catalog #105553-AAV1; titer=$1.9x10^{13}$ GC/mL; hereafter: “AAV1-Cre”) was bilaterally injected into the anterior insula (from bregma: A/P +2.7, M/L +/-4.3 D/V -5.6) and a cre-dependent, AAV containing hM4Di (pAAV5-hSyn-DIO-hM4D(G_i)-mCherry; Addgene catalog #44362-AAV5; titer=$2.4x10^{13}$ GC/mL), a gene encoding a modified receptor that is coupled to the $G\alpha_i$ G-protein receptor signaling cascade(Roth, 2016), was bilaterally injected into the PL (from bregma: A/P +3.2, M/L +/-0.6 D/V -3.5). Another set of rats received bilateral injections of AAV1-Cre into the posterior insula (from bregma: A/P -1.8, M/L +/6.5 D/V -7) and the same viral injections in the PL as the first set of rats. Five minutes were allowed for diffusion for all viruses. After surgery, rats were subcutaneously injected with meloxicam (1 mg/kg, Eloxiject; Henry Schein) as well as the antibiotic penicillin (1 mg/kg, Combi-Pen; Henry Schein). 10 mL lactated Ringer’s solution was delivered in two doses of 5 mL subcutaneously on the right and left side of the body. Rats were allowed 3 weeks of recovery before undergoing behavioral procedures as described below. 1 week prior to behavioral testing, test rats were wrapped in a towel and handled by an experimenter for 1 minute each weekday for a total of 5 days of handling. This was done to ensure rats were fully

habituated to handling prior to drug injection. The viral approach taken here transfers cre-recombinase to all monosynaptic target cells of the insula and, in this case, induces expression of hM4Di receptors in these cells in the PL (Zingg et al., 2017), allowing for reversible inhibition of PL_{alC} and PL_{plC} neurons via systemic administration of the hM4Di actuator, clozapine-N-oxide (CNO)(Armbruster et al., 2007; Roth, 2016). To inhibit these specific cell populations, rats received either an intraperitoneal (I.P.) vehicle (DMSO in Saline) or CNO (3 mg/kg; Tocris) injection 45 minutes prior to SAP testing. We used a CNO dose of 3 mg/kg previously to inhibit BLA with behavioral effects in hM4Di expressing rats and no effect in sham controls (Djerdjaj et al., 2022) which is consistent with similar approaches reported by others (Mahler et al., 2014; Stachniak et al., 2014).

3.2.6 Social Affective Preference (SAP) Test

This procedure allows for the quantification of a rodent's behavior when interacting with conspecifics of varying social affect. The SAP paradigm takes place in a plastic arena (76.2cm × 20.3cm × 17.8 cm, Length × Width × Height) with beta chip bedding and a transparent plastic lid. Conspecifics were placed into individual clear acrylic chambers (18 x 21 x 10cm; L x W x H) made up of acrylic rods spaced 1 cm apart that allows for direct interaction. During testing, these chambers containing conspecifics are placed at opposite ends of the arena. Testing in this experiment consisted of 2 habituation days followed by 6 test days. On day 1, experimental rats were placed in the testing room for 1 hr

and exposed to the testing arena for 15 minutes before being returned to their home cage. On day 2, experimental rats were placed in the plastic testing arena for 5 minutes of behavior testing where they were presented with two naïve juvenile conspecifics in the acrylic chambers. Days 3 and 4 tested the experimental rats' social affective preference for juvenile conspecifics after injection of vehicle or CNO, as described above. Day 4 was counterbalanced to day 3, with the same rats receiving the opposite treatment to the prior day. Days 5 and 6 took place 48 h after Day 4 and tested the experimental rats' social affective preference for adult conspecifics after injection of vehicle or CNO. Finally, days 7 and 8 tested the experimental rats' preference for opposite sex adult conspecifics. On each testing day, rats were placed in the testing room for 1 h before testing began. Upon being placed in the testing arena, experimental rats were presented with a pair of unfamiliar conspecifics. On testing days 3-6, one conspecific received an acute stressor of 2 footshocks immediately preceding placement in its chamber of the testing arena (5s, 1 mA, inter-shock interval of 50s); the other conspecific was naïve to any treatment. On all testing days, both conspecifics were always novel and unfamiliar to the experimental rat. A trained observer quantified the amount of time the experimental rat spent investigating each conspecific. Social investigation was defined as time spent sniffing or touching the conspecific through the acrylic bars. All testing followed a counterbalanced, within-subjects design, with the same rats receiving the opposite injection to the day prior. All tests were recorded in digital video. Recordings were scored by a trained observer blind to the experimental

conditions to establish inter-rater reliability. For muscimol experiments, experimental rats underwent SAP testing with juvenile conspecifics only, as described above. In an attempt to mitigate confounding order effects of muscimol, which has a relatively long half-life, SAP testing days were conducted 48 hours apart (Djerdjaj et al., 2023). However, order effects of muscimol were observed on the second day of SAP testing and data from the first day was analyzed using a between-subjects two way ANOVA.

3.2.7 Tissue collection

Experimental rats from the PL_{pIC} chemogenetic inhibition study were overdosed with tribromoethanol and perfused with cold 0.01 M heparinized PBS followed by 4% paraformaldehyde. Experimental rats from the retrograde tracing study were perfused similarly 10 days after surgeries. Dissected brains were stored in 4% paraformaldehyde for 24 h and then transferred to 30% sucrose for at least 2 days. For all rats across experiments, 40 µm coronal sections were obtained in series on a freezing cryostat (Leica). For the tracer study, PL and insula sections from along the rostral-caudal axis were directly mounted onto slides and coverslipped with Vectashield containing DAPI (Vector Laboratories) to visualize Fluorogold under a fluorescent microscope (Zeiss Axiomanger Z.2) in the PL for verification, and the anterior, medial, and posterior insula for quantification of projection patterns. For the PL_{IC} cellular inhibition study, PL sections were directly mounted onto slides and coverslipped with Vectashield containing DAPI to visualize mCherry, a genetically encoded fluorescent protein fused to hM4Di,

under a fluorescent microscope to determine the spread of hM4Di. Additional sections containing either anterior or posterior insula were collected and stored in cryoprotectant to verify expression of cre-recombinase in the appropriate regions with immunohistochemical staining, as described below.

3.2.8 Immunohistochemistry

To verify expression of cre recombinase in the anterior or posterior insula, tissue sections were washed in PBS-T (0.01% Triton-X 100), blocked in 5% normal donkey serum in PBS-T, and then incubated overnight in mouse anti-cre recombinase primary antibody (1:5,000; EMD Millipore, Product #MAB3120). Sections were then washed in PBS-T and incubated in AlexaFluor 488 AffiniPure donkey anti-mouse fluorescent secondary antibody (1:500; Jackson Immunoresearch, Cat #715-545-150). Sections were then floated onto glass slides and coverslipped with Vectashield containing DAPI. To identify and quantify PL_{IC} cell-type as glutamatergic or GABAergic, tissue from chemogenetic experiments that was verified for viral expression in the PL and either the anterior or posterior insula was used for IHC staining. Due to the inability to find primary antibodies for CaMKII and GAD67 that were produced in different hosts, two separate IHC stains were performed. All tissue was washed in PBS-T and blocked in 5% normal donkey serum. One set of tissue (n=30) was then incubated overnight in mouse anti-CaMKII alpha primary antibody (1:5,000; Invitrogen, Cat #MA1-048) and a second set of tissue (n=30) in mouse anti-GAD67 primary antibody (1:5,000; Sigma, Cat #MAD5406). Sections were then

washed in PBS-T and incubated in AlexFluor 647 AffiniPure donkey anti-mouse fluorescent secondary antibody (1:500; Jackson Immunoresearch, Cat #715-605-150). Sections were then floated onto glass slides and coverslipped with Vectashield containing DAPI.

3.2.9 Imaging and cell-type quantification

For all studies, tissue was imaged on a Zeiss Axioimager Z2 fluorescent microscope in the Boston College Imaging Core. Z-stacked, tiled images containing the ROIs (anterior, medial, or posterior insula for retrograde tracing study; PL for neuroanatomy study) were taken using a Zeiss Hammamatsu Orca Flash4.0 digital camera through a 20X objective lens. Using ImageJ software, individual fluorescent channel images were stacked, ROIs were traced with reference to the rat brain atlas, and the cell counter plug-in was used to count labeled cells. For the retrograde tracing study, cells that expressed Fluorogold were quantified and expressed as a percentage of DAPI cells. For the neuroanatomy study, superficial (cortical layer 2/3) and deep (cortical layers 5 & 6) layer ROIs of the PL were counted within each image. For each ROI, the total number of DAPI, mCherry-expressing PL_{IC} cells, CaMKII or GAD67 cells, and PL_{IC} cells coexpressing either CaMKII or GAD67 was quantified within this population to determine whether PL_{IC} cells were primarily glutamatergic or GABAergic.

3.2.10 One-on-one social interaction tests and fos analysis

Four days after SAP testing, rats from the PL_{alC} chemogenetic manipulation studies underwent one-on-one social interaction tests to elicit c-Fos activation in the PL. Here we sought to confirm CNO inhibited PL activity as intended by comparing c-Fos activation between experimental rats that received vehicle vs. CNO injections. Each experimental rat was placed into a standard plastic tub cage with beta chip bedding and a wire lid 1 h prior to testing. Experimental rats then received vehicle or CNO injections 45 minutes prior to testing. Testing consisted of a stressed juvenile being introduced into the experimental rat's cage for 5 minutes. In our prior work with both males and females we quantified a range of behaviors apparent in the social interaction and SAP contexts and found that only social exploratory behaviors (sniffing, pinning, allogrooming) initiated by the experimental rat were sensitive to conspecific age and stress (Rogers-Carter et al., 2018b, 2018a). These were timed by an observer. 90 minutes after testing, rats were perfused and brains were collected and sectioned as described above.To verify inhibition of the PL via CNO administration, tissue sections were washed in PBS-T (0.01% Triton-X 100), blocked in 5% normal donkey serum in PBS-T, and then incubated overnight in rabbit polyclonal antibody to c-fos (1:5,000; EnCor RPCA-c-Fos-AP, Lot #:241-102320). Sections were then washed in PBS-T and incubated in AlexaFluor 488 AffiniPure donkey anti-mouse fluorescent secondary antibody (1:500; Jackson Immunoresearch, Cat #715-545-150). Sections were then floated onto glass slides and coverslipped with Vectashield containing DAPI. The PL was imaged as described above and for

each image the total number of PL_{aIC}, c-Fos, and PL_{aIC} cells colocalized with c-Fos were counted.

3.2.11 Statistical Analysis

Data from rats receiving stereotaxic injections were only included when site-specificity criteria were met. For muscimol studies, rats were only included if cannula terminated within the PL. For chemogenetic studies, inclusion required both mCherry expression in the PL and cre recombinase expression in the anterior or posterior insula. These strict parameters necessitated the exclusion of a number of rats within each cohort. Due to this, and to minimize the number of animals used, rats with unilateral viral expression in the PL and insula were included. Sample sizes were determined by conducting a priori power analyses in G*Power using effect sizes observed previously (Rogers-Carter, 2018) and N = 12 was determined to be appropriate to achieve power ≥ 0.70. Several cohorts of rats went through these procedures to reach the target sample size. Datasets were tested for normality and sphericity prior to analysis and found to be suitable for t-test and analysis of variance (ANOVA). For the retrograde tracing, fluorogold expression was extremely variable across subjects despite consistent targeting of the PL. Due to this study being confirmatory rather than descriptive in nature,

data was analyzed from one representative rat that displayed Fluorogold expression in the PL and adequate retrograde expression along the insula. An ordinary one-way ANOVA was used to compare fluorogold and DAPI co-labeled cells across insular subregions (anterior vs. medial vs. posterior). This method was also used to compare co-labeled cells as a percentage of DAPI cells. Post-hoc analysis consisted of Tukey's multiple comparisons test. For muscimol experiments, order effects of muscimol were observed on the second day of SAP testing so data from the first day was analyzed using a between-subjects two way ANOVA. For chemogenetic experiments, social exploration times were compared using repeated measures two-way ANOVA with conspecific stress and drug treatment (vehicle or CNO) as within-subjects variables. Main effects and interaction effects were deemed significant at $p < 0.05$ and followed by Sidak post-hoc tests to maintain experiment-wise type 1 error rate to $\alpha < 0.05$. Preference for the stressed conspecific in each condition was calculated as a percentage of the total time spent investigating both conspecifics (time

interacting with stressed / (time interacting with naïve + time interacting with stressed) x 100). In muscimol experiments, preference scores were compared using an unpaired t-test. In chemogenetic experiments, preference scores were compared using repeated-measures two-way ANOVA with age (juvenile or adult) and drug (vehicle or CNO) as within-subjects factors. Two-way ANOVA was used to analyze differences in PL_{IC} population size and cell-type distribution across cortical layers (layer 2/3 vs. layers 5&6) and between insula projection origin (anterior vs. posterior). Three-way ANOVA was used to determine the primary cell-type of PL_{IC} neurons (GAD67 vs. CaMKii) and whether this varied based on cortical layer (layer 2/3 vs. layers 5&6) or insula projection origin (anterior vs. posterior). Statistical analyses were conducted in Prism 8 (Graphpad Software).

3.3. Results

3.3.1. Anterior and posterior subregions of the insula project to the PL.

Insula projections to the mPFC, and to the PL specifically, are well-documented in rodent models (Vertes, 2004; Hoover and Vertes, 2007; Mathiasen et al., 2023). To confirm the extent of these projections in Sprague-Dawley rats, fluorogold, a retrograde tracer, was deposited into the PL of adult male rats (Figure 3.1A). Ten days later, rats were perfused, brains were extracted, and tissue was collected for cell quantification. Due to the variability observed in fluorogold retrograde expression across subjects and the

confirmatory, rather than descriptive, nature of this study, data from one representative rat with fluorogold expression in the PL were analyzed. Fluorescent images were taken along the extent of the insula. Cells expressing DAPI, a generic nuclear stain, and GFP, which expressed in insula afferents to the PL, were counted in the anterior, medial, and posterior portions of the insula (Figure 3.1B). A one-way ANOVA with insula ROI (anterior vs. medial vs. posterior) as a within-subject factor was performed to analyze differences in both the raw cell counts and the density of PL-projectors as a percent of total DAPI across the insula. This revealed a main effect of ROI in both the number of colabeled cells ($F(2,25) = 18.9$, $p < 0.0001$) and the percentage of colabeled cells ($F(2,25) = 9.85$, $p = 0.0007$), with anterior insula having significantly more projections to the PL than both the medial ($p < 0.0001$) and posterior ($p = 0.0003$) insula (Figure 3.1C&D). In sum, both the anterior and posterior portions of the insula send projections to the PL, with a denser projection coming from the anterior insula.

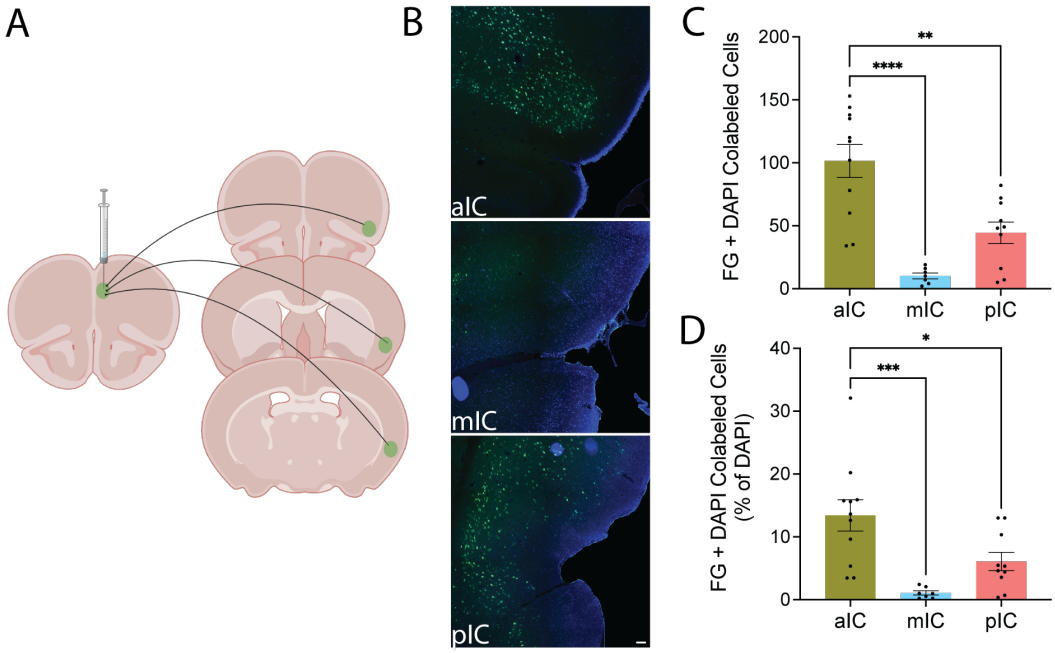


Figure 3.1 Anterior and posterior portions of the insula project to the PL. A. Schematic of experimental design. Fluorogold was unilaterally deposited into the PL of male test rats. Retrograde expression of fluorogold was quantified along the rostral-caudal gradient of the insula. **B.** Representative fluorescent images of anterior (TOP), medial (MIDDLE), and posterior (BOTTOM) insula (blue = DAPI, green = fluorogold). Scale bar = 200 uM. **C.** Mean (with standard error) number of colabeled cells (fluorogold + DAPI). Each dot represents data from one fluorescent image. The anterior portion of the insula had significantly more colabeled cells than both the medial ($p < 0.0001$) and posterior ($p = 0.001$) insula. **D.** Data from **C** expressed as a percentage of DAPI cells. Anterior insula had a higher percentage of PL-projecting cells than both the medial ($p = 0.0006$) and posterior ($p = 0.024$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$. Diagram in A created with BioRender.com.

3.3.2. The PL is necessary for social affective preference behavior toward stressed juveniles.

To determine the effect of PL inactivation on social affective behavior, adult male rats received bilateral cannula implants in the PL and later underwent SAP testing with juvenile conspecifics after vehicle or muscimol injections (Figure

3.2A). After cannula verifications, 22 rats met inclusion criteria (Figure 3.2B). The amount of time the experimental rat spent investigating the naïve and stressed conspecific was analyzed with ANOVA. Due to observed order effects of muscimol, drug treatment (vehicle vs. muscimol) was analyzed as a between-subjects factor while conspecific affect (naïve vs. stressed) was treated as a within-subjects factor. Experimental rats in the vehicle condition preferred interactions with stressed juveniles while rats that received muscimol injections had no preference (Figure 3.2). There was a drug by affect interaction ($F(1,20) = 12.0$, $p = 0.0025$, $\eta^2 = 16.8$). Post-hoc comparison revealed a significant difference between social investigation of naïve and stressed juveniles in the vehicle condition ($p = 0.004$) that was not present in the muscimol condition ($p = 0.376$, Figure 3.2C). The preference for the stressed conspecific was calculated as a percentage of the total time spent investigating both conspecifics (Figure 3.2D). An unpaired t-test revealed a significant preference for the stressed juvenile in the vehicle rats compared to the rats that received muscimol ($t(20) = 3.52$, $p = 0.002$). In sum, inactivation of the PL via muscimol administration interfered with a rat's preference for stressed juveniles in the SAP test.

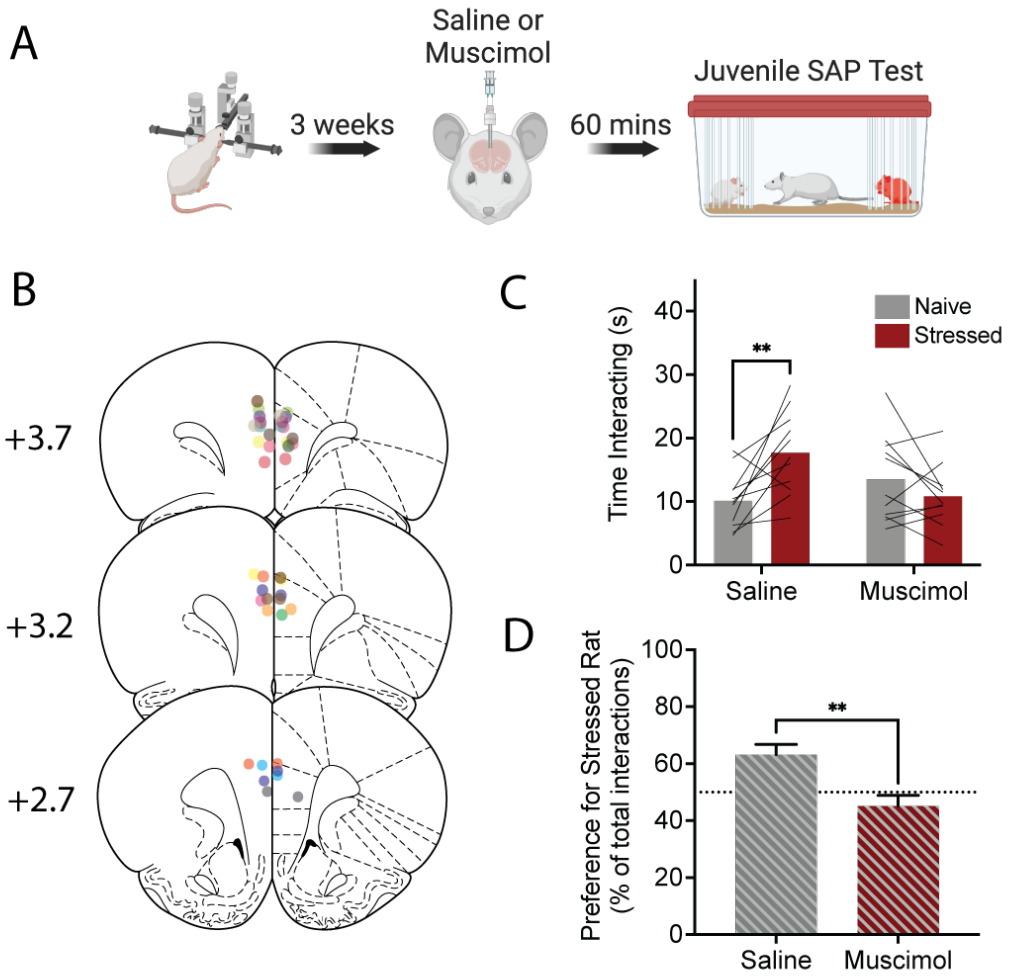


Figure 3.2 PL inactivation abolishes preference for stressed juveniles. A. Schematic diagram of the experimental design. Bilateral cannula were implanted into the PL of male test rats and, 3 weeks later, rats received vehicle (V) or muscimol injections one hour prior to SAP tests with juvenile conspecifics. **B.** Schematic of cannula implants into the PL (individual rats are represented by different colors). **C.** Mean (with individual replicates) time spent exploring the naïve and stressed conspecifics during the 5 min. SAP test. Rats in the vehicle condition preferred social investigation of stressed juveniles compared to naïve juveniles ($p = 0.004$), while rats that received muscimol injections showed no difference in social investigation. **D.** Data from **C** presented as a preference for the stressed conspecific as a percentage of total social interaction. Experimental rats in the vehicle condition preferred interaction with stressed juveniles, while rats in the muscimol condition displayed no preference ($p = 0.002$). $^{**}p < 0.01$. Diagram in A created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998), use pending permission.

3.3.3. PL_{alC} neurons are necessary for social affective behavior.

To determine the effect of inhibition of PL_{alC} cells, adult male experimental rats received bilateral injections of an anterograde, transsynaptic viral vector encoding cre-recombinase into the anterior insula and bilateral injections of a cre-dependent viral vector encoding hM4Di into the PL, allowing for reversible inhibition of the specific population of PL neurons that are postsynaptic to anterior insula. Three weeks later, experimental rats underwent SAP testing with juvenile conspecifics, receiving systemic I.P. injections of vehicle (DMSO in saline) or CNO (3 mg/kg) 45 minutes prior to testing (Figure 3.3A). After verification of viral expression, 15 rats were found to meet criteria for inclusion, with 9 of these rats displaying unilateral PL or insula expression (Figure 3.3B&C). The amount of time the experimental rat spent investigating the naïve and stressed conspecific was analyzed with ANOVA where drug treatment (vehicle vs. CNO) and conspecific affect (naïve vs. stressed) were treated as within-subjects factors. Experimental rats preferred interaction with stressed juveniles after vehicle injection but appeared to lose this preference after CNO administration (Figure 3.3D). 2-way ANOVA revealed a drug by affect interaction ($F(1,14) = 7.24$, $p = 0.018$, $\eta^2 = 6.24$). Post-hoc comparison revealed a significant difference between social investigation of naïve and stressed juveniles in the vehicle condition ($p = 0.038$) that was not present in the CNO condition ($p = 0.461$, Figure 3.3D). These same experimental rats then underwent SAP testing with adult conspecifics, again receiving vehicle or CNO 45 minutes prior to testing. Experimental rats spent more time investigating naïve adults after vehicle

injection but appeared to lose this preference after CNO administration (Figure 3.3E) Data were analyzed as described above, with a 2-way ANOVA once again revealing a drug by affect interaction ($F(1,14) = 8.89$, $p = 0.0099$, $\eta^2 = 6.53$). Post-hoc comparisons revealed no difference in social investigation of naïve and stressed adults in either the vehicle ($p = 0.092$) or CNO ($p = 0.12$) condition. Notably, in this experiment 4 of 15 males preferred the stressed adult in the vehicle condition which is a slightly larger portion than typically observed but consistent with our prior work where preference fell along a normal distribution with some animals approaching adults (Rogers-Carter et al., 2018b); it could be that SAP testing experimental rats with adults conspecific after juveniles shifts behavioral preferences in some cases.

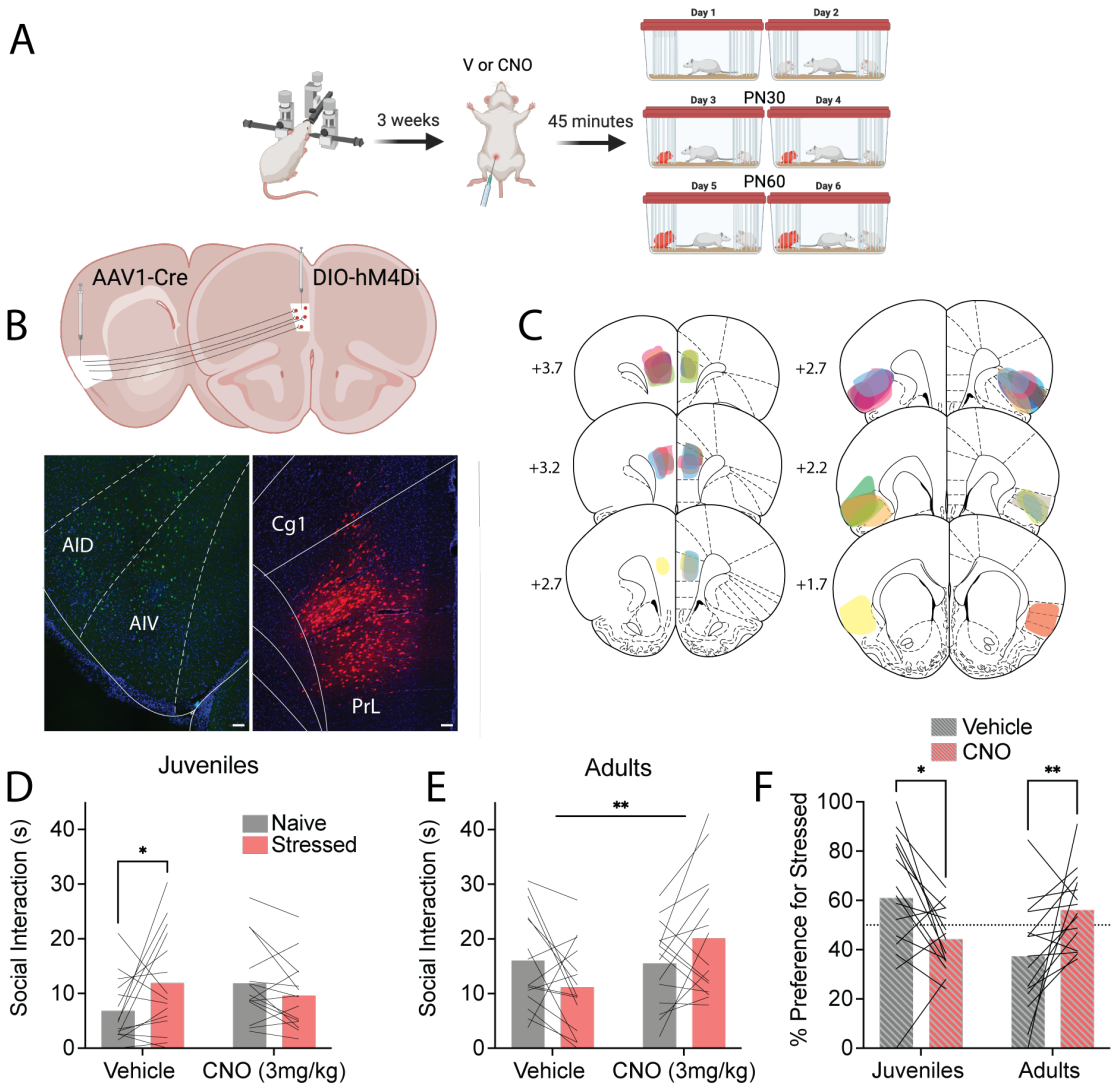


Figure 3.3 Inhibition of PL_{aIC} neurons interferes with social affective behavior. A. Schematic diagram of experimental approach. Anterograde, transsynaptic AAV encoding cre-recombinase was bilaterally injected into the anterior insula and cre-dependent AAV encoding hM4Di was bilaterally injected into the PL and 3 weeks later rats received systemic vehicle (V) or CNO (3mg/kg) injections 45 min prior to SAP tests with juvenile or adult conspecifics. **B.** TOP: Schematic of viral injections into the anterior insula and PL. BOTTOM (left-to-right): Representative fluorescent images of i) AAV1-Cre expression in the anterior insula and ii) DIO-hM4Di expression in the PL (green = GFP, red = native mCherry, blue = DAPI). Scale bar = 200 uM **C.** Maps of viral expression in PL (left side) and the anterior insula (right side, individual rats are represented by different colors) **D.** Mean (with individual replicates) time spent interacting with the naïve and stressed juvenile conspecifics during the 5 min SAP tests after vehicle or CNO injection. In the vehicle condition, rats spent more time investigating stressed juvenile conspecifics than naïve juvenile conspecifics ($p = 0.038$). In the CNO condition rats explored the stressed and naïve conspecifics equally. **E.** Mean (with individual replicates) time spent interacting with the naïve and stressed adult conspecifics during 5 min SAP tests after vehicle or CNO injection. A drug by social affect interaction effect was observed ($p = 0.0099$), with CNO

injections increasing the time spent investigating the stressed adult ($p = 0.118$) compared to vehicle injections ($p = 0.092$). **F.** Data from **D** and **E** expressed as the mean (with individual replicates) preference for the stressed conspecific as a percentage of the total social interaction. CNO injections reduced preference for the stressed juvenile ($p = 0.015$) and increased preference for the stressed adult ($p = 0.007$). *$p < 0.05$, **$p < 0.01$. Diagrams in A and B were created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998), use pending permission.

To account for the individual variability seen in SAP behavior, a percent preference score is calculated. The preference for the stressed conspecific in each experiment was calculated as a percentage of the total time spent investigating both conspecifics. A 2-way ANOVA was performed with drug treatment (vehicle vs. CNO) and age (juvenile vs. adult) as within-subjects variables. This revealed a drug by age interaction effect ($F(1,14) = 21.8$, $p = 0.0004$, $\eta^2 = 17.02$). Post-hoc comparisons revealed a significant change in preference for stressed juveniles ($p = 0.015$) and naïve adults ($p = 0.0073$) when comparing vehicle to CNO (Figure 3.3F). In summary, chemogenetic inhibition of PL_{alC} neurons interfered with social affective preference behaviors toward stressed juveniles and naïve adults.

To confirm inhibition of PL_{alC} neuronal activity via CNO binding of hM4Di receptors, rats from the SAP studies received either vehicle or CNO injections and underwent one-on-one social interaction tests with stressed juveniles to elicit c-fos activation (Figure 3.4). The amount of time rats spent interacting with stressed juveniles was recorded and analyzed using an unpaired t-test with drug treatment (V vs. CNO) as a between-subjects factor. There was no significant effect of drug on social interaction ($p = 0.283$, Figure 3.4B). Rats were perfused 90 minutes after behavioral testing and their brains were collected, sectioned, and stained for c-fos using immunohistochemistry. Sections with mCherry expression within either hemisphere of the PL were imaged and PL_{alC} cells, c-fos, and co-labeled cells were counted. Sections that did not contain mCherry expression were excluded and cell counts from rats that had multiple images

from the left or right hemisphere were averaged. There was a final N of 7 sections from vehicle rats and 6 sections from CNO rats that met inclusion criteria for analysis. Colabeled cells were calculated as a percentage of total PL_{alC} cells and analyzed using an unpaired t-test with drug treatment (V vs. CNO) as a between subjects factor. Rats that received vehicle injections prior to social interaction tests had significantly more PL_{alC} and c-fos colabeled cells ($t(11) = 5.33$, $p = 0.0002$, Figure 3.4C), indicating that CNO effectively inhibited PL_{ilC} neurons.

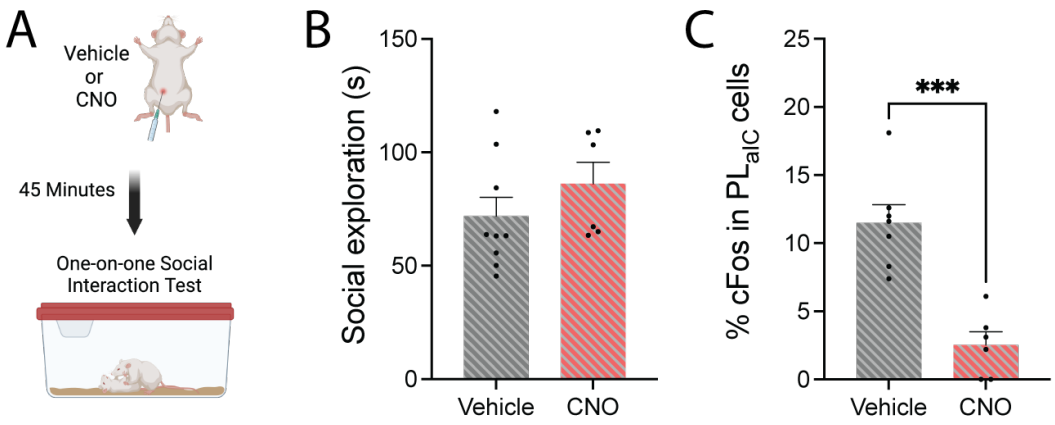


Figure 3.4 Inhibition of PL_{aIC} neurons via CNO injection had no effect on social interaction with stressed juveniles but reduced cFos activation. A. Schematic diagram of experimental approach. Rats received either vehicle or CNO 45 minutes prior to one-on-one social interaction tests with stressed juveniles. Rats were perfused and brains were collected 90 minutes after testing. **B.** Mean (with individual replicates and SEM) time spent interacting with a stressed juvenile. Inhibition of PL_{aIC} neurons had no effect on social interaction. **C.** Mean (with individual replicates and SEM) percentage of PL_{aIC} cells co-labeled with cFos. Rats that received vehicle injections had significantly higher cFos expression in PL_{aIC} neurons than rats that received CNO ($p = 0.0002$). ***$p < 0.001$. Diagram in A created with BioRender.com.

3.3.4. PL_{pIC} neurons are necessary for social affective behavior.

To determine the effect of inhibition of PL_{pIC} cells on social affective behavior, adult male experimental rats received bilateral injections of AAV1-Cre into the posterior insula and bilateral injections of a cre-dependent viral vector encoding hM4Di into the PL, allowing for reversible inhibition of the specific population of PL neurons that are postsynaptic to posterior insula (Figure 3.5A). Three weeks later, experimental rats underwent SAP testing with juvenile conspecifics, receiving systemic intraperitoneal injections of vehicle (DMSO in saline) or CNO (3mg/kg) 45 minutes prior to testing. After verification of viral expression, 15 rats were found to meet criteria for inclusion, with 6 of these rats displaying unilateral PL or insula expression (Figure 3.5B). The amount of time the experimental rat spent investigating the naïve and stressed conspecific was analyzed with ANOVA where drug treatment (vehicle vs. CNO) and conspecific affect (naïve vs. stressed) were treated as within-subjects factors. Experimental rats spent more time interacting with stressed juveniles after vehicle injection but spent equivalent amounts of time investigating both conspecifics after CNO inection (Figure 3.5C). 2-way ANOVA revealed no significant results, although a main effect of social affect ($p = 0.057$) and a drug by affect interaction effect ($p = 0.069$) approached significance. Post-hoc comparison also revealed no significant differences, although the difference in time spent investigating the stressed vs. the naïve juvenile in the vehicle condition approached significance ($p = 0.052$). Again, in this experiment 5 of 15 males either preferred the naïve juvenile or displayed no preference at all in the vehicle condition, which is a

slightly larger portion than typically observed but consistent with our prior work where preference fell along a normal distribution with some animals avoiding stressed juveniles(Rogers-Carter et al., 2018b). These same experimental rats then underwent SAP testing with adult conspecifics, again receiving vehicle or CNO 45 minutes prior to testing. Experimental rats spent more time investigating naïve adults after vehicle injection but appeared to lose this preference after CNO administration (Figure 3.5D). Data were analyzed as described above, with a 2-way ANOVA once again revealing a drug by affect interaction ($F(1,14) = 13.1$, $p = 0.0028$, $\eta^2 = 12.2$). Post-hoc comparison revealed a significant difference between social investigation of naïve and stressed adults in the vehicle condition ($p = 0.0021$) that was not present in the CNO condition ($p = 0.547$).

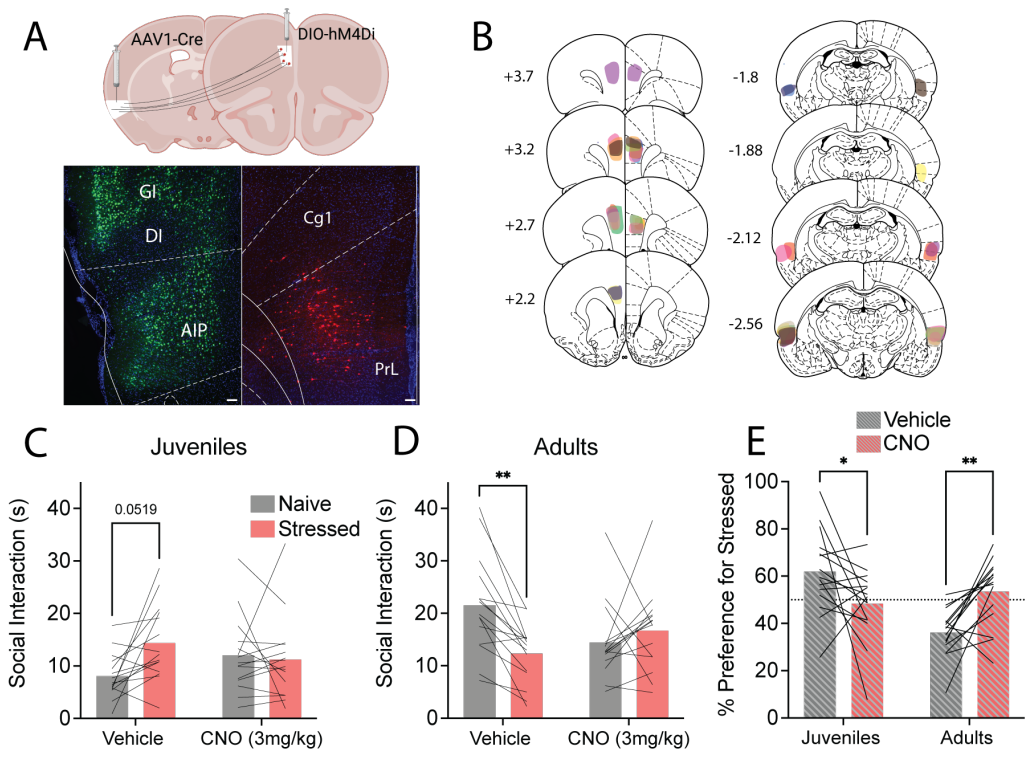


Figure 3.5 Inhibition of PL_{pIC} neurons interferes with social affective behavior. A. TOP: Schematic diagram of viral injections. Anterograde, transsynaptic AAV encoding cre-recombinase was bilaterally injected into the posterior insula and cre-dependent AAV encoding hM4Di was bilaterally injected into the PL and 3 weeks later rats received systemic vehicle (V) or CNO (3 mg/kg) injections 45 min prior to SAP tests with juvenile or adults conspecifics. BOTTOM (left-to-right): Representative fluorescent images of i) AAV1-cre expression in the posterior insula and ii) DIO-hM4Di expression in the PL (green = GFP, red = native mCherry, blue = DAPI). Scale bar = 200 uM **B.** Maps of viral expression in PL (left side) and the posterior insula (right side, individual rats are represented by different colors) **C.** Mean (with individual replicates) time spent interacting with the naïve and stressed juvenile conspecifics during the 5 min SAP tests after vehicle or CNO injection. In the vehicle condition, rats spent more time investigating stressed juvenile conspecifics than naïve juvenile conspecifics ($p = 0.052$). In the CNO condition rats explored the stressed and naïve conspecifics equally. **D.** Mean (with individual replicates) time spent interacting with the naïve and stressed adult conspecifics during 5 min SAP tests after vehicle or CNO injection. In the vehicle condition, rats spent more time investigating naïve adult conspecifics than stressed adult conspecifics ($p = 0.002$). In the CNO condition rats explored the stressed and naïve conspecifics equally. **E.** Data from **C** and **D** expressed as the mean (with individual replicates) preference for the stressed conspecific as a percentage of the total social interaction. CNO injections reduced preference for the stressed juvenile ($p = 0.016$) and naïve adult ($p = 0.003$). *$p < 0.05$, **$p < 0.01$. Diagram in A was created with BioRender.com. Atlas images recreated from Paxinos & Watson (1998), use pending permission.

The percent preference for the stressed conspecific in each experiment was calculated as described above. A 2-way ANOVA was performed with drug treatment (vehicle vs. CNO) and age (juvenile vs. adult) as within-subjects variables. This revealed a main effect of age ($F(1,14) = 6.08$, $p = 0.027$, $\eta^2 = 9.41$) and a drug by age interaction effect ($F(1,14) = 24.7$, $p = 0.0002$, $\eta^2 = 20.9$). Post-hoc comparisons revealed a significant change in preference for stressed juveniles ($p = 0.016$) and naïve adults ($p = 0.0029$) when comparing vehicle to CNO (Figure 3.5E). In summary, chemogenetic inhibition of PL_{pIC} neurons interfered with social affective preference behaviors toward stressed juveniles and naïve adults.

3.3.5. Opposite sex preference does not require PL_{IC} neurons.

To determine whether PL_{IC} neurons are specifically recruited during interactions where affective discrimination is necessary, male rats underwent opposite sex preference testing where no stressful manipulations were performed. Here, experimental males that had undergone SAP testing in the experiments described above underwent opposite sex preference tests 45 minutes after receiving vehicle or CNO injections (Figure 3.6A). In sum, 30 rats with selective hM4Di expression in PL_{IC} neurons underwent this testing: 15 with PL_{aIC} expression and 15 with PL_{pIC} expression (see Figures 3.3C and 3.5B for viral verifications). Time spent investigating male and female conspecifics over the course of a 5 minute test was recorded. Experimental male rats spent more time investigating female conspecifics regardless of drug treatment. A 3-way

ANOVA with drug treatment, conspecific sex, and insula origin (anterior vs. posterior) was performed, revealing a main effect of conspecific sex ($F(1,28) = 37.4$, $p < 0.0001$, $\eta^2 = 37.2$). Therefore, data from PL_{aIC} and PL_{pIC} inhibition experiments were pooled together. A 2-way ANOVA revealed a main effect of conspecific sex ($F(1,29) = 38.0$, $p < 0.0001$, $\eta^2 = 37.2$, figure). Post-hoc comparisons revealed a significant difference between social investigation of female vs. male conspecifics in both the vehicle and drug condition ($p < 0.0001$, Figure 3.6B). Additionally, there was no difference in percent preference for the opposite sex conspecific, with male rats preferring female conspecifics under both vehicle and CNO conditions (Figure 3.6C). In summary, chemogenetic inhibition of PL_{IC} neurons had no effect on preference for an opposite sex conspecific, regardless of projection origin.

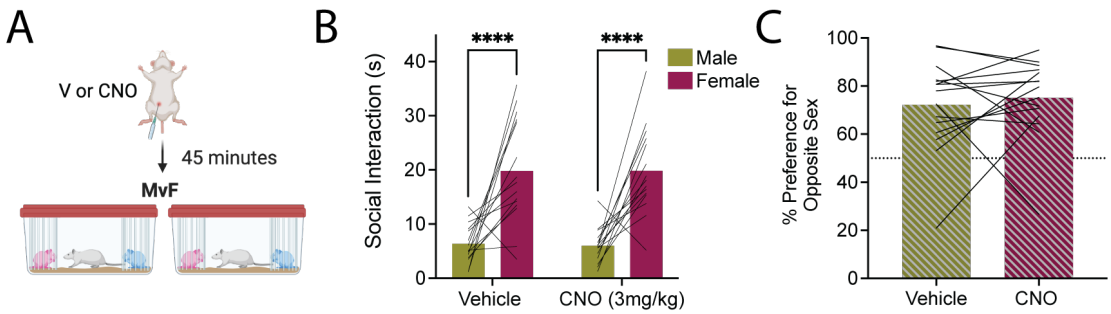


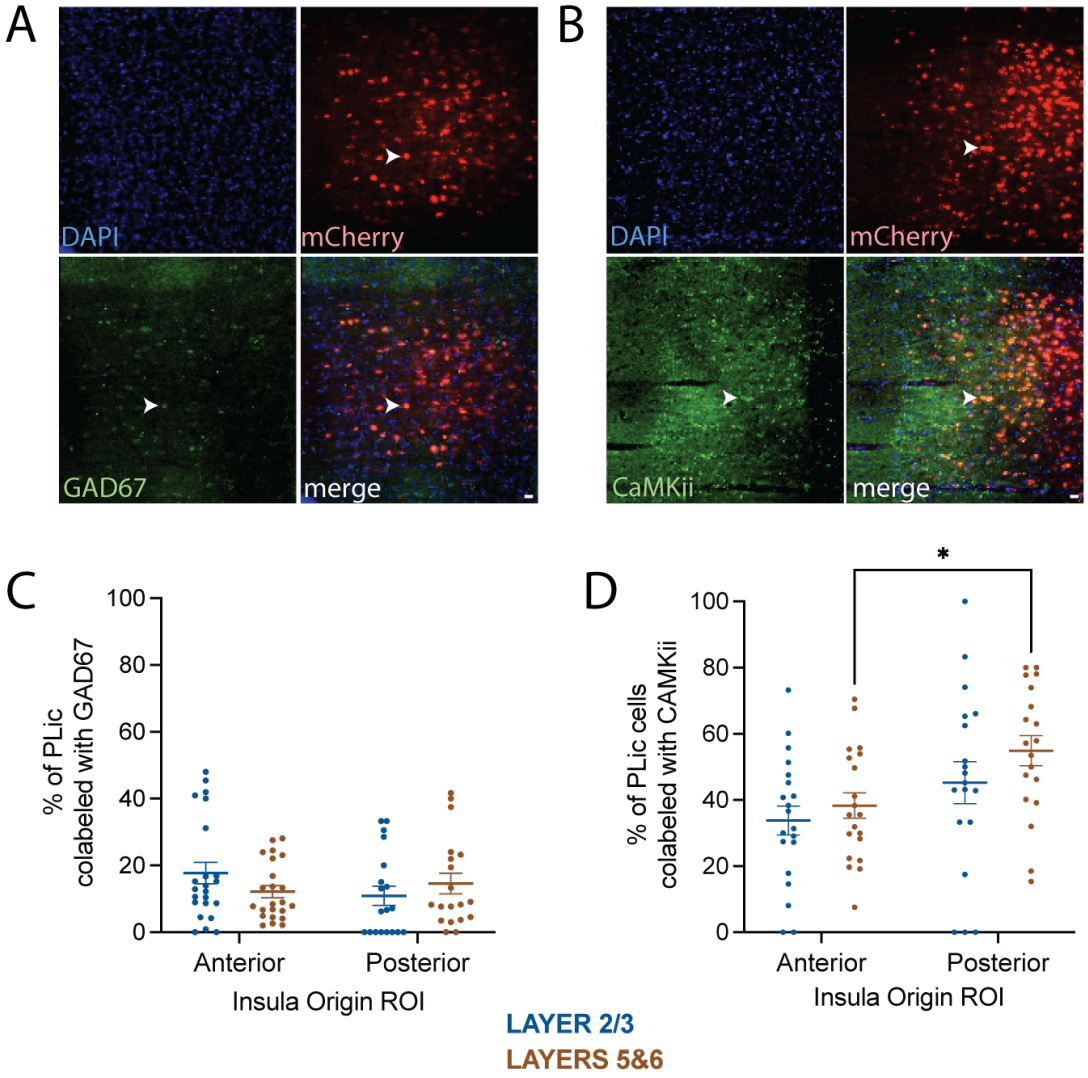
Figure 3.6 Inhibition of PL_{IC} neurons has no effect preference for opposite sex conspecifics. A. Schematic of experimental design. After SAP testing, experimental male rats underwent 2 days of opposite sex preference tests, receiving vehicle or CNO (3mg/kg) 45 minutes prior to testing (see Figure 3.3C and 3.4B for viral verifications). **B.** Mean (with individual replicates) time spent interacting with male and female conspecifics during the 5 min tests after vehicle or CNO injection. Male rats spent more time investigating female conspecifics after both vehicle ($p < 0.0001$) and CNO ($p < 0.0001$) injections, regardless of whether PL_{IC} neurons were postsynaptic to anterior or posterior insula. **C.** Data from **B** expressed as the mean (with individual replicates) preference for the opposite sex conspecific as a percentage of the total social interaction. Rats displayed a preference for opposite sex conspecifics regardless of whether they received vehicle or CNO injections. ****$p < 0.0001$. Diagram in A was created with BioRender.com.

3.3.6. PL_{IC} neurons are primarily glutamatergic regardless of insula origin.

To classify PL_{IC} neurons as GABAergic or glutamatergic, tissue from rats that underwent behavioral testing described above was stained for GAD67, a marker for inhibitory neurons, or CaMKii, a marker of excitatory neurons, and imaged under a fluorescence microscope. PL images were taken from both the left and right hemispheres. Cells expressing DAPI, mCherry (PL_{IC} neurons), GFP (GAD67 or CaMKii), and both mCherry and GFP were counted across shallow and deep cortical layers (Figure 3.7A&B). Sections that did not contain mCherry expression were excluded. Cell counts from rats that had multiple images from the left or right hemisphere were averaged. For tissue stained with GAD67, there was a final N of 23 sections from PL_{aIC} tissue and 19 sections from PL_{pIC} tissue that met inclusion criteria for analysis. For tissue stained with CaMKii, 20 sections were obtained from PL_{aIC} tissue and 19 from PL_{pIC} tissue. The percentage of PL_{IC} neurons colabeled with either GAD67 or CaMKii was calculated as a percent of total PL_{IC} neurons. A 2-way ANOVA with cortical layer (Layer ⅔ vs. Layers 5&6) as a within-subjects factor and insula origin (anterior vs. posterior) as a between-subjects factor was conducted separately for GAD67 and CaMKii counts. There were no significant differences found in the percentage of PL_{IC} neurons colabeled with GAD67 across cortical layer or between insula origin (Figure 3.7C). In regards to the percentage of PL_{IC} neurons colabeled with CaMKii, 2-way ANOVA revealed a main effects of cortical layer ($F(1,37) = 4.18$, $p = 0.048$, $\eta^2 = 2.54$,) and insula origin ($F(1,37) = 5.69$, $p = 0.022$, $\eta^2 = 9.95$). Post-hoc analysis using Sidak's multiple comparisons test revealed

significantly more colabeled PL_{pIC} neurons in layers 5&6 than colabaled PL_{aIC} neurons in layers 5&6 ($p = 0.035$, Figure 3.7D). A 3-way ANOVA (cell-type x cortical layer x insula origin) was conducted to determine differences in PL_{IC} cell-type across cortical layer and insula origin. This revealed a main effect of cell-type (GAD67 vs. CaMKii) ($F(1,77) = 77.9$, $p < 0.0001$, $\eta^2 = 39.6$) and both a cell-type by cortical layer interaction ($F(1,77) = 4.23$, $p = 0.043$, $\eta^2 = 0.84$) and cell-type by insula origin interaction ($F(1,77) = 6.65$, $p = 0.012$, $\eta^2 = 3.38$, Figure 3.6E). Post-hoc analysis using Sidak's multiple comparisons test revealed significantly more PL_{aIC} neurons colabeled with CaMKii than GAD67 in layers 5&6 ($p < 0.0001$), significantly more PL_{pIC} neurons colabeled with CaMKii than GAD67 in both layers ⅔ ($p < 0.0001$) and layers 5&6 ($p < 0.0001$), and significantly more PL_{pIC} neurons than PL_{aIC} neurons colabeled with CaMKii in layers 5&6 ($p = 0.032$). Finally, the percentage of PL_{IC} neurons within the PL was calculated as a percent of total DAPI cells counted and a 2-way ANOVA (cortical layer by insula origin) was conducted to determine whether PL_{IC} neuronal populations differed in size depending on the origin of insula input (anterior vs. posterior). This revealed a main effect of both cortical layer ($F(1,49) = 40.6$, $p < 0.0001$, $\eta^2 = 9.41$) and insula origin ($F(1,49) = 46.5$, $p < 0.0001$, $\eta^2 = 37.0$) and a cortical layer by insula origin interaction effect ($F(1,49) = 13.3$, $p = 0.0006$, $\eta^2 = 3.08$, Figure 3.7F). Post-hoc analysis using Sidak's multiple comparisons test revealed significantly more PL_{aIC} neurons in layers 5&6 than layer ⅔ ($p < 0.0001$) and significantly more PL_{aIC} neurons than PL_{pIC} neurons in layer ⅔ ($p < 0.0001$) and layers 5&6 ($p < 0.0001$). Taken together, PL_{IC} neuronal populations were

primarily glutamatergic neurons residing in layers 5&6, with PL_{alC} neurons representing a significantly larger population of cells and PL_{plC} neurons consisting of more glutamatergic neurons relative to PL_{alC} neuronal populations.


A
DAPI
mCherry
GAD67
merge
B
DAPI
mCherry
CaMKii
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C
% of PLic colabeled with GAD67
Anterior
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Insula Origin ROI
D
% of PLic cells colabeled with CAMKii
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Anterior
Posterior
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LAYER 2/3
LAYERS 5&6
E
Anterior
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Anterior
Posterior
Insula Origin ROI

Figure 3.7. PL_{IC} neuronal population size varies based on insula origin but neurons within these populations are primarily glutamatergic. A. Representative fluorescent images of nuclei (TOP LEFT, DAPI), PL_{IC} neurons (TOP RIGHT, mCherry), GAD67 neurons (BOTTOM LEFT, GFP), and colabeled PL_{IC} + GAD67 neurons (BOTTOM RIGHT, merge). White arrow indicates the same cell across channels. **B.** Representative fluorescent images of nuclei (TOP LEFT, DAPI), PL_{IC} neurons (TOP RIGHT, mCherry), CaMKii neurons (BOTTOM LEFT, GFP), and colabeled PL_{IC} + CaMKii neurons (BOTTOM RIGHT, merge). White arrow indicates the same cell across channels. Scale bar = 200 uM. **C.** Mean (with SEM) percentage of PL_{IC} neurons colabeled with GAD67. Each dot represents an individual replicate. The percentage of PL_{IC} neurons colabeled with GAD67 did not differ across cortical layer or between insula projection origin. **D.** Mean (with SEM) percentage of PL_{IC} neurons colabeled with CaMKii. Each dot represents an individual replicate. Significantly more PL_{pIC} cells than PL_{aIC} cells were colabeled with CaMKii in cortical layers 5&6. **E.** Mean (with SEM) percentage of PL_{IC} cells colabeled with either GAD67 or CaMKii. Significantly more PL_{IC} neurons were colabeled with CaMKii regardless of cortical layer or insula origin. **F.** Mean (with SEM and individual replicates) percentage of PL_{IC} neurons within the PL. PL_{aIC} cells represent a larger population of neurons within the PL than PL_{pIC} cells. *$p < 0.05$, ****$p < 0.0001$.

3.4 Discussion

Here, we investigated insula-PL circuitry and its involvement in social affective behaviors. PL inactivation via muscimol abolished preference for stressed juveniles, indicating a role for this region in social affective decision making. Projections from the insula to the PL arise along the extent of its rostral-caudal axis but are most concentrated in the anterior portion. Chemogenetic inhibition of PL_{IC} neurons abolished preference behavior in male rats, regardless of whether these neurons were postsynaptic to anterior or posterior insula. However, inhibition of these neurons had no effect on preference for the opposite sex, indicating that these populations may be selectively recruited to navigate social interaction when there is an affective component. Additionally, these insula-innervated PL populations were largely glutamatergic neurons localized to layers 5 and 6 of this region, suggesting that PL_{IC} neurons are primarily projection neurons. These results contribute to a growing understanding of the neural circuits underlying social affective decision making by identifying a cortical pathway necessary for approach to or avoidance of stressed others.

Given its anatomical connections with limbic structures and sensory cortices and its functional significance to various aspects of social behavior, including social novelty (Zhao et al., 2022), approach (Lee et al., 2016; Liang et al., 2018), and short-term recognition memory (Yashima et al., 2023), it's unsurprising that the PL is implicated in social affective behavior. Specifically, the mPFC is critical to the discrimination of affective states in rodents (Scheggia et al., 2020). SOM interneurons within the PL of mice are selectively engaged when

investigating conspecifics in either a positive or negative state and photoinhibition of these neuronal populations prevents affective state discrimination (Scheggia et al., 2020). Inactivation of the PL via administration of a GABA agonist, muscimol, resulted in similar behavior in the SAP test. Thus, application of muscimol likely interfered with the balance of local excitation and inhibition in PL microcircuits, subsequently disrupting preference for stressed juveniles in the SAP test.

The PL's role in executive function and adaptive behavior requires both moment-to-moment tracking of environmental and internal changes and evaluation of optimal action selection. Numerous *in vivo* electrophysiology and calcium imaging studies reveal that the mPFC dynamically tracks changes in social environments (Liang et al., 2018; Zhao et al., 2022), encodes various social sensory cues (Levy et al., 2019), and alters activity in anticipation of potential threats (Kim et al., 2018), indicating that this region is responsible for updating representations of social contexts. The mPFC also drives behaviors via its downstream projections: afferents to the BLA, dorsal raphe nucleus, and PAG bidirectionally modulate social preference, induce active escape-like behavior, and promote social interaction, respectively (Warden et al., 2012; Franklin et al., 2017; Huang et al., 2020). The SAP test presents a social context where the choice to approach or avoid a conspecific is informed by multiple factors including age, affect, familiarity, and internal stress, making behavioral flexibility necessary. It's likely the computational processes that contribute to action selection within the PL are dependent on information provided by the insula, which is also involved in sensory integration and interoception. Here, PL neurons

targeted by projections from both the anterior and posterior insula were necessary for social affective behavior but not preference for an opposite sex conspecific. It's possible that the insula is responsible for the integration of interoceptive and exteroceptive information which shapes social motivation and informs an appraisal or decision-making process that involves the PL, leading to approach or avoidance. Importantly, there are social scenarios where the motivation for interaction is fundamentally so strong, or innate, that the social behaviors that emerge do not require the sort of executive functions ascribed to the insula and PL. In two extremes, we could consider the escape and defensive responses evoked by a predator or the appetitive, consummatory behaviors associated with reproductive behavior aimed at opposite sex conspecifics. Here, we sought to determine if the population of PL neurons innervated by the insula contributes to opposite sex social preference. In contrast to the findings in social affective discrimination, inhibition of PL_{IC} neurons had no effect on preference for female conspecifics. Despite reports that mPFC ensembles encode conspecific sex (Kingsbury et al., 2020), our data suggest that action selection in a sex preference task occurs independent of the PL. The mPFC's intrinsic mixed selectivity, along with the inherent multisensory nature of social interactions, implies that all social interactions should have prefrontal neural correlates, regardless of whether these correlates are necessary for behavior.

Cell-type identification of PL_{IC} populations was conducted due to the importance of the excitatory/inhibitory balance within the mPFC (Yizhar et al., 2011). The PL receives input from a variety of long-range afferents and this

information is further processed via local microcircuitry (Anastasiades and Carter, 2021; Yang et al., 2021). Specifically, interneuron populations within the PL contribute to disinhibitory mechanisms that mediate conditioned social fear (Xu et al., 2019), goal-directed behaviors (Mederos et al., 2021), and social competition (Zhang et al., 2022a) among other functions. These interneurons can be excited by long-range afferents that help drive feedforward inhibition. For example, inputs from the mediodorsal thalamus (MD) to the mPFC activate parvalbumin (PV) interneurons and reduction of MD activity contributes to increased excitatory/inhibitory balance that results in reduced social behavior in the three-chamber sociability test (Ferguson and Gao, 2018). Local computations within the PL therefore influence behavioral outcomes in certain contexts, contributing to the need for more detailed descriptions of cellular organization within this region. Here, PL_{IC} neurons were primarily glutamatergic, regardless of insula origin or cortical layer. These neurons include a mix of intratelencephalic cells projecting to cortical, striatal, claustral, and amygdalar regions, pyramidal tract cells that target subcortical regions, and corticothalamic cells which project to thalamic relay nuclei and the thalamic reticular nucleus (Anastasiades and Carter, 2021), suggesting that insula input to the PL could influence sensory, motivational and motor processes. It's also possible that certain PL tracts are preferentially recruited to drive approach or avoidance behavior in the SAP test. Future studies should aim to further classify these PL_{IC} neurons based on their projection target and assess how exposure to stressed juveniles or adults differentially engages PL outputs. The existing anatomy includes several tracks

that are plausible for mediating approach or avoidance, including the NAc and BLA, respectively (Murugan et al., 2017; Diehl et al., 2020). Though significantly fewer PL_{IC} neurons were colabeled with GAD67, differences in interneuron subtype function within the PL warrants finer classification of these cells and their specific contributions to social affective behavior.

The functional diversity of the insula along its rostral-caudal gradient warranted separate investigation of the anterior and posterior insula tracts to the PL. Inhibition of PL_{IC} neurons abolished social affective preference behavior regardless of insula origin and despite a larger PL population postsynaptic to the anterior insula. One potential interpretation of this is that these insula subregions target the same population of PL neurons, with cells postsynaptic to posterior insula residing within the larger anterior insula-PL_{IC} population. It's also possible that these represent separate, distinct populations of neurons. In both cases, the anterior and posterior insula may be contributing distinct streams of information to PL computational processes. The anterior insula is broadly involved in social emotional awareness, integrating cognitive, emotional, and sensory processes to inform affective states (Gu et al., 2013b). In rodents, neuronal activation of anterior insula increases during cohabitation with a mouse in chronic pain (Benassi-Cezar et al., 2021) and inhibition contributes to a reduction in helping behavior (Cox et al., 2022a). Based on these findings, it's possible that the anterior insula evaluates the affective state of others and shares this information with the PL to inform motivated behaviors. On the other hand, the posterior insula's connectivity with somatosensory regions informs its role in interoceptive

and exteroceptive sensory integration (Centanni et al., 2021). Photoactivation of posterior insula increases defensive behaviors and associated autonomic functions in mice while neural activity in this region is responsive to a number of acute sensory stimuli and representative of one's affective state (Gehrlach et al., 2019a). In this case, it's possible that the posterior insula integrates internal and external sensory signals to establish bodily states and this interoceptive information is relayed to the PL. Taken together, PL receives processed information pertaining to one's environment and internal state from the extent of the insula and can then selectively guide approach or avoidance behavior via top-down control of subcortical and thalamic regions.

An important consideration here is that the anterior insula's role in SAP testing has not been previously investigated. Due to this unknown function and the significantly larger population of PL_{IC} neurons postsynaptic to the anterior insula, it's possible that the effects of chemogenetic inhibition observed here were a result of a general inhibition of the PL rather than a specific silencing of anterior insula input. Future experiments aim to address these gaps in the research, first investigating whether inhibition of the anterior insula interferes with SAP behavior, then using a cre-dependent, retrograde approach to selectively inhibit PL-projecting anterior insula neurons.

Social dysfunction characteristic of ASD and schizophrenia arises, in part, from the disruption of network connectivity between brain regions (Brunet-Gouet and Decety, 2006; Volkmar, 2011; Gotts et al., 2012). To understand how impairment of brain network communication contributes to these disorders, more

research into the circuitry driving social behaviors is necessary. The social affective behavior investigated here is dependent on the activity of and communication between a number of different brain regions, including the insula (Rogers-Carter et al., 2018b), BLA (Djerdjaj et al., 2022), and NAc (Rogers-Carter et al., 2019). Here, we've identified the insula-PL pathway as another crucial circuit mediating social affective decision-making. Continued identification and investigation into connections between nodes within this social affective brain network will inform targeted treatments for social affective disorders.

Chapter 4: Discussion

4.1 Summary of findings

The aims of this dissertation were to 1) determine whether BLA-insula circuitry is necessary for social affective behavior, 2) expand our BLA-insula findings to include female subjects, and 3) define PL_{IC} neurons by their cell-type and functional significance to social affective preference behavior. Here, I report that chemogenetic inhibition of the BLA and its projections to the insula interfere with social affective preference for stressed juveniles, suggesting that information the insula receives from the BLA contributes to a rat's decision to approach a stressed juvenile over a naïve one. Regarding aim 2, handling female rats more frequently and allowing for longer time to recover post-surgery enabled successful replication of SAP results in female rats. Utilizing these adjustments, pharmacological inhibition of the BLA or the insula of female rats abolished preference for stressed juveniles and naïve adults. Interestingly, oxytocin administration into the BLA, but not the insula, increased social exploration of naïve conspecifics regardless of age, indicating a potential sex-specific role of insular oxytocin in male rats. Finally, aim 3 presented a comprehensive description of the PL_{IC} neuronal anatomy and its necessity to appropriate social affective behavior. Pharmacological inhibition of the PL abolished preference for stressed juveniles. Retrograde tracing confirmed insula PL projections originate from both the anterior and posterior portion of this region. Chemogenetic inhibition of these PL_{IC} neurons, regardless of insular origin (anterior or posterior) interfered with social affective preference behavior toward stressed juveniles and naïve adults. However, inhibition had no effect on male rats' preference for

conspecifics of the opposite sex, implying that these PL_{IC} cells may be specifically recruited in social contexts where the affective discrimination is necessary. Quantification of glutamatergic and GABAergic cells within PL_{IC} populations revealed that insula inputs preferentially target glutamatergic neurons regardless of insula origin or cortical layer. These findings identify and classify PL_{IC} cells that mediate social affective behavior. More broadly, this work describes two social affective circuits that include structures within the greater SDMN and CSB and that hinge on insula function.

In the following sections, I will discuss the implications and limitations of these results on our understanding of how neural mechanisms guide social affective behavior, specifically focusing on what information the BLA, insula, and PL share with each other and what each of these regions and circuits may be contributing to the detection and appraisal of external, affective signals, the integration of sensory cues with internal motivations, and the selection and execution of an optimal behavioral response. Additionally, I will briefly discuss how these circuits may interact with other nodes within the broader SDMN and CSB and how oxytocin may modulate activity within this tri-circuit to further guide social affective behavior. Finally, I will discuss what this work adds to our growing understanding of the social brain and the clinical implications in regards to social affective disorders.

4.2 How is valence detection within the BLA shaping SAP behavior?

Detection of affective cues and inference of social affective states are essential first steps in social decision making. The SAP test rests on the idea that

an experimental rat is influenced by some stimuli emitted by a stressed conspecific. In rodents, affective state is conveyed via ultrasonic vocalizations (USVs), chemosignals, and bodily states (Sterley and Bains, 2021). While sensory systems in the brain are responsible for recognizing these various stimuli, they are meaningless without an understanding of their value or valence. Valence can help drive behavior, with negatively and positively valenced stimuli eliciting aversive or appetitive behavior, respectively. As discussed in Chapter 2, the BLA receives input from the thalamus, a region essential to sensory relay, and sensory cortices and is responsible for recognizing the valence of these stimuli (O'Neill et al., 2018; Ju and Beyeler, 2021). Additionally, social stimuli, such as conspecific urine, are prioritized and discriminated by the BLA (Song et al., 2021), indicating that salient conspecific features may be classified within this region. In the SAP test, it's likely that the BLA is involved in recognizing each conspecific's affective state as negative or neutral based on the signals they are emitting. For example, stressed juvenile and adult conspecifics emit fewer appetitive "rising" and "trill" calls than their naïve counterparts when interacting with a test rat (Rogers-Carter et al., 2018b). These USVs may be evaluated in the BLA, where firing rates decrease in response to appetitive 50-Hz vocalizations (Parsana et al., 2012). BLA inhibition, therefore, may prevent social affective discrimination in the SAP test via a rat's inability to assign social valence based on USVs.

The BLA's ability to encode positive and negative valence contributes to its role in both fear and reward circuitry. This is because the valence-encoding

properties of BLA neurons are defined, in part, by their projection targets. For example, photoactivation of BLA terminals in the PL, which is critical to fear learning, increases freezing behavior (Burgos-Robles et al., 2017). Similarly, inhibition of BLA terminals within the CeA, another node in fear circuitry, impairs fear conditioning (Namburi et al., 2015). Alternatively, NAc-projecting BLA neurons encoding positively valenced cues exhibit increases in synaptic plasticity after appetitive conditioning and activation of these projectors supports positive reinforcement (Namburi et al., 2015). These findings demonstrate that BLA circuitry is indissociable from its valence encoding properties and may inform its role in social affective behavior. Taking this perspective into account when considering the findings presented in Chapter 2.1, it's possible that the BLA-insula tract is necessary for the integration of valence with other environmental stimuli. While the BLA recognizes the negative affective state of the stressed juvenile, it doesn't appear to be sufficient to drive avoidance behavior away from this conspecific. Instead, it's likely that this information is relayed to the insula, where it is integrated with other external stimuli, potentially including conspecific age, to produce approach behavior. Without this input, the insula lacks the affective information needed to create an accurate representation of one's environment.

An important caveat is that, though adult SAPS were conducted in our BLA-insula experiments, inconsistent vehicle data due to on-campus construction at the time prevented us from drawing any concrete conclusions about this pathway's role in this behavior. Extrapolating from results presented in Chapter

2.2 where BLA inactivation in female rats abolished preference for naïve adults, it's possible that inhibition of this BLA-insula pathway would've produced similar results in males and females. This would be consistent with the interpretation presented above, that integration of affective valence relayed from the BLA with age-related information occurs in the insula. If inhibition of this pathway had no effect on a rat's preference to interact with a naïve adult conspecific, it's possible that age and affect information are integrated within the BLA itself to determine the ultimate valence of a conspecific. Stressed juveniles may be perceived as rewarding within the BLA and its projections to the insula could be specifically involved in driving appetitive, consummatory behaviors. Prior work shows that stressed adults, but not stressed juveniles, emitted significantly more 22-Hz vocalizations, which signal aversive states, than their naïve counterparts (Rogers-Carter et al., 2018b) and BLA activity increases in response to these calls (Parsana et al., 2012). Together, the integration of these USVs with conspecific age could produce a negative valence assignment in the BLA that is relayed to insula, which can generate aversive behaviors via its outputs to CeA and BNST (Gehrlach et al., 2019a; Luchsinger et al., 2021). Currently, it's unknown whether these insula targets are solely responsible for driving avoidance in the SAP test, necessitating further research into other outputs of the BLA and insula that could be involved in this behavior, including the PL.

Though no studies conducted here specifically manipulated BLA-PL projections during SAP testing, ample research allows for the speculation of what this pathway may contribute to this behavior. This circuitry is bidirectional

(Reppucci and Petrovich, 2016) and regulates a number of behaviors including fear- (Arruda-Carvalho and Clem, 2015), anxiety-related (Tye et al., 2011), and social behaviors (Gangopadhyay et al., 2021). In a task where competing shock- and sucrose-predicting cues were presented, correlated firing to the shock-associated cue showed BLA-to-PL directionality. While the BLA was responsive to both reward- and shock-predicting cues, more PL neurons were responsive to the aversive cue and these were more strongly correlated with BLA activity (Burgos-Robles et al., 2017). Photoactivation of this pathway also increased freezing behavior during a pavlovian discrimination task (Burgos-Robles et al., 2017), produced anxiogenic effects in the elevated plus maze and open-field test, and reduced social interaction in a resident-intruder test (Felix-Ortiz et al., 2016). Together, these findings support the interpretation that negative valence information is relayed from the BLA to the PL, where subsequent aversive behavior is produced. In SAP testing, this interpretation may be confirmed if inhibition of BLA projections to the PL interferes with preference for naïve adults but has no effect on preference for stressed juveniles. In this case, positive or negative valence information encoded within the BLA may be preferentially routed to either the insula or PL to drive approach or avoidance behavior, respectively.

In sum, the findings presented in this dissertation along with prior work suggest that the BLA's involvement in social affective behavior stems from its role in valence detection. Affective discrimination is a critical component of SAP testing, as this social decision first requires the accurate identification of affective

states of others. The BLA is capable of differentiating between unconditioned positive and negative sensory stimuli (Gore et al., 2015; Kim et al., 2016; Zhang and Li, 2018), responds to conspecific emitted cues like USVs (Parsana et al., 2012; Schönfeld et al., 2020), and projects to a number of nodes within the SDMN (O'Connell and Hofmann, 2011a) and CSB, including the insula and PL (Mcdonald, 1991; McDonald, 1998; Shi and Cassell, 1998; Reppucci and Petrovich, 2016). Future studies may aim to untangle which conspecific features apart from USVs are recognized and appraised by the BLA, as olfactory cues, overt behaviors, and facial expressions are also known to convey affective states (Dolensek et al., 2020; Sterley and Bains, 2021). For example, *in vivo* calcium imaging could be used to record BLA neurons at either the single cell or population level during exposures to discrete, isolated positive, negative, or neutral chemosignals to determine whether specific subsets of neurons preferentially respond to any of these cues. Additionally, mechanistic studies focused on the role of the BLA-PL circuit in SAP testing may inform whether social affective valence neurons in the BLA are defined by their projection targets.

4.3 How does exteroceptive and interoceptive information engage the insula during SAP testing?

Findings presented in Chapters 2 and 3 provide further evidence of the insula's essential role in social affective behavior. As a node where multiple sensory inputs converge and outputs to a number of SDMN regions originate

(Rogers-Carter and Christianson, 2019), the insula is positioned to integrate social sensory stimuli, like conspecific valence relayed from the BLA (Djerdjaj et al., 2022), with interoceptive state information, like hunger (Barretto-de-Souza et al., 2023), creating an internal model of one's social context and motivational state. In this view, interoceptive signals interact with conspecific affective information to produce a behavioral response. A conspecific's affective state can act as an emotional contagion influencing the internal state of an observer (Keysers et al., 2022) and this is mediated in part by insula projections to the BLA. Photoactivation of these projections increases the intensity of observational pain in mice (Zhang et al., 2022b), indicating that the insula promotes changes in internal state dependent on conspecific affect via connections to nodes within the SDMN. Taking this finding into consideration, it's possible that inhibition of BLA terminals within the insula prevented relevant valence information from being integrated with other sensory stimuli, interfering with potential empathic processes mediated by the insula. Interestingly, this also suggests a feedback loop where sensory inputs from the BLA inform shifts in internal state representations in the insula that then sends signals back to the BLA to promote behavioral expressions of this updated internal state. Activation of this insula-BLA pathway may facilitate or exaggerate approach and avoidance behaviors in the SAP test.

The functional heterogeneity of the insula along its rostral-caudal axis and the organization of its projection to the PL warranted separate investigations of anterior and posterior insula tracts to the PL. While both pathways proved

necessary in the SAP test, it's possible, given the differences in PL_{IC} population sizes, that these insula subregions provide separate, distinct streams of information that are both essential to social affective decision making. Broadly, the posterior insula, with its extensive connectivity with somatosensory regions, is thought to encode sensory and interoceptive information (Gehrlach et al., 2019a). On the other hand, the anterior insula is involved in emotional awareness (Gu et al., 2013a), potentially linking it to affective processes (Choi and Jeong, 2017). In this view, posterior insula could be sharing integrated sensory information about social context and interoceptive state while the anterior insula is providing a motive to approach or avoid based on affective processing. While substantial evidence points to the necessity of posterior insula in SAP behavior (Rogers-Carter et al., 2018b, 2019; Djerdjaj et al., 2022, 2023; Rieger et al., 2022c, 2022a), the role of the anterior insula in this behavior remains unknown. Current studies seek to determine whether local inhibition of this region interferes with SAP behavior similarly to posterior insula, which may lend further credence to functional specialization across the insula.

It's also important to acknowledge that insula subregions communicate with each other, sharing information to determine a motivational state based on environmental context. Adolescents with ASD display reduced functional connectivity between anterior and posterior insula (Ebisch et al., 2011). Thermo-nociceptive stimuli trigger posterior insular and amygdalar responses in humans and these inputs converge in the anterior insula, contributing to the expression of pain (Bastuji et al., 2018). Further, interhemispheric disconnection of anterior

insula produced a reduction in social interest (Glangetas et al., 2022). To further elucidate insular contributions to social affective preference behavior, studies aimed at manipulating insular inter-subregion and interhemispheric connectivity should be conducted. Additionally, *in vivo* recordings of anterior and posterior insula during SAP testing would grant insight into what particular aspects of behavior each subregion is encoding. The view of posterior-to-anterior flow of information may suggest that posterior insula would be most responsive at the beginning of testing, as rat's orient themselves to their new environment, while anterior insula activity increases as rats begin initiating a consistent behavioral response informed by their motivational state.

One potentially relevant functional difference across the insula is taste coding. Within the insula, sweet and bitter tastes activate anterior and posterior cortical fields, respectively. Photoactivation of each of these regions in a place-preference test drives appetitive and aversive responses (Peng et al., 2015). Further, facial expressions of pleasure and disgust can be facilitated in a mouse via stimulation of the anterior and posterior insula, respectively (Dolensek et al., 2020). While the role, if any, taste plays in social affective behavior is unknown the ability of insular subregions to drive opposing behaviors based on the valence of stimuli may inform how its projections to the PL influence SAP behavior. The appetitive drive to approach stressed juveniles may be formed in the anterior insula based on interoceptive and affective sensory information relayed by the posterior insula. Similarly, the aversive drive to avoid a stressed adult could be motivated by cortical fields in the posterior insula integrating these

negative affective stimuli with internal signals promoting threat response. These separate streams of information can be conveyed to the PL, where executive control over subcortical and brainstem structures allows for the production of appropriate behavior. However, hedonic reactivity also differs along the insula in a way that seems to contradict these findings. Activation of anterior insula facilitates expressions of disgust, indicating this subregion may serve as a hedonic “coldspot,” while posterior insula activation leads to expressions of pleasure or liking, suggesting this subregion serves as a hedonic “hotspot” (Castro and Berridge, 2017). These seemingly contradictory findings indicate that the anterior and posterior insula can both mediate negative and positive behaviors. It’s here where studies into the dynamics of neural activity in these subregions would prove beneficial to our understanding of how certain behavioral tasks or contexts influence computational processes within the insula. In the study conducted by Dolesek, et al (2020) mentioned above, individual neurons within the posterior insula responded to positive and negative stimuli while also encoding facial expressions conveying disgust or pleasure (Dolensek et al., 2020), indicating that neuronal population dynamics within the anterior or posterior insula, in addition to environmental context, may dictate when these regions are recruited for appetitive or aversive behavior. As it stands, the capacity to drive opposing behaviors in each subregion may contribute to their necessity in both juvenile and adult SAP behavior.

A growing body of work investigating insula’s role in physiological homeostasis may also provide insight into its ability to encode both appetitive

and aversive stimuli along its axis. During a visual discrimination task where mice learned to associate a specific visual cue with food delivery, *in vivo* imaging within the insula revealed a dense, distributed population of neurons that responded to this cue. Interestingly, these neural responses increased in food-deprived mice and were attenuated during satiety, suggesting that the motivational salience of a cue can be modulated within the insula based on physiological state (Livneh et al., 2017). Similarly, two-photon imaging of the insula during various physiological states, including thirsty and quenched states, revealed distinct patterns of ongoing activity. Interestingly, in thirsty mice, cues associated with water delivery shifted insular activity toward patterns associated with future satiety, indicating that representations of future homeostasis can bias motivations for salient stimuli (Livneh et al., 2020). Together these findings suggest that internal states can switch the valence or direction of behavior for a given stimulus. Considering this in the context of the SAP test, it's possible that the stress of an adult conspecific coupled with the stress of the test itself contributes to a higher degree of emotion contagion than the stress of a juvenile. In this case, social affective stimuli may be appraised differently within the insula, resulting in avoidance or approach behavior.

A limitation to the work presented here is its focus on manipulations of circuit mechanisms and conspecific affect. Much has been discussed about the insula's role in bodily awareness and homeostasis and how that may shape behavior. In the SAP test, changes in internal states of the test rat lead to disordered preference behavior. Hunger abolishes social affective behavior, in

part through the transmission of orexin and melanin concentrating hormone (MCH) in the insula (Barretto-de-Souza et al., 2023). Receptor antagonism of CRF, a neuromodulator released upon exposure to stressed conspecifics (Sterley et al., 2018), within the insula interferes with social interactions with stressed others (Rieger et al., 2022a), suggesting that CRF is critical to driving social transfer of stress. Maternal stress via an immune challenge also interfered with SAP behavior in male offspring (Rieger et al., 2023), suggesting that disruptions during cortical development contribute to long-lasting changes in sociability. Prior stress in experimental rats, along with social transfer of stress via a stressed cagemate, drives preference toward stressed adult conspecifics (Toyoshima et al., 2021, 2022), though whether this is mediated by the insula is unknown. Future studies should aim to investigate how other changes in internal states and disruption of interoceptive processes or homeostatic signals within the insula affect SAP behavior.

4.4 How is PL activity contributing to action selection in the SAP test?

In Chapter 3, both general PL inactivation and specific inhibition of PL_{IC} neurons interfered with social affective preference behavior. The PL, a subregion of the mPFC, has long been considered an essential substrate for executive control (Kesner and Churchwell, 2011; Kamigaki, 2019) and behavioral adaptability (Howland et al., 2022). Extensive research has also identified this region as a key node in social decision-making (Kietzman and Gourley, 2023), likely due to its processing of social information and modulation of goal-directed

behavior (Woon et al., 2020). In the SAP test, the decision to approach or avoid a conspecific is informed by a number of factors including age, affect (Rogers-Carter et al., 2018b), sickness (Rieger et al., 2022c), and familiarity (Rogers-Carter et al., 2018a). Broadly, the PL preferentially responds to social over non-social cues (Levy et al., 2019), with certain neuronal populations selectively responding to some of the factors modulating SAP behavior. GABAergic neuronal populations within the PL encode social novelty (Zhao et al., 2022) while somatostatin interneurons within these GABAergic populations preferentially respond to affective conspecific states (Scheggia et al., 2020). During SAP testing, the PL likely monitors changes in social contexts by tracking these socially salient stimuli. The PL can then orchestrate appropriate motivated behavior based on this social information.

The encoding of various aspects of social stimuli by interneuronal PL populations highlights the importance of the excitatory/inhibitory balance within the PL and presents a potential mechanism by which this region can exert top-down control of behavior. Shifts in the ratio of excitatory and inhibitory transmission within the PL contributes to social dysfunction (Yizhar et al., 2011). In the studies discussed in the preceding paragraph, inhibition of specific GABAergic populations contributed to disrupted social novelty behavior (Zhao et al., 2022) and affective state discrimination(Scheggia et al., 2020). In both cases, disinhibition of pyramidal neurons contributes to deficits in social behavior, suggesting that tight control of excitatory transmission via local microcircuitry within the PL is critical to social decision making. In the SAP test, administration

of a $GABA_A$ agonist contributed to disordered social affective behavior towards stressed juveniles. In this case, increasing inhibitory activity within the PL also results in social dysfunction, potentially disrupting the PL's ability to discriminate affective state and preventing projection neurons from signaling an appropriate behavioral response.

Inhibition of PL_{IC} neurons, which were largely deep layer glutamatergic neurons, interfered with SAP behavior regardless of insula projection origin. A recent study investigating the role of the insula in itching behavior showed that inhibition of an anterior insula-to-PL pathway suppressed scratching induced by an intradermal 5-HT injection. This finding supports the view that insula carries interoceptive information to the PL that contributes to action selection. This information is likely integrated with relevant social stimuli within the PL, where inputs from long-range afferents are processed via local microcircuitry, an appropriate behavioral response is selected and efferent projections to subcortical structures execute this behavior (Anastasiades and Carter, 2021; Yang et al., 2021). Taking the importance of the E/I balance into consideration, decreasing excitatory transmission of PL_{IC} neurons, which potentially act on interneuron populations within the PL, likely contributes to disordered computational processes that ultimately results in a lack of preference for either conspecific due to insufficient information about internal state or social context.

Further classification of PL_{IC} cells is necessary to gain a better understanding of how these inputs are contributing to various aspects of social behavior. PL_{IC} neurons colabaled with CaMKii are likely projection neurons that

could target any number of downstream regions including other cortical regions, the striatum, amygdala, thalamus, or NAc. Classification of these PL_{IC} neurons based on their projection target would indicate how insula input to the PL is modulating decision-making behavior. These glutamatergic PL_{IC} may also contribute to local excitation between cortical layers, engaging interneuron populations that may selectively inhibit or promote excitatory transmission to downstream regions (Anastasiades and Carter, 2021), adding further refinement to information received from the insula. Though significantly more glutamatergic PL_{IC} neurons were identified, about 10-15% of these PL_{IC} neurons were colabled with GAD67. Given the functional heterogeneity of GABAergic populations within the PL, future work should classify these neurons as PV, SOM, or vasoactive intestinal peptide cells.

PL_{IC} neurons postsynaptic to the anterior insula represented a significantly larger population than those postsynaptic to the posterior insula. While both populations proved necessary for social affective behavior, this could be an indicator that these populations are in fact the same, meaning that posterior insula inputs exist within the larger PL_{IC} population postsynaptic to anterior insula. Given that our knowledge of the insula's role in SAP behavior up to this point is limited to the posterior insula, these populations being the same could mean that PL_{pIC} neurons alone are influencing social affective behavior. On the other hand, if PL_{pIC} and PL_{aIC} neurons are separate populations, taking the functional heterogeneity of the insula into account it could be possible that, within the PL, information related to interoception or visceral sensations coming from the

posterior insula are being integrated independently from the more socioemotional-related information being conveyed by the anterior insula. More work is needed to confirm whether or not these populations are one in the same or whether they represent separate groups of neurons responsible for sharing distinct streams of information with the PL.

To explore how the PL guides action selection and behavioral adaptability, it's useful to look at its projection targets and the function of these anatomical connections. Specifically, circuitry between the BLA and the PL has repeatedly been implicated in fear learning, anxiety-related, and social behaviors. Recordings from the mPFC and BLA during fear discrimination revealed increased synchrony between the two regions in mice that successfully discriminated between safe and danger cues, with BLA firing selectively tuning to mPFC input (Likhtik et al., 2014). This suggests that mPFC input to the BLA is essential for cue discrimination and the resultant adaptive behavior. Additionally, photoinhibition of PL-BLA neurons reduced anxiety-like behaviors in mice with chronic pain (Gao et al., 2023) while activation increased avoidance behaviors in an active avoidance task. This further suggests that PL-to-BLA transmission modulates behavioral responses to fear and anxiety, and hyperactivation of this pathway may interfere with response inhibition and contribute to abnormal behaviors. In regards to social behavior, photoactivation of PL-BLA projections reduced social interactions and abolished social preference. Further, activation of PL-BLA projectors that had previously responded to an aversive event produced deficits in social preference behavior (Huang et al., 2020). Taken together, these

studies indicate that hyperactivity of PL-BLA projections contributes to behavioral dysfunction. In SAP testing, inhibition of PL_{IC} neurons may have led to an increase in PL-BLA projector activity, resulting in disordered social affective behavior.

4.5 How do these regions interact with each other and with other nodes within the SDMN to orchestrate social affective behavior?

Though investigations into the PL-BLA pathway are beyond the scope of this dissertation, prior work described here provides compelling evidence for a potential BLA-insula-PL circuit mediating social affective preference behavior. Briefly, conspecific valence, defined through the appraisal of various social signals, is identified within the BLA, which sends projections to the posterior insula, where affective information is integrated with other sensory modalities and internal signals along the rostral-caudal gradient to generate a context-dependent motivational state informed by socioemotional processes. The insula then relays this to the PL, where an appropriate behavioral response informed by this social contextual and motivational information is selected and top-down projections to subcortical regions execute this adaptive behavior. In the case of insula-PL connectivity, it's possible that a threshold of salient information must be met before the insula engages the PL. Prior studies have demonstrated the insula's necessity to social decision-making in instances where external factors such as conspecific age, stress, and health vary (Rogers-Carter et al., 2018c; Rieger et al., 2022c) or internal factors such as satiety and neuromodulatory

transmission are manipulated (Rieger et al., 2022a; Barretto-de-Souza et al., 2023; Ng et al., 2023). There are also circumstances when rats would exhibit approach or avoidance behavior that do not reach this threshold to become "insula-dependent". For example, inhibition of the insula and its targets within the PL had no effect on social novelty or opposite sex preference, respectively (Rogers-Carter et al., 2019), suggesting that certain social stimuli or motives may be so salient that they are mediated entirely by subcortical systems. More work is needed to investigate whether this applies to interactions in the aversive realm, as it's unknown whether insula or PL recruitment is necessary for avoidance in situations where danger may be more imminent.

While studies discussed here focus on BLA-insula and insula-PL projections in social affective behavior, this circuitry exists within and interacts with a larger network of brain regions also implicated in social decision-making. Though space precludes a thorough review of all the potential circuits involved in this behavior, prior work done in our lab allows for speculation into a few candidate regions likely to be engaged during SAP testing. One such region is the NAc, a canonical node in reward circuitry (Wise, 2002) that also receives projections from the BLA, insula, and PL (Wright and Groenewegen, 1996; Shih and Chang, 2024). Inhibition of this region interferes with both approach and avoidance behaviors in a cue-elicited decision task (Hamel et al., 2017), suggesting that the NAc is involved in value-based decision making. Salient valence information may enter the NAc for valuation via the amygdala. Basal amygdala neurons that project to the NAc are engaged by both appetitive and

aversive social stimuli (Poggi et al., 2024). Optogenetic stimulation of the BLA-NAc circuit results in social deficits and increased social avoidance (Folkes et al., 2020), suggesting that increasing excitatory transmission of this tract may bias negative valuation of social interaction within the NAc. On the other hand, chemogenetic silencing of insula terminals within the NAc prevented preference for stressed juveniles in the SAP test (Rogers-Carter et al., 2019), indicating that this tract may be involved in driving appetitive social behaviors via integration of conspecific affective and interoceptive information with reward valuation. In terms of PL-NAc circuitry, inhibition of this tract increased responding to non-reinforced appetitive, aversive, and combined cues in a discriminative cue task (Hamel et al., 2022) and reduced preference for food paired with a novel conspecific in a social incentivization for future choice task (Kietzman et al., 2022). Together, these findings suggest that PL-NAc projections may drive behavior in scenarios where the risk of aversive outcomes are reduced or the appropriate valuation of reward requires synthesis of co-occurring stimuli. In the case of SAP testing, the NAc may serve as a locus for determining the value of approaching or avoiding conspecifics, evaluations that are informed by its efferent connections with the BLA, insula, and PL.

When considering other subcortical structures, the PAG stands out as a potential substrate for orchestrating avoidant behavior in the SAP test. This region is classically associated with fear-evoked freezing and defensive behaviors (Koutsikou et al., 2014; Lefler et al., 2020), while its role in social behavior remains unknown. A recent pilot study inhibiting PAG via muscimol

injection prior to SAP testing abolished preference for stressed juveniles, suggesting this region is involved in social affective preference behavior. The PAG signals threat probability in a fear discrimination task where an uncertainty cue predicts a foot shock only 37.5% of the time (Wright and McDannald, 2019) and changes its firing rate in response to different levels of predatory threat (Bindi et al., 2022), indicating that this region may be involved in evaluating threatening stimuli. Given the fact that amygdala lesions interfered with contextual fear learning but not unconditioned freezing elicited by PAG stimulation (Oliveira et al., 2004), it's possible that the amygdala shares contextual threat-related information with the PAG that influences behavioral responses. Insula-PAG circuit function remains understudied, but could be further involved in threat evaluation, with the insula sharing relevant interoceptive information that could inform threat assessment. Finally, prefrontal input to the PAG could bias behavioral output. Afferents from this region target both dorsal, defense-related and ventral, approach-related columns of the PAG and inhibition of PFC-dorsal PAG projections increased social avoidance, resulting in a social defeat phenotype (Franklin et al., 2017). These findings suggest a mechanism by which the PFC can drive both appetitive and aversive social behaviors via PAG inhibition and may be essential to threat-coping strategies. Much more work is needed to define the PAG's role in this behavior but one potential function may be serving as a "threat detection" center during SAP testing, utilizing information received from the BLA and insula to classify stressed juveniles or adults as less

or more threatening, respectively, with prefrontal input driving the ultimate behavioral response to this social context.

4.6 Neuromodulation of BLA-insula-PL circuitry: spotlight on oxytocin

A number of neuromodulators are likely involved in social affective preference. We know that insular CRF (Rieger et al., 2022a), MCH, orexin (Barretto-de-Souza et al., 2023), and oxytocin (Rogers-Carter et al., 2018b) modulate SAP behavior. Oxytocin is of particular interest here, as findings presented in Chapter 2 identified sex specific effects of insular oxytocin in social investigation. Administration of oxytocin to the BLA, but not the insula, of female rats increased social exploration of naïve juvenile and adult conspecifics. Prior work identified oxytocin within the insula as an essential component of male social affective behavior, indicating a sex difference in insular oxytocin activity. Implications and limitations of these experiments are discussed in depth in Chapter 2. Here, I'd like to briefly highlight the known prosocial role of oxytocin in each region investigated above to encourage future studies into the neuromodulation of this circuitry in SAP behavior.

Oxytocin neurons are mainly found within the paraventricular and supraoptic nuclei of the hypothalamus, where they project to various cortical, subcortical, and brainstem regions, including the BLA, insula, and PL (Gimpl and Fahrenholz, 2001). Across species, oxytocin seems to modulate socially salient brain networks (Brodmann et al., 2017; Johnson et al., 2017; Parr et al., 2018; Rilling et al., 2018), contributing to the view that oxytocin promotes social behavior via the reinforcement or enhancement of socially salient stimuli within

the brain (Shamay-Tsoory and Abu-Akel, 2016; Menon and Neumann, 2023). Consistent with this view, in humans intranasal oxytocin administration enhanced BLA activation and functional connectivity while viewing emotional faces (Procyshyn et al., 2022). In rodents, oxytocin receptor antagonist delivered to the insula or mPFC impaired SAP behavior (Rogers-Carter et al., 2018b) and short term social recognition memory (Yashima and Sakamoto, 2024), respectively. Interestingly, oxytocin receptor antagonism within the mPFC rescued social behavior deficits in male rats that had undergone chronic chemogenetic activation of this region (Janz et al., 2023). At the circuit level, glutamatergic oxytocin receptor-expressing neurons in the mPFC project to the BLA and optogenetic stimulation of these axons within the BLA impairs social recognition memory (Tan et al., 2019). Together, these two findings suggest that hyperactivity of mPFC and increased oxytocin signaling may be linked and contribute to social dysfunction.

The studies highlighted above along with the results discussed in Chapter 2 support a potential role for oxytocin in modulating social affective behavior. In the BLA, oxytocin may modulate valence processing of conspecific affect, resulting in the increased social exploration observed after oxytocin administration to the BLA (Djerdjaj et al., 2023). Within the insula, oxytocin potentially signals changes in social salience that inform representations of one's social environment. This could also increase the incentive value of social interaction with certain conspecifics, contributing to motivational processes generated within the insula. Finally, fine-tuned control of oxytocin signaling within

the PL may be essential to the execution of adaptive social behaviors, with abnormal increases in oxytocin transmission leading to potential hyperactivation of projection neurons within the PL and subsequent disordered sociality. Much more work is needed to test these various hypotheses and sexually dimorphic functions of oxytocin only emphasize the importance of characterizing the role this neuromodulator plays in social affective behavior.

4.7 Relevance to clinical research

The work presented in this dissertation highlights circuitry underlying social affective behavior and has implications for clinical treatments of social affective disorders. Neuropsychiatric disorders, including ASD and schizophrenia, are often characterized by deficits in affective processes, including emotion recognition (Kuusikko et al., 2009; Gao et al., 2021), and empathy (Bonfils et al., 2016; Trimmer et al., 2017). Numerous human neuroimaging studies have linked activity in the amygdala (Sergerie et al., 2008), insula (Gu et al., 2012), and PFC (Seitz et al., 2006) to empathic processes that inform affective behaviors, with impairments in each of these regions contributing to social or emotional dysfunction (Adolphs et al., 2002; Mah et al., 2004; Cho et al., 2012; Bjorkquist and Herbener, 2013). Further, these impairments contribute to aberrant functional connectivity patterns across a brain-wide social network (Shen et al., 2016; Francis et al., 2019; Guo et al., 2019; Ibrahim et al., 2019; Sato et al., 2023). Specifically, connectivity between amygdala, insula, and prefrontal regions is altered in individuals with ASD or schizophrenia (Chai et al.,

2011; Ibrahim et al., 2019; Odriozola et al., 2019). For example, intrinsic functional connectivity between the insula and amygdala is reduced in patients with ASD (Ebisch et al., 2011) while schizophrenic patients display reduced connectivity between amygdala and insula regions when making social judgements (Mukherjee et al., 2014). Decreased resting state connectivity between insula and ventromedial PFC is also observed in patients with ASD (Guo et al., 2019). The findings presented here provide support for these human studies that have identified the importance of these regions and their connectivity to social affective processes. Social behaviors are the product of coordinated activity between a network of brain regions. Here, mechanistic studies have revealed a causal link between deficits in BLA-insula and insula-PL circuitry and social affective dysfunction that could inform clinical research into therapeutic targets for ASD and schizophrenia treatment. Future work can consider these circuits as central pathways integrating external and internal affective information and executing adaptive social behaviors.

4.8 Conclusion

In this dissertation, I have investigated the circuit-specific mechanisms underlying social affective behaviors. Using pharmacological and chemogenetic techniques, I have identified two circuits, BLA-insula and insula-PL, that are essential for approach and avoidance behaviors toward stressed others. To my knowledge, these findings are the first to implicate these tracts in social affective behavior. Taken together, they provide a framework for how social emotional

information is detected, appraised, and relayed throughout the brain and how social context-dependent behaviors are generated in response to these cues. These behaviors arise from the combined activity of a large network of brain regions, so much more work is needed to determine what other nodes of this social brain contribute to this social affective processing and how they interact with this circuitry to influence motivated social behaviors.

References

Adolphs R (2001) The neurobiology of social cognition. Current Opinion in Neurobiology 11:231–239.

Adolphs R (2010) What does the amygdala contribute to social cognition? Ann N Y Acad Sci 1191:42–61.

Adolphs R, Baron-Cohen S, Tranel D (2002) Impaired Recognition of Social Emotions following Amygdala Damage. Journal of Cognitive Neuroscience 14:1264–1274.

Aguilar-Rivera M, Kim S, Coleman TP, Maldonado PE, Torrealba F (2020) Interoceptive insular cortex participates in sensory processing of gastrointestinal malaise and associated behaviors. Scientific Reports 10:21642.

Ajram LA, Horder J, Mendez MA, Galanopoulos A, Brennan LP, Wichers RH, Robertson DM, Murphy CM, Zinkstok J, Ivin G, Heasman M, Meek D, Tricklebank MD, Barker GJ, Lythgoe DJ, Edden R a. E, Williams SC, Murphy DGM, McAlonan GM (2017) Shifting brain inhibitory balance and connectivity of the prefrontal cortex of adults with autism spectrum disorder. Transl Psychiatry 7:e1137–e1137.

Alcaro A, Huber R, Panksepp J (2007) Behavioral functions of the mesolimbic dopaminergic system: An affective neuroethological perspective. Brain Research Reviews 56:283–321.

Alexander WH, Brown JW (2011) Medial prefrontal cortex as an action-outcome predictor. Nat Neurosci 14:1338–1344.

Allen GV, Saper CB, Hurley KM, Cechetto DF (1991) Organization of visceral and limbic connections in the insular cortex of the rat. J Comp Neurol 311:1–16.

Alvares GA, Balleine BW, Whittle L, Guastella AJ (2016) Reduced goal-directed action control in autism spectrum disorder. Autism Research 9:1285–1293.

Ambroggi F, Ishikawa A, Fields HL, Nicola SM (2008) Basolateral Amygdala Neurons Facilitate Reward-Seeking Behavior by Exciting Nucleus Accumbens Neurons. Neuron 59:648–661.

Amir A, Kyriazi P, Lee S-C, Headley DB, Paré D (2019) Basolateral amygdala neurons are activated during threat expectation. Journal of Neurophysiology 121:1761–1777.

Amodio DM, Frith CD (2006) Meeting of minds: the medial frontal cortex and social cognition. Nature Reviews Neuroscience 7:268–277.

Anastasiades PG, Carter AG (2021) Circuit organization of the rodent medial prefrontal cortex. Trends in Neurosciences 44:550–563.

Anders S, Eippert F, Weiskopf N, Veit R (2008) The human amygdala is sensitive to the valence of pictures and sounds irrespective of arousal: an fMRI study. Social Cognitive and Affective Neuroscience 3:233–243.

Anholt RRH, O'Grady P, Wolfner MF, Harbison ST (2020) Evolution of Reproductive Behavior. Genetics 214:49–73.

Armbruster BN, Li X, Pausch MH, Herlitze S, Roth BL (2007) Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proc Natl Acad Sci USA 104:5163–5168.

Arruda-Carvalho M, Clem RL (2015) Prefrontal-amygdala fear networks come into focus. Frontiers in Systems Neuroscience 9 Available at: https://www.frontiersin.org/articles/10.3389/fnsys.2015.00145 [Accessed February 14, 2024].

Avery JA, Drevets WC, Moseman SE, Bodurka J, Barcalow JC, Simmons WK (2014) Major Depressive Disorder Is Associated With Abnormal Interoceptive Activity

and Functional Connectivity in the Insula. Biological Psychiatry 76:258–266.
Avery JA, Liu AG, Ingeholm JE, Riddell CD, Gotts SJ, Martin A (2020) Taste Quality Representation in the Human Brain. J Neurosci 40:1042–1052.
Baas D, Aleman A, Vink M, Ramsey NF, de Haan EHF, Kahn RS (2008) Evidence of altered cortical and amygdala activation during social decision-making in schizophrenia. NeuroImage 40:719–727.
Báez-Mendoza R, Mastrobattista EP, Wang AJ, Williams ZM (2021) Social agent identity cells in the prefrontal cortex of interacting groups of primates. Science 374:eabb4149.
Bangasser DA, Cuarenta A (2021) Sex differences in anxiety and depression: circuits and mechanisms. Nat Rev Neurosci 22:674–684.
Bao AM, Swaab DF (2010) Sex differences in the brain, behavior, and neuropsychiatric disorders. Neuroscientist 16:550–565.
Baron-Cohen S, Ring HA, Bullmore ET, Wheelwright S, Ashwin C, Williams SCR (2000) The amygdala theory of autism. Neuroscience & Biobehavioral Reviews 24:355–364.
Barrash J, Tranel D, Anderson SW (2000) Acquired Personality Disturbances Associated With Bilateral Damage to the Ventromedial Prefrontal Region. Developmental Neuropsychology 18:355–381.
Barretto-de-Souza L, Joseph SA, Lynch FM, Ng AJ, Crestani CC, Christianson JP (2023) Melanin-concentrating hormone and orexin shape social affective behavior via action in the insular cortex of rat. Psychopharmacology (Berl).
Bartal IB-A, Decety J, Mason P (2011) Empathy and Pro-Social Behavior in Rats. Science 334:1427–1430.
Baslet G, Termini L, Herbener E (2009) Deficits in emotional awareness in schizophrenia and their relationship with other measures of functioning. J Nerv Ment Dis 197:655–660.
Bastuji H, Frot M, Perchet C, Hagiwara K, Garcia-Larrea L (2018) Convergence of sensory and limbic noxious input into the anterior insula and the emergence of pain from nociception. Sci Rep 8:13360.
Beattie L, Kyle SD, Espie CA, Biello SM (2015) Social interactions, emotion and sleep: A systematic review and research agenda. Sleep Medicine Reviews 24:83–100.
Beery AK, Kaufer D (2015) Stress, social behavior, and resilience: Insights from rodents. Neurobiology of Stress 1:116–127.
Belova MA, Paton JJ, Salzman CD (2008) Moment-to-Moment Tracking of State Value in the Amygdala. Journal of Neuroscience 28:10023–10030.
Benarroch EE (2019) Insular cortex. Neurology 93:932–938.
Benassi-Cezar G, Carmona IM, Baptista-de-Souza D, Nunes-de-Souza RL, Canto-de-Souza A (2021) Differential modulation of the anterior cingulate and insular cortices on anxiogenic-like responses induced by empathy for pain. Neuropharmacology 192:108413.
Berendse HW, Graaf YG-D, Groenewegen HJ (1992) Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. Journal of Comparative Neurology 316:314–347.
Bernhardt BC, Singer T (2012) The neural basis of empathy. Annu Rev Neurosci 35:1–23.
Beyeler A, Chang C-J, Silvestre M, Lévêque C, Namburi P, Wildes CP, Tye KM (2018) Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. Cell Reports 22:905–918.
Beyeler A, Namburi P, Glober GF, Simonnet C, Calhoon GG, Conyers GF, Luck R,

Wildes CP, Tye KM (2016) Divergent Routing of Positive and Negative Information from the Amygdala during Memory Retrieval. Neuron 90:348–361.
Bickart KC, Dickerson BC, Feldman Barrett L (2014) The amygdala as a hub in brain networks that support social life. Neuropsychologia 63:235–248.
Bicks LK, Koike H, Akbarian S, Morishita H (2015) Prefrontal Cortex and Social Cognition in Mouse and Man. Frontiers in Psychology 6 Available at: https://www.frontiersin.org/articles/10.3389/fpsyg.2015.01805 [Accessed September 14, 2022].
Bicks LK, Yamamuro K, Flanigan ME, Kim JM, Kato D, Lucas EK, Koike H, Peng MS, Brady DM, Chandrasekaran S, Norman KJ, Smith MR, Clem RL, Russo SJ, Akbarian S, Morishita H (2020) Prefrontal parvalbumin interneurons require juvenile social experience to establish adult social behavior. Nat Commun 11:1003.
Bindi RP, Maia RGO, Pibiri F, Baldo MVC, Poulter SL, Lever C, Canteras NS (2022) Neural correlates of distinct levels of predatory threat in dorsal periaqueductal grey neurons. European Journal of Neuroscience 55:1504–1518.
Bjorkquist OA, Herbener ES (2013) Social perception in schizophrenia: Evidence of temporo-occipital and prefrontal dysfunction. Psychiatry Research: Neuroimaging 212:175–182.
Björnsdotter M, Löken L, Olausson H, Vallbo Å, Wessberg J (2009) Somatotopic Organization of Gentle Touch Processing in the Posterior Insular Cortex. J Neurosci 29:9314–9320.
Blanchard RJ, Blanchard DC, Rodgers J, Weiss SM (1990) The characterization and modelling of antipredator defensive behavior. Neuroscience & Biobehavioral Reviews 14:463–472.
Bloem B, Poorthuis R, Mansvelder H (2014) Cholinergic modulation of the medial prefrontal cortex: the role of nicotinic receptors in attention and regulation of neuronal activity. Frontiers in Neural Circuits 8 Available at: https://www.frontiersin.org/articles/10.3389/fncir.2014.00017 [Accessed January 29, 2024].
Bonfils KA, Lysaker PH, Minor KS, Salyers MP (2016) Affective empathy in schizophrenia: a meta-analysis. Schizophrenia Research 175:109–117.
Boucher O, Rouleau I, Lassonde M, Lepore F, Bouthillier A, Nguyen DK (2015) Social information processing following resection of the insular cortex. Neuropsychologia 71:1–10.
Braver TS, Cohen JD, Nystrom LE, Jonides J, Smith EE, Noll DC (1997) A Parametric Study of Prefrontal Cortex Involvement in Human Working Memory. NeuroImage 5:49–62.
Bridges RS (1996) Biochemical Basis of Parental Behavior in the Rat. In: Advances in the Study of Behavior (Rosenblatt JS, Snowdon CT, eds), pp 215–242 Parental Care: Evolution, Mechanisms, and Adaptive Significance. Academic Press. Available at: https://www.sciencedirect.com/science/article/pii/S0065345408603344 [Accessed January 17, 2023].
Brodmann K, Gruber O, Goya-Maldonado R (2017) Intranasal Oxytocin Selectively Modulates Large-Scale Brain Networks in Humans. Brain Connectivity 7:454–463.
Brothers L (1990) The Social Brain: A Project for Integrating Primate Behavior and Neurophysiology in a New Domain. Concepts in Neuroscience 1:27–51.
Bruchey AK, Jones CE, Monfils M-H (2010) Fear conditioning by-proxy: Social

transmission of fear during memory retrieval. Behavioural Brain Research 214:80–84.
Brudzynski SM (2013) Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. Current Opinion in Neurobiology 23:310–317.
Brüne M (2005) Emotion recognition, 'theory of mind,' and social behavior in schizophrenia. Psychiatry Research 133:135–147.
Brunet-Gouet E, Decety J (2006) Social brain dysfunctions in schizophrenia: A review of neuroimaging studies. Psychiatry Research: Neuroimaging 148:75–92.
Bs JS, Bs TZ, Azar T, Lawson D (2003) Are "By-stander" Female Sprague-Dawley Rats Affected by Experimental Procedures? 42.
(Bud) Craig A (2003) Interoception: the sense of the physiological condition of the body. Current Opinion in Neurobiology 13:500–505.
(Bud) Craig, D A (2009) How do you feel — now? The anterior insula and human awareness. Nat Rev Neurosci 10:59–70.
Burgos-Robles A, Kimchi EY, Izadmehr EM, Porzenheim MJ, Ramos-Guasp WA, Nieh EH, Felix-Ortiz AC, Namburi P, Leppla CA, Presbrey KN, Anandalingam KK, Pagan-Rivera PA, Anahtar M, Beyeler A, Tye KM (2017) Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. Nat Neurosci 20:824–835.
Bzdok D, Langner R, Schilbach L, Engemann D, Laird A, Fox P, Eickhoff S (2013) Segregation of the human medial prefrontal cortex in social cognition. Frontiers in Human Neuroscience 7 Available at: https://www.frontiersin.org/articles/10.3389/fnhum.2013.00232 [Accessed January 24, 2024].
Caldwell HK (2018) Oxytocin and sex differences in behavior. Current Opinion in Behavioral Sciences 23:13–20.
Carnevali L, Montano N, Statello R, Coudé G, Vacondio F, Rivara S, Ferrari PF, Sgoifo A (2017) Social stress contagion in rats: Behavioural, autonomic and neuroendocrine correlates. Psychoneuroendocrinology 82:155–163.
Castro DC, Berridge KC (2017) Opioid and orexin hedonic hotspots in rat orbitofrontal cortex and insula. Proceedings of the National Academy of Sciences 114:E9125–E9134.
Cea Salazar VI, Perez M, Robison AJ, Trainor BC (2024) Impacts of sex differences on optogenetic, chemogenetic, and calcium-imaging tools. Current Opinion in Neurobiology 84:102817.
Ceceli AO, Tricomi E (2018) Habits and goals: a motivational perspective on action control. Current Opinion in Behavioral Sciences 20:110–116.
Centanni SW, Janes AC, Haggerty DL, Atwood B, Hopf FW (2021) Better living through understanding the insula: Why subregions can make all the difference. Neuropharmacology 198:108765.
Chai XJ, Whitfield-Gabrieli S, Shinn AK, Gabrieli JDE, Nieto Castañón A, McCarthy JM, Cohen BM, Öngür D (2011) Abnormal Medial Prefrontal Cortex Resting-State Connectivity in Bipolar Disorder and Schizophrenia. Neuropsychopharmacol 36:2009–2017.
Chang SWC, Fagan NA, Toda K, Utevsky AV, Pearson JM, Platt ML (2015) Neural mechanisms of social decision-making in the primate amygdala. Proc Natl Acad Sci USA 112:16012–16017.
Chen BK, Murawski NJ, Cincotta C, McKissick O, Finkelstein A, Hamidi AB, Merfeld E, Doucette E, Grella SL, Shpokayte M, Zaki Y, Fortin A, Ramirez S (2019) Artificially Enhancing and Suppressing Hippocampus-Mediated Memories.

Current Biology 29:1885-1894.e4.
Chen C, Lee Y-H, Cheng Y (2014a) Anterior insular cortex activity to emotional salience of voices in a passive oddball paradigm. Front Hum Neurosci 8 Available at: https://www.frontiersin.org/articles/10.3389/fnhum.2014.00743/full [Accessed July 21, 2020].
Chen C, Lee Y-H, Cheng Y (2014b) Anterior insular cortex activity to emotional salience of voices in a passive oddball paradigm. Front Hum Neurosci 8 Available at: https://www.frontiersin.org/articles/10.3389/fnhum.2014.00743/full [Accessed July 21, 2020].
Chen K, Kogan JF, Fontanini A (2021a) Spatially Distributed Representation of Taste Quality in the Gustatory Insular Cortex of Behaving Mice. Current Biology 31:247-256.e4.
Chen VM, Foilb AR, Christianson JP (2016) Inactivation of ventral hippocampus interfered with cued-fear acquisition but did not influence later recall or discrimination. Behav Brain Res 296:249–253.
Chen WG, Schloesser D, Arensdorf AM, Simmons JM, Cui C, Valentino R, Gnadt JW, Nielsen L, Hillaire-Clarke CS, Spruance V, Horowitz TS, Vallejo YF, Langevin HM (2021b) The Emerging Science of Interoception: Sensing, Integrating, Interpreting, and Regulating Signals within the Self. Trends in Neurosciences 44:3–16.
Cho H-J, Kim S-J, Hwang SJ, Jo M-K, Kim H-J, Seeley WW, Kim E-J (2012) Social-emotional dysfunction after isolated right anterior insular infarction. J Neurol 259:764–767.
Choi J, Jeong Y (2017) Elevated emotional contagion in a mouse model of Alzheimer's disease is associated with increased synchronization in the insula and amygdala. Sci Rep 7:46262.
Chouchou F, Mauguière F, Vallayer O, Catenoix H, Isnard J, Montavont A, Jung J, Pichot V, Rheims S, Mazzola L (2019) How the insula speaks to the heart: Cardiac responses to insular stimulation in humans. Human Brain Mapping 40:2611–2622.
Christianson JP, Ragole T, Amat J, Greenwood BN, Strong PV, Paul ED, Fleshner M, Watkins LR, Maier SF (2010) 5-Hydroxytryptamine 2C Receptors in the Basolateral Amygdala Are Involved in the Expression of Anxiety After Uncontrollable Traumatic Stress. Biological Psychiatry 67:339–345.
Chudasama Y, Robbins TW (2004) Dopaminergic Modulation of Visual Attention and Working Memory in the Rodent Prefrontal Cortex. Neuropsychopharmacol 29:1628–1636.
Contreras M, Ceric F, Torrealba F (2007) Inactivation of the Interoceptive Insula Disrupts Drug Craving and Malaise Induced by Lithium. Science 318:655–658.
Cox SS, Kearns AM, Woods SK, Brown BJ, Brown SJ, Reichel CM (2022a) The role of the anterior insula during targeted helping behavior in male rats. Sci Rep 12:3315.
Cox SS, Kearns AM, Woods SK, Brown BJ, Brown SJ, Reichel CM (2022b) The role of the anterior insula during targeted helping behavior in male rats. Sci Rep 12:3315.
Craig AD, Chen K, Bandy D, Reiman EM (2000) Thermosensory activation of insular cortex. Nat Neurosci 3:184–190.
Curtis CE, D'Esposito M (2003) Persistent activity in the prefrontal cortex during working memory. Trends in Cognitive Sciences 7:415–423.
D'Argembeau A, Ruby P, Collette F, Degueldre C, Balteau E, Luxen A, Maquet P,

Salmon E (2007) Distinct Regions of the Medial Prefrontal Cortex Are Associated with Self-referential Processing and Perspective Taking. Journal of Cognitive Neuroscience 19:935–944.
Davidovic M, Starck G, Olausson H (2019) Processing of affective and emotionally neutral tactile stimuli in the insular cortex. Developmental Cognitive Neuroscience 35:94–103.
Davis M, Shi C (2000) The amygdala. Current Biology 10:R131.
de Giambattista C, Ventura P, Trerotoli P, Margari F, Margari L (2021) Sex Differences in Autism Spectrum Disorder: Focus on High Functioning Children and Adolescents. Frontiers in Psychiatry 12 Available at: https://www.frontiersin.org/articles/10.3389/fpsyt.2021.539835 [Accessed January 16, 2023].
de Waal FBM (2008) Putting the Altruism Back into Altruism: The Evolution of Empathy. Annu Rev Psychol 59:279–300.
de Waal FBM, Preston SD (2017) Mammalian empathy: behavioural manifestations and neural basis. Nat Rev Neurosci 18:498–509.
Decety J (2011) Dissecting the Neural Mechanisms Mediating Empathy. Emotion Review 3:92–108.
Decety J, Moriguchi Y (2007) The empathic brain and its dysfunction in psychiatric populations: implications for intervention across different clinical conditions. Biopsychosoc Med 1:22.
Decety J, Skelly LR, Kiehl KA (2013) Brain response to empathy-eliciting scenarios involving pain in incarcerated individuals with psychopathy. JAMA Psychiatry 70:638–645.
DeLeo JA, Rutkowski MD (2000) Gender differences in rat neuropathic pain sensitivity is dependent on strain. Neuroscience Letters 282:197–199.
Diehl MM, Iravedra-Garcia JM, Morán-Sierra J, Rojas-Bowe G, Gonzalez-Diaz FN, Valentín-Valentín VP, Quirk GJ (2020) Divergent projections of the prelimbic cortex bidirectionally regulate active avoidance Schoenbaum G, Frank MJ, Schoenbaum G, McDannald MA, eds. eLife 9:e59281.
Dielenberg RA, McGregor IS (2001) Defensive behavior in rats towards predatory odors: a review. Neuroscience & Biobehavioral Reviews 25:597–609.
Djerdjaj A, Ng AJ, Rieger NS, Christianson JP (2022) The basolateral amygdala to posterior insular cortex tract is necessary for social interaction with stressed juvenile rats. Behavioural Brain Research 435:114050.
Djerdjaj A, Rieger NS, Brady BH, Carey BN, Ng AJ, Christianson JP (2023) Social affective behaviors among female rats involve the basolateral amygdala and insular cortex. PLOS ONE 18:e0281794.
Dolensek N, Gehrlach DA, Klein AS, Gogolla N (2020) Facial expressions of emotion states and their neuronal correlates in mice. Science 368:89–94.
Dulka BN, Bress KS, Grizzell JA, Cooper MA (2018) Social Dominance Modulates Stress-induced Neural Activity in Medial Prefrontal Cortex Projections to the Basolateral Amygdala. Neuroscience 388:274–283.
Dumais KM, Bredewold R, Mayer TE, Veenema AH (2013) Sex differences in oxytocin receptor binding in forebrain regions: Correlations with social interest in brain region- and sex- specific ways. Hormones and Behavior 64:693–701.
Dumais KM, Veenema AH (2016) Vasopressin and oxytocin receptor systems in the brain: Sex differences and sex-specific regulation of social behavior. Frontiers in Neuroendocrinology 40:1–23.
Ebisch SJH, Gallese V, Willems RM, Mantini D, Groen WB, Romani GL, Buitelaar JK,

Bekkering H (2011) Altered intrinsic functional connectivity of anterior and posterior insula regions in high-functioning participants with autism spectrum disorder. Human Brain Mapping 32:1013–1028.
Eisenberger NI, Moieni M, Inagaki TK, Muscatell KA, Irwin MR (2017) In Sickness and in Health: The Co-Regulation of Inflammation and Social Behavior. Neuropsychopharmacol 42:242–253.
Euston DR, Gruber AJ, McNaughton BL (2012) The Role of Medial Prefrontal Cortex in Memory and Decision Making. Neuron 76:1057–1070.
Felix-Ortiz AC, Burgos-Robles A, Bhagat ND, Leppla CA, Tye KM (2016) Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. Neuroscience 321:197–209.
Felix-Ortiz AC, Tye KM (2014) Amygdala Inputs to the Ventral Hippocampus Bidirectionally Modulate Social Behavior. Journal of Neuroscience 34:586–595.
Ferguson BR, Gao W-J (2018) Thalamic Control of Cognition and Social Behavior Via Regulation of Gamma-Aminobutyric Acidergic Signaling and Excitation/Inhibition Balance in the Medial Prefrontal Cortex. Biological Psychiatry 83:657–669.
Fernández M, Mollinedo-Gajate I, Peñagarikano O (2018) Neural Circuits for Social Cognition: Implications for Autism. Neuroscience 370:148–162.
Ferri SL, Abel T, Brodkin ES (2018) Sex Differences in Autism Spectrum Disorder: a Review. Curr Psychiatry Rep 20:9.
Folkes OM, Báldi R, Kondev V, Marcus DJ, Hartley ND, Turner BD, Ayers JK, Baechle JJ, Misra MP, Altemus M, Grueter CA, Grueter BA, Patel S (2020) An endocannabinoid-regulated basolateral amygdala–nucleus accumbens circuit modulates sociability. J Clin Invest 130:1728–1742.
Forster SE, Brown JW (2011) Medial prefrontal cortex predicts and evaluates the timing of action outcomes. NeuroImage 55:253–265.
Francis SM, Camchong J, Brickman L, Goelkel-Garcia L, Mueller BA, Tseng A, Lim KO, Jacob S (2019) Hypoconnectivity of insular resting-state networks in adolescents with Autism Spectrum Disorder. Psychiatry Research: Neuroimaging 283:104–112.
Frank SM, Baumann O, Mattingley JB, Greenlee MW (2014) Vestibular and visual responses in human posterior insular cortex. Journal of Neurophysiology 112:2481–2491.
Franklin TB, Silva BA, Perova Z, Marrone L, Masferrer ME, Zhan Y, Kaplan A, Greetham L, Verrechia V, Halman A, Pagella S, Vyssotski AL, Illarionova A, Grinevich V, Branco T, Gross CT (2017) Prefrontal cortical control of a brainstem social behavior circuit. Nat Neurosci 20:260–270.
Fujii N, Hihara S, Nagasaka Y, Iriki A (2009) Social state representation in prefrontal cortex. Social Neuroscience 4:73–84.
Funahashi S (2017) Working Memory in the Prefrontal Cortex. Brain Sciences 7:49.
Gabbott PLA, Warner TA, Jays PRL, Salway P, Busby SJ (2005) Prefrontal cortex in the rat: Projections to subcortical autonomic, motor, and limbic centers. Journal of Comparative Neurology 492:145–177.
Gangopadhyay P, Chawla M, Dal Monte O, Chang SWC (2021) Prefrontal-amygdala circuits in social decision-making. Nat Neurosci 24:5–18.
Gao F, Huang J, Huang G-B, You Q-L, Yao S, Zhao S-T, Liu J, Wu C-H, Chen G-F, Liu S-M, Yu Z, Zhou Y-L, Ning Y-P, Liu S, Hu B-J, Sun X-D (2023) Elevated prelimbic cortex-to-basolateral amygdala circuit activity mediates comorbid anxiety-like behaviors associated with chronic pain. J Clin Invest 133 Available at: https://www.jci.org/articles/view/166356 [Accessed January 2, 2024].

Gao Z, Zhao W, Liu S, Liu Z, Yang C, Xu Y (2021) Facial Emotion Recognition in Schizophrenia. Frontiers in Psychiatry 12 Available at: https://www.frontiersin.org/journals/psychiatry/articles/10.3389/fpsyt.2021.633717 [Accessed February 18, 2024].
Garavan H, Pendergrass JC, Ross TJ, Stein EA, Risinger RC (2001) Amygdala response to both positively and negatively valenced stimuli. NeuroReport 12:2779.
Gehrlach DA, Dolensek N, Klein AS, Chowdhury RR, Matthys A, Junghänel M, Gaitanos TN, Podgornik A, Black TD, Vaka NR, Conzelmann K-K, Gogolla N (2019a) Aversive state processing in the posterior insular cortex. Nat Neurosci 22:1424–1437.
Gehrlach DA, Dolensek N, Klein AS, Roy Chowdhury R, Matthys A, Junghänel M, Gaitanos TN, Podgornik A, Black TD, Reddy Vaka N, Conzelmann K-K, Gogolla N (2019b) Aversive state processing in the posterior insular cortex. Nat Neurosci 22:1424–1437.
Gehrlach DA, Weiand C, Gaitanos TN, Cho E, Klein AS, Hennrich AA, Conzelmann K-K, Gogolla N (2020a) A whole-brain connectivity map of mouse insular cortex Wassum KM, Livneh Y, eds. eLife 9:e55585.
Gehrlach DA, Weiand C, Gaitanos TN, Cho E, Klein AS, Hennrich AA, Conzelmann K-K, Gogolla N (2020b) A whole-brain connectivity map of mouse insular cortex Wassum KM, Livneh Y, eds. eLife 9:e55585.
Gentry RN, Roesch MR (2018) Neural Activity in Ventral Medial Prefrontal Cortex Is Modulated More Before Approach Than Avoidance During Reinforced and Extinction Trial Blocks. J Neurosci 38:4584–4597.
Gilbert SJ, Bird G, Brindley R, Frith CD, Burgess PW (2008) Atypical recruitment of medial prefrontal cortex in autism spectrum disorders: An fMRI study of two executive function tasks. Neuropsychologia 46:2281–2291.
Gimpl G, Fahrenholz F (2001) The Oxytocin Receptor System: Structure, Function, and Regulation. Physiological Reviews 81:629–683.
Glangetas C, Ladevèze E, Guillaumin A, Gauthier M, Doudnikoff E, Bézard E, Taupignon A, Baufreton J, Georges F (2022) Encoding social preference by interhemispheric neurons in the Insula. :2022.12.15.520538 Available at: https://www.biorxiv.org/content/10.1101/2022.12.15.520538v1 [Accessed February 7, 2023].
Gogolla N (2017) The insular cortex. Current Biology 27:R580–R586.
Gogolla N, Takesian AE, Feng G, Fagiolini M, Hensch TK (2014) Sensory Integration in Mouse Insular Cortex Reflects GABA Circuit Maturation. Neuron 83:894–905.
Gogos A, Ney LJ, Seymour N, Van Rheenen TE, Felmingham KL (2019) Sex differences in schizophrenia, bipolar disorder, and post-traumatic stress disorder: Are gonadal hormones the link? British Journal of Pharmacology 176:4119–4135.
Goodson JL (2005) The vertebrate social behavior network: evolutionary themes and variations. Horm Behav 48:11–22.
Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA, Salzman CD, Axel R (2015) Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. Cell 162:134–145.
Gorka SM, Fitzgerald DA, Labuschagne I, Hosanagar A, Wood AG, Nathan PJ, Phan KL (2015) Oxytocin Modulation of Amygdala Functional Connectivity to Fearful Faces in Generalized Social Anxiety Disorder. Neuropsychopharmacology 40:278–286.

Gotts SJ, Simmons WK, Milbury LA, Wallace GL, Cox RW, Martin A (2012) Fractionation of social brain circuits in autism spectrum disorders. Brain 135:2711–2725.
Gourley SL, Taylor JR (2016) Going and stopping: dichotomies in behavioral control by the prefrontal cortex. Nat Neurosci 19:656–664.
Grabenhorst F, Báez-Mendoza R, Genest W, Deco G, Schultz W (2019) Primate Amygdala Neurons Simulate Decision Processes of Social Partners. Cell 177:986-998.e15.
Grossman M, Eslinger PJ, Troiani V, Anderson C, Avants B, Gee JC, McMillan C, Massimo L, Khan A, Antani S (2010) The role of ventral medial prefrontal cortex in social decisions: Converging evidence from fMRI and frontotemporal lobar degeneration. Neuropsychologia 48:3505–3512.
Gruber AJ, Calhoon GG, Shusterman I, Schoenbaum G, Roesch MR, O'Donnell P (2010) More Is Less: A Disinhibited Prefrontal Cortex Impairs Cognitive Flexibility. J Neurosci 30:17102–17110.
Gu X, Gao Z, Wang X, Liu X, Knight RT, Hof PR, Fan J (2012) Anterior insular cortex is necessary for empathetic pain perception. Brain 135:2726–2735.
Gu X, Hof PR, Friston KJ, Fan J (2013a) Anterior insular cortex and emotional awareness. Journal of Comparative Neurology 521:3371–3388.
Gu X, Liu X, Van Dam NT, Hof PR, Fan J (2013b) Cognition–Emotion Integration in the Anterior Insular Cortex. Cerebral Cortex 23:20–27.
Gunther Moor B, van Leijenhorst L, Rombouts SARB, Crone EA, Van der Molen MW (2010) Do you like me? Neural correlates of social evaluation and developmental trajectories. Social Neuroscience 5:461–482.
Guo X, Duan X, Suckling J, Chen H, Liao W, Cui Q, Chen H (2019) Partially impaired functional connectivity states between right anterior insula and default mode network in autism spectrum disorder. Human Brain Mapping 40:1264–1275.
H. Rodrigo A, Domenico SID, Ayaz H, Gulrajani S, Lam J, Ruocco AC (2014) Differentiating functions of the lateral and medial prefrontal cortex in motor response inhibition. NeuroImage 85:423–431.
Haaranen M, Scuppa G, Tambalo S, Järvi V, Bertozzi SM, Armirotti A, Sommer WH, Bifone A, Hyytiä P (2020) Anterior insula stimulation suppresses appetitive behavior while inducing forebrain activation in alcohol-preferring rats. Transl Psychiatry 10:150.
Haggerty DL, Grecco GG, Reeves KC, Atwood B (2020) Adeno-Associated Viral Vectors in Neuroscience Research. Molecular Therapy - Methods & Clinical Development 17:69–82.
Hahn AC, Xiao D, Sprengelmeyer R, Perrett DI (2013) Gender differences in the incentive salience of adult and infant faces. Q J Exp Psychol (Hove) 66:200–208.
Hamel L, Cavdaroglu B, Yeates D, Nguyen D, Riaz S, Patterson D, Khan N, Kirolos N, Roper K, Ha QA, Ito R (2022) Cortico-Striatal Control over Adaptive Goal-Directed Responding Elicited by Cues Signaling Sucrose Reward or Punishment. J Neurosci 42:3811–3822.
Hamel L, Thangarasa T, Samadi O, Ito R (2017) Caudal Nucleus Accumbens Core Is Critical in the Regulation of Cue-Elicited Approach-Avoidance Decisions. eNeuro 4 Available at: https://www.eneuro.org/content/4/1/ENEURO.0330-16.2017 [Accessed March 6, 2024].
Haroush K, Williams ZM (2015) Neuronal Prediction of Opponent's Behavior during Cooperative Social Interchange in Primates. Cell 160:1233–1245.
Harris LT, McClure SM, van den Bos W, Cohen JD, Fiske ST (2007) Regions of the MPFC differentially tuned to social and nonsocial affective evaluation. Cognitive,

Affective, & Behavioral Neuroscience 7:309–316.
Hartley ND, Gaulden AD, Báldi R, Winters ND, Salimando GJ, Rosas-Vidal LE, Jameson A, Winder DG, Patel S (2019) Dynamic remodeling of a basolateral-to-central amygdala glutamatergic circuit across fear states. Nat Neurosci 22:2000–2012.
Hasselmo ME (2005) A Model of Prefrontal Cortical Mechanisms for Goal-directed Behavior. Journal of Cognitive Neuroscience 17:1115–1129.
Hayton SJ, Lovett-Barron M, Dumont EC, Olmstead MC (2010) Target-Specific Encoding of Response Inhibition: Increased Contribution of AMPA to NMDA Receptors at Excitatory Synapses in the Prefrontal Cortex. J Neurosci 30:11493–11500.
Hayton SJ, Olmstead MC, Dumont ÉC (2011) Shift in the Intrinsic Excitability of Medial Prefrontal Cortex Neurons following Training in Impulse Control and Cued-Responding Tasks. PLOS ONE 6:e23885.
Hernandez-Lallement J, Gómez-Sotres P, Carrillo M (2020) Towards a unified theory of emotional contagion in rodents—A meta-analysis. Neuroscience & Biobehavioral Reviews Available at: https://www.sciencedirect.com/science/article/pii/S0149763420305674 [Accessed May 11, 2021].
Hintiryan H, Foster NN, Bowman I, Bay M, Song MY, Gou L, Yamashita S, Bienkowski MS, Zingg B, Zhu M, Yang XW, Shih JC, Toga AW, Dong H-W (2016) The mouse cortico-striatal projectome. Nat Neurosci 19:1100–1114.
Hiser J, Koenigs M (2018) The Multifaceted Role of the Ventromedial Prefrontal Cortex in Emotion, Decision Making, Social Cognition, and Psychopathology. Biological Psychiatry 83:638–647.
Hoffman KL, Gothard KM, Schmid MC, Logothetis NK (2007) Facial-Expression and Gaze-Selective Responses in the Monkey Amygdala. Current Biology 17:766–772.
Hoover WB, Vertes RP (2007) Anatomical analysis of afferent projections to the medial prefrontal cortex in the rat. Brain Struct Funct 212:149–179.
Howland JG, Ito R, Lapish CC, Villaruel FR (2022) The rodent medial prefrontal cortex and associated circuits in orchestrating adaptive behavior under variable demands. Neuroscience & Biobehavioral Reviews 135:104569.
Hsueh B et al. (2023) Cardiogenic control of affective behavioural state. Nature 615:292–299.
Hu L, Zhang L, Chen R, Yu H, Li H, Mouraux A (2015) The primary somatosensory cortex and the insula contribute differently to the processing of transient and sustained nociceptive and non-nociceptive somatosensory inputs. Human Brain Mapping 36:4346–4360.
Huang W-C, Zucca A, Levy J, Page DT (2020) Social Behavior Is Modulated by Valence-Encoding mPFC-Amygdala Sub-circuitry. Cell Reports 32:107899.
Ibrahim K, Eilbott JA, Ventola P, He G, Pelphrey KA, McCarthy G, Sukhodolsky DG (2019) Reduced Amygdala–Prefrontal Functional Connectivity in Children With Autism Spectrum Disorder and Co-occurring Disruptive Behavior. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 4:1031–1041.
Insel TR, Fernald RD (2004) HOW THE BRAIN PROCESSES SOCIAL INFORMATION: Searching for the Social Brain. Annual Review of Neuroscience 27:697–722.
Isoda M, Noritake A (2013) What makes the dorsomedial frontal cortex active during reading the mental states of others? Frontiers in Neuroscience 7 Available at: https://www.frontiersin.org/articles/10.3389/fnins.2013.00232 [Accessed January 25, 2024].

Iwaoki H, Nakamura K (2022) Neuronal Encoding of Emotional Valence and Intensity in the Monkey Amygdala. J Neurosci 42:7615–7623.
Jabarin R, Netser S, Wagner S (2022) Beyond the three-chamber test: toward a multimodal and objective assessment of social behavior in rodents. Molecular Autism 13:41.
Janz P, Knoflach F, Bleicher K, Belli S, Biemans B, Schnider P, Ebeling M, Grundschober C, Benekareddy M (2023) Selective oxytocin receptor activation prevents prefrontal circuit dysfunction and social behavioral alterations in response to chronic prefrontal cortex activation in male rats. Front Cell Neurosci 17:1286552.
Jaramillo AA, Randall PA, Stewart S, Fortino B, Van Voorhies K, Besheer J (2018a) Functional role for cortical-striatal circuitry in modulating alcohol self-administration. Neuropharmacology 130:42–53.
Jaramillo AA, Van Voorhies K, Randall PA, Besheer J (2018b) Silencing the insular-striatal circuit decreases alcohol self-administration and increases sensitivity to alcohol. Behavioural Brain Research 348:74–81.
Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin S-Y, Rabah D, Kinet J-P, Shin H-S (2010) Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. Nat Neurosci 13:482–488.
Jiang M, Wang M, Shi Q, Wei L, Lin Y, Wu D, Liu B, Nie X, Qiao H, Xu L, Yang T, Wang Z (2021) Evolution and neural representation of mammalian cooperative behavior. Cell Reports 37:110029.
Jin J, Zelano C, Gottfried JA, Mohanty A (2015) Human Amygdala Represents the Complete Spectrum of Subjective Valence. Journal of Neuroscience 35:15145–15156.
Johnson ZV, Walum H, Xiao Y, Riefkohl PC, Young LJ (2017) Oxytocin receptors modulate a social salience neural network in male prairie voles. Hormones and Behavior 87:16–24.
Ju A, Beyeler A (2021) A new player in neural circuits of emotions. Nat Neurosci:1–2.
Kadakia F, Chhetri AK, Davidson S (2022) Chemogenetic Modulation of the Posterior Insular Cortex Alters Both Affective and Sensory Pain-related Behavior. The Journal of Pain 23:26–27.
Kahn JB, Ward RD, Kahn LW, Rudy NM, Kandel ER, Balsam PD, Simpson EH (2012) Medial prefrontal lesions in mice impair sustained attention but spare maintenance of information in working memory. Learn Mem 19:513–517.
Kamigaki T (2019) Prefrontal circuit organization for executive control. Neuroscience Research 140:23–36.
Kamiński J, Sullivan S, Chung JM, Ross IB, Mamelak AN, Rutishauser U (2017) Persistently active neurons in human medial frontal and medial temporal lobe support working memory. Nat Neurosci 20:590–601.
Keller M, Pelz H, Perlitz V, Zweerings J, Röcher E, Baqapuri HI, Mathiak K (2020) Neural correlates of fluctuations in the intermediate band for heart rate and respiration are related to interoceptive perception. Psychophysiology 57:e13594.
Kesner RP, Churchwell JC (2011) An analysis of rat prefrontal cortex in mediating executive function. Neurobiology of Learning and Memory 96:417–431.
Keysers C, Knapska E, Moita MA, Gazzola V (2022) Emotional contagion and prosocial behavior in rodents. Trends in Cognitive Sciences 26:688–706.
Kietzman HW, Gourley SL (2023) How social information impacts action in rodents and humans: the role of the prefrontal cortex and its connections. Neuroscience & Biobehavioral Reviews 147:105075.

Kietzman HW, Trinoskey-Rice G, Blumenthal SA, Guo JD, Gourley SL (2022) Social incentivization of instrumental choice in mice requires amygdala-prelimbic cortex-nucleus accumbens connectivity. Nat Commun 13:4768.
Kim A, Keum S, Shin H-S (2019) Observational fear behavior in rodents as a model for empathy. Genes, Brain and Behavior 18:e12521.
Kim C, Johnson NF, Cilles SE, Gold BT (2011) Common and Distinct Mechanisms of Cognitive Flexibility in Prefrontal Cortex. J Neurosci 31:4771–4779.
Kim EJ, Kong M-S, Park SG, Mizumori SJY, Cho J, Kim JJ (2018) Dynamic coding of predatory information between the prelimbic cortex and lateral amygdala in foraging rats. Science Advances 4:eaar7328.
Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S (2016) Antagonistic negative and positive neurons of the basolateral amygdala. Nat Neurosci 19:1636–1646.
Kingsbury L, Huang S, Raam T, Ye LS, Wei D, Hu RK, Ye L, Hong W (2020) Cortical Representations of Conspecific Sex Shape Social Behavior. Neuron 107:941-953.e7.
Kingsbury L, Huang S, Wang J, Gu K, Golshani P, Wu YE, Hong W (2019) Correlated Neural Activity and Encoding of Behavior across Brains of Socially Interacting Animals. Cell 178:429-446.e16.
Kiyokawa Y, Kikusui T, Takeuchi Y, Mori Y (2005) Mapping the neural circuit activated by alarm pheromone perception by c-Fos immunohistochemistry. Brain Research 1043:145–154.
Kiyokawa Y, Shimozuru M, Kikusui T, Takeuchi Y, Mori Y (2006) Alarm pheromone increases defensive and risk assessment behaviors in male rats. Physiology & Behavior 87:383–387.
Klein AS, Dolensek N, Weiand C, Gogolla N (2021) Fear balance is maintained by bodily feedback to the insular cortex in mice. Science 374:1010–1015.
Klumpp H, Angstadt M, Phan KL (2012) Insula reactivity and connectivity to anterior cingulate cortex when processing threat in generalized social anxiety disorder. Biological Psychology 89:273–276.
Knapska E, Nikolaev E, Boguszewski P, Walasek G, Blaszczyk J, Kaczmarek L, Werka T (2006) Between-subject transfer of emotional information evokes specific pattern of amygdala activation. Proc Natl Acad Sci U S A 103:3858–3862.
Knutson B, Burgdorf J, Panksepp J (20021016) Ultrasonic vocalizations as indices of affective states in rats. Psychological Bulletin 128:961.
Kobayashi M (2011) Macroscopic Connection Of Rat Insular Cortex: Anatomical Bases Underlying Its Physiological Functions. In: International Review of Neurobiology (Kobayashi M, Koshikawa N, Iwata K, Waddington JL, eds), pp 285–303 Translating Mechanisms Orofacial Neurological Disorder. Academic Press. Available at: http://www.sciencedirect.com/science/article/pii/B9780123851987000114 [Accessed November 29, 2020].
Kohl J, Dulac C (2018) Neural control of parental behaviors. Current Opinion in Neurobiology 49:116–122.
Konu D, Turnbull A, Karapanagiotidis T, Wang H-T, Brown LR, Jefferies E, Smallwood J (2020) A role for the ventromedial prefrontal cortex in self-generated episodic social cognition. NeuroImage 218:116977.
Koutsikou S, Crook JJ, Earl EV, Leith JL, Watson TC, Lumb BM, Apps R (2014) Neural substrates underlying fear-evoked freezing: the periaqueductal grey–cerebellar link. The Journal of Physiology 592:2197–2213.
Krajbich I, Adolphs R, Tranel D, Denburg NL, Camerer CF (2009) Economic Games

Quantify Diminished Sense of Guilt in Patients with Damage to the Prefrontal Cortex. J Neurosci 29:2188–2192.
Kudryavtseva NN (2000) Agonistic behavior: A model, experimental studies, and perspectives. Neurosci Behav Physiol 30:293–305.
Kuehn E, Mueller K, Lohmann G, Schuetz-Bosbach S (2016) Interoceptive awareness changes the posterior insula functional connectivity profile. Brain Struct Funct 221:1555–1571.
Kurth F, Zilles K, Fox PT, Laird AR, Eickhoff SB (2010) A link between the systems: functional differentiation and integration within the human insula revealed by meta-analysis. Brain Struct Funct 214:519–534.
Kuusikko S, Haapsamo H, Jansson-Verkasalo E, Hurtig T, Mattila M-L, Ebeling H, Jussila K, Bölte S, Moilanen I (2009) Emotion Recognition in Children and Adolescents with Autism Spectrum Disorders. J Autism Dev Disord 39:938–945.
Kyriazi P, Headley DB, Pare D (2018) Multi-dimensional Coding by Basolateral Amygdala Neurons. Neuron 99:1315-1328.e5.
Lai M-C, Lombardo MV, Baron-Cohen S (2014) Autism. The Lancet 383:896–910.
Lamm C, Singer T (2010) The role of anterior insular cortex in social emotions. Brain Struct Funct 214:579–591.
Laubach M, Amarante LM, Swanson K, White SR (2018) What, If Anything, Is Rodent Prefrontal Cortex? eNeuro 5:ENEURO.0315-18.2018.
LeDoux JE, Cicchetti P, Xagoraris A, Romanski LM (1990) The lateral amygdaloid nucleus: sensory interface of the amygdala in fear conditioning. J Neurosci 10:1062–1069.
Lee E, Rhim I, Lee JW, Ghim J-W, Lee S, Kim E, Jung MW (2016) Enhanced Neuronal Activity in the Medial Prefrontal Cortex during Social Approach Behavior. Journal of Neuroscience 36:6926–6936.
Lefler Y, Campagner D, Branco T (2020) The role of the periaqueductal gray in escape behavior. Current Opinion in Neurobiology 60:115–121.
Levy DR, Tamir T, Kaufman M, Parabucki A, Weissbrod A, Schneidman E, Yizhar O (2019) Dynamics of social representation in the mouse prefrontal cortex. Nat Neurosci 22:2013–2022.
Li L-F, Li Z-L, Song B-L, Jiang Y, Wang Y, Zou H-W, Yao L-G, Liu Y-J (2023) Dopamine D2 receptors in the dorsomedial prefrontal cortex modulate social hierarchy in male mice. Current Zoology 69:682–693.
Li SW, Zeliger O, Strahs L, Báez-Mendoza R, Johnson LM, McDonald Wojciechowski A, Williams ZM (2022) Frontal neurons driving competitive behaviour and ecology of social groups. Nature 603:661–666.
Liang B, Zhang L, Barbera G, Fang W, Zhang J, Chen X, Chen R, Li Y, Lin D-T (2018) Distinct and Dynamic ON and OFF Neural Ensembles in the Prefrontal Cortex Code Social Exploration. Neuron 100:700-714.e9.
Likhtik E, Stujenske JM, A Topiwala M, Harris AZ, Gordon JA (2014) Prefrontal entrainment of amygdala activity signals safety in learned fear and innate anxiety. Nat Neurosci 17:106–113.
Lim MM, Young LJ (2006) Neuropeptidergic regulation of affiliative behavior and social bonding in animals. Hormones and Behavior 50:506–517.
Limanowski J, Lopes P, Keck J, Baudisch P, Friston K, Blankenburg F (2020) Action-Dependent Processing of Touch in the Human Parietal Operculum and Posterior Insula. Cerebral Cortex 30:607–617.
Livneh Y, Ramesh RN, Burgess CR, Levandowski KM, Madara JC, Fenselau H, Goldey GJ, Diaz VE, Jikomes N, Resch JM, Lowell BB, Andermann ML (2017)

Homeostatic circuits selectively gate food cue responses in insular cortex. Nature 546:611–616.
Livneh Y, Sugden AU, Madara JC, Essner RA, Flores VI, Sugden LA, Resch JM, Lowell BB, Andermann ML (2020) Estimation of Current and Future Physiological States in Insular Cortex. Neuron 105:1094-1111.e10.
Lonstein JS, De Vries GJ (2000) Sex differences in the parental behavior of rodents. Neuroscience & Biobehavioral Reviews 24:669–686.
Luchsinger JR, Fetterly TL, Williford KM, Salimando GJ, Doyle MA, Maldonado J, Simerly RB, Winder DG, Centanni SW (2021) Delineation of an insula-BNST circuit engaged by struggling behavior that regulates avoidance in mice. Nat Commun 12:3561.
Luo Y-F, Lu L, Song H-Y, Xu H, Zheng Z-W, Wu Z-Y, Jiang C-C, Tong C, Yuan H-Y, Liu X-X, Chen X, Sun M, Tang Y-M, Fan H-Y, Han F, Lu Y-M (2023) Divergent projections of the prelimbic cortex mediate autism- and anxiety-like behaviors. Mol Psychiatry:1–12.
Maggio JC, Whitney G (1985) Ultrasonic vocalizing by adult female mice (Mus musculus). J Comp Psychol 99:420–436.
Mah L, Arnold MC, Grafman J (2004) Impairment of Social Perception Associated With Lesions of the Prefrontal Cortex. AJP 161:1247–1255.
Mahler SV, Vazey EM, Beckley JT, Keistler CR, McGlinchey EM, Kaufling J, Wilson SP, Deisseroth K, Woodward JJ, Aston-Jones G (2014) Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. Nat Neurosci 17:577–585.
Mandy W, Chilvers R, Chowdhury U, Salter G, Seigal A, Skuse D (2012) Sex differences in autism spectrum disorder: evidence from a large sample of children and adolescents. J Autism Dev Disord 42:1304–1313.
Maren S, Aharonov G, Stote DL, Fanselow MS (1996) N-Methyl-D-Aspartate Receptors in the Basolateral Amygdala Are Required for Both Acquisition and Expression of Conditional Fear in Rats. :10.
Marlin BJ, Mitre M, D'amour JA, Chao MV, Froemke RC (2015) Oxytocin enables maternal behaviour by balancing cortical inhibition. Nature 520:499–504.
Martel G, Nishi A, Shumyatsky GP (2008) Stathmin reveals dissociable roles of the basolateral amygdala in parental and social behaviors. Proceedings of the National Academy of Sciences 105:14620–14625.
Masten CL, Morelli SA, Eisenberger NI (2011) An fMRI investigation of empathy for 'social pain' and subsequent prosocial behavior. NeuroImage 55:381–388.
Mather M, Lighthall NR, Nga L, Gorlick MA (2010) Sex differences in how stress affects brain activity during face viewing. Neuroreport 21:933–937.
Mathiasen ML, Aggleton JP, Witter MP (2023) Projections of the insular cortex to orbitofrontal and medial prefrontal cortex: A tracing study in the rat. Frontiers in Neuroanatomy 17 Available at: https://www.frontiersin.org/articles/10.3389/fnana.2023.1131167 [Accessed August 17, 2023].
Mazzola L, Isnard J, Mauguière F (2006) Somatosensory and Pain Responses to Stimulation of the Second Somatosensory Area (SII) in Humans. A Comparison with SI and Insular Responses. Cerebral Cortex 16:960–968.
Mazzola L, Royet J-P, Catenoix H, Montavont A, Isnard J, Mauguière F (2017) Gustatory and olfactory responses to stimulation of the human insula. Annals of Neurology 82:360–370.
Mcdonald AJ (1991) Organization of amygdaloid projections to the prefrontal cortex and

associated striatum in the rat. Neuroscience 44:1–14.
McDonald AJ (1998) Cortical pathways to the mammalian amygdala. Progress in Neurobiology 55:257–332.
Mederos S, Sánchez-Puelles C, Esparza J, Valero M, Ponomarenko A, Perea G (2021) GABAergic signaling to astrocytes in the prefrontal cortex sustains goal-directed behaviors. Nat Neurosci 24:82–92.
Menon R, Neumann ID (2023) Detection, processing and reinforcement of social cues: regulation by the oxytocin system. Nat Rev Neurosci 24:761–777.
Menon V, Uddin LQ (2010a) Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct 214:655–667.
Menon V, Uddin LQ (2010b) Saliency, switching, attention and control: a network model of insula function. Brain Struct Funct 214:655–667.
Meyza KZ, Bartal IB-A, Monfils MH, Panksepp JB, Knapska E (2017) The roots of empathy: Through the lens of rodent models. Neuroscience & Biobehavioral Reviews 76:216–234.
Mikosz M, Nowak A, Werka T, Knapska E (2015) Sex differences in social modulation of learning in rats. Sci Rep 5:18114.
Min J-Y, Park S, Cho J, Huh Y (2023) The anterior insular cortex processes social recognition memory. Sci Rep 13:10853.
Miura I, Sato M, Overton ETN, Kunori N, Nakai J, Kawamata T, Nakai N, Takumi T (2020) Encoding of social exploration by neural ensembles in the insular cortex. PLOS Biology 18:e3000584.
Mizoguchi N, Kobayashi M, Muramoto K (2016) Integration of olfactory and gustatory chemosignals in the insular cortex. Journal of Oral Biosciences 58:81–84.
Möhrle D, Fernández M, Peñagarikano O, Frick A, Allman B, Schmid S (2020) What we can learn from a genetic rodent model about autism. Neuroscience & Biobehavioral Reviews 109:29–53.
Moles A, Costantini F, Garbugino L, Zanettini C, D'Amato FR (2007) Ultrasonic vocalizations emitted during dyadic interactions in female mice: A possible index of sociability? Behavioural Brain Research 182:223–230.
Moles A, D'amato FR (2000) Ultrasonic vocalization by female mice in the presence of a conspecific carrying food cues. Anim Behav 60:689–694.
Molnar-Szakacs I, Uddin LQ (2022) Anterior insula as a gatekeeper of executive control. Neuroscience & Biobehavioral Reviews:104736.
Monfils MH, Agee LA (2019) Insights from social transmission of information in rodents. Genes, Brain and Behavior 18:e12534.
Mony TJ, Hong M, Lee HJ (2018) Empathy Study in Rodent Model of Autism Spectrum Disorders. Psychiatry Investig 15:104–110.
Morici JF, Bekinschtein P, Weisstaub NV (2015) Medial prefrontal cortex role in recognition memory in rodents. Behavioural Brain Research 292:241–251.
Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ, Dolan RJ (1996) A differential neural response in the human amygdala to fearful and happy facial expressions. Nature 383:812–815.
Mosher CP, Zimmerman PE, Gothard KM (2014) Neurons in the monkey amygdala detect eye-contact during naturalistic social interactions. Curr Biol 24:2459–2464.
Motomura K, Terasawa Y, Natsume A, Iijima K, Chalise L, Sugiura J, Yamamoto H, Koyama K, Wakabayashi T, Umeda S (2019) Anterior insular cortex stimulation and its effects on emotion recognition. Brain Struct Funct 224:2167–2181.
Mukherjee P, Whalley HC, McKirdy JW, Sprengelmeyer R, Young AW, McIntosh AM, Lawrie SM, Hall J (2014) Altered Amygdala Connectivity Within the Social Brain

in Schizophrenia. Schizophrenia Bulletin 40:152–160.
Murray EA (2007) The amygdala, reward and emotion. TRENDS in Cognitive Sciences 11:491–497.
Murugan M, Jang HJ, Park M, Miller EM, Cox J, Taliaferro JP, Parker NF, Bhave V, Hur H, Liang Y, Nectow AR, Pillow JW, Witten IB (2017) Combined Social and Spatial Coding in a Descending Projection from the Prefrontal Cortex. Cell 171:1663-1677.e16.
Mutschler I, Wankerl J, Seifritz E, Ball T (2011) The role of the human insular cortex in pain processing. European Psychiatry 26:1001–1001.
Nagahama Y, Okada T, Katsumi Y, Hayashi T, Yamauchi H, Oyanagi C, Konishi J, Fukuyama H, Shibasaki H (2001) Dissociable Mechanisms of Attentional Control within the Human Prefrontal Cortex. Cerebral Cortex 11:85–92.
Nagai M, Kishi K, Kato S (2007) Insular cortex and neuropsychiatric disorders: A review of recent literature. European Psychiatry 22:387–394.
Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, Anahtar M, Felix-Ortiz AC, Wickersham IR, Gray JM, Tye KM (2015) A circuit mechanism for differentiating positive and negative associations. Nature 520:675–678.
Naqvi NH, Gaznick N, Tranel D, Bechara A (2014) The insula: a critical neural substrate for craving and drug seeking under conflict and risk. Ann N Y Acad Sci 1316:53–70.
Newman SW (1999) The Medial Extended Amygdala in Male Reproductive Behavior A Node in the Mammalian Social Behavior Network. Annals of the New York Academy of Sciences 877:242–257.
Newman SW (1999) The Medial Extended Amygdala in Male Reproductive Behavior A Node in the Mammalian Social Behavior Network. Annals of the New York Academy of Sciences.
Ng AJ, Vincelette LK, Li J, Brady BH, Christianson JP (2023) Serotonin modulates social responses to stressed conspecifics via insular 5-HT2C receptors in rat. Neuropharmacology 236:109598.
Ochoa S, Usall J, Cobo J, Labad X, Kulkarni J (2012) Gender Differences in Schizophrenia and First-Episode Psychosis: A Comprehensive Literature Review. Schizophr Res Treatment 2012:916198.
O'Connell LA, Hofmann HA (2011a) The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. Journal of Comparative Neurology 519:3599–3639.
O'Connell LA, Hofmann HA (2011b) The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J Comp Neurol 519:3599–3639.
O'Doherty J, Rolls ET, Francis S, Bowtell R, McGlone F (2001) Representation of Pleasant and Aversive Taste in the Human Brain. Journal of Neurophysiology 85:1315–1321.
Odriozola P, Dajani DR, Burrows CA, Gabard-Durnam LJ, Goodman E, Baez AC, Tottenham N, Uddin LQ, Gee DG (2019) Atypical frontoamygdala functional connectivity in youth with autism. Developmental Cognitive Neuroscience 37:100603.
Oh SW et al. (2014) A mesoscale connectome of the mouse brain. Nature 508:207–214.
Oliveira LC, Nobre MJ, Brandão ML, Landeira-Fernandez J (2004) Role of amygdala in conditioned and unconditioned fear generated in the periaqueductal gray. NeuroReport 15:2281.

O'Neill P-K, Gore F, Salzman CD (2018) Basolateral amygdala circuitry in positive and negative valence. Curr Opin Neurobiol 49:175–183.
Ostlund SB, Balleine BW (2005) Lesions of Medial Prefrontal Cortex Disrupt the Acquisition But Not the Expression of Goal-Directed Learning. J Neurosci 25:7763–7770.
Padilla-Coreano N et al. (2022) Cortical ensembles orchestrate social competition through hypothalamic outputs. Nature 603:667–671.
Paine TA, Swedlow N, Swetschinski L (2017) Decreasing GABA function within the medial prefrontal cortex or basolateral amygdala decreases sociability. Behav Brain Res 317:542–552.
Panksepp J, Panksepp JB (2013) Toward a cross-species understanding of empathy. Trends Neurosci 36:10.1016/j.tins.2013.04.009.
Parkinson JA, Robbins TW, Everitt BJ (2000) Dissociable roles of the central and basolateral amygdala in appetitive emotional learning. European Journal of Neuroscience 12:405–413.
Parr LA, Mitchell T, Hecht E (2018) Intranasal oxytocin in rhesus monkeys alters brain networks that detect social salience and reward. American Journal of Primatology 80:e22915.
Parsana AJ, Li N, Brown TH (2012) Positive and negative ultrasonic social signals elicit opposing firing patterns in rat amygdala. Behavioural Brain Research 226:77–86.
Paulus MP, Stein MB (2010) Interoception in anxiety and depression. Brain Struct Funct 214:451–463.
Paxinos G (1998) The Rat Brain in Stereotaxic Coordinates. Academic Press.
Peng Y, Gillis-Smith S, Jin H, Tränkner D, Ryba NJP, Zuker CS (2015) Sweet and bitter taste in the brain of awake behaving animals. Nature 527:512–515.
Pettijohn TF, Ahmed SF, Pettijohn TF (2012) Hunger and Social Motivation: Hungry People are Less Interested in Social Activities than Satiated People. Curr Psychol 31:1–5.
Phelps EA, LeDoux JE (2005) Contributions of the Amygdala to Emotion Processing: From Animal Models to Human Behavior. Neuron 48:175–187.
Pignatelli M, Beyeler A (2019) Valence coding in amygdala circuits. Current Opinion in Behavioral Sciences 26:97–106.
Pinto L, Dan Y (2015) Cell-Type-Specific Activity in Prefrontal Cortex during Goal-Directed Behavior. Neuron 87:437–450.
Poggi G, Bergamini G, Dulinskas R, Madur L, Greter A, Ineichen C, Dagostino A, Kúkeľová D, Sigrist H, Bornemann KD, Hengerer B, Pryce CR (2024) Engagement of basal amygdala-nucleus accumbens glutamate neurons in the processing of rewarding or aversive social stimuli. European Journal of Neuroscience n/a Available at: https://onlinelibrary.wiley.com/doi/abs/10.1111/ejn.16272 [Accessed February 28, 2024].
Pohl A, Anders S, Schulte-Rüther M, Mathiak K, Kircher T (2013) Positive Facial Affect – An fMRI Study on the Involvement of Insula and Amygdala. PLOS ONE 8:e69886.
Posner J, Russell JA, Peterson BS (2005) The circumplex model of affect: An integrative approach to affective neuroscience, cognitive development, and psychopathology. Development and psychopathology 17:715.
Pratt WE, Mizumori SJY (2001) Neurons in rat medial prefrontal cortex show anticipatory rate changes to predictable differential rewards in a spatial memory task. Behavioural Brain Research 123:165–183.

Preckel K, Kanske P, Singer T (2018) On the interaction of social affect and cognition: empathy, compassion and theory of mind. Current Opinion in Behavioral Sciences 19:1–6.
Preis MA, Schmidt-Samoa C, Dechent P, Kroener-Herwig B (2013) The effects of prior pain experience on neural correlates of empathy for pain: An fMRI study. PAIN® 154:411–418.
Preuss TM (1995) Do Rats Have Prefrontal Cortex? The Rose-Woolsey-Akert Program Reconsidered. Journal of Cognitive Neuroscience 7:1–24.
Procyshyn T, Lombardo M, Lai MC, Jassim N, Auyeung B, Crockford S, Deakin J, Soubramanian S, Sule A, Terburg D, Baron-Cohen S, Bethlehem R (2022) Oxytocin enhances basolateral amygdala activation and functional connectivity while processing emotional faces: preliminary findings in autistic versus non-autistic women. Available at: https://www.repository.cam.ac.uk/handle/1810/334737 [Accessed January 16, 2023].
Prounis GS, Ophir AG (2020a) One cranium, two brains not yet introduced: Distinct but complimentary views of the social brain. Neurosci Biobehav Rev 108:231–245.
Prounis GS, Ophir AG (2020b) One cranium, two brains not yet introduced: Distinct but complementary views of the social brain. Neuroscience & Biobehavioral Reviews 108:231–245.
Pushparaj A, Kim AS, Musiol M, Trigo JM, Le Foll B (2015) Involvement of the rostral agranular insular cortex in nicotine self-administration in rats. Behavioural Brain Research 290:77–83.
Qi J, Li B-Z, Zhang Y, Pan B, Gao Y-H, Zhan H, Liu Y, Shao Y-C, Zhang X (2021) Altered insula-prefrontal functional connectivity correlates to decreased vigilant attention after total sleep deprivation. Sleep Medicine 84:187–194.
Qiu S, Chen T, Koga K, Guo Y, Xu H, Song Q, Wang J, Descalzi G, Kaang B-K, Luo J, Zhuo M, Zhao M (2013) An Increase in Synaptic NMDA Receptors in the Insular Cortex Contributes to Neuropathic Pain. Science Signaling 6:ra34–ra34.
Qu C, Ligneul R, Henst J-BV der, Dreher J-C (2017) An Integrative Interdisciplinary Perspective on Social Dominance Hierarchies. Trends in Cognitive Sciences 21:893–908.
Ratto AB, Kenworthy L, Yerys BE, Bascom J, Wieckowski AT, White SW, Wallace GL, Pugliese C, Schultz RT, Ollendick TH, Scarpa A, Seese S, Register-Brown K, Martin A, Anthony LG (2018) What About the Girls? Sex-Based Differences in Autistic Traits and Adaptive Skills. J Autism Dev Disord 48:1698–1711.
Reppucci CJ, Petrovich GD (2016) Organization of connections between the amygdala, medial prefrontal cortex, and lateral hypothalamus: a single and double retrograde tracing study in rats. Brain Struct Funct 221:2937–2962.
Rieger NS, Ng AJ, Lee S, Brady BH, Christianson JP (2023) Maternal immune activation alters social affective behavior and sensitivity to corticotropin releasing factor in male but not female rats. Hormones and Behavior 149:105313.
Rieger NS, Varela JA, Ng AJ, Granata L, Djerdjaj A, Brenhouse HC, Christianson JP (2021) Insular cortex corticotropin-releasing factor integrates stress signaling with social decision making. bioRxiv:2021.03.23.436680.
Rieger NS, Varela JA, Ng AJ, Granata L, Djerdjaj A, Brenhouse HC, Christianson JP (2022a) Insular cortex corticotropin-releasing factor integrates stress signaling with social affective behavior. Neuropsychopharmacol:1–13.
Rieger NS, Varela JA, Ng AJ, Granata L, Djerdjaj A, Brenhouse HC, Christianson JP (2022b) Insular cortex corticotropin-releasing factor integrates stress signaling

with social affective behavior. Neuropsychopharmacology 47:1156–1168.
Rieger NS, Worley NB, Ng AJ, Christianson JP (2022c) Insular cortex modulates social avoidance of sick rats. Behavioural Brain Research 416:113541.
Rigney N, de Vries GJ, Petrulis A, Young LJ (2022) Oxytocin, Vasopressin, and Social Behavior: From Neural Circuits to Clinical Opportunities. Endocrinology 163:bqac111.
Rilling JK, Chen X, Chen X, Haroon E (2018) Intranasal oxytocin modulates neural functional connectivity during human social interaction. American Journal of Primatology 80:e22740.
Rilling JK, DeMarco AC, Hackett PD, Chen X, Gautam P, Stair S, Haroon E, Thompson R, Ditzen B, Patel R, Pagnoni G (2014) Sex differences in the neural and behavioral response to intranasal oxytocin and vasopressin during human social interaction. Psychoneuroendocrinology 39:237–248.
Rilling JK, Sanfey AG (2011) The Neuroscience of Social Decision-Making. Annual Review of Psychology 62:23–48.
Ripoll LH, Snyder R, Steele H, Siever LJ (2013) The neurobiology of empathy in borderline personality disorder. Curr Psychiatry Rep 15:344.
Risterucci C, Terramorsi D, Nieoullon A, Amalric M (2003) Excitotoxic lesions of the prelimbic-infralimbic areas of the rodent prefrontal cortex disrupt motor preparatory processes. European Journal of Neuroscience 17:1498–1508.
Rodgers KM, Benison AM, Klein A, Barth DS (2008) Auditory, Somatosensory, and Multisensory Insular Cortex in the Rat. Cereb Cortex 18:2941–2951.
Rodríguez M, Ceric F, Murgas P, Harland B, Torrealba F, Contreras M (2020) Interoceptive Insular Cortex Mediates Both Innate Fear and Contextual Threat Conditioning to Predator Odor. Frontiers in Behavioral Neuroscience 13:283.
Rogers-Carter MM, Christianson JP (2019) An insular view of the social decision-making network. Neurosci Biobehav Rev 103:119–132.
Rogers-Carter MM, Djerdjaj A, Culp AR, Elbaz JA, Christianson JP (2018a) Familiarity modulates social approach toward stressed conspecifics in female rats. PLOS ONE 13:e0200971.
Rogers-Carter MM, Djerdjaj A, Gribbons KB, Varela JA, Christianson JP (2019) Insular Cortex Projections to Nucleus Accumbens Core Mediate Social Approach to Stressed Juvenile Rats. J Neurosci 39:8717–8729.
Rogers-Carter MM, Varela JA, Gribbons KB, Pierce AF, McGoey MT, Ritchey M, Christianson JP (2018b) Insular cortex mediates approach and avoidance responses to social affective stimuli. Nat Neurosci 21:404–414.
Rogers-Carter MM, Varela JA, Gribbons KB, Pierce AF, McGoey MT, Ritchey M, Christianson JP (2018c) Insular cortex mediates approach and avoidance responses to social affective stimuli. Nat Neurosci 21:404–414.
Rosenberger LA, Eisenegger C, Naef M, Terburg D, Fourie J, Stein DJ, Van Honk J (2019) The Human Basolateral Amygdala Is Indispensable for Social Experiential Learning. Current Biology 29:3532-3537.e3.
Rossi AF, Pessoa L, Desimone R, Ungerleider LG (2009) The prefrontal cortex and the executive control of attention. Exp Brain Res 192:489–497.
Roth BL (2016) DREADDs for Neuroscientists. Neuron 89:683–694.
Roth RH, Ding JB (2020) From Neurons to Cognition: Technologies for Precise Recording of Neural Activity Underlying Behavior. BME Frontiers 2020:7190517.
Royet J-P, Plailly J, Delon-Martin C, Kareken DA, Segebarth C (2003) fMRI of emotional responses to odors:: influence of hedonic valence and judgment, handedness, and gender. NeuroImage 20:713–728.

Sah P, Faber ESL, Lopez De Armentia M, Power J (2003) The Amygdaloid Complex: Anatomy and Physiology. Physiological Reviews 83:803–834.
Sakai N, Imada S (2003) Bilateral lesions of the insular cortex or of the prefrontal cortex block the association between taste and odor in the rat. Neurobiology of Learning and Memory 80:24–31.
Sakamoto T, Yashima J (2022) Prefrontal cortex is necessary for long-term social recognition memory in mice. Behavioural Brain Research 435:114051.
Sanchez-Larsen A, Principe A, Ley M, Vaquerizo B, Langohr K, Rocamora R (2023) Insular Role in Blood Pressure and Systemic Vascular Resistance Regulation. Neuromodulation: Technology at the Neural Interface Available at: https://www.sciencedirect.com/science/article/pii/S1094715923000065 [Accessed January 14, 2024].
Sander K, Frome Y, Scheich H (2007) FMRI activations of amygdala, cingulate cortex, and auditory cortex by infant laughing and crying. Hum Brain Mapp 28:1007–1022.
Sangha S, Chadick JZ, Janak PH (2013) Safety Encoding in the Basal Amygdala. Journal of Neuroscience 33:3744–3751.
Santos S, Almeida I, Oliveiros B, Castelo-Branco M (2016) The Role of the Amygdala in Facial Trustworthiness Processing: A Systematic Review and Meta-Analyses of fMRI Studies. PLOS ONE 11:e0167276.
Sarlitto MC, Foilb AR, Christianson JP (2018) Inactivation of the Ventrolateral Orbitofrontal Cortex Impairs Flexible Use of Safety Signals. Neuroscience 379:350–358.
Sato M, Nakai N, Fujima S, Choe KY, Takumi T (2023) Social circuits and their dysfunction in autism spectrum disorder. Mol Psychiatry 28:3194–3206.
Sato W, Yoshikawa S, Kochiyama T, Matsumura M (2004) The amygdala processes the emotional significance of facial expressions: an fMRI investigation using the interaction between expression and face direction. NeuroImage 22:1006–1013.
Scheggia D, Managò F, Maltese F, Bruni S, Nigro M, Dautan D, Latuske P, Contarini G, Gomez-Gonzalo M, Requie LM, Ferretti V, Castellani G, Mauro D, Bonavia A, Carmignoto G, Yizhar O, Papaleo F (2020) Somatostatin interneurons in the prefrontal cortex control affective state discrimination in mice. Nat Neurosci 23:47–60.
Scholl JL, Afzal A, Fox LC, Watt MJ, Forster GL (2019) Sex differences in anxiety-like behaviors in rats. Physiology & Behavior 211:112670.
Schönfeld L-M, Zech M-P, Schäble S, Wöhr M, Kalenscher T (2020) Lesions of the rat basolateral amygdala reduce the behavioral response to ultrasonic vocalizations. Behavioural Brain Research 378:112274.
Schuster R (2002) Cooperative coordination as a social behavior. Hum Nat 13:47–83.
Seffer D, Schwarting RKW, Wöhr M (2014) Pro-social ultrasonic communication in rats: Insights from playback studies. Journal of Neuroscience Methods 234:73–81.
Segerdahl AR, Mezue M, Okell TW, Farrar JT, Tracey I (2015) The dorsal posterior insula subserves a fundamental role in human pain. Nat Neurosci 18:499–500.
Seitz RJ, Nickel J, Azari NP (2006) Functional modularity of the medial prefrontal cortex: Involvement in human empathy. Neuropsychology 20:743–751.
Selimbeyoglu A, Kim CK, Inoue M, Lee SY, Hong ASO, Kauvar I, Ramakrishnan C, Fenno LE, Davidson TJ, Wright M, Deisseroth K (2017) Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Science Translational Medicine 9:eaah6733.
Sepahvand T, Power KD, Qin T, Yuan Q (2023) The Basolateral Amygdala: The Core of

a Network for Threat Conditioning, Extinction, and Second-Order Threat Conditioning. Biology 12:1274.
Sergerie K, Chochol C, Armony JL (2008) The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. Neuroscience & Biobehavioral Reviews 32:811–830.
Servonnet A, Hernandez G, Hage CE, Rompré P-P, Samaha A-N (2020) Optogenetic Activation of the Basolateral Amygdala Promotes Both Appetitive Conditioning and the Instrumental Pursuit of Reward Cues. J Neurosci 40:1732–1743.
Shamay-Tsoory SG, Abu-Akel A (2016) The Social Salience Hypothesis of Oxytocin. Biological Psychiatry 79:194–202.
Shen MD, Li DD, Keown CL, Lee A, Johnson RT, Angkustsiri K, Rogers SJ, Müller R-A, Amaral DG, Nordahl CW (2016) Functional Connectivity of the Amygdala Is Disrupted in Preschool-Aged Children With Autism Spectrum Disorder. Journal of the American Academy of Child & Adolescent Psychiatry 55:817–824.
Shi CJ, Cassell MD (1998) Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. J Comp Neurol 399:440–468.
Shih C-W, Chang C (2024) Anatomical analyses of collateral prefrontal cortex projections to the basolateral amygdala and the nucleus accumbens core in rats. Brain Struct Funct 229:97–114.
Shipley MT, Geinisman Y (1984) Anatomical evidence for convergence of olfactory, gustatory, and visceral afferent pathways in mouse cerebral cortex. Brain Research Bulletin 12:221–226.
Siegal N, Dow-Edwards D (2009) Isoflurane anesthesia interferes with the expression of cocaine-induced sensitization in female rats. Neuroscience Letters 464:52–56.
Silberberg A, Allouch C, Sandfort S, Kearns D, Karpel H, Slotnick B (2014) Desire for social contact, not empathy, may explain “rescue” behavior in rats. Anim Cogn 17:609–618.
Singer T, Critchley HD, Preuschoff K (2009) A common role of insula in feelings, empathy and uncertainty. Trends in Cognitive Sciences 13:334–340.
Singer T, Seymour B, O’Doherty J, Kaube H, Dolan RJ, Frith CD (2004) Empathy for Pain Involves the Affective but not Sensory Components of Pain. Science 303:1157–1162.
Sivaselvachandran S, Acland EL, Abdallah S, Martin LJ (2018) Behavioral and mechanistic insight into rodent empathy. Neurosci Biobehav Rev 91:130–137.
Small DM (2010) Taste representation in the human insula. Brain Struct Funct 214:551–561.
Small DM, Gregory MD, Mak YE, Gitelman D, Mesulam MM, Parrish T (2003) Dissociation of Neural Representation of Intensity and Affective Valuation in Human Gustation. Neuron 39:701–711.
Smith CJW, Poehlmann ML, Li S, Ratnaseelan AM, Bredewold R, Veenema AH (2017) Age and sex differences in oxytocin and vasopressin V1a receptor binding densities in the rat brain: focus on the social decision-making network. Brain Struct Funct 222:981–1006.
Smith CJW, Wilkins KB, Mogavero JN, Veenema AH (2015) Social Novelty Investigation in the Juvenile Rat: Modulation by the μ-Opioid System. J Neuroendocrinol 27:752–764.
Smith DM, Torregrossa MM (2021) Valence encoding in the amygdala influences motivated behavior. Behavioural Brain Research 411:113370.
Smith R, Lane RD, Alkozei A, Bao J, Smith C, Sanova A, Nettles M, Killgore WDS (2018) The role of medial prefrontal cortex in the working memory maintenance

of one's own emotional responses. Sci Rep 8:3460.
Song Z, Swarna S, Manns JR (2021) Prioritization of social information by the basolateral amygdala in rats. Neurobiology of Learning and Memory:107489.
Spunt RP, Adolphs R (2019) The neuroscience of understanding the emotions of others. Neuroscience Letters 693:44–48.
Stachniak TJ, Ghosh A, Sternson SM (2014) Chemogenetic Synaptic Silencing of Neural Circuits Localizes a Hypothalamus→Midbrain Pathway for Feeding Behavior. Neuron 82:797–808.
Staub E (1974) Helping a Distressed Person: Social, Personality, and Stimulus Determinants. In: Advances in Experimental Social Psychology (Berkowitz L, ed), pp 293–341. Academic Press. Available at: https://www.sciencedirect.com/science/article/pii/S0065260108600404 [Accessed January 16, 2023].
Stein MB, Simmons AN, Feinstein JS, Paulus MP (2007) Increased Amygdala and Insula Activation During Emotion Processing in Anxiety-Prone Subjects. AJP 164:318–327.
Sterley T-L, Baimoukhametova D, Füzesi T, Zurek AA, Daviu N, Rasiah NP, Rosenegger D, Bains JS (2018) Social transmission and buffering of synaptic changes after stress. Nat Neurosci 21:393–403.
Sterley T-L, Bains JS (2021) Social communication of affective states. Current Opinion in Neurobiology 68:44–51.
Stoesz BM, Hare JF, Snow WM (2013) Neurophysiological mechanisms underlying affiliative social behavior: Insights from comparative research. Neuroscience & Biobehavioral Reviews 37:123–132.
Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, Zhang F, Deisseroth K, Bonci A (2011) Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature 475:377–380.
Sun Y, Gooch H, Sah P (2020) Fear conditioning and the basolateral amygdala. F1000Res 9:F1000 Faculty Rev-53.
Swain JE (2011) The human parental brain: In vivo neuroimaging. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35:1242–1254.
Swanson LW, Petrovich GD (1998) What is the amygdala? Trends in Neurosciences 21:323–331.
Tan Y, Singhal SM, Harden SW, Cahill KM, Nguyen D-TM, Colon-Perez LM, Sahagian TJ, Thinschmidt JS, Kloet AD de, Febo M, Frazier CJ, Krause EG (2019) Oxytocin Receptors Are Expressed by Glutamatergic Prefrontal Cortical Neurons That Selectively Modulate Social Recognition. J Neurosci 39:3249–3263.
Tassy S, Oullier O, Duclos Y, Coulon O, Mancini J, Deruelle C, Attarian S, Felician O, Wicker B (2012) Disrupting the right prefrontal cortex alters moral judgement. Social Cognitive and Affective Neuroscience 7:282–288.
Tavares V, Fernandes LA, Antunes M, Ferreira H, Prata D (2022) Sex Differences in Functional Connectivity Between Resting State Brain Networks in Autism Spectrum Disorder. J Autism Dev Disord 52:3088–3101.
Thioux M, Keysers C (2010) Empathy: shared circuits and their dysfunctions. Dialogues Clin Neurosci 12:546–552.
Thoma P, Zalewski I, von Reventlow HG, Norra C, Juckel G, Daum I (2011) Cognitive and affective empathy in depression linked to executive control. Psychiatry Res

189:373–378.
Tiedemann LJ, Alink A, Beck J, Büchel C, Brassen S (2020) Valence Encoding Signals in the Human Amygdala and the Willingness to Eat. J Neurosci 40:5264–5272.
Tomek SE, Stegmann GM, Leyrer-Jackson JM, Piña J, Olive MF (2020) Restoration of prosocial behavior in rats after heroin self-administration via chemogenetic activation of the anterior insular cortex. Social Neuroscience 15:408–419.
Toth I, Neumann ID (2013) Animal models of social avoidance and social fear. Cell Tissue Res 354:107–118.
Toyoshima M, Mitsui K, Yamada K (2021) Prior stress experience modulates social preference for stressed conspecifics in male rats. Neurosci Lett 765:136253.
Toyoshima M, Okuda E, Hasegawa N, Kaseda K, Yamada K (2022) Socially Transferred Stress Experience Modulates Social Affective Behaviors in Rats. Neuroscience 502:68–76.
Tremblay S, Sharika KM, Platt ML (2017) Social Decision-Making and the Brain: A Comparative Perspective. Trends Cogn Sci 21:265–276.
Trimmer E, McDonald S, Rushby JA (2017) Not knowing what I feel: Emotional empathy in autism spectrum disorders. Autism 21:450–457.
Tsuda MC, Mahdi S, Namchuk A, Wu TJ, Lucki I (2020) Vendor differences in anxiety-like behaviors in female and male Sprague Dawley rats. Physiology & Behavior 227:113131.
Tye KM, Prakash R, Kim S-Y, Fenno LE, Grosenick L, Zarabi H, Thompson KR, Gradinaru V, Ramakrishnan C, Deisseroth K (2011) Amygdala circuitry mediating reversible and bidirectional control of anxiety. Nature 471:358–362.
Uddin LQ (2015) Salience processing and insular cortical function and dysfunction. Nat Rev Neurosci 16:55–61.
Uylings HBM, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behavioural Brain Research 146:3–17.
Vaccaro AG, Heydari P, Christov-Moore L, Damasio A, Kaplan JT (2022) Perspective-taking is associated with increased discriminability of affective states in the ventromedial prefrontal cortex. Social Cognitive and Affective Neuroscience 17:1082–1090.
Valenta JG, Rigby MK (1968) Discrimination of the Odor of Stressed Rats. Science 161:599–601.
Vasconcelos M, Stein DJ, Gallas-Lopes M, Landau L, de Almeida RMM (2020) Corticotropin-releasing factor receptor signaling and modulation: implications for stress response and resilience. Trends Psychiatry Psychother 42:195–206.
Venniro M, Caprioli D, Zhang M, Whitaker LR, Zhang S, Warren BL, Cifani C, Marchant NJ, Yizhar O, Bossert JM, Chiamulera C, Morales M, Shaham Y (2017) The Anterior Insular Cortex→Central Amygdala Glutamatergic Pathway Is Critical to Relapse after Contingency Management. Neuron 96:414-427.e8.
Vereczki VK, Müller K, Krizsán É, Máté Z, Fekete Z, Rovira-Esteban L, Veres JM, Erdélyi F, Hájos N (2021) Total Number and Ratio of GABAergic Neuron Types in the Mouse Lateral and Basal Amygdala. J Neurosci 41:4575–4595.
Vertes RP (2004) Differential projections of the infralimbic and prelimbic cortex in the rat. Synapse 51:32–58.
Vestergaard M, Carta M, Güney G, Poulet JFA (2023) The cellular coding of temperature in the mammalian cortex. Nature:1–7.
Volkmar FR (2011) Understanding the social brain in autism. Developmental

Psychobiology 53:428–434.
von dem Hagen EAH, Stoyanova RS, Baron-Cohen S, Calder AJ (2013) Reduced functional connectivity within and between 'social' resting state networks in autism spectrum conditions. Soc Cogn Affect Neurosci 8:694–701.
Wager TD, Barrett LF (2017) From affect to control: Functional specialization of the insula in motivation and regulation. :102368 Available at: https://www.biorxiv.org/content/10.1101/102368v1 [Accessed January 10, 2024].
Walker SC, McGlone FP (2013) The social brain: Neurobiological basis of affiliative behaviours and psychological well-being. Neuropeptides 47:379–393.
Wang Z, Yueh H, Chau M, Veenstra-VanderWeele J, O'Reilly KC (2023) Circuits underlying social function and dysfunction. Autism Research 16:1268–1288.
Warden MR, Selimbeyoglu A, Mirzabekov JJ, Lo M, Thompson KR, Kim S-Y, Adhikari A, Tye KM, Frank LM, Deisseroth K (2012) A prefrontal cortex–brainstem neuronal projection that controls response to behavioural challenge. Nature 492:428–432.
Wassum KM, Izquierdo A (2015) The basolateral amygdala in reward learning and addiction. Neurosci Biobehav Rev 57:271–283.
Wei D, Talwar V, Lin D (2021) Neural circuits of social behaviors: Innate yet flexible. Neuron 109:1600–1620.
Weinberg J, Krahn EA, Levine S (1978) Differential effects of handling on exploration in male and female rats. Developmental Psychobiology 11:251–259.
Weisz E, Zaki J (2018) Motivated empathy: a social neuroscience perspective. Current Opinion in Psychology 24:67–71.
Wellman LL, Forcelli PA, Aguilar BL, Malkova L (2016) Bidirectional Control of Social Behavior by Activity within Basolateral and Central Amygdala of Primates. J Neurosci 36:8746–8756.
Wickens JR, Budd CS, Hyland BI, Arbuthnott GW (2007) Striatal Contributions to Reward and Decision Making. Annals of the New York Academy of Sciences 1104:192–212.
Wicker B, Keysers C, Plailly J, Royet J-P, Gallese V, Rizzolatti G (2003) Both of Us Disgusted in My Insula: The Common Neural Basis of Seeing and Feeling Disgust. Neuron 40:655–664.
Williams JM, Mohler EG, Givens B (1999) The role of the medial prefrontal cortex in attention: Altering predictability of task difficulty. Psychobiology 27:462–469.
Winston JS, Gottfried JA, Kilner JM, Dolan RJ (2005) Integrated Neural Representations of Odor Intensity and Affective Valence in Human Amygdala. J Neurosci 25:8903–8907.
Wise RA (2002) Brain Reward Circuitry: Insights from Unsensed Incentives. Neuron 36:229–240.
Wolf RC, Philippi CL, Motzkin JC, Baskaya MK, Koenigs M (2014) Ventromedial prefrontal cortex mediates visual attention during facial emotion recognition. Brain 137:1772–1780.
Woon EP, Sequeira MK, Barbee BR, Gourley SL (2020) Involvement of the rodent prelimbic and medial orbitofrontal cortices in goal-directed action: A brief review. Journal of Neuroscience Research 98:1020–1030.
Wright CI, Groenewegen HJ (1996) Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. Neuroscience 73:359–373.
Wright KM, McDannald MA (2019) Ventrolateral periaqueductal gray neurons prioritize threat probability over fear output Schoenbaum G, Colgin L, Ikemoto S, Bradfield L, eds. eLife 8:e45013.

Wrighten SA, Hall CR (2016) Support for Altruistic Behavior in Rats. Open Journal of Social Sciences 4:93–102.
Xi C, Zhu Y, Niu C, Zhu C, Lee TMC, Tian Y, Wang K (2011) Contributions of subregions of the prefrontal cortex to the theory of mind and decision making. Behavioural Brain Research 221:587–593.
Xing B, Mack NR, Guo K-M, Zhang Y-X, Ramirez B, Yang S-S, Lin L, Wang DV, Li Y-C, Gao W-J (2021) A Subpopulation of Prefrontal Cortical Neurons Is Required for Social Memory. Biological Psychiatry 89:521–531.
Xu H, Liu L, Tian Y, Wang J, Li J, Zheng J, Zhao H, He M, Xu T-L, Duan S, Xu H (2019) A Disinhibitory Microcircuit Mediates Conditioned Social Fear in the Prefrontal Cortex. Neuron 102:668-682.e5.
Yang S-S, Mack NR, Shu Y, Gao W-J (2021) Prefrontal GABAergic Interneurons Gate Long-Range Afferents to Regulate Prefrontal Cortex-Associated Complex Behaviors. Frontiers in Neural Circuits 15 Available at: https://www.frontiersin.org/articles/10.3389/fncir.2021.716408 [Accessed January 29, 2024].
Yang S-T, Shi Y, Wang Q, Peng J-Y, Li B-M (2014) Neuronal representation of working memory in the medial prefrontal cortex of rats. Mol Brain 7:61.
Yang Y, Mailman RB (2018) Strategic neuronal encoding in medial prefrontal cortex of spatial working memory in the T-maze. Behavioural Brain Research 343:50–60.
Yashima J, Sakamoto T (2024) Oxytocin receptors in the prefrontal cortex play important roles in short-term social recognition in mice. Behavioural Brain Research 456:114706.
Yashima J, Uekita T, Sakamoto T (2023) The prelimbic cortex but not the anterior cingulate cortex plays an important role in social recognition and social investigation in mice. PLOS ONE 18:e0284666.
Yi L, Sun H, Ge C, Chen Y, Peng H, Jiang Y, Wu P, Tang Y, Meng Q, Xu S (2014) Role of insular cortex in visceral hypersensitivity model in rats subjected to chronic stress. Psychiatry Research 220:1138–1143.
Yiannakas A, Rosenblum K (2017) The Insula and Taste Learning. Frontiers in Molecular Neuroscience 10 Available at: https://www.frontiersin.org/articles/10.3389/fnmol.2017.00335 [Accessed January 17, 2024].
Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K (2011) Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171–178.
Young L, Bechara A, Tranel D, Damasio H, Hauser M, Damasio A (2010) Damage to Ventromedial Prefrontal Cortex Impairs Judgment of Harmful Intent. Neuron 65:845–851.
Young L, Koenigs M (2007) Investigating emotion in moral cognition: a review of evidence from functional neuroimaging and neuropsychology. British Medical Bulletin 84:69–79.
Zaki J, Davis JI, Ochsner KN (2012) Overlapping activity in anterior insula during interoception and emotional experience. NeuroImage 62:493–499.
Zalla T, Sperduti M (2013) The amygdala and the relevance detection theory of autism: an evolutionary perspective. Front Hum Neurosci 7 Available at: https://www.frontiersin.org/articles/10.3389/fnhum.2013.00894/full [Accessed November 19, 2020].

Zaniboni CR, Pelarin V, Baptista-de-Souza D, Canto-de-Souza A (2018) Empathy for Pain: Insula Inactivation and Systemic Treatment With Midazolam Reverses the Hyperalgesia Induced by Cohabitation With a Pair in Chronic Pain Condition. Front Behav Neurosci 12 Available at: https://www.frontiersin.org/articles/10.3389/fnbeh.2018.00278/full [Accessed May 11, 2021].

Zhang C, Zhu H, Ni Z, Xin Q, Zhou T, Wu R, Gao G, Gao Z, Ma H, Li H, He M, Zhang J, Cheng H, Hu H (2022a) Dynamics of a disinhibitory prefrontal microcircuit in controlling social competition. Neuron 110:516-531.e6.

Zhang M-M et al. (2022b) Glutamatergic synapses from the insular cortex to the basolateral amygdala encode observational pain. Neuron 110:1993-2008.e6.

Zhang X, Guan W, Yang T, Furlan A, Xiao X, Yu K, An X, Galbavy W, Ramakrishnan C, Deisseroth K, Ritola K, Hantman A, He M, Josh Huang Z, Li B (2021) Genetically identified amygdala–striatal circuits for valence-specific behaviors. Nat Neurosci 24:1586–1600.

Zhang X, Li B (2018) Population coding of valence in the basolateral amygdala. Nat Commun 9:5195.

Zhang Y, Zhou W, Wang S, Zhou Q, Wang H, Zhang B, Huang J, Hong B, Wang X (2019) The Roles of Subdivisions of Human Insula in Emotion Perception and Auditory Processing. Cerebral Cortex 29:517–528.

Zhao Z, Zeng F, Wang H, Wu R, Chen L, Wu Y, Li S, Shao J, Wang Y, Wu J, Feng Z, Gao W, Hu Y, Wang A, Cheng H, Zhang J, Chen L, Wu H (2022) Encoding of social novelty by sparse GABAergic neural ensembles in the prelimbic cortex. Science Advances 8:eabo4884.

Zhou Y, Fan L, Qiu C, Jiang T (2015) Prefrontal cortex and the dysconnectivity hypothesis of schizophrenia. Neurosci Bull 31:207–219.

Zingg B, Chou X, Zhang Z, Mesik L, Liang F, Tao HW, Zhang LI (2017) AAV-Mediated Anterograde Transsynaptic Tagging: Mapping Corticocollicular Input-Defined Neural Pathways for Defense Behaviors. Neuron 93:33–47.

Zingg B, Hintiryan H, Gou L, Song MY, Bay M, Bienkowski MS, Foster NN, Yamashita S, Bowman I, Toga AW, Dong H-W (2014) Neural Networks of the Mouse Neocortex. Cell 156:1096–1111.

Zlotnick C, Zimmerman M, Wolfsdorf BA, Mattia JI (2001) Gender Differences in Patients With Posttraumatic Stress Disorder in a General Psychiatric Practice. AJP 158:1923–1925.