The Role of the Insular Cortex in Rodent Social Affective Behavior:

Author: Morgan M. Rogers-Carter

THE ROLE OF THE INSULAR CORTEX IN RODENT SOCIAL AFFECTIVE BEHAVIOR

MORGAN M. ROGERS-CARTER

A dissertation

submitted to the Faculty of

the department of Psychology

in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

Boston College
Morrissey College of Arts and Sciences
Graduate School

March 2019

Title: The Role of the Insular Cortex in Rodent Social Affective Behavior

By: Morgan M. Rogers-Carter

Advisor: John P. Christianson, Ph.D.

Abstract: In social species, animals must detect, evaluate and respond to the states of other individuals in their group. A constellation of gestures, vocalizations, and chemosignals enable animals to convey affect and arousal to others in nuanced, multisensory ways. Observers integrate such social information with environmental cues and internal physiology to general social behavioral responses via a process called social decision-making. The mechanisms and anatomical correlates of social decision-making, particularly those that allow behavioral responses to others' emotional states, are not fully known. Therefore, the objective of this dissertation is to broaden the anatomical understanding of social decision-making by investigating the role of the insular cortex in social behaviors that depend upon others' emotional state. Using a novel behavioral paradigm, I present causal evidence that implicates the insular cortex and its projections to the nucleus accumbens in social affective behavior. These findings are consistent with evidence from the literature that suggests insular cortex is positioned to convey sensory cues to social brain structures to produce flexible and appropriate behavioral responses to social affective cues.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AH	Anterior Hypothalamus
BLA	Basolateral Amygdala
BNST	Bed Nucleus of the Stria Terminalis
CNO	Clozapine-N-Oxide
CTb	Cholera Toxin B
fEPSP	Field Excitatory Post-Synaptic Potential
Hipp	Hippocampus
IC	Insular Cortex
LGN	Lateral Geniculate Nucleus
LS	Lateral Septum
MeA	Medial Amygdala
MGN	Medial Geniculate Nucleus
mPFC	Medial Prefrontal Cortex
mPOA	Medial Preoptic Area
NAc	Nucleus Accumbens Core
OFC	Orbitofrontal Cortex
OT	Oxytocin
OTRa	Oxytocin Receptor Antagonist
PAG	Periaqueductal Grey
pIC	Posterior Insular Cortex
PKC	Protein Kinase C
PN	Post-natal

PVN	Paraventricular nucleus of the hypothalamus
SAP	Social Affective Preference
SBN	Social Brain Network
SDMN	Social Decision-Making Network
TTX	Tetrodotoxin
VMH	Ventromedial Hypothalamus
VP	Ventral Pallidum
VPL	Ventroposterior Lateral Nucleus of the Thalamus
VPM	Ventropostrior Medial Nucleus of the Thalamus
VTA	Ventral Tegmental Area

ACKNOWLEDGEMENTS

I would like to first thank those in my personal life who have provided endless support. Mom and Dad – it is difficult to articulate everything you have done for me, but I am most thankful you taught me to forge my own path. You told me to seize every opportunity that presents itself, to stand up for myself, and most importantly, to work hard. I wouldn't be where I am today without you, and your love and support means the world to me. To my husband, Matt – I have a lot of support and patience to thank you for, and I am so grateful for your light-hearted approach to life. I have fun with you every single day and you have made a grueling program some of the best years of my life (so far). This is just one phase of a long and exiting journey and I look forward to every single step of it. To the rest of my family and friends – thank you for your support, and I am truly so fortunate to have you in my life.

I would also like to thank my advisor, Dr. John Christianson. You have dedicated an exorbitant amount of time to my personal and professional growth and I am forever grateful for your genuine devotion to mentorship. Thank you for your inspiration, patience, and great scientific conversations over the years. This relationship is one I will value forever and I thank you for being a lifelong resource, mentor and friend. I am so proud of the line of work we established, and I cannot wait to hear where it goes next.

To my dissertation committee members, Drs. Michael McDannald, Liane Young, & Marcy Kingsbury. Your feedback has been seminal to my growth as a student, writer, and scientist. To listen and converse with excellent scientists has been the greatest source of growth throughout my graduate career, and I thank you for doing so with me.

Lastly, thank you to current and past Christianson lab members. Allie, Nick, Mary, Anthony and Juan, together we were able to accomplish great work and had tons of fun along the way. Just remember, when the going gets tough, #NFGs. To my excellent undergraduates, Bates, Christina, Morgan, Meghan, Amelia, and Josh, without whom this work would not be possible, thank you. You truly inspire me to be the best mentor I can be.

Chapter 1: Introduction to the insular cortex and social decision-making

Portions of this chapter are currently under review:

Rogers-Carter, M.M., Christianson, J.P. (2019). An insular view of the social decision-making network in rodents. Under Review

1.1. Social Decision Making

It is nearly impossible to overstate the importance of sociality. Social behaviors are necessary for survival and reproductive success across many species (Wilson, 1992) and the ability to make flexible social decisions underlies sophisticated social behavior in humans (Tremblay et al., 2017) and non-human primates (Sueur & Pelé, 2016). Emotion is tightly interwoven with behavior and sharing one's emotional state can evoke adaptive behavioral responses from other group members. This capacity is observed in fundamental social actions across species, including parenting, mate selection and aggression (Ranote et al., 2004; Rendon et al., 2015), as well as more complex and multidimensional facets of social interactions like empathy, perspective-taking and compassion (Marsh, 2018; Shamay-Tsoory, 2011). In humans, impaired emotion recognition and the subsequent inability to generate appropriate social responses may underlie the aberrant social function characteristic of psychiatric disorders like autism and schizophrenia (Edwards et al., 2002; Lozier et al., 2014). Thus, the work presented in this dissertation was inspired by the need to identify the neural mechanisms by which social animals detect and respond to socioemotional cues in order to understand both healthy and aberrant social function.

Emotion, of course, is a complex and multi-faceted construct with varying definitions and applications (Cabanac, 2002). While it is difficult to come to a consensus about the exact definition of emotion, there are several agreed-upon foundational ideas about emotion and its purpose, which provide the conceptual framework underlying the experiments to be discussed in this dissertation. One element of emotion is "core affect" which varies from moment to moment and is a reflection of an individual's arousal level

and hedonic state (Russell, 2003). An individual's core affect is shaped by external stimuli and fluctuations in internal state, and falls along two dimensions than span from sedating to arousing, and from aversive to pleasing. Emotional words, such as happiness, reflect a core affect with modest arousal and pleasure, whereas fear describes a core affect that is aroused and aversive. To outwardly communicate these internal states likely evolved for myriad social functions that promote individual or group survival. Here, I use the term "social affect" to describe the expression of one's core affect, which may be appraised by observers. The behavioral responses to appraising another's' affect state are considered "social affective behaviors," and, despite their importance in social species, the underlying mechanisms remain elusive.

Many species rely on expressions like vocalizations, touch, chemosignals, olfactory cues, and behavioral gestures to communicate changes in their core affect to other members of the group (Darwin, 1976; Insel, 2010). For example, an infant's cry prompts maternal attention, and a distressed call may elicit help from others in both humans (Marsh, 2018) and rodents (Brudzynski, 2013; Ihnat et al., 1995). Responses to social affective cues require more than simple reflexes and are generated through a processed called social decision-making, which utilizes sensory and perceptive systems in the brain to appraise and integrate social information with situational factors, past experiences and internal physiology to shape specific, context-appropriate social behaviors. Further, these factors help determine the reward value of a social behavior (Gil et al., 2013; Vanderschuren et al., 2016; Weiss et al., 2015) which is calculated to inform one's decision to execute a certain behavior (Caldú & Dreher, 2007). It is unknown whether social decision-making is truly a conscious evaluation of how one

should respond to a given situation with regard to the aforementioned factors, or rather is merely the computational product of integrating such factors. However, in either instance, social decision-making enables animals to react within social settings with flexible and adaptive behaviors (O'Connell & Hofmann, 2011).

An obstacle to understanding the anatomical correlates underlying social decision-making is its complexity; several parallel neural processes must coordinate across a network of brain regions to produce an observable social behavior. These include salience detection and sensory integration, which function to encode the external environment, memory processes that determine context and the identity of another individual, and motivation, reward, and ultimately, motor systems which enable animals to generate social responses to social affective cues (Insel & Fernald, 2004a; O'Connell & Hofmann, 2011). While these processes are not specific to social function, they are necessary to generate flexible, nuanced and context-appropriate social actions. Thus, to construct a complete neurobiological account of social cognition we must ask: how do processes like salience detection and sensory integration interact with the processes that generate social behaviors?

Rodents are a suitable non-human model in which the anatomical correlates of social decision-making can be studied because neural circuits are readily tractable. Importantly, a number of studies identify that social signals emitted by one animal, referred to as the "demonstrator," can elicit behavioral changes in an "observer," which show rodents do in fact detect and response to social affective cues. For example, rats will more readily press a lever to release a squealing rat than a toy block from a harness (Rice & Gainer, 1962) and to spare a conspecific from a painful shock (Green, 1969).

More recent findings report that rodents transfer negative affective states, including fear, pain and stress, which elicit social responses. Observer rats (Knapska et al., 2006), mice (Meyza et al., 2015), and voles (Burkett et al., 2016) spend more time exploring a cagemate who underwent tone-shock conditioning, and observers display increased exploration of the demonstrator (Knapska et al., 2010; Mikosz et al., 2015). Further, observer rats who witness or interact with footshocked or fear conditioned demonstrators show observational fear learning, increased sensitivity to pain, and a greater propensity to avoid of noxious stimuli (Jeon et al., 2010; Kim et al., 2010). These phenomena highlight the importance of social affect in prosocial behaviors, which are comprehensively reviewed by (Meyza et al., 2017).

Importantly, social behaviors that result from detecting a stressed demonstrator are influenced by a range of situational factors including social rank, age, sex, familiarity, and previous stress experience, several of which will be discussed in the upcoming sections. Regarding age, I will report on a set of experiments in which adult rats will show approach toward a stressed juvenile, but avoidance of a stressed adult in Chapter 2 and 3(Rogers-Carter et al., 2018b). Familiarity is an important determinant of social actions in rodent paradigms: familiarity is either necessary for, or augments, vicarious fear (Jones et al., 2014), pain empathy (Langford et al., 2006; Li et al., 2014), social approach (Rogers-Carter et al., 2018a) and consoling behavior (Burkett et al., 2016). Similarly, rats will work to press a lever to release a restrained conspecific of the same, but not different, strain (Ben-Ami Bartal et al., 2014) but see (Silberberg et al., 2014). In Chapter 2 I will discuss how familiarity between conspecifics alters approach behavior between adult rats.

These findings indicate that social responses to distressed conspecifics are sensitive to a diverse range of factors. Exactly how this occurs, however, is not completely understood. The next sections will review where external factors, social cues, and physiological state converge in the brain to shape behavioral responses to social affective cues. First, I present the existing anatomical models of social behaviors (Insel & Fernald, 2004a; Newman, 1999; O'Connell & Hofmann, 2011). Next, I discuss the mechanisms by which social affective cues are detected and processed in the brain, while considering the flow of sensory information to the social decision-making network. Lastly, I synthesize evidence from anatomical, functional and behavioral studies and describe the insular cortex (IC) as a site where social sensory stimuli, internal physiological state, and reward value converge. These features suggest IC is equipped to contribute to some of the processes currently unaccounted for in the existing anatomical models of social decision-making.

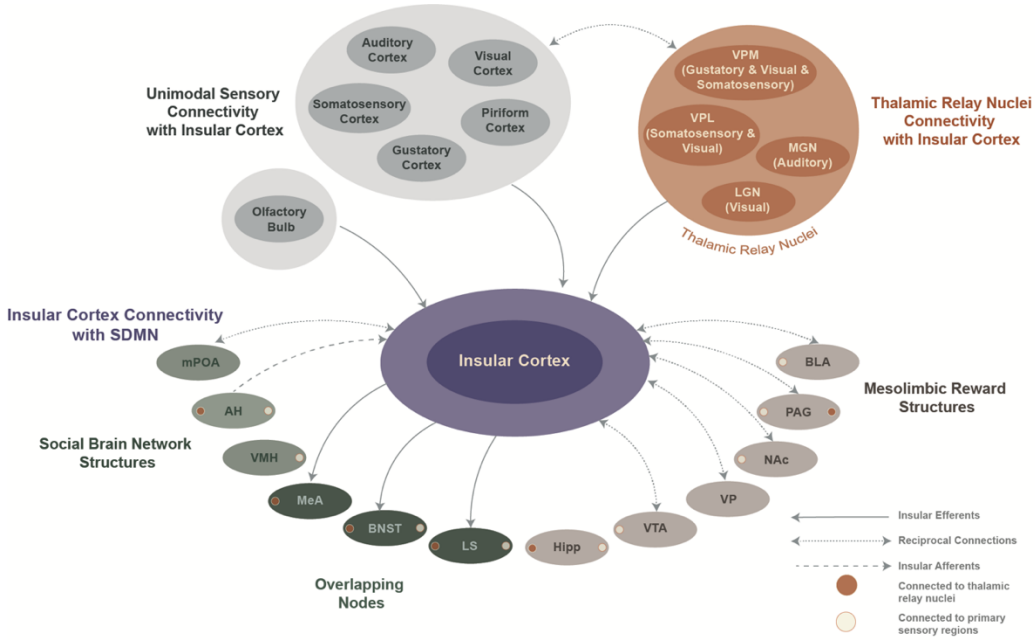


Figure 1.1. Schematic of insula connectivity with unimodal thalamic relay nuclei, primary sensory areas, and the social decision-making network. Thalamic nuclei encode and relay sensory information that conveys affect from the external environment to primary sensory areas where sensory information is decoded and assigned meaning. This information is also relayed via parallel projections to the insular cortex, where social cues are contextualized with internal physiology and other factors to produce unique output patterns in insula efferents that influence SDMN network activity and generate flexible behaviors. Few connections exist between thalamic nuclei and SDMN nodes, except for the following: medial geniculate nucleus projects to AH, MeA and PAG; ventroposterior medial nucleus projections to BNST and LS; lateral geniculate nucleus projects to Hipp (orange circles) and, likewise, unimodal cortical connectivity with SDMN is sparse (beige circles; see section 1.3 for anatomical connections) so sensory information must reach the SDMN via other projections. Insular cortex is well-connected with thalamic relay nuclei, primary sensory areas, and most SDMN structures, suggesting IC is positioned to integrate sensory cues to influence SDMN activity and, therefore, contribute to the integration of social affective information with social behaviors. Abbreviations: **MeA**: Medial amygdala; **AH**: Anterior hypothalamus; **VMH**: Ventromedial hypothalamus; **mPOA**: Medial preoptic area; **BNST**: Bed nucleus stria terminalis; **LS**: Lateral septum; **Hipp**: Hippocampus; **VTA**: Ventral tegmental area; **VP**: Ventral pallidum; **NAc**: Nucleus accumbens core; **PAG**: Periaqueductal grey; **BLA**: Basolateral amygdala; **LGN**: Lateral geniculate nucleus; **MGN**: Medial geniculate nucleus; **VPL**: ventroposterior lateral nucleus of the thalamus; **VPM**: ventroposterior medial nucleus of the thalamus.

1.2. Anatomical Substrates of Social Decision-Making

1.2.1 The Social Brain Network

That humans and mammals can detect and react to social affective cues is a product of neurohormonal circuity that initially evolved in mammals to execute parenting behavior, but has since expanded to support prosocial responses to others via social hormones and reward circuitry (Marsh, 2018). A network model of brain structures that can execute all social behaviors in rodents was first introduced in the Social Brain Network (SBN; Newman, 1999). This system includes the LS, mPOA, AH, VMH, PAG, VTA, MeA and BNST (See Fig. 1.1 caption for list of abbreviations). These structures were included in the SBN because they are all reciprocally connected, contain receptors for gonadal hormones and implicated in at least one mammalian social behavior.

Newman, (1999) posited that specific social behaviors arise from unique patterns of network activity. Accordingly, recent reports support this notion because cell type- and tract-specific optogenetic manipulations clearly indicate that different nodes of the SBN underlie specific behaviors, and together give rise to a broad repertoire of social actions. For example, in the MeA, GABAergic neurons can produce aggressive, grooming, or mounting behavior depending on the strength of optogenetic excitation (Hong et al., 2014). This findings may support the idea that specific features of a social stimulus differently drive MeA activity, and thus beavhior. Consistently, the strenght of MeA neural activation during a social interaction varied with conspecific sex. (Yao et al., 2017). A similar phemenon is observed in the VMH where estrogen receptor 1 neurons promote mounting and sniffing behavior at low stimulation strength, but attack behavior at higher levels of stimulation (Lee et al., 2014), and in the BLA where inputs to mPFC

have bidirectional control to increase or decrease social investigation (Felix-Ortiz et al., 2016). Moreover, the social cues that drive activity pattners in the SDMN may also induce long-last changes in neurons; in the SBN social cues may induce long-lasting effects on neural activity in the SBN as progesterone-expressing neurons in the VMH mediate aggression in single-housed, but not socially-housed male mice (Yang et al., 2017b). That optogenetic manipulations were able to 1) modulate specific aspects of social behaviors and 2) generate different behaviors depending upon stimulation protocols reinforces the SBN view that social behaviors are the product of coordinated activity within the network. To summarize, there is evidence that specific behaviors correspond to neural activity in specific SBN nodes, and that the behavior emerges when the network shifts to a weighted activity pattern strongly driven by the corresponding SBN node.

1.2.2 The Social Decision-Making Network

While the SBN captures the essential loci underlying the gamt of social behavioral expressions, this network lacks the systems needed to detect and appraise potentially rewarding aspects of social interactions. Indeed, social interactions are reinforced by the mesolimbic dopamine system (Chelnokova et al., 2014; Dölen et al., 2013; Hong et al., 2014; Ramos et al., 2015; Smith et al., 2018) and O'Connell and Hofmann (2011, 2012) made the keen observation that the key components of the mesolimbic dopamine reward system evolved to express many of the same receptors that were used to define the SBN. The integration of the SBN with the mesolimbic reward system led to the formation of the social decision-making network (SDMN). This

system contains the elements necessary for responding to social cues including salience detection, stimulus evaluation and reward value calculations in order to influence social actions.

The SDMN consists of the NAc, VP, BLA, Hipp, and VTA, with LS, BNST, and MeA (see Fig. 1.1 caption for abbreviations) as overlapping nodes in both reward and SBN circuits, and each are known to execute important roles in motivated behaviors. First, sensory information from the environment is detected as salience and evaluated by the VTA (Bromberg-Martin et al., 2010; Root et al., 2018), and integrated with valence information in the BLA (Beyeler et al., 2018; LeDoux, 2000). This information is contextualized by Hipp, which can translate social information into memory and identify familiar conspecifics (Mizumori, 2013; Okuyama, 2018), and NAc, which reinforces rewarding behaviors (Chen et al., 2015; Higa et al., 2017) and drives social approach or avoidance (Hamel et al., 2017; Hoebel et al., 2007) through connections with limbic and motor structures (Mogenson & Yang, 1991; Smith et al., 2009).

There are several overlapping nodes of the SBN and mesolimbic system, including the LS and BNST, both of which are implicated in goal-directed actions (Faser et al., 1991), as well as sexual behaviors (Gulia et al., 2002; Maejima et al., 2015; Petrulis, 2013) and aggression (Consiglio et al., 2005; Wong et al., 2016). Furthermore, mesolimbic structures, similar to the SBN, express genes associated with dopaminergic signaling, gonadal hormones, and oxytocin and vasopressin, all of which regulate behaviors and decision-making across many species (Creutz & Kritzer, 2004; O'Connell & Hofmann, 2011; Zhang et al., 2008).

The SDMN was articulated from a comparative and evolutionary biological approach that identified homologous systems across a range of telostian, amphibian, reptilian, avian and mammalian species (O'Connell & Hofmann, 2012). This approach necessarily omitted systems that are not found across these species, like the neocortex, and so the SDMN does not account for processes executed by structures unique to "higher order" species. Specifically, the SMDN does not capture how the sensory information that communicates social cues converges with internal physiology and external envionrmentals factors, and how this informtion interacts with the neural systems that are direclty attributed to the control of specific social behaviors. In mammals, this likely involves the cerebral cortex (Adolphs, 2001; Amodio & Frith, 2006; Hiser & Koenigs, 2018) which receives social information from primary sensory relays (i.e. thalamic nuclei) and integrates this information to produce higher-order representations of sensory information through cortico-cortical network interactions. In the next sections, we review evidence from rodent social behaviors in which cortical structures appear to be necessary for many aspects of social decision making.

1.2.3 The Cortex in Social Affective Behaviors

In humans and primates, the cortex is implicated in myriad social processing: distinguishing self from other, deciding one's motivation to engage in a social interaction, and deciphering the intention of another's motives, among other social cognitions (Amodio & Frith, 2006), and evidence from non-human primates implicates specific cortial areas in unique processes. Broadly, the orbitofrontal cortex (OFC) executes self-other distinction and responses to emotional stimuli. OFC neurons

preferentially respond to faces and emotional expressions (Barat et al., 2018), signal social reward value, and track if a reward was received by oneself or another (Azzi et al., 2012). OFC is also implicated in deciphering threatening versus affiliative social cues (Machado & Bachevalier, 2007), and lesions to the OFC reduce freezing to threatening stimuli (Kalin et al., 2007) and produced aberrant social approach and facial expressions in monkeys (Babineau et al., 2011). Lastly, subpopulations of neurons in OFC respond to social cues and inhibit feeding, which demonstrates this region can mediate internally-motivated behaviors in response to external social stimuli (Jennings et al., 2019).

The medial prefrontal cortex (mPFC) is also widely implicated in social cognitive processes in monkeys and humans (Amodio & Frith, 2006), including detection of social status (Mason et al., 2014), perspective taking, action-outcome prediction (Alexander & Brown, 2011) and pair-bonding (Bales et al., 2007). In rodents, mPFC neurons show increased firing during social approach (Lee et al., 2016) and strength of synaptic efficacy positively correlates with social rank in mice (Wang et al., 2011). In voles that interact with a recently stressed cagemate, elevated Fos expression is observed in ACC and ACC activation by oxytocin is necessary for social consoling behavior (Burkett et al., 2016).

There is also evidence of contributions to social behavior in sensory cortex. Piriform cortex, which is involved in olfaction, is necessary for learning both appetitive and aversive social odor information (Choe et al., 2015) and oxytocin in auditory cortex increases the signal-to-noise ratio of pup calls which prompt maternal behaviors (Marlin et al., 2015). Other areas with sensory functions, including anterior insular cortex and

inferior parietal cortex have been shown in humans to correlate with emotion processing (Boucher et al., 2015) and perception of observable social actions like eye gaze (Saitovitch et al., 2012), respectively. These cortical regions enable the appraisal and integration of another's actions or intentions with reward evaluation, perceived effort, and other factors to produce flexible, informed behavioral responses during the social interaction (Lee, 2013). Human neuroimaging studies also identifies the insular cortex as one of the most consistently-activated regions in emotion-related neuroimaging research (Phan et al., 2002). IC activity increases in empathic tasks (Bernhardt & Singer, 2012), emotional awareness (Gu et al., 2013), and deficits are related to impairments in emotion recognition (Green et al., 2016; Yamada et al., 2016). Mechanistic support for these human neuroimaging findings is generally lacking, but the results to be discussed in this thesis compliment these human neuroimaging findings by identifying a role for insular cortex in social affective behavior (Rogers-Carter et al., 2018b).

While a complete account of cortical regulation of social cognition is beyond the scope of chapter, these findings highlight evidence from human, non-human primate, and rodent research that support a role for the cortex in complex social functions. This begets the question, what is the contribution of the cortex to social decision-making across species? In rodent models where circuit-specific tools allow precise investigation of social behaviors, recent findings implicate cortical structures in the neural processes that appear to be related to some of the components underlying sophisticated social cognition that have been elaborated in humans. The goal the next section is to examine

the anatomy of cortical systems as they relate to the SDMN and, when available, review mechanistic studies in cortical regions in social decision making.

1.3 Primary Loci of Sensory Processes Related to Social Affective Information

1.3.1 Unimodal Sensory Processes

1.3.1.1 Odor and Piriform Cortex

Odor communication is vital for rodent social behaviors (Johnston, 2003) and olfactory cues and chemosignals are used to communicate and identify one's species, sex, reproductive state, social status, sickness, and familiarity (Arakawa et al., 2008; Liberles, 2014). Exposure to specific odors can elicit myriad social behaviors in rodents, such as mating (Coria-Avila et al., 2005; Sakuma, 2008), aggression (Stowers et al., 2013), social learning (Choe et al., 2015), maternal behavior (Fleming & Rosenblatt, 1974), and social buffering of fear (Kiyokawa et al., 2012; Takahashi et al., 2013). Odor information is processed in the piriform cortex, which receives primary odor afferents from the olfactory bulb and is modulated by cortical structures including the orbitofrontal and entorhinal cortices (Illig & Wilson, 2009). This anatomical arrangement distinguishes piriform from the other unimodal sensory cortices, which are primarily innervated by thalamic relay nuclei. The piriform cortex can distinguish the identity, concentration and intensity of odorants (Bolding & Franks, 2017) and accordingly, the piriform cortex is implicated in odor-driven social behaviors. Mice who scent-mark in the presence of a conspecific expressed elevated Fos immunoreactivity in the piriform cortex (Borelli et al., 2009), and female mice exposed to juvenile play sessions with a lemon-scented partner later preferred to mate with a lemon-scented male, over an

almond-scented male, and expressed greater piriform cortex Fos immunoreactivity than females who underwent the experiment without odor-pairing (Paredes-Ramos et al., 2014). Piriform cortex also receives oxytocinergic inputs from the paraventricular nucleus (Mitre et al., 2016), which contribute to enhanced odor discrimination and behavioral responses or odors by increasing the signal-to-noise ratio of social olfactory cues and chemosignals (Oettl & Kelsch, 2017). Piriform activation during social tasks likely refects the detection of specifically social doors because piriform can distinguish social from non-social odors; pharmacological and optogenetic inhibition of piriform oxytocin receptors abolished learning of both appetitive and aversive social, but not non-social, stimuli suggesting a role for this region in distinguishing social from non-social odors (Choe et al., 2015).

Piriform cortex has dense reciprocal connectivity with the MeA, BLA, Hipp and the VTA (Aransay et al., 2015; Coolen & Wood, 1998; Cádiz-Moretti et al., 2016; De La Rosa-Prieto et al., 2015; Johnson et al., 2000) but connectivity with other SDMN nodes is sparse as known projections include only the BNST, NAc and Hipp (Brog et al., 1993; Dong & Swanson, 2006; Weller & Smith, 1982), and SDMN inputs to piriform are only found in AH and LS (Roeling et al., 1994; Siegel & Tassoni, 1971). Whether these connections are sufficient to integrate odor-driven activity from piriform cortex to inform social decision-making is currently unknown. However, several studies demonstrate an interplay between olfactory cues and other sensory inputs in regard to mediating behavior (Moyaho et al., 2015; Rossier & Schenk, 2003), suggesting that separate anatomical regions capable of multimodal integration combine odor with other social stimuli and contextual factors to drive social behaviors.

1.3.1.2 Tactile Contact and Somatosensory Cortex

Tactile information is represented in the somatosensory cortex. In rodents, the majority of somatosensory information is detected via the whiskers, which relay physical touch to the ventral posterior medial and posterior medial thalamic nuclei (Erzurumlu & Gaspar, 2012; Viaene et al., 2011). These regions send efferents to barrel cortex, a distinct somatosensory cortex subregion that reflects the somatotopic organization of whisker positioning on the face (Li & Crair, 2011). Barrel cortex neurons respond to tactile behaviors during rodent social interactions, including pinning, anogenital sniffing, allogrooming, and nape-biting. Following rough and tumble play behavior, juvenile rats express elevated Fos immunoreactivity in the barrel cortex (Gordon et al., 2002) and pharmacological blockade of the barrel cortex during social interaction reduced pinning behavior (Charles Lawrence et al., 2008). Rodents also frequently engage in "social face touch," a form of head-to-head contact utilizing the whiskers (Wolfe et al., 2011), and the necessity of both whiskers and barrel cortex are well-documented in this behavior. Whisker activity changes as a function of distance from a conspecific (Wolfe et al., 2011), and during bouts of social face touch, at least 40% of barrel cortex neurons increased firing at a rate significantly higher than that observed during contact with non-social stimuli (Bobrov et al., 2014). Moreover, social isolation, and therefore lack of social contact, results in impaired whisker sensitivity and disrupted AMPA receptor trafficking in the barrel cortex (Miyazaki et al., 2012). Neonatal mice with trimmed whiskers performed poorly on a tactile sensitivity assay and displayed aberrant social behaviors in adulthood (Soumiya et al., 2016). Whisker-driven neural activation in

barrel cortex was disrupted in NMDA receptor knock-out mice and correlated with social behavioral deficits (Arakawa et al., 2014), and de-whiskered pups were less successful at nursing than whisker-intact littermates and were less active during social interactions with siblings (Sullivan et al., 2003). These findings signify that somatosensory cortex contains distinct subregions that are capable of uniquely responding to social stimuli.

The somatosensory cortex does not receive inputs from any SDMN structures. However, it projects to several SDMN structures and therefore provides an anatomical pathway for tactile experience to shape social behaviors. Projections from somatosensory cortex to the VMH may code tactile events during mating (Angoa-Pérez & Kuhn, 2015; Stanzani & Russo, 1980), whereas projections to NAc and VTA (Faget et al., 2016; Lenschow & Brecht, 2018; Tai & Kromer, 2014; Wilson, 2014) may convey information about social contact or position to inform reward value and reinforcement (Báez-Mendoza et al., 2013; Dölen et al., 2013). Sparse somatosensory cortex projections to PAG (Lenschow & Brecht, 2018) may allow social touch to inform adaptive and flexible physical responses in a social interaction (Skuse & Gallagher, 2009). However, none of these tracts have been investigated in rodent social behaviors.

1.3.1.3 Visible Social Cues and Visual Cortex

Rodents display facial and body expressions during social interactions (Brecht & Freiwald, 2012), yet the role of these gestures in coordinating social behavior remains enigmatic. During social interactions and expsoure to aversive stimuli, rats display facial tightening of the eyes, welling of the nose and cheeks, and flattening of the ears (Defensor et al., 2012), and changes in ear posture correspond to changes in general

arousal state (Lecorps & Féron, 2015). When rats are tickled to induce a positive affect, ears become dark pink and flex at a wider angle (Finlayson et al., 2016), and mice display a “grimace” during negative affective states, like pain (Sotocinal et al., 2011), which correlates to a shorter and more curved spinal stance (Mittal et al., 2016). Facial expressions are observed during social interactions as well. Naked mole rats show aggressive mouth gaping and incisor fencing (Lacey et al., 1991) to establish social dominance (Brecht & Freiwald, 2012), and similarly, mice pluck the whiskers of others, rendering them unable to display aggressive or dominant faces (Sarna et al., 2000). Whisker position also signals if an interaction is aggressive or prosocial: during aggressive bouts, whiskers are protracted and whisk with high amplitude (Wolfe et al., 2011). Moreover, facial displays, like earwigging in females, are associated with courting behavior (Vreeburg & Ooms, 1985). Despite a limited understanding of the meaning of rodent visual cues, they appear to be important contributors to social decisions.

To our knowledge there are no neurobiological accounts of visual cortex neural activity during social interaction or exposure to social stimuli, but the importance of social experience on visual cortex development is fairly well-documented. Rats raised in social isolation displayed increased higher-order dendritic branching (Volkmar & Greenough, 1972) and aberrant expression of dendritic spines in visual cortex pyramidal cells (Connor & Diamond, 1982), whereas rearing in an enriched social environment increased synaptic strength in visual cortex (Mainardi et al., 2010). Lastly, socially impaired BTBR mice displayed impaired functional connectivity with visual cortex and other sensory cortical areas (Sforazzini et al., 2016). Visual cortex projects to

the PAG, NAc, and VTA (Dinopoulos & Parnavelas, 1991; Newman et al., 1989; Wilson, 2014) but does not receive inputs from the SDMN. This connectivity may provide the anatomical framework for visual information to inform reward valuation during a social interaction, and also suggests that corticocortical interactions are necessary to further process social visual cues and relay them to SDMN.

1.3.1.4 Vocal Communication and Auditory Cortex

Rodents emit audible and ultrasonic vocalizations to convey negative and positive affective states. The acoustic characteristics, functional purpose and neural correlates of ultrasonic vocalizations are reviewed in (Brudzynski, 2013; Portfors & Perkel, 2014). Ultrasonic vocalizations are observed in prosocial (Burke et al., 2017; Pultorak et al., 2016), reproductive (Matsumoto & Okanoya, 2016; Neunuebel et al., 2015) and territorial behaviors (Hammerschmidt et al., 2012; Pasch et al., 2011) and are encoded by auditory cortex. Auditory cortex neurons are active during playbacks of vocalizations (Carruthers et al., 2013). Further, rats expressed elevated Fos in auditory cortex after exposure to recordings of 22kHz calls (Ouda et al., 2016), and calcium imaging revealed a subset of auditory cortex neurons that respond to the frequency sweep rate of modulating calls (Issa et al., 2017). Ultrasonic vocalizations can modulate social behaviors as well; rats displayed approach behavior when played recordings of 55kHz calls and expressed Fos in the auditory cortex (Sadananda et al., 2008), and auditory neurons in female dams undergo plasticity to improve the signal-to-noise ratio of pup calls (Tasaka et al., 2018), which drive maternal behaviors (Valtcheva & Froemke, 2018).

Auditory cortex projects to the PAG, BLA, Hipp, and VTA, which may allow acoustic information to be incorporated into context and reward valuation (Budinger et al., 2008; Lindvall & Stenevi, 1978; Newman et al., 1989; Romanski & LeDoux, 1993). Further, projections from auditory cortex to LS (Lindvall & Stenevi, 1978) and BNST (Shin et al., 2008) may integrate vocal cues with affective (Singewald et al., 2011) and physiological states (Herman et al., 2003), and vocal inputs may evoke oxytocin release via projections to the PVN during social interaction (Peñagarikano et al., 2015). Interestingly, auditory cortex has reciprocal connectivity with NAc, an important hub for vocal song learning in birds (Prather, 2013), and so this pathway may help encode social sounds for later recognition or performance. Moreover, NAc projections in the visual cortex also project to the lateral amygdala, which may allow for the integration of acoustic inputs with affective state (LeDoux et al., 1991). However, like several other unimodal sensory cortical regions, none of the projections from auditory cortex to SDMN structures have been causally examined in a social behavior.

1.3.1.5 Taste Communication and Gustatory Cortex

Evidence for gustatory communication in social behavior comes from social transmission of food preference. Rats will prefer to consume a certain food if they had recently explored a conspecific who consumed the same food (Galef & Wigmore, 1983; Galef et al., 1985). Similarly, hamsters preferred to consume the diet their mother was fed, but failed to show a preference for a diet fed to a either a littermate or novel conspecific (Lupfer et al., 2003). Lastly, in rats, presentation of an olfactogustatory cue prompted nicotine self-administration during social interaction (Chen et al., 2011). Non-

volatile chemosignals are detected through sniffing and licking (Lehman et al., 1980), and so it's possible that chemosensory information involves gustatory processing to some extent. Regarding the SDMN, gustatory cortex is only connected to this network via inputs from the BLA, which may combine taste with affective state, such as in conditioned taste aversion acquisition (Gallo et al., 1992). However, gustatory cortex is a subregion of insular cortex and anatomical studies of IC may not distinguish gustatory from the broader insular cortex, the connectivity of which with the SDMN will be discussed below.

1.3.2 Multimodal Sensory Processing of Social Affective Cues

Concurrent unimodal sensory information must be integrated to assemble a complete representation of the external environment. To do so requires multisensory convergence and multisensory integration, two processes that are observed in both individual neurons and populations of neurons. Multisensory convergence occurs where inputs from multiple sensory modalities overlap in time, and the resulting neural response is a direct sum of the response that each unimodal input evokes individually. Multisensory integration, however, is identified when the neural response to multisensory inputs is distinctly different (i.e. non-linear) than the sum of the neural responses to converging unimodal signals and, therefore, each individual modality cannot be delineated from the resulting activity (Stein et al., 2014). This binding of information via multisensory integration converts simple sensory representations into higher order abstractions that inform behavioral decisions (Siemann et al., 2014). There are several examples in which concurrent cues take on a unique social meaning (Partan

& Marler, 1999). For example, dogs signal threat to one another via growling, but growling in combination with a bowed stance initiates play behavior (Bekoff, 1972). Animals trained to approach a visual cue do so more readily if the cue is paired with an auditory stimulus, which indicates an effect of multisensory presentation on attention (Stein et al., 1989). Furthermore, rat mating behavior (Beach, 1942) and maternal behaviors (Beach & Jaynes, 1956; Smotherman et al., 1974) are elicited by exposure to combined olfactory, visual, and tactile cues, which do not elicit as robust behavioral responses on their own (but see Stern, 1990).

Only a few studies have examined the neurobiology underlying the integration of multimodal social cues. In the rat auditory cortex, ultrasonic vocalizations emitted during social interactions evoke fast-spiking neural activity that correspond to the start and end of a tactile contact bout. However, this activity is nhibited by social face touch, which suggests that the salience of auditory information can depend on concurrent somatosensory inputs (Rao et al., 2014). Similarly, in lactating female rats, pup odor increases auditory tone-evoked activity of neurons in auditory cortex, which may function to increase maternal attention to pups (Cohen et al., 2011). Consistent with the idea that unimodal sensory cues influence neural responses to stimuli of various modalities, several findings report neural activity that reflects multisensory integration in primary sensory areas (Duhamel, 2002; Kayser & Logothetis, 2007; Ma et al., 2016; Maier et al., 2015; Samuelsen & Fontanini, 2017) which suggests that sensory inputs may already begin to undergo integration in these regions.

Known sites of multisensory integration are also likely involved, and a number of regions including the superior colliculus (Stein et al., 1989) and perirhinal (Furtak et al.,

2007; Otto & Eichenbaum, 1992), retrosplenial (Vann et al., 2009), orbitofrontal (Schoenbaum et al., 2003), medial prefrontal (Martin-Cortecero & Nuñez, 2016) cortices are implicated in both multisensory integration and social behaviors. The insular cortex is one region of interest where multimodal social information may converge to inform social decisions. The next section will discuss how the unique anatomical arrangement and functional role of the insular cortex warrants consideration of this region as a key hub in social decision-making.

1.4 Insular Cortex in Social Decision-Making

1.4.1 The Insular Cortex Integrates External Sensory Stimuli

The IC is a well-documented site of multisensory processing (Gogolla et al., 2014; Rodgers et al., 2008) and is reciprocally connected with thalamocortical structures (Guldin & Markowitsch, 1983), which suggest IC is positioned to integrate sensory information with affective and motivational states (Krushel & van der Kooy, 1988). Unimodal visual, sensory, auditory, gustatory and nociceptive inputs first converge in posterior IC (pIC) by way of dense connections from somatosensory cortices and the supragenicular, posterior intralaminar, peripeduncular nucleus, medial geniculate, and reticular thalamic nuclei, all of which relay sensory information from spinal afferents to the brain (Fig. 1.1; Hanamori et al., 1998; Linke & Schwegler, 2000; Shi & Cassell, 1998). pIC can then integrate unimodal sensory inputs; supralinear responses to multimodal sensory stimuli are observed in pIC (Rodgers et al., 2008). Similarly, single unit recordings of neurons in the insular auditory field receive thalamic afferents and show co-responsivity of pIC neurons to both forepaw stimulation and

white noise bursts (Kimura et al., 2010) and extracellular recordings in pIC reveal overlapping neural responses tail pinch, baroreceptor stimulation, appetitive and aversive tastes, and arterial chemoreceptor stimulation (Hanamori et al., 1998). Human neuroimaging studies indentify IC activity in response to facial expressions (Boucher et al., 2015) and voice recognition (Andics et al., 2010). These properties suggest IC has the capacity to encode both internal and external sensory stimuli of various modalities.

1.4.2 Insular Cortex Integrates External Stimuli with Changes in Physiological State

In addition to integrating external sensory stimuli, IC is thought to be a locus of interoception because inputs from visceral thalamic nuclei allow IC to detect internal physiological changes (Strigo & Craig, 2016). Homeostatic changes profoundly impact brain function, attention, motivation and behavior, and rodent studies of affect contagion have identified several instances where exposure to a recently stressed demonstrator can cause physiological changes in an observer: In mice, 22kHz alarm calls emitted by demonstrators activate the sympathetic nervous system of observers (Chen et al., 2009), and after exposure to biting horse flies, demonstrator mice display conditioned analgesia, which is transferred to observers via NMDA-receptor activity (Kavaliers et al., 2001). Further, both observer and demonstrator voles express elevated plasma corticosterone levels (Burkett et al., 2016), and observer mice display potentiation of corticotropin-release hormone neurons in the paraventricular nucleus of the hypothalamus following footshock (Sterley et al., 2018). This begets the question, do observers have a mechanism to detect these bodily changes in order to generate an appropriate social response? Information about internal state is relayed to the brain via

visceral afferents from spinal and cranial nerves, which allow the brain to appraise the body's physiological state and generate subsequent behavioral responses (Critchley & Harrison, 2013). These signals are detected by a "general visceral IC" (Cechetto & Saper, 1987) which receives inputs from ventroposterior medial and ventroposterior parvocellular thalamic nuclei and responds to diverse visceral input from gastric mechanoreceptors, arterial chemoreceptors, cardiovascular baroreceptors, as well as changes in respiration and vagal nerve activity (Barnabi & Cechetto, 2001). Moreover, IC is known to have top-down control of visceral function via efferent projections to the nucleus of the solitary tract, parabrachial nucleus, and ventrobasal and mediodorsal thalamus (Krushel & van der Kooy, 1988), and stimulation of IC neurons that project to the dorsal vagal complex or lateral hypothalamic area increases blood pressure and airflow (Bagaev & Aleksandrov, 2006), and sympathetic factors like heart rate, renal nerve activation, and blood pressure (Cechetto & Chen, 1990), respectively. These findings position the IC to be involved in either the response to or initiation of behavioral responses to various stressors: IC may detect changes in the internal state caused by exposure to a stressed demonstrator. Alternatively, IC might detect social affective information and initiates physiological changes that mimic stress to prompt a behavioral response.

Regarding social function, visceral vagal inputs are hypothesized to initiate the neurophysiological processes underlying stress and subsequent social behaviors (Porges, 2001) and in IC, exposure to stress increases Fos immunoreactivity (Yokoyama & Sasaki, 1999) and induces hypersensitivity to visceral function (Sun et al., 2016). Further, there may be overlapping neural populations in posterior IC that respond

to, and therefore integrate, both internal and external information (Shinder & Newlands, 2014), which may give rise to the “self-other” distinction, and subsequent empathic abilities attributed to the IC in human neuroimaging experiments (Singer et al., 2009; Uddin et al., 2008). Thus, if exposure to a stressed demonstrator induces physiological changes in an observer, IC may integrate visceral information with limbic and motivational states via efferents to PFC (Fujita et al., 2010), amygdala (Shi & Cassell, 1998), hypothalamus (Reep & Winans, 1982) and NAc (Wright & Groenewegen, 1996), all of which are implicated in various social behaviors (Yizhar, 2012).

1.4.3 The Insular Cortex, Rodent Social Behaviors, and Connectivity with the Social Decision-Making Network

Recent studies implicat IC in rodent social functions. Our lab has reported that oxytocin receptors in IC mediated both approach toward stressed juvenile rats and avoidance of stressed adult rats (Rogers-Carter et al., 2018b), to be discussed in chapter 3. Further, aberrant GABAergic circuit maturation is observed in IC in several strains of autism-model mice with known social impairments (Gogolla et al., 2014) and IC microinfusions of a NMDA receptor antagonist are sufficient to decrease wrestling, social exploration, and ultrasonic vocalizations in male rats exposed to alcohol during development (Bird et al., 2017). Regarding social memory, dopaminergic, adrenergic, and serotonergic receptors in IC are involved in social recognition memory (Cavalcante et al., 2017).

These social roles for IC may result from its extensive circuitry with SDMN structures, a feature that is not observed in other higher-order sensory processing sites in the cortex. IC is reciprocally connected with the VTA, BLA, VP, NAc, MeA and PAG (Cádiz-Moretti et al., 2016; Dong & Swanson, 2006; Faget et al., 2016; Groenewegen et al., 1993; Herrero et al., 1991; Neafsey et al., 1986; Pardo-Bellver et al., 2012; Reep & Winans, 1982; Reynolds & Zahm, 2005; Shi & Cassell, 1998; Wright & Groenewegen, 1996), projects to LS, BNST and mPOA (Dong & Swanson, 2004; Dong & Swanson, 2006; Simerly & Swanson, 1986) and receives afferents from AH (Reep & Winans, 1982). The only SDMN structures with which IC lacks connectivity are Hipp and VMH (Fig. 1.1). These connections position IC to integrate internal and external stimuli sensory information to influence SDMN activity.

1.4.4 Valence Coding in the Insular Cortex

Decisions are influenced by reward value (Ploeger et al., 1991), and IC is implicated in a number of reward-related tasks in rodents. IC neurons respond to cues that predict a water reward, and increase activity after a negative outcome (Jo & Jung, 2016), suggesting IC encodes stimulus value. Consistnely, in a conditioned place preference test, rats favored the maze compartment in which they received electrical stimulation of IC (Hurtado & Puerto, 2018), and unilateral stimulation of pIC was sufficient to induce the conditioned place preference (García et al., 2013). Moreover, reward valuation in the IC may underlie its known role in decision-making. Pattij and colleagues (2014) demonstrated that pharmacological inactivation of IC dopamine receptors impaired performance in a delay-discounting task. In rat gambling tasks,

infusion of methamphetamine to IC caused rats to opt for a high-risk, high-reward option (Mizoguchi et al., 2015). Congruent with this evidence, (Ishii et al., 2012) observed a decrease in risk-taking during a rat gambling task when IC was pharmacologically inactivated. These findings are consistent with human studies in which insular lesions were associated with impaired risky decision-making (Clark et al., 2008).

The foregoing suggests that IC also contributes to value coding in social settings. A major output of the IC is the NAc, with anterior IC efferents terminating in the dorsomedial aspects and NAc core; terminals arising from the mid and caudal IC shift to target the dorsolateral aspects and NAc core (Wright & Groenewegen, 1996). NAc encodes reward value and decision-making, and, as noted, both IC and NAc are implicated in rodent and human social behaviors (Lamm & Singer, 2010; Lichtenberg et al., 2018; Singer et al., 2006; Trezza et al., 2011). In one experiment investigating this tract, silencing anterior IC⇢NAc neurons reduced reward seeking behavior (Jaramillo et al., 2018b). Interestingly, while glutamatergic inputs to the NAc tend to reinforce instrumental behaviors (Britt et al., 2012), the consequence of NAc glutamate is a product of interactions between glutamate and co-transmitters like dopamine and oxytocin (Dölen et al., 2013), and the specific postsynaptic NAc cell types (Tye, 2012). Accordingly, glutamatergic inputs to NAc might contribute to both rewarding and aversively motivated social behaviors.

1.4.5 Insular Cortex in Social Decision Making

In the foregoing sections we present an anatomical foundation for thinking about the contributions of IC to social cognition. Social cognition arises from a coherent

understanding of one's social environment, and to generate context-appropriate behaviors requires multimodal sensory integration and convergence so that affective cues can be bound with relevant external cues and internal physiological signals. The literature suggests the IC is well situated to perform this function as an anatomical waypoint for social information to influence the SDMN. Importantly, IC seems to relate to a range of social behaviors, from approach to avoidance, possibly due to its unique anatomical position to integrate multisensory social and internal information. To account for these phenomena, we present a hypothetical account for how IC contributes to social cognition via interactions with the SDMN. Inputs to the IC from primary sensory regions and BLA convey the valence of social stimuli, which are bound in a multimodal sensory representation by IC. Thus, the IC encodes, or attributes affect to either ones self or another animal, which is critical to social decision-making. This leads to a prediction that removing either the BLA or sensory inputs to the IC would render social behavior insensitive to the affective states of others. Based on our finding that IC activity, in the absence of social affective factors, was sufficient to generate either approach or avoidance behaviors (to be discussed in Chapter 3), we suggest that within IC, ensembles of neurons must contribute in some way to selecting a social behavioral response (i.e. approach or avoid). Finally, IC outputs to SDMN, such as to the NAc, shape the pattern of activity across the network and it is via IC connections with the SDMN that a large range of social processes might be shaped by the affective cues, situational factors and other features present in social encounters.

1.5 Dissertation Aims and Synopsis

The overarching goal of this dissertation is to test if the insular cortex is necessary for social responses to other's affective states, which requires experimental manipulations of the IC that can only be performed in rodent models. Therefore, the first goal of this work was to construct a behavioral paradigm in which rats display social responses to other's affective states. The second goal was to test if these behaviors depend on the insular cortex, which set the stage for the third goal, which was to test if IC projections to the SDMN produce social affective behaviors.

Chapter 2 will present behavioral results from our novel paradigm, the social affective preference (SAP) test (Rogers-Carter et al., 2018a; Rogers-Carter et al., 2018b). In this paradigm, an experimental adult rat is provided two *novel* conspecifics to explore; one conspecific is naïve to any treatment, and the other is stressed immediately prior to testing to induce a negative affect. The experimental rat's preference depends upon both the age and stress state of the conspecific; male and female rats prefer to explore stressed juvenile conspecifics, but avoid stressed adults. Moreover, familiarity is a factor that influences whether an experimental rat approaches or avoids a conspecific; if presented familiar conspecifics, female experimental rats will approach stressed adults whereas male experimental rats still avoid stressed adults. These findings established that behavior in the social affective preference test is sensitive to affect, familiarity and age, which represent a subset of factors that influence social decision-making.

Chapter 3 will build upon the main finding established in chapter 2, that experimental rats approach stressed juveniles but avoid stressed adults, by testing if the

insular cortex is necessary for both approach and avoidance. Pharmacological and optogenetic inhibition of the insular cortex blocked the preference to explore stressed juvenile conspecifics and naïve adult conspecifics, and multielectrode array recordings of acute brain slices of insular cortex revealed that increased excitability may underlie the ability of IC neurons to detect social affective cues. Together these studies demonstrate that IC can initiate both approach and avoidance behaviors depending on affective stimuli, possibly as a result of neuromodulation of IC neurons.

Considering the unique responses to stressed adult versus juvenile conspecifics, I next questioned if the same efferent projections from IC were necessary for both juvenile- and adult-directed SAP behavior. These projections were unknown, but network analyses revealed identified the nucleus accumbens core (NAc) as a region that may have an age-specific role in social affective behavior (Rogers-Carter et al., 2018b). Chapter 4 will review a set of experiments that demonstrate IC projections to NAc are necessary for interactions with juvenile, but not adult conspecifics.

These findings reflect the initial goals of this work; using a novel and valid animal model of social affective behavior, I report that insular cortex is necessary to detect the social affective state of others', and influences the decision to approach or avoid a conspecific via projections to the social decision-making network. These results lend strong support to the hypothesis that IC is an indispensable component of the circuitry underlying rodent social affective behavior.

Chapter 2: Social Affective Behavior in Rat

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

Rogers-Carter, M.M., Varela, J., Gribbons, K.B., Pierce, A., McGoey, M.T., Ritchey, M., and Christianson, J.P. (2018). Insular cortex mediates approach and avoidance responses to social affective stimuli2. *Nature Neuroscience, 21,* 404-414.

Rogers-Carter, MM, Djerdjaj, A., Culp, A., Elbaz, J.A., Christianson, J.P. (2018). Familiarity modulates social approach toward stressed conspecifics in female rats. *PLoS ONE* 13(10): e0200971.doi.org/10.1371/journal.pone.0200971

2.1 Introduction

There is great potential for animal models of social affective behavior to elucidate the mechanisms by which the affect of one animal can influence the social behaviors of another. As discussed in the introduction, sensory and perceptive systems in the SDMN allow one to appraise social affective stimuli and integrate them with past experiences and situational, and somatic factors to shape behavioral responses to socioemotional cues (O'Connell & Hofmann, 2011), and a number of studies in rodents demonstrate observer rodents show prosocial behavioral responses to stressed demonstrators (Meyza et al., 2017). These models capture well aspects of social cognition, including emotion contagion and social buffering (de Waal & Preston, 2017). However, some paradigms require learning or direct exposure to a conspecific in pain, making it difficult to isolate social affective processes from other motives (Burkett et al., 2016; Silberberg et al., 2014). To address this, we developed a rat social affective preference (SAP) test in which social affective behaviors were objectively quantified as a preference to approach or avoid interaction with a conspecific that received a mild stressor. Because the experimental rat in the SAP test was not exposed to, nor witness to, the stressor itself, the unconditioned behavior of the experimental subject toward the conspecifics can be interpreted as a response to the affective state of the target.

In this chapter I present behavioral findings from the SAP test in which conspecific age and affective state mediate approach and avoidance behavior in experimental rats, and discuss the possible role of ultrasonic vocalizations as a modality by which rats communicate affect. Further, I report on a set of experiments that tested if familiarity between conspecifics produces difference patterns of approach and avoidance behavior

in the SAP test than the preference patterns observed with unfamiliar conspecifics. Together these findings demonstrate that the preference to approach or avoid a conspecific in this paradigm depends on affect, age, and familiarity.

2.2 Materials and Methods

2.2.1 Animals

Male and Female Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). For experiments 2.3.1 through 2.3.3, rats were allowed a minimum of 7 days to acclimate to the vivarium after arrival and housed in groups of 2-3 with free access to food and water on a 12 h light/dark cycle. For experiments 2.3.4 and 2.3.5, rats were housed in isosexual groups of 4 with free access to food and water on a 12hr light/dark cycle and experimental rats were randomly assigned to either a "Familiar" or "Unfamiliar" group, which produced 4 experimental conditions: females interacting with familiar conspecifics (Female-Cagemate), females with unfamiliar conspecifics (Female-Unfamiliar), males with familiar conspecifics (Male-Cagemate), and males with unfamiliar conspecifics (Male-Unfamiliar, n = 10 per group). The remaining 32 rats in experiments 2.3.4 and 2.3.5 were used as conspecific targets during social exploration testing. To establish familiarity, rats in the Female-Cagemate and Male-Cagemate conditions were cohoused with isosexual conspecifics as follows: each cage contained 2 experimental rats and 2 conspecifics; 1 of the conspecifics was used as the naïve, and the other as the stressed, conspecific for SAP testing. Thus, each conspecific cagemate pair was used for a total of 2 SAP tests, each of which was conducted with one of their experimental rat cagemates. The rats used for targets in

SAP tests with unfamiliar conspecifics were housed separately in groups of 4. All behavioral experiments were conducted within the first 4 h of the light phase. All procedures were conducted in accordance with the Public Health Service *Guide for the Care and Use of Laboratory Animals* and were approved by the Boston College Institutional Animal Care and Use Committee.

2.2.2 Social Affective Preference Test

The SAP test allowed quantification of social interactions initiated by an adult experimental when presented with two unfamiliar conspecific stimuli simultaneously. To begin the test, the adult test subject was placed into a clear plastic cage (50 x 40 x 20 cm, L x W x H) with a wire lid. Pairs of stimuli rats were either juvenile (PN 30 +/- 2 days old) or adult (PN 50 +/- 2 days old) and were placed inside one of two clear acrylic plastic enclosures (18 x 21 x 10 cm, L x W x H) on either end of the arena. Interaction between the experimental and stimuli rats was permitted on one side of the enclosure, which consisted of clear acrylic rods, spaced 1 cm apart center-to-center (Fig. 2.1 A). To habituate subjects to the procedure, on days 1 and 2 the experimental rat was placed in the arena for 60 min and then empty enclosures (Day 1) or enclosures containing experimentally naïve, unfamiliar stimuli (Day 2) were added to the test arena for 5 min. To assess social affective preference, on Day 3 two unfamiliar conspecifics were added, one of which was exposed to 2 footshocks (1mA, 5 sec duration, 60 sec inter-shock-interval, Precision Regulated Animal Shocker, Coulbourn Instruments, Whitehall, PA) immediately prior to the 5 min test to induce a stressed affective state. Shock occurred in a separate room and shock parameters were selected because they

were sufficient to produce a conditioned fear in our laboratory (data not shown). The 5 min test length was selected after pilot studies in which we observed a reliable decrease in social preference behavior after the first 5 min of test. In experiments involving optogenetics or intracerebral injections, a within-subjects design was employed such that each adult test subject was exposed to both vehicle and experimental treatments in SAP tests on consecutive days. A trained observer quantified the time spent socially interacting with each of the stimuli. Social interaction consisted of nose-nose and nose-body sniffing, and reaching into the enclosure to contact the stimulus rat. Digital video recordings were made for each test for later determination of inter-rater-reliability by a second observer completely blind to the experimental manipulations and hypotheses. Across experiments included in (Rogers-Carter et al., 2018b) we observed very high inter-rater reliability, $r(80) = 0.966$, $p < 0.0001$, and for experiments in (Rogers-Carter et al., 2018b) we report an inter-rater reliability of $r(30) = 0.8528$, $p < 0.0001$.

2.2.3 One-on-One Social Exploration Tests

Each experimental subject was placed into a plastic cage with shaved wood bedding and a wire lid to acclimate the rat to the arena for 60 min before testing. To begin the test, a juvenile or adult conspecific was introduced in the test arena for 5 min and exploratory behaviors (sniffing, pinning, and allogrooming) initiated by the adult experimental rat were timed by an observer blind to treatment. Juvenile and adult conspecifics were used for multiple tests but were never tested more than once with the same adult experimental rat. Each experimental adult was tested on consecutive days, once with an unfamiliar naïve conspecific and once with an unfamiliar stressed

conspecific (2 foot shocks, exactly as above); test order was counterbalanced (see Fig. 2.4 A).

2.2.4 Behavior Assessment

For some tests we quantified a number of additional behaviors displayed by the experimental adult rat from digital video recordings. This included time spent: 1) sniffing or investigating the test arena (chamber exploration), 2) immobile, 3) digging in the bedding, 4) self-grooming, 5) biting or pulling at the conspecific, 6) escape-oriented behavior, and 7) bedding and perimeter investigation. Operational definitions for these behaviors are provided in the table below (Table 2.1). Videos were selected blind to SAP test results. Chamber exploration, and investigative behaviors likely assess general locomotor function. Immobility is expressed in states of fear or anxiety (Bouton & Bolles, 1979), self-grooming may reflect emotion contagion (Burkett et al., 2016) and biting and pulling may reflect aggression. For a randomly selected group of rats in experiments 2.2.1-2.2.2 we also quantified the behaviors of conspecifics, including 1) chamber sniffing, 2) time inactive, 3) self-grooming, and 4) social interaction, to ensure the stress manipulation did not induce behavioral differences.

Behavior	Description
Social Exploration	Reaching or direct nose contact with any part of the conspecific's body
Chamber Exploration	Direct nose contact with the conspecific restraint chambers.
Immobility	Complete absence of locomotor activity except for that related to respiration
Digging	Digging bedding away from the restraint chamber
Self-grooming	Stereotypical wiping of the face with front paws or licking/wiping of coat
Biting/Pulling	Using the mouth to bite at the conspecific, or using forepaws to grab at the conspecific target, through the restraint chamber
Escape-oriented Behavior	Placing forepaws on the top of restraint chamber or rearing to cage sidewall with sniffing directed outside of the arena
Bedding and Perimeter Investigation	Sniffing the bedding of the test arena or the perimeter where the bedding meets the cage wall

Table 2.1: Description of behaviors quantified from experimental rats during SAP tests.

2.2.5 Ultrasonic Vocalization Recordings and Analysis

44 adult male rats were habituated for 60 min to the test arena, and 24h later randomly assigned to 1 of 4 treatment conditions that determined which conspecifics they would explore during testing: Naïve-Juvenile, Naïve-Adult, Stressed-Juvenile or

Stressed-Adult in a 2 by 2 (Stress by Age) design (*n* = 11/group). Rats were given 5 min, one-on-one social interaction tests with the conspecific corresponding to their assigned group. To record USVs, an acrylic lid was placed over the arena with a 192kHz USB microphone (Ultramic192K, www.Dodotronic.com) placed directly in the center of the lid. Recordings were made using Audacity 2.1 (www.audacityteam.org), exported as .wav files, and audio spectrograms were generated in Raven Pro 1.5 (The Cornell Lab of Ornithology https://store.birds.cornell.edu/Raven_Pro_p/ravenpro.htm). USVs were identified using the Band Limited Energy Detector function within Raven Pro. High frequency "Trill" calls were found in the range of 8-20ms, from 55-80kHz; frequency modulating "Rising" calls were 30-100ms, from 35-68kHz; and 22kHz "flat" calls were greater than 100ms, from 18-28kHz. These ranges were drawn from the literature (Brudzynski, 2013) and detection parameters were refined for each recording based on visual inspection by a trained observer who was blind to treatment condition.

2.2.6 Data Analysis

Sample sizes were initially determined based on prior work using social interaction (Vetere et al., 2017), no formal statistical methods were used to pre-determine sample sizes. In all behavioral experiments, rats were randomly assigned to groups. To compare differences between mean scores of social interactions we used t-tests and analysis of variance (ANOVA). To account for individual differences in social exploration, a preference score was computed by dividing the time spent exploring the stressed conspecific by the total social exploration time (stress plus naïve) multiplied by 100. Individual replicate data are provided in the figures. In most experiments, there

were within-subjects variables, which were treated as such in the analysis (paired samples t-test or repeated measures ANOVA). The data distributions were visually inspected (all replicates are shown) and appeared to meet the assumptions for normality and equal variance; but these were not tested formally. Data were either collected by observers blind to treatment, or collected by an experimenter and later rescored by an observer blind to treatment from digital video recordings. Final data analyses were not performed blind to the conditions of the experiments. Main effects and interactions were deemed significant when $p < 0.05$ and all reported post hoc test p values are Sidak-adjusted, to maintain an experiment-wise risk of type I errors at $\alpha = 0.05$. ANOVA analyses were conducted in Prism 7.0c (GraphPad Software) and SPSS Statistics 24 (IBM).

2.3 Summary of Experiments and Results

2.3.1 The social affective preference (SAP) test: age and stress exposure of conspecific determine social approach

As described in section 2.2.2, adult male rats (PN60-80) underwent SAP testing and their preference to explore either the naïve or stressed conspecific was recorded. Because social approach behaviors are shaped, in part, by features of the target including age (Staub & Baer, 1974), we hypothesized that rats may differentially respond to stressed conspecifics as a function of the target's age. To test this, 8 experimentally naïve, adult male rats were tested with a pair of unfamiliar PN 30 male juvenile conspecifics, and 12 were tested with a pair of unfamiliar PN 50 male adult conspecifics (Fig. 2.1 A & B). The PN 30 and PN 50 timepoints represent pre- and post-

pubescent ages in rats, respectively (Spear, 2015). The design was a 2 by 2 with conspecific Age (PN 30 vs. PN 50) as a between-subjects factor and conspecific Affect (naïve or stress) as a within-subjects factor. Analysis of the social interaction times compared with the naïve versus stressed conspecifics revealed an effect of conspecific age and affect on social preference behavior ($F_{AGE}(1, 18) = 27.93$, $p < 0.001$; $F_{AFFECT}(1, 18) = 9.965$, $p = 0.006$; $F_{AGE*AFFECT}(1, 18) = 46.05$, $p < 0.001$). We observed that experimental rats tested with PN 30 conspecifics prefer to explore the stressed PN 30 conspecifics (Fig. 2.1 C, $p < 0.001$), whereas experimental rats tested with PN 50 conspecifics prefer to explore the naïve conspecific (Fig. 2.1 C, $p < 0.05$). The preference scores (Fig. 2.1 D) summarize the main findings from the raw social exploration data; experimental rats tested with PN 30 conspecifics have a significantly greater preference score than experimental rats tested with PN 50 conspecifics ($t(18) = 5.783$, $p < 0.001$, 2-tailed), indicating that experimental rats who were presented PN 30 conspecifics spent more time exploring the stressed conspecific, compared to the experimental rats presented PN 50 conspecifics.

The SAP procedure was replicated several times in (Rogers-Carter et al., 2018b, to be discussed in Chapter 3), which provided a large sample (n = 51 for PN 30, n = 46 for PN 50) to evaluate the reliability and generality of these phenomena and so these data were pooled and converted to percent preference scores (Fig. 2.1 E) and the scores were found to fit a normal distribution (D'Agostino and Pearson normality test, for PN 30: $K2 = 2.52$, $p = 0.28$, for PN 50: $K2 = 2.14$, $p = 0.34$). PN 30 and PN 50 preference scores to the hypothetical value of 50% (equal time exploring both naïve and stressed conspecifics, one sample t-tests, 2-tailed) revealed a preference for the

stressed PN 30 and the naïve PN 50 conspecific (PN 30: $t_{\text{one-sample}}(50) = 6.49$, $p < 0.0001$, 2-tailed; PN 50: $t_{\text{one-sample}}(45) = 4.39$, $p < 0.0001$, 2-tailed). Approximately 82% (42 of 51) of rats tested with PN 30 conspecifics exhibited a preference for the stressed target, while only 21% (10 of 46) of rats tested with PN 50 conspecifics exhibited preference for the stressed target. The preference scores from the PN 30 group significantly differed from the PN 50 group, ($t(95) = 7.66$, $p < 0.0001$, 2-tailed), reflecting the robustness of this phenomenon across replications.

We also report that female experimental rats demonstrated the same social affective preference pattern. 8 regularly cycling experimental adult female rats (PN 60-80 days) underwent SAP tests with male conspecifics as above. A significant 2-way interaction, $F_{\text{AGE*AFFECT}}(1, 14) = 15.52$ showed that female experimental rats, like males, also preferred to explore the stressed juvenile (Fig. 2.1 F, $p < 0.001$) but avoided interacting with the stressed adult ($p < 0.05$). When the raw social exploration times were converted to a percent preference score, a t-test revealed that female experimental rats tested with juvenile conspecifics showed a greater preference score than female experimental rats tested with adult conspecifics, which was consistent with the preference to explore the stressed juvenile and naïve adult (Fig. 2.1 G; $t(7) = 3.51$, $p = 0.002$, 2-tailed).

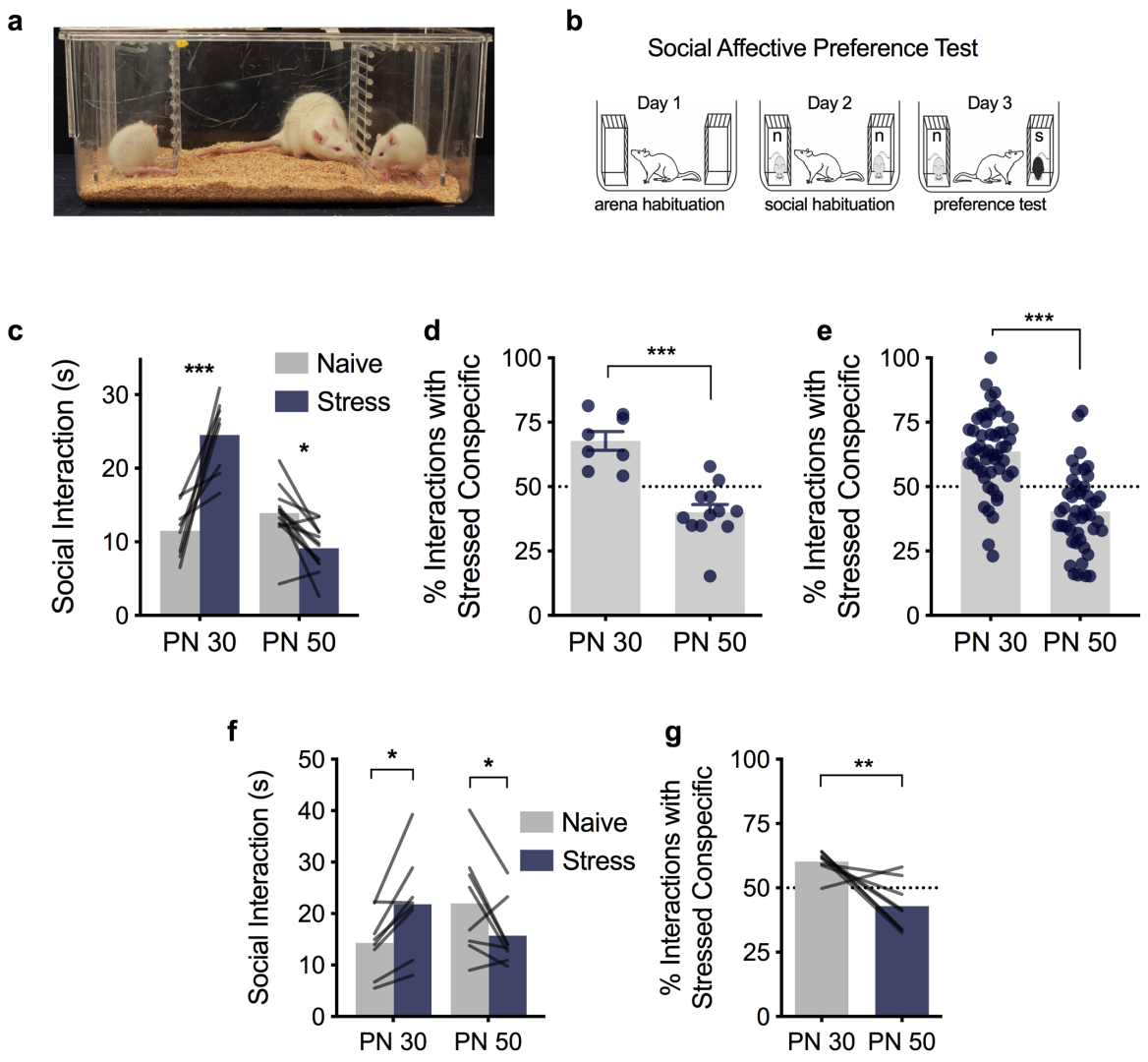


Figure 2.1 Social Affective Preference (SAP) Test. (A) The SAP arena containing juvenile conspecifics on the left and right with an experimental adult in the center. **(B)** Diagram of SAP test procedure. **(C)** Mean (individual replicates shown as connecting lines) time spent in social interaction with the naïve or stressed conspecific by age (n = 8, PN 30; n = 12, PN 50). Although conspecific age did not alter time spent interacting with the naïve conspecific, a bidirectional effect of age was apparent in time spent interacting with the stressed conspecifics ($F_{AGE}(1, 18) = 27.93$, $p < 0.001$; $F_{AFFECT}(1, 18) = 9.965$, $p = 0.006$; $F_{AGE*AFFECT}(1, 18) = 46.05$, $p < 0.001$). Experimental rats spent more time exploring the stressed PN 30 conspecific compared to the PN 30 naïve, but spent less time exploring the stressed PN 50 conspecific compared to the PN 50 naïve. **(D)** Mean (+ SEM with individual replicates) data in **C** expressed as the percent of time spent interacting with the stressed conspecifics relative to the total time spent interacting. Here, experimental adults showed a marked preference (values greater than 50%) for interaction with stressed conspecifics and avoidance (values less than 50%) of

stressed adults ($t(18) = 5.783$, $p < 0.001$, 2-tailed). **(E)** Mean (+ SEM, $n = 51$ for PN 30, $n = 46$ for PN 50) percent preference for interacting with the stressed conspecific pooled from all of the subjects in the experimental control groups included in (Rogers-Carter and Varela et al., 2018). Percent preference scores for PN 30 and PN 50 interactions were significantly different ($t(95) = 7.66$, $p < 0.0001$, 2-tailed) from each other, and in both conditions the mean percent preference differed from 50% (PN 30: $t_{\text{one-sample}}(50) = 6.49$, $p < 0.0001$, 2-tailed; PN 50: $t_{\text{one-sample}}(45) = 4.39$, $p < 0.0001$, 2-tailed). **(F)** Mean social interaction time. SAP tests were performed on adult female, regularly cycling rats with pairs of male juvenile or adult conspecifics as interaction stimuli (N=8 females). A significant 2-way interaction, $F(1, 14) = 15.52$, $p = 0.002$ indicated that the females also exhibit preference to interact with stressed juvenile conspecifics but avoid stressed adults, *p<0.05 (Sidak). **(G)** Results in c. shown as Mean % time interacting with stressed conspecific for comparison, **$t(7) = 3.51$, $p = 0.009$ (2-tailed).

2.3.2 Analysis of experimental rat and conspecific behavior during SAP testing.

To evaluate whether exposure to the stressed conspecific in the SAP tests altered any other aspect of experimental adult behavior, we quantified behaviors of 10 experimental adult rats tested with PN 30 conspecifics, and 7 experimental adult rats tested with PN 50 conspecifics, during both naïve-naïve habituation (day 3) and SAP testing (day 4; Fig. 2.2 A) as described in section 2.2.4. While most behaviors measured were equal across tests, a significant Age by Behavior by Test (habituation vs. SAP) interaction ($F_{\text{AGE*BEHAVIOR*TEST}}(4, 60) = 3.014$, $p = 0.025$) reflected increases in arena investigation ($p = 0.05$) and self-grooming ($p = 0.035$) during SAP tests with PN 50 conspecifics (Fig 2.2 A).

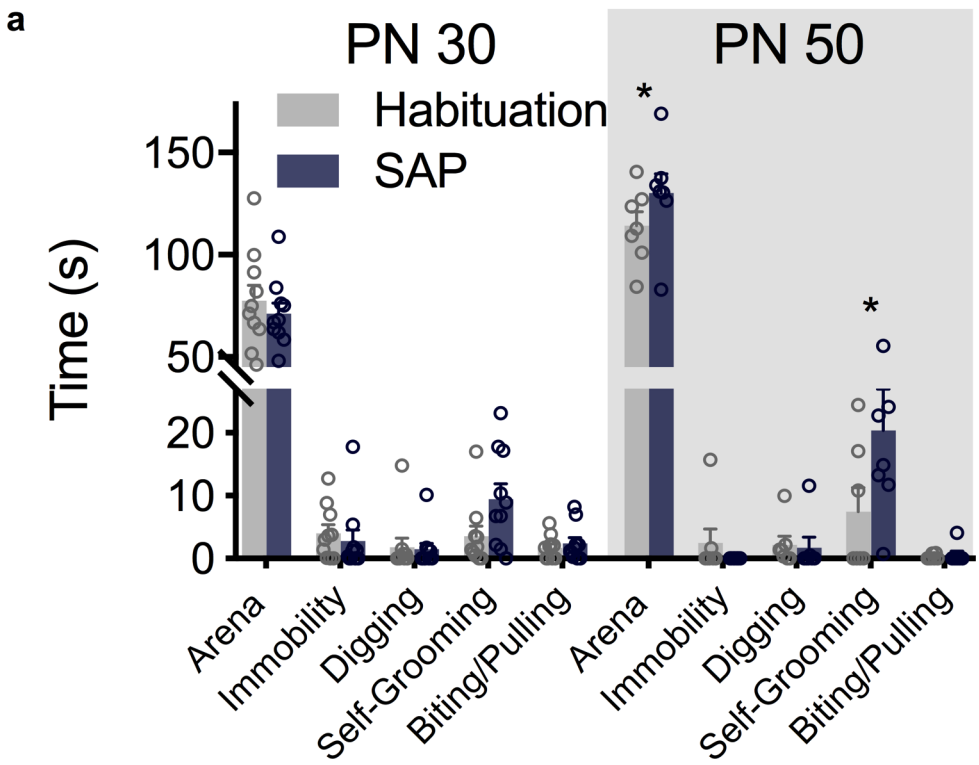
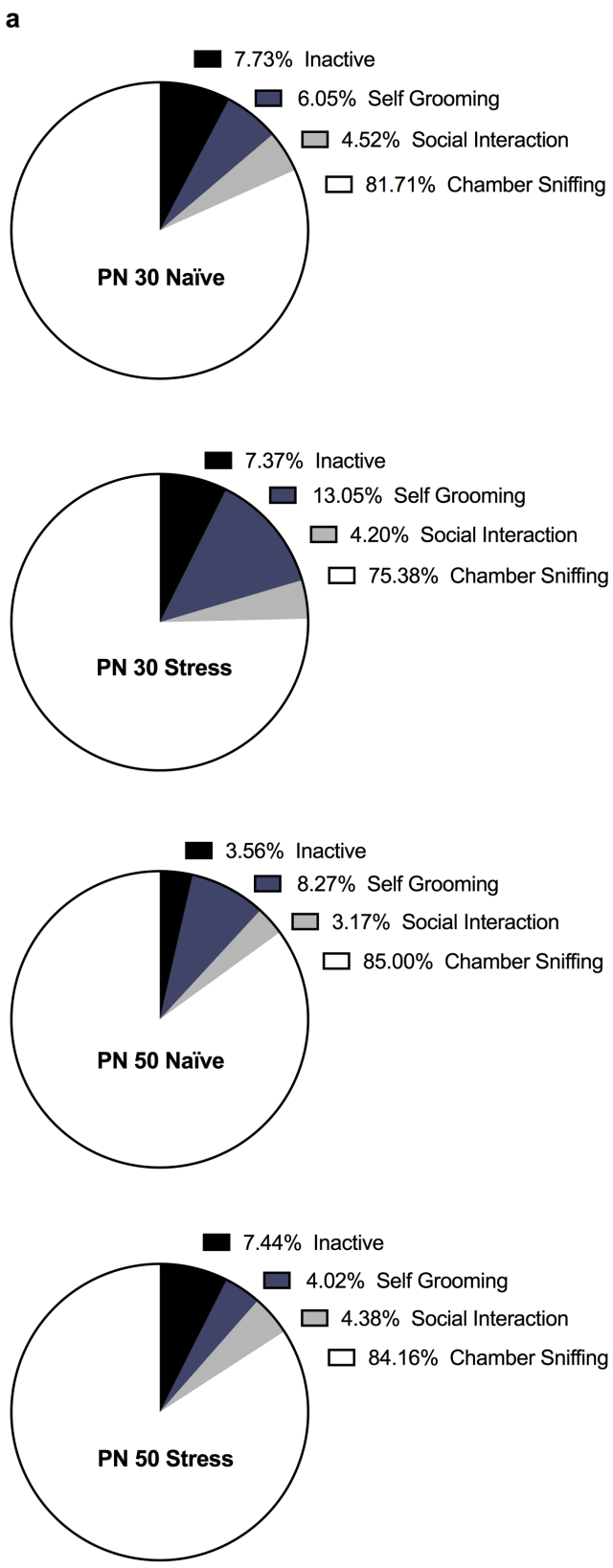


Figure 2.2 Analysis of experimental rat behavior during habituation and SAP testing. (A) Mean (+ S.E.M. with individual replicates) time spent in non-social behaviors during habituation tests and SAP tests (n = 10, PN 30; n = 7 PN 50). More investigation of the arena and time spent self-grooming was observed in the PN 50 rats ($F_{AGE*BEHAVIOR*TEST}(4, 60) = 3.014$, $p = 0.025$).

An analysis of *conspecific* behavior was also conducted as described in section 2.2.4. For each behavioral measure, the percentage of the 5 min test that the naïve versus stress conspecific engaged in each behavior was analyzed as separate t-tests, and these analyses revealed that the footshock procedure was associated with an increase in self-grooming behavior in PN 30 conspecifics, but did not mediate any behaviors in PN 50 conspecifics (Fig. 2.3 A & B). Further, these findings reveal that

conspecifics spent most of the trial engaging in activate behaviors (sniffing the chamber and arena).


a
7.73% Inactive
6.05% Self Grooming
4.52% Social Interaction
81.71% Chamber Sniffing
PN 30 Naïve
7.37% Inactive
13.05% Self Grooming
4.20% Social Interaction
75.38% Chamber Sniffing
PN 30 Stress
3.56% Inactive
8.27% Self Grooming
3.17% Social Interaction
85.00% Chamber Sniffing
PN 50 Naïve
7.44% Inactive
4.02% Self Grooming
4.38% Social Interaction
84.16% Chamber Sniffing
PN 50 Stress

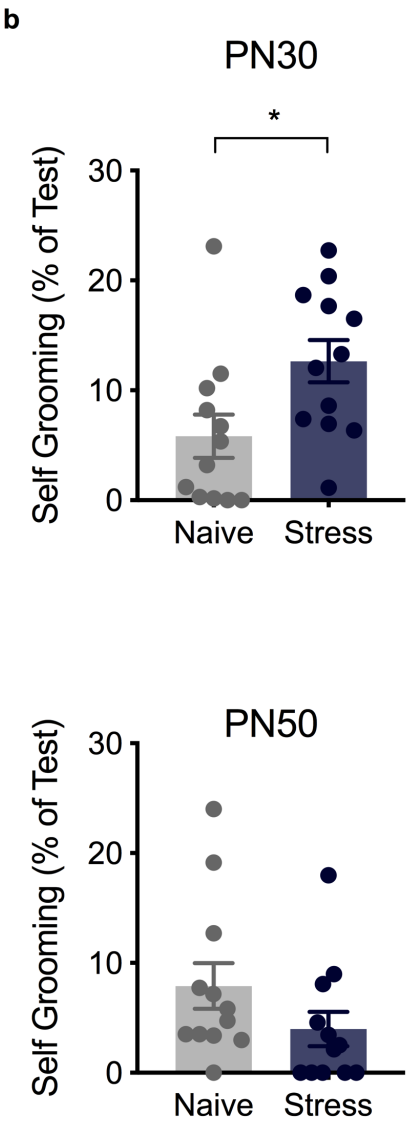

b
PN30
*
Self Grooming (% of Test)
30
20
10
0
Naive
Stress
PN50
Self Grooming (% of Test)
30
20
10
0
Naive
Stress

Figure 2.3 Analysis of conspecific behaviors during SAP testing. (A) An observer blind to conspecific treatment tallied the time spent inactive, self-grooming, engaged in social interaction with the experimental adult and time spent sniffing and exploring the conspecific chamber of 12 pairs of target rats from each of the PN 30 and PN 50 treatment groups. The average portion (% of total) time spent in each behavior of the 5 minute test are shown for each treatment. The analyses indicate that the conspecifics spent the majority of time in active behaviors, namely sniffing and exploring the chamber. **(B)** Mean (+/- SEM) time spent self-grooming. Separate t-tests were conducted to compare time in each behavior and revealed a significant increase in self grooming after stress in the PN 30 group (left panel). *$t(22) = 2.47$, $p = 0.022$ (2-tailed). No other comparisons reached significance, and self-grooming did not correlate with behavior of the experimental adult rats.

2.3.3 One-on-one testing and ultrasonic vocalizations.

From the SAP test results, we predicted that, in one-on-one interactions, an experimental adult should engage in more social interaction with stressed juveniles than naïve juveniles, but should display the opposite pattern with adult conspecifics. 8 experimental adult rats were given a series of 2 “one-on-one” social exploration tests (5 min duration, one test per day, as described in section 2.2.3) with either 1 unfamiliar naïve PN 30 juvenile, and later 1 unfamiliar stressed PN 30 juvenile as the social interaction targets (Fig. 2.4 A); a separate set of 8 experimental adults received the same series of tests with PN 50 adult conspecifics. A within-subjects design was used with test order counter balanced, and the time spent exploring each conspecific was converted to the percent of time spent in social interaction to control for individual differences in sociality. A 2-way ANOVA ($F_{AGE}(1, 14)$ 103.10, $p < 0.001$; $F_{AGE*AFFECT}(1, 14) = 31.34$, $p < 0.001$) revealed that experimental adults spent more time interacting with the stressed juvenile ($p < 0.001$; Fig. 2.4 B) and less time with the stressed adult ($p = 0.043$; Fig. 2.4 B), as observed in SAP tests (Fig. 2.1). There was no difference in conspecific-initiated social investigation at either age.

Ultrasonic vocalizations (USVs) convey affective states in rats (Brudzynski, 2013). To explore whether stress altered USVs during social interactions, a separate set of rats was given 5 min one-on-one social interaction tests in a chamber equipped with an ultrasonic microphone. Social interaction and USVs were quantified during exposure to 1 of 4 conspecific stimuli: naïve PN 30, naïve PN 50, stressed PN 30 or stressed PN 50 (ns = 11/group). Three types of vocalizations were present in our recordings: 22kHz flat vocalizations, ~30-60kHz rising calls, and ~60-80kHz trills (Fig. 2.4 C). Rising and trill calls, which are emitted during and in anticipation of rewarding stimuli, were quite frequent during naïve interactions but reduced during stressed interactions (Fig. 2.4 D; ($F_{STRESS}(1, 40) = 26.16$, $p < 0.001$; $F_{CALL\ TYPE}(2, 80) = 60.86$, $p < 0.001$; $F_{STRESS*CALL\ TYPE}(2, 80) = 20.18$, $p < 0.001$). 22kHz vocalizations are thought to convey negative affect, and these were observed primarily during interactions with stressed adults (Fig. 2.4 E; $F_{CALL\ TYPE*AGE}(2, 80) = 3.43$, $p = 0.37$). We observed more 22kHz calls from PN 50 stressed rat interactions than any other condition (ps < 0.007). Fewer rising calls in PN 30 and PN 50 stressed rats were observed compared to naïve PN30 and PN 50 conspecifics (ps < 0.045), more rising calls were evident in the stressed PN 30 than stressed PN 50 conspecifics($p = 0.03$), fewer trills were found in the PN 30 and PN 50 stress conditions than naïve (ps < 0.02) and a trend for more trills in the naïve PN 50 compared to PN 30 ($p = 0.055$). Although it is impossible to determine whether stress reduced USVs emitted by conspecifics, reduced USVs emitted by the experimental rat toward the stressed conspecific, or some combination of both, it appeared that stressor exposure dramatically shifted the patterns of USVs consistent with a state of negative affect.

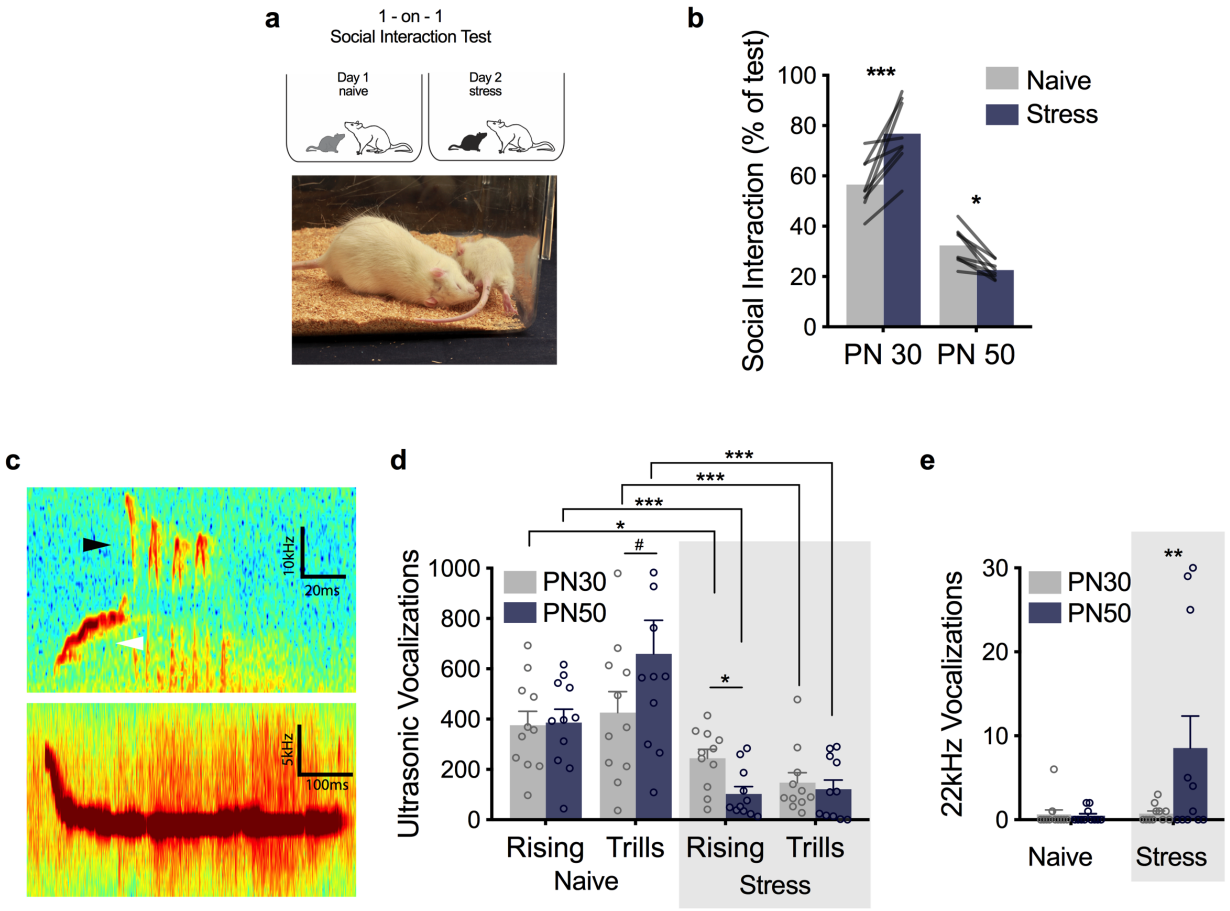


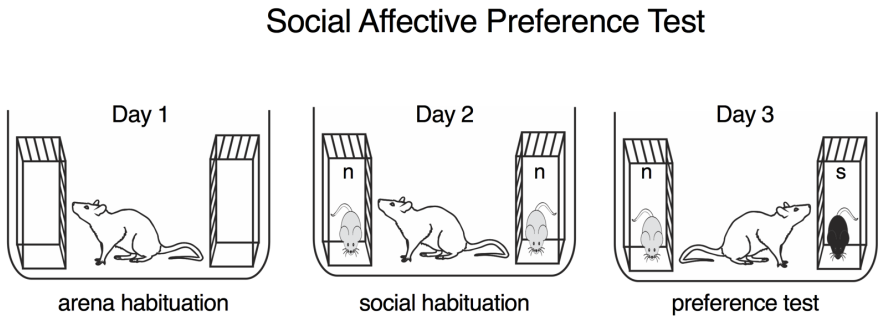
Figure 2.4 One-on-One testing and ultrasonic vocalizations. (A) Diagram of 1-on-1 social interaction test and photo of typical adult-initiated interactions. **(B)** Mean (individual replicates, *n*s = 8/ age) time interacting with the naïve or stressed conspecific in a 1-on-1 test shown as percent of test time. Experimental adults spent significantly more time interacting with the stressed PN 30 conspecific but significantly less time with the stressed PN 50 conspecific compared to the respective naïve conspecific targets ($F_{AGE}(1, 14)$ 103.10, $p < 0.001$; $F_{AGE*AFFECT}(1, 14) = 31.34$, $p < 0.001$). **(C)** Representative audio spectrograms depicting rising (top, white arrow), trills (top, black arrow) and 22kHz ultrasonic vocalizations (USVs). Scale bars indicate Y-axis ranges: 60-70kHz (top) and 30-35kHz (bottom). **(D-E)** Mean (+SEM with individual replicates, *n*s = 11/group) number of rising and trill USVs recorded during 5 min one-on-one social interactions. Fewer rising and trill calls were observed during interactions with stressed conspecifics with fewer rising calls observed in stressed adults compared to stressed juveniles but more 22kHz calls observed in stressed adults than stressed juveniles ($F_{STRESS}(1, 40) = 26.16$, $p < 0.001$; $F_{CALL\ TYPE}(2, 80) = 60.86$, $p < 0.001$; $F_{STRESS*CALL\ TYPE}(2, 80) = 20.18$, $p < 0.001$; $F_{CALL\ TYPE*AGE}(2, 80) = 3.43$, $p = 0.37$). *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Sidak).

2.3.4 Familiarity mediates SAP behavior with adult conspecifics in females.

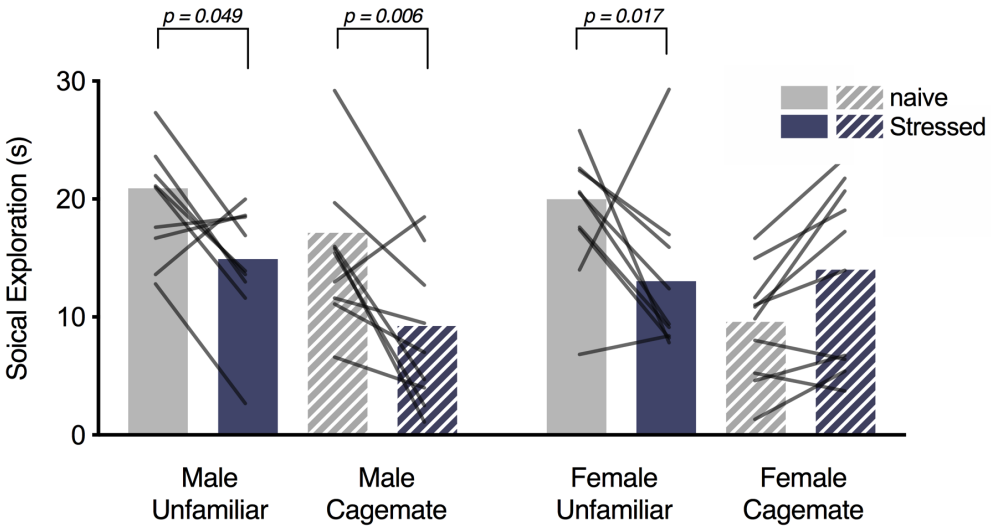
Whether or not conspecifics are familiar with one another can dictate social affective responses (Chen, 2018; Meyza et al., 2017; Staub & Baer, 1974). Here we replicated the social affective preference test with familiar conspecifics to see if familiarity would mediate preference behaviors in experimental rats tested with PN 50 conspecifics. 1 cohort of male experimental rats and 1 cohort of female experimental rats underwent SAP test with cagemates as the conspecifics, whereas another 2 cohorts of male and female experimental rats underwent SAP test with unfamiliar conspecifics (ns = 10/group; Fig. 2.5 A) Consistent with our previous findings experimental rats spent less time exploring the unfamiliar stressed adult conspecifics compared to the naïve, but female rats spent more time exploring familiar stressed conspecifics (Fig. 2.5 B). This pattern produced a significant interaction ($F_{\text{AFFECT*FAMILIARITY*SEX}}(1,36) = 8.479$, $p < 0.01$). Male experimental rats spent less time exploring the stressed conspecific compared to the naïve for both unfamiliar ($p < 0.05$) and cagemate ($p < 0.01$) pairs. Female rats spent less time exploring unfamiliar stressed conspecifics ($p < 0.01$), but not familiar stressed cagemates($p < 0.01$), when compared to naïve counterparts. To control for the range of individual variation in social exploration, we again computed a preference score for the stressed conspecific (Fig. 2.5 C). The preference scores were compared to 50%, the score at which there is no observable social affective preference. One-sample t-tests revealed preference scores significantly less than 50% for the Male Cagemate ($t(9) = 3.55$, $p < 0.01$), Male Unfamiliar ($t(9) = 2.579$, $p < 0.05$), and Female Unfamiliar ($t(9) = 2.519$, $p < 0.05$)

groups but significantly greater than 50% for the Female Cagemate ($t(9)=2.613$, $p < 0.05$) group. In sum, familiarity altered experimental female reactions to stressed conspecifics leading to greater social interaction.

a


Social Affective Preference Test
Day 1
Day 2
Day 3
n
n
n
s
arena habituation
social habituation
preference test

b


p = 0.049
p = 0.006
p = 0.017
naive
Stressed
Soical Exploration (s)
30
20
10
0
Male Unfamiliar
Male Cagemate
Female Unfamiliar
Female Cagemate

c

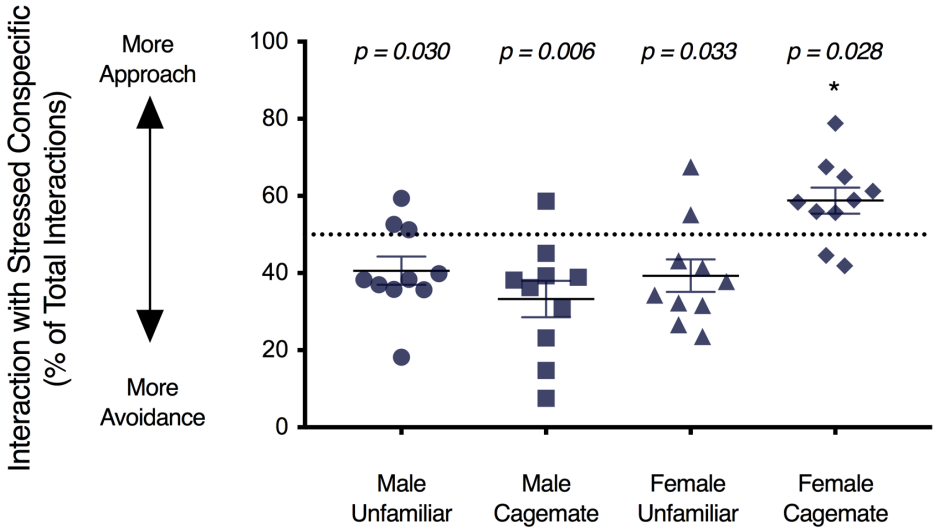

Interaction with Stressed Conspecific (% of Total Interactions)
More Approach
More Avoidance
100
80
60
40
20
0
p = 0.030
p = 0.006
p = 0.033
p = 0.028
*
Male Unfamiliar
Male Cagemate
Female Unfamiliar
Female Cagemate

Figure 2.5 Familiarity mediates social avoidance of stressed conspecifics in female rats. (A) Schematic of the SAP test procedure. **(B)** Mean (with individual replicates) social exploration time the experimental rats in each group engaged with naïve or stressed conspecifics on Day 3. A sex-specific effect of familiarity was observed ($F_{SEX}(1,36) = 8.479$, $p < 0.01$): Rats in the Male-Unfamiliar ($p < 0.05$) , Male-Cagemate ($p < 0.01$), and Female-Unfamiliar groups spent less time exploring the stressed conspecific compared to the naïve ($p = 0.052$), whereas Female-Cagemate rats spent more time exploring the stressed conspecifics ($p < 0.01$). **(C)** Mean (individual replicates with ± s.e.m.) data from (B) expressed as the percent of social exploration time directed toward the stressed conspecific. Male-Unfamiliar (one-sample $t(9) = 2.579$, $p < 0.05$), Male-Cagemate (one-sample $t(9) = 3.550$, $p < 0.05$), and Female-Unfamiliar (one-sample $t(9) = 2.519$, $p < 0.05$) percent preference scores were significantly less than 50%, indicating avoidance of the stressed conspecific. The Female-Cagemate percent preference score was greater than 50% (one-sample $t(9) = 2.613$, $p < 0.05$) indicating approach to the stressed conspecific; the Female-Cagemate preference score was significantly greater than the preference score of all other groups. *** $p < 0.001$.

2.3.5 Comparison of experimental rat behaviors between habituation and SAP test days.

Several findings report a change in observer behavior in the presence of a stressed conspecific (Burkett et al., 2016). To assess if the presence of a stressed conspecific caused any behavioral changed in the experimental rat, we compared several additional behaviors on the naïve-naïve habituation day to the same behaviors on the SAP test day. Time spent engaged in chamber exploration, escape-oriented behaviors, bedding exploration, immobility, self-grooming, digging, and social exploration during the social habituation and SAP test days was quantified from digital video recordings as described in section 2.2.4 and 3-way ANOVAs were performed for each quantified behavior. A significant interaction ($F_{\text{TEST DAY*FAMILIARITY*SEX}}(1,56) = 5.522$, $p < 0.05$) and post-hoc comparisons on chamber exploration revealed that Male-Unfamiliar rats engaged in greater conspecific chamber exploration during habituation

than any other group on either test day (Fig. 2.6 A) which may reflect more social interest in this group compared to others. A main effect of Test Day ($F_{\text{TEST DAY}}(1,56) = 9.404$, $p = 0.003$) was found for self-grooming which reflected an increase in self-grooming during SAP testing than the social habituation test day in all treatment conditions (Fig. 2.6 B). Similarly, a main effect of Test Day ($F_{\text{TEST DAY}}(1,56)= 4.941$, $p < 0.050$) and a Test Day x Sex interaction ($F_{\text{TEST DAY*SEX}}(1,56) = 5.454$, $p = 0.231$) on immobility indicated that experimental rats exhibit more immobility in the presence of a stressed conspecific than in the presence of naïve conspecifics (Fig. 2.6 C). For bedding and perimeter sniffing, the test revealed a main effect of Familiarity ($F_{\text{FAMILIARITY}}(1,16) =19.74$, $p < 0.001$), indicating that experimental rats spent more time exhibiting non-social behaviors in the presence of cagemates, regardless of sex or test day (Fig. 2.6 D).

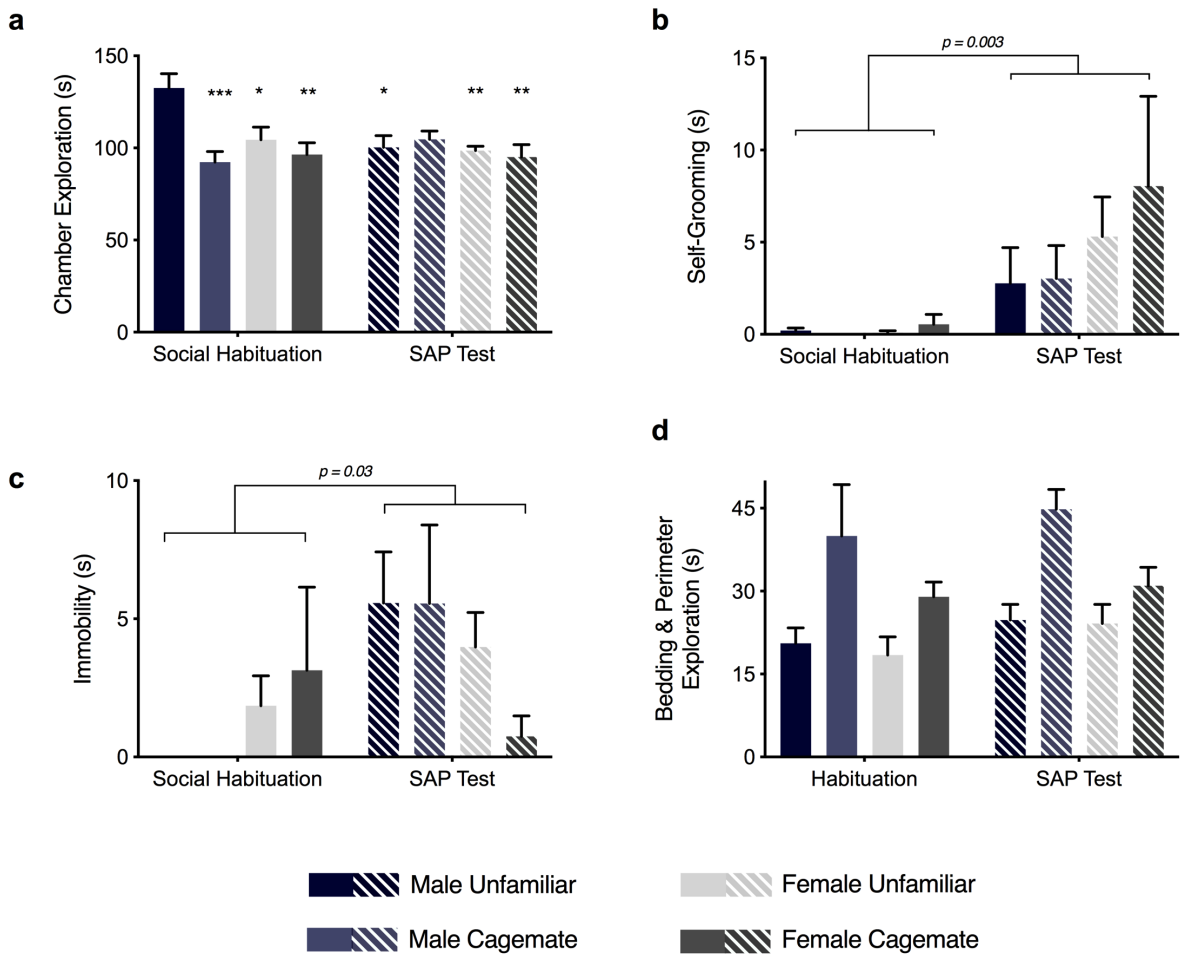


Figure 2.6 Comparison of experimental rat behaviors between habituation and SAP test days. (A) Mean (± s.e.m) time spent exploring the conspecific chambers on social habituation and SAP test days. Greater time spent in chamber exploration was observed in the Male-Unfamiliar group during habituation than any other group ($F_{\text{TEST DAY*FAMILIARITY*SEX}}(1,56) = 5.522$, $p < 0.05$). **(B)** Mean (± s.e.m) time spent self-grooming. In the presence of a stressed conspecific during SAP tests, experimental rats exhibited more self-grooming than during social habituation ($F_{\text{TEST DAY}}(1,56) = 9.404$, $p < 0.01$). **(C)** Mean (± s.e.m) time experimental rats spent immobile, which was greater during SAP tests than social habituation ($F_{\text{TEST DAY}}(1,56) = 4.941$, $p < 0.050$). **(D)** Mean (± s.e.m) time experimental rats spent exploring the bedding or perimeter of the cage. Experimental rats spent more time exploring the bedding and perimeter during interactions with cagemates regardless of sex or day ($F_{\text{FAMILIARITY}}(1,16) = 19.74$, $p = 0.000$). *p < 0.05, **p < 0.01, ***p<0.001

2.4 Discussion

To assess the influence of stress on social interactions, we developed a social affective preference (SAP) test in which the choice to approach or avoid a stressed conspecific could be quantified. In the SAP test, behaviors depended upon both the age and affective state of the conspecific, which may reflect a species-specific adaptation in which social stress signals are prosocial cues when generated by juveniles, but danger cues when generated by an unfamiliar adult. Also, familiarity mediated SAP behavior between experimental rats and adult PN 50 conspecifics, which may be the result of survival mechanisms in group-living species. These results add to the range of behaviors previously identified to influence behaviors directed toward stressed conspecifics (Meyza et al., 2017), which set the stage for further mechanistic investigation.

While the exact motivations to approach or avoid a conspecific are intractable, the decision to avoid a stressed adult may be adaptive if the stressed conspecific is perceived as a social danger cue. Consistent with this idea, we found that behaviors associated with states of fear and emotion contagion, namely immobility and self-grooming, were evident in SAP tests with both experimental male and female rats paired with unfamiliar conspecifics (Fig. 2.6 B & C). In both of these cohorts, we observed avoidance of the stressed conspecifics (Fig. 2.5 B). Therefore, avoiding the stressed conspecific may be a product of a conserved mechanism by which rodents use social communication to warn others of threat or harm

On the other hand, it is difficult to pinpoint the motivation to approach the stressed juvenile, though it is possible that the evolutionarily-conserved circuitry that

gives rise to parenting behavior has expanded to also enable social species to generate prosocial behaviors in response to juvenile-like affective cues from non-offspring conspecifics (Marsh, 2018). In human social behavior, features of the target stimulus, including age, are critical determinants to whether or not someone will approach, help or avoid another in distress (Staub & Baer, 1974) and there are examples of prosocial responses to stressed conspecifics in both vole (Burkett et al., 2016; Sterley et al., 2018) and mouse (Langford et al., 2010). However, these prosocial examples appear to have boundary conditions. First, prosocial effects are evident in pair-bonded voles and familiar female mice, but not between strangers or in male mice. This phenomenon may generalize to rats (Ben-Ami Bartal et al., 2014) but see (Silberberg et al., 2014), who will work to release a conspecific from a restraint chamber if they are of the same strain. An effect of the conspecific's age has not previously been investigated, and our results suggest that prosocial behaviors may occur towards unfamiliar conspecifics if they are juveniles. How a rat determines if a conspecific is juvenile or adult to inform their social response, however, is not fully clear.

Both rodents and humans utilize socioemotional cues to convey their affective states. In rodents, social communication of emotion occurs via chemosignals (Valenta & Rigby, 1968), vocalizations (Brudzynski, 2013), and overt behaviors (Sotocinal et al., 2011). Foot-shocked rats emit social odors that can either attract conspecifics in social buffering or serve as social alarm signals (Kiyokawa, 2017). Although we did not investigate chemosignals, these likely contribute to the behavior of the experimental rats in the SAP test. Regarding vocalizations, 22kHz USVs are emitted as alarm signals, and frequency modulating USVs are thought to convey positive affect (Burgdorf et al.,

2008). Accordingly, 22kHz vocalizations were nearly undetectable during naïve-naïve interactions but increased dramatically when one of the conspecifics was a stressed adult. Frequency modulating (rising and trill) calls preceded bouts of social interaction and were abundant in naïve-naïve interactions but dropped considerably when one of the conspecifics had received footshock, a pattern consistent with a negative affective state in the stressed conspecifics. Regarding overt behaviors, stress increased the amount of time juvenile conspecifics engaged in self-grooming. Thus, the experimental rat may draw from a constellation of vocalizations, visible cues and chemical signals to compute the age and emotional state of the conspecific.

Of course, it is not possible to determine if age-, affect-, and familiarity-specific behaviors are the product of a decision-making process or, alternatively, reflexive responses to specific combinations of social information. Despite this limitation, that rodents display preferences that depend on external factors allows for speculation on possible decision-making processes. Regarding the sex-specific effect of familiarity, Female-Cagemate rats will approach stressed conspecifics, whereas Male-Cagemate rats still avoid the stressed conspecific. To our knowledge, this is the first evidence of a sex difference regarding the role of familiarity on rat social affective behaviors, which suggests that males and females may appraise social stress signals in fundamentally different ways. This may be a consequence of familiarity modulating the way by which affective cues are integrated with other information during social decision-making in sex-specific ways. This pattern raises three questions regarding familiar females in the SAP test. First, do experimental females perceive or appraise the social stress signals of familiar rats differently than those of unfamiliar rats? Although insufficient data are

available to completely address this question, female rats engaged in more rough and tumble play behavior with familiar than unfamiliar conspecifics but familiarity did not influence male behavior (Argue & McCarthy, 2015), consistent with a sex-specific effect of familiarity on the observer. (Mikosz et al., 2015) reported increased amygdala and prefrontal cortex Fos expression in males and females in diestrus, but not females in estrus, after exposure to stressed conspecifics suggesting a neural substrate for different perceptions of conspecific stress in males and females which, in turn, would provide a different neural milieu to integrate familiarity in females than in males.

Second, do stressed rats emit different social signals to familiar observers than to unfamiliar? To our knowledge, this question has never been addressed but it is possible that the stress signals present in familiar female interactions, such as vocalizations or chemosignals, promote affiliative behaviors. In addition to the 22kHz danger signals, rodents emit a number of higher frequency vocalizations that are thought to facilitate social interaction, including maternal pup retrieval, and, in adults, reflect positive affective states (Brudzynski, 2013). Interestingly, Kiyokawa and colleagues have isolated chemosignal compounds released by stressed rats that are sufficient promote either affiliation or avoidance (Kiyokawa, 2017). Thus, it is possible that the combination of vocalizations, overt behaviors, and chemosignals generated by familiar female conspecifics is qualitatively different and therefore results in approach behavior.

Finally, does the presence of a familiar female observer buffer the stress of the demonstrator thus changing many aspects of the interaction? Demonstrators and observers have reciprocal effects on one another (Sterley et al., 2018) ahd this idea is an important factor in regard to social buffering of fear, which is evident when the

presence of a conspecific mitigates fear or stress. Social buffering occurs with several different stressors in many experimental contexts and has been observed in both male (Kiyokawa et al., 2007) and female (Ishii et al., 2016) rats. Interestingly, social buffering is more robust between familiar rats (Kiyokawa et al., 2014) and animals of the same strain (Nakamura et al., 2016). Although sex differences in social buffering have not been thoroughly explored (Kiyokawa & Hennessy, 2018; Meyza et al., 2017), it is possible that females are more effective than males at buffering fear in familiar conspecifics, which might give way to increased social exploration.

The work presented in this chapter identified age, affect and familiarity as factors that influence social responses in the social affective preference test. Chapter 3 will introduce mechanistic studies that begin to identify the neural correlates of social affective behavior.

Chapter 3: Insular Cortex Oxytocin Receptors mediate Social Affective Behavior in Rat

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

Rogers-Carter, M.M., Varela, J., Gribbons, K.B., Pierce, A., McGoey, M.T., Ritchey, M., and Christianson, J.P. (2018). Insular cortex mediates approach and avoidance responses to social affective stimuli. *Nature Neuroscience, 21,* 404-414.

3.1 Introduction

3.1.1 Human neuroimaging evidence implicating IC in emotion recognition

In humans, sophisticated social cognitions like empathy and perspective taking arise from the ability to recognize the emotional state of others. In fact, de Waal argued that “one party is affected by another’s emotional or arousal state” is the most elementary component of empathy. The SAP test captures well this phenomenon and therefore, we can draw from human neuroimaging experiments of empathy and emotion recognition to help identify potential neural anatomical correlates of social affective behavior. This expansive literature identified the insular cortex (IC) as one region of interest. In fact, a review of PET and fMRI studies found IC activation during emotional tasks in 60% of the reviewed literature (Phan et al., 2002) and a meta-analysis of empathy-related fMRI data concluded that the right anterior IC is recruited by emotional-empathic tasks (Fan et al., 2011). More specifically, the strength of IC activation positively correlated to performance during an empathic task (Kober et al., 2008) and IC activation has been reported as a neural correlate of mindfulness, discerning between in-and out-group members, and social pain from exclusion (Friedel et al., 2015; Molapour et al., 2015). The necessity of the IC for empathic and emotion-related behaviors is also exemplified in clinical populations; IC thinning (Castro et al., 2015), resection (Boucher et al., 2015), or lesion (Campanella et al., 2014; Terasawa et al., 2015b) have all been implicated in socio-cognitive impairments. Furthermore, autism is characterized in part by socio-emotional abnormalities, and aberrant IC resting state connectivity (Green et al., 2016), and abnormal IC activation in response to sensorimotor, cognitive and affect-related information have been observed in autistic

patients (Yamada et al., 2016). These findings implicate the IC in social affective processing and thus beget the question: what anatomical and functional properties of IC result in such consistent involvement across socio-emotional tasks?

The anatomical connectivity of the IC may help address this question as it is well positioned to process sensory information. Social information, including emotion, affect and arousal, is conveyed across multiple sensory modalities, and the perception of such social cues drives behavioral responses (Insel, 2010). IC responds to primary sensory inputs from all unimodal sensory modalities (Lucas et al., 2015; Mazzola et al., 2014) and is coupled to structures included in the social brain network. Furthermore, sensory information processed in the IC has emotional value; IC has been extensively studied for its role in processing disgust (Calder et al., 2000; Croy et al., 2016; Papagno et al., 2016; Phillips et al., 1997) and has been implicated as the anatomical locus for pain empathy (Botvinick et al., 2005; Corradi-Dell'Acqua et al., 2011; Eres et al., 2015; Lamm & Singer, 2010; Singer et al., 2004). Moreover, a meta-analysis of human IC neuroimaging work identified overlapping function subregions of IC that detect both sensory information and response to socioemotional stimuli, suggesting IC may serve as an anatomical locus for functional integration of sensory and emotional information to produce one's coherent experience of a social environment (Kurth et al., 2010). Output regions of IC include the amygdala, ventral tegmental area, and orbitofrontal cortex (Chang et al., 2013) and this anatomical arrangement may allow salient sensory information that is processed in IC to be integrated with emotion and reward value to inform social behavioral responses. However, despite ample evidence of IC involvement

during both sensory integration and emotion recognition-related processes, there has yet to be casual examination of this relationship.

3.1.2 Rodent evidence implicating IC in sensory processing and social affective behavior

The anatomical connections and functional roles of IC that likely pertain to social affective behavior is, importantly, conserved across species. Posterior IC is a well-documented site of sensory integration, which is important in regard to social affective behavior because stressed conspecifics likely communicate their affective state via a constellation of sensory cues. IC is equipped to detect such sensory information via direct inputs from thalamic relay nuclei (Guldin & Markowitsch, 1983).
and multisensory integrative abilities (Gogolla et al., 2014; Rodgers et al., 2008). Furthermore, rodent IC is connected to the prefrontal cortex and amygdala (Shi & Cassell, 1998) and these two targets of IC efferents are known to mediate social behaviors (Felix-Ortiz et al., 2016). Together, these findings are congruent with those from human neuroimaging which demonstrate IC is positioned to integrate social sensory information with emotional state and motivational signals (Krushel & van der Kooy, 1988). Moreover, visceral changes that occur after exposure to a stressed demonstrator (Burkett et al., 2016; Chen, 2018; Sterley et al., 2018) can mediate social behaviors (Porges, 2001). This suggests observers have a mechanism to detect the physiological changes that result from emotion transmission in order to generate appropriate social responses, a process IC may execute due to its sensitivity to fluctuations in visceral function (Cechetto & Saper, 1987; Critchley & Harrison, 2013).

However, there are no mechanistic studies in rodents that have causally examined sensory integration, emotion and social behaviors and so we hypothesized IC would be an interesting region of interest to target in social affective behavior.

3.1.3 Oxytocin, sensory processing, and social behaviors

Oxytocin (OT) is a critical modulator of numerous social behaviors across species (Marlin & Froemke, 2017). In humans, oxytocin is known to mediate processing of facial emotional expressions (Ellenbogen, 2018) and social decision-making with regard to emotional information (Di Simplicio & Harmer, 2016). Moreover, polymorphisms in the OTR gene are associated with deficits in emotion recognition (Puglia et al., 2015) and increase the risk of autism-like symptoms (LoParo & Waldman, 2015), and there is a conflicting but promising body of work which suggests that intranasal administration of OT improves socio-emotional performance (Guastella et al., 2012) and emotion recognition (Timmermann et al., 2017). While there is little mechanistic from humans as to how oxytocin increases emotion recognition (Evans et al., 2014) the existing literature prompted a number of studies in rodents, which demonstrate that oxytocin can modulate the salience and subsequent behavioral responses to socioemotional cues. In piriform cortex, administration of an oxytocin receptor antagonist (OTRa) blocked the formation of a social odor memory, but did not impact non-social odor memory formation (Choe et al., 2015). Similarly, neurons in the auditory cortex of mouse dams are tuned to pup calls, and administration of oxytocin decreased the latency for pup retrieval by increasing the salience of auditory pup calls

via modulation of the excitatory/inhibitory balance in auditory cortex (Marlin et al., 2015).

Given the known role of both oxytocin and the IC in emotion recognition in humans, we speculated that oxytocinergic activity in IC may be important for social behaviors that require sensory processing. In rat, the IC contains a dense distribution of OTRs relative to neighboring cortical areas (Dumais et al., 2013), which suggests IC is a target for neuromodulation by OT. Importantly, OT-ergic axons originating from the hypothalamus terminate in the IC (Knobloch et al., 2012) and in rats there is evidence that OT mediates excitatory transmission (Oettl et al., 2016). Further, OT can alter cortical circuit tone *in vivo* to increase neuronal excitability (Marlin et al., 2015). The OT receptor is coupled to the heterotrimeric G protein subunit $G_{\alpha q11}$ (Gimpl & Fahrenholz, 2001) which activates phospholipase Cβ (PLCβ) to hydrolyze the membrane phospholipid phosphatidylinositol,4,5-bisphosphate (PIP_2) and two second messengers: diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). DAG initiates protein kinase C (PKC), which mediates activity in numerous cellular pathways. Moreover, downstream targets of OT signaling cascades have been implicated in rat social interactions in IC (Cao et al., 2013) and thus, the effect of OT on processing of social sensory cues may result from intracellular signaling that modulates the excitability of neurons to sensory inputs.

The literature suggests that, if OT is present in the IC, it may increase the salience of social sensory cues by augmenting neural circuit function. In this chapter I will discuss findings from behavioral, pharmacological and optogenetic manipulations that implicate insular cortex OT in rat social affective behavior. Further, I present data

from Fos immunohistochemistry that suggests stressed juveniles elicit greater IC activation than exposure to stressed adult conspecifics. Lastly, I report on a set of pharmacological and electrophysiological findings that oxytocin may drive social affective behaviors by increasing the excitability of IC, possibly via an intracellular signaling cascade. These studies are the first to implicate oxytocinergic activity in IC and offer mechanistic insight into social decision-making during the SAP test.

3.2 Materials and Methods

3.2.1 Animals

Male Sprague-Dawley rats were obtained from Charles River Laboratories (Wilmington, MA). Rats were allowed a minimum of 7 days to acclimate to the vivarium after arrival and housed in groups of 2-3 with free access to food and water on a 12 h light/dark cycle. Behavioral procedures were conducted within the first 4 h of the light phase. All reagents and chemicals were purchased from Fisher Scientific, Tocris or Sigma unless otherwise noted. All procedures were conducted in accordance with the Public Health Service *Guide for the Care and Use of Laboratory Animals* and were approved by the Boston College Institutional Animal Care and Use Committee.

3.2.2 Behavior testing `

Rats underwent one-on-one or social affective preference testing as in Chapter 2; please reference section 2.2.2 and 2.2.3 for methodological details. However, for the experiments presented in this chapter, rats underwent an additional SAP testing day so that experiments with pharmacological and optogenetic manipulations were of a within-subjects and counterbalanced design. Thus, the 4 day SAP procedure was conducted

as follows: day 1 was habituation to the test arena, day 2 was acclimation to the arena with two naïve conspecifics presented in the restraint chambers, and days 3 and 4 were SAP tests, where one naïve and one stressed conspecific were presented to the experimental rats. The only exception to this design is experiment 3.3.1 in which muscimol was microinfused to IC prior to testing; we discovered an order effect of drug treatment (data not shown) and so this experiment was conducted as a 3-day between-subjects SAP procedure. Half of the experimental rats in experiment 3.3.1 were randomly assigned to the vehicle condition and half to the drug treatment condition.

3.2.3: Insular cortex cannula placements and microinjection

Under inhaled isoflurane anesthesia (2-5% v/v in O_2), cannula (26g, Plastics One, Roanoke, VA) were inserted bilaterally into IC (from Bregma: AP: -1.8mm, ML: +/-6.5mm, DV: -6.2mm from skull surface) and fixed in place with acrylic cement and stainless steel screws. Rat were administered the analgesic meloxicam (1mg/kg, Eloxiject, Henry Schein) and antibiotic penicillin G (12,000 Units, Combi-pen 48, Henry Schein) after surgery and allowed between 7-10 days recovery prior to experimentation. The OTR antagonist (OTRa) desGly-NH_2-d$(CH_2)_5$[Tyr(Me)^2,Thr^{4}OVT^{56} as in (Lukas et al., 2013) and OT were dissolved in sterile 0.9% saline vehicle, the pan-PKC inhibitor Gö 6983 was first dissolved in 100% DMSO and then diluted to 200nM in a vehicle of 10% DMSO and water. All injections were 0.5µL per side and infused at a rate of 1µL/min with an additional minute for diffusion. At the conclusion of the experiment, rats were overdosed with tribromoethanol (Sigma) and brains were dissected and sectioned at 40 µm to verify the microinjector tip location using cresyl violet stain in comparison to

the stereotaxic atlas (Paxinos et al., 1980). Rats with occluded injectors or having cannula located outside of the IC were excluded from all analyses (see Fig. 3.7). To control for nonspecific effects of OTRa on social behavior, we conducted a control experiment in which OTRa had no effect on interaction between experimentally naïve adult and PN 30 rats (see Fig. 3.5 G).

3.2.4 Effects of social interactions with naïve juvenile, stressed juvenile, naïve adult and stressed adult conspecifics on Fos expression in IC

After testing for USVs, the experimental rats from experiment 2.3.3 were left in their test cage alone and placed in a quiet room for 90 min, at which point the rat was overdosed with tribromoethanol and perfused with 0.01M ice-cold heparinized phosphate buffered saline (PBS) followed by 4% paraformaldehyde for later Fos analysis as previously reported (Christianson et al., 2011): Brains were dissected and post-fixed in 4% paraformaldehyde at 4°C for 24h and transferred to 30% sucrose for 2 days. 40µm coronal slices of the entire rostral-caudal extent of the brain were collected via a freezing cryostat at -19°C and stored in 24-well plates containing cryoprotectant at 4°C. To visualize Fos, floating sections quenched for endogenous peroxidase with 3% H_20_2, blocked with 2% normal donkey serum in PBS-T (0.01% Triton-X100), and then incubated overnight in rabbit anti-c-fos antibody (1:500, Cat. No. sc-52, Santa Cruz). Sections were then washed and incubated in biotinylated donkey anti-rabbit secondary antibody (1:200, Cat. No. 711-065-152, Jackson ImmunoResearch) using the avidin-biotin complex method (ABC Elite Kit, Cat. No. PK-6100, Vector Labs) with chromogen precipitate (NovaRed Kit, Cat. No. SK-4800, Vector Laboratories). Sections were floated

onto glass slides, dehydrated, cleared, and coverslipped with Permount (Fischer Scientific). All steps were conducted at room temperature. To quantify Fos immunoreactive nuclei, tissue was imaged on a Zeiss Axioimager Z2 light microscope in the Boston College Imaging Core. Tiled images containing the ROIs, including IC, were taken using a Zeiss AxioCam HRc digital camera through a 10x objective (N.A. 0.45). Using ImageJ software, images were converted to 16-bit, IC was traced with reference to the rat brain atlas, and Fos immunoreactivity was quantified using the cell counter plug-in using parameters that were validated by comparison to manual counts by a trained observer. Cell density was computed the number of Fos immunoreactive cells divided by the ROI area (in pixels) for ANOVA and network analyses. A network analysis of Fos immunoreactivity across 29 ROIs can be found in (Rogers-Carter et al., 2018b); this chapter will only report Fos expression in IC. However, the results of the network analyses will be briefly addressed in the discussion.

3.2.5 Optogenetic manipulations of IC in SAP testing

Adult male rats underwent stereotaxic surgery to be implanted with bilateral guide cannula designed to fit a 200µm optical fiber (Plastics One). After the cannula was secured, 250nL of a viral vector containing the neuronal silencing halorhodopsin eNpHr3.0 under the CamKIIα promoter (AAV5-CamKIIα-eNpHR3.0-mcherry; (Tye et al., 2011) or a sham virus (AAV5-CamKIIα-YFP) was microinjected at a depth 1mm below the termination of the guide cannula at a rate of 50nL/min and allowed 5 min for diffusion. Optogenetic transductions were evaluated by fluorescence microscopy and *in vitro* whole cell recordings (Fig. 3.4). During testing, a multimodal fiber optic wire

(200µm core, 0.39NA, Model FT200EMT, Thorlabs) extending 1mm below the cannula tip was affixed to the stylet via a screw top ferrule (Plastics One) and connected to a laser (GL523T3-100, Shanghai Laser & Optics Century). Throughout the length of a social test green light (l = 523nm) at a power of ~10-15mW/mm^2 was administered to maintain insular inhibition for the light ON conditions. During the light OFF condition, rats underwent the SAP test while connected to the las without light. Functional photo inhibition was verified in whole cell recordings of mCherry positive IC pyramidal neurons in acute brain slices before, during, after green light administration (10mW/mm^2) delivered through the objective of the electrophysiology microscope (Fig. 3.4 C & D). The extent of transfection was determined by imaging mCherry expression with widefield fluorescent microscopy (Zeiss AxioImager Z2). Locations of transfections are provided in Fig. 3.4 A & B.

3.2.6 Effects of oxytocin on excitatory field potentials in IC

Adult male rats were anesthetized with isofluorane, intracardially perfused with chilled (4°C), oxygenated aCSF cutting solution and quickly decapitated. 300 µm coronal slices including the IC were taken using a vibratome (VT-1000S, Leica Microsystems, Nussloch, Germany). The slices were placed in oxygenated aCSF cutting solution (95% O^2 and 5% CO^2) at 37°C for 30 min and then at room temperature for a minimum of 30 min before slices were used for electrophysiological recordings.

Evoked field excitatory postsynaptic potentials (fEPSPs) were recorded on a 6 x 10 perforated multiple electrode array (Model: MCSMEA-S4-GR, Multichannel Systems) with integrated acquisition hardware (Model: MCSUSB60) and analyzed with MC_Rack

Software (Version 3.9). Slices were placed on the array and adhered by suction of the perfusion through the perforated substrate. Bath solutions were as above and perfused through the slice from above. A stimulating electrode was selected in the deep layers of IC, and fEPSPs were recorded after stimulation (0 to 5V, biphasic 220μs, 500mV increments) before, during application of 500nM OT, and after (Wash). Each step in the I/O curve was repeated 3 times (20s inter-stimulus-interval) and each family of steps was replicated 3 times in each phase of the experiment. fEPSPs from channels displaying clear synaptic responses (as in Fig. 3.6 D) and in the vicinity of the stimulating electrode (Fig. 3.6 C) were normalized to the individual channel's maximum response to 5V stimulation at baseline; channels from the same slice were averaged for group analysis.

The electrophysiology experiments were replicated to test the dependence of OT effects on PKC. A few key aspects of the experiments were different. First, a between-groups design was used such that baseline measures were taken from all neurons after a stable recording was achieved. Then, drugs were bath applied in aCSF that contained 0.5% DMSO to permit solubility of the pan-PKC inhibitor Gö 6983 (200nM; (Gschwendt et al., 1996). The conditions were as follows: aCSF, aCSF with Gö 6983, OT (1μM for fEPSPs), or OT with Gö 6983. The OT dose was increased to 1μM in the fEPSP experiment as 500nM did not reliably replicate the increase in fEPSPs when DMSO was added to the aCSF. All dependent measures were normalized to pre-drug baselines for analysis.

3.2.7 Data analysis

Sample sizes were initially determined based on prior work using social interaction tests (Christianson et al., 2008; Christianson et al., 2011) and intrinsic physiology and intrinsic physiology (Varela et al., 2012), no formal statistical methods were used to pre-determine sample sizes. In all behavioral experiments, rats were randomly assigned to treatments. In electrophysiology experiments, cells were treated according to a Latin square design to achieve even representation of cells in treatment groups from different donor rats. For all of the experiments that entailed a mechanistic manipulation of the IC including optogenetic and OT, OTRa, and PKC inhibitor pharmacological infusions, we observed a portion of rats that did not exhibit the expected behavior towards stress conspecifics. Therefore, rats were excluded from the statistical analysis if any of the following conditions were met: 1) they did not express the expected preference for stressed juveniles or avoidance of stressed adults was greater or less than 50% in the control condition, 2) the cannula were occluded or found to be outside of the insula, and/or 3) virus expression was found to be unilateral or outside of the insula. The data from rats excluded due to item 1 above are provided for inspection in Fig. 3.7 B.

To compare differences between mean scores of social interaction and electrophysiological endpoints we used t-tests and analysis of variance (ANOVA). Individual replicate data are provided in the figures. In most experiments, there were within-subjects variables, which were treated as such in the analysis (paired samples t-test or repeated measures ANOVA). The data distributions were visually inspected (all replicates are shown) and appeared to meet the assumptions for normality and equal

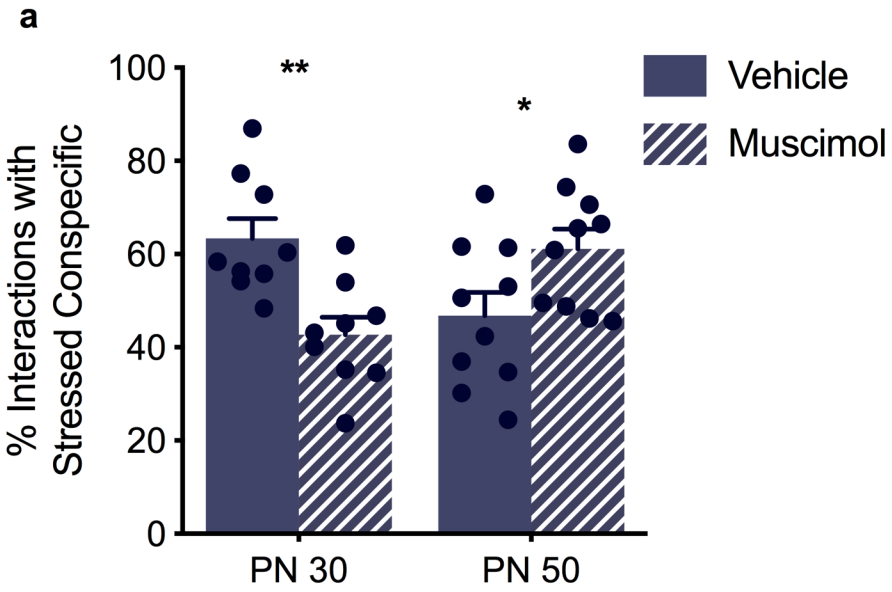
variance; but these were not tested formally. Data were collected by observers blind to treatment. Final data analyses were not performed blind to the conditions of the experiments. Main effects and interactions were deemed significant when $p < 0.05$ and all reported post hoc test p values are Sidak-adjusted, to maintain an experiment-wise risk of type I errors at alpha = 0.05. ANOVA analyses were conducted in Prism 7.0c (GraphPad Software) and SPSS Statistics 24 (IBM).

3.3 Summary of Experiments and Results

3.3.1 Pharmacological inhibition of IC with muscimol in SAP tests with PN 30 conspecifics

Because the aforementioned literature implicates IC in sensory processing and emotion recognition, and preference behavior in the SAP test likely requires the experimental rat to detect social sensory cues that convey affect, we hypothesized that inactivating IC would disrupt social affective preference behaviors. To test the necessity of IC, we first used the drug Muscimol in inactivate IC. Muscimol is a $GABA_a$ agonist used to pharmacologically inactivate specific brain region by hyperpolarizing neurons to achieve widespread and robust inhibition (Akhondzadeh & Stone, 1995). For this experiment, experimental adult rats were bilaterally implanted with guide cannula as described and tested with either PN 30 (ns = 9 per treatment group) or PN 50 (ns = 10 per treatment group) conspecifics 1 week after surgery. 60 min prior to SAP testing on day 3, experimental rats in the vehicle or drug treatment conditions received bilateral IC injections of 0.9% saline or the $GABA_a$ agonist muscimol, respectively, and the amount

of time each experimental rat explored the stressed and naïve conspecifics was recorded. This experiment was different in design from the others in chapters 2-4 because rats received only 1 SAP test, precluding the more powerful within-subject comparisons of drug effect. Exploration times were converted to a preference score for



the stressed conspecific as in section 2.2.6, and a 2-way ANOVA revealed a significant interaction ($F_{AGE*DRUG}$ (1, 34) = 16.27, $p < 0.001$) that indicated the social affective preference behavior of experimental rats in both the PN 30 and PN 50 groups was sensitive to muscimol; experimental rats showed a reduction in the percentage of exploration time spent with the stressed PN 30 conspecifics following muscimol injections($p < 0.01$), whereas experimental rats tested with PN 50 conspecifics showed an increase in the portion of social exploration time directed toward the stressed conspecific following muscimol injections ($p < 0.05$; Fig. 3.1)

Figure 3.1 Pharmacological inhibition of IC with muscimol during SAP testing. (A) Rats were implanted with bilateral IC cannula for microinjections and received SAP tests with either PN 30 (n = 9) or PN 50 (n = 10) conspecifics. Rats were tested after either Vehicle (0.9% saline) or muscimol (100ng/side in saline) was microinfused to IC 60 minutes prior to SAP tests on day 3. SAP behavior is shown as the mean (+/- SEM) percent of time spent interacting with the stressed conspecific of the total time spent in social interaction during the SAP test. A 2-way between subjects ANOVA revealed a significant Age by Drug interaction, $F(1, 34) = 16.27$, $p < 0.001$. Post hoc comparisons between drug conditions within each age revealed a significant decrease in time spent interacting with the stressed PN 30 juvenile conspecific after microinjections of muscimol ($p < 0.01$) and a significant increase in the portion of time spent interacting with the stressed PN 50 adult conspecific ($p < 0.05$).

3.3.2 Analysis of Fos expression in IC after interactions with naïve *and stressed conspecifics.*

Experiment 3.3.1 revealed that inactivating IC caused experimental rats to behave in a way that was agonistic to affect, which suggested IC is able to detect stressed affect. We therefore hypothesized that exposure to stressed conspecifics would cause different levels of activation in IC than exposure to naïve conspecifics. To test if conspecific affect could influence IC activity, experimental rats were sacrificed 90 min after the one-on-one social interaction tests in section 2.2.3, where rats explored a naïve PN 30, stressed PN 30, naïve PN 50 or stressed PN 50 conspecific. Sections containing IC were stained for Fos immunoreactivity (Fig. 3.2 A). Fos counts in each IC subregion (agranular, dysgranular, and granular) were collected using image J and converted to a measure of cell counts per area in arbitrary units. All regions in all conditions contained Fos expression, and a 3-way ANOVA revealed a significant interaction age by stressed interaction ($F_{STRESS*AGE}(1, 36) = 6.21$, $p = 0.017$); Fos immunoreactivity was higher in the IC of rats that had interacted with stressed PN 30 conspecifics than in rats that had interacted with stressed PN 50 conspecifics (Fig. 3.2

B, p = 0.009), and IC Fos immunoreactivity was lower in rats after interaction with PN 50 stressed rats than in rats after interaction with PN 50 naïve rats (p = 0.038; pooled across region). A main effect of subregion ($F_{SUBREGION}(2, 72) = 7.90$, p = 0.001) revealed that experimental rats who interacted with stressed PN 30 conspecifics have greater Fos immunoreactivity in the agranular ($p < 0.01$) and dysgranular ($p < 0.05$) IC subregions, compared to experimental rats who explored stressed PN 50 conspecifics (Fig. 3.2 B). A linear regression analysis of mean Fos immunoreactivity pooled across all IC subregions predicted the amount of time the experimental rat engaged in exploration and thus reflected both the age and stress state of the conspecifics (Fig.3.2 C). The analysis most strongly reflected social interaction by Fos level, Age, and most interactions between Stress, Fos and Age ($F_{FOS}(1, 34) = 19.72$, $p < 0.001$, $F_{AGE}(1, 34) = 36.93$, $p < 0.001$; $F_{FOS*STRESS}(1, 34) = 3.97$, p = 0.055; $F_{STRESS*AGE}(1, 34) = 4.09$, p = 0.051).

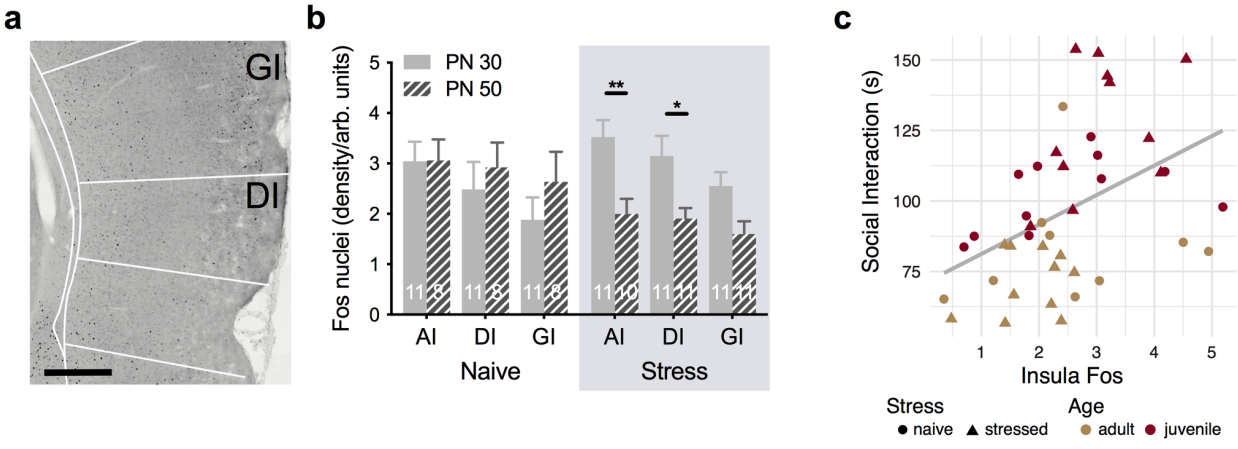


Figure 3.2 Fos expression in IC after one-on-one social interactions. (A) Representative digital photomicrograph containing IC subregions and Fos immunoreactive nuclei (black ovoid particles). Scale bar = 500mm. **(B)** Mean (+SEM, numbers indicate number of replicates) Fos immunoreactive nuclei by IC subregion (AI = Agranular, DI = Dysgranular, GI = Granular) quantified 90 min after social interaction

with a naïve PN 30, naïve PN 50, stressed PN 30 or stressed PN 50 conspecific (5 min test). Fos was found in all regions but there was an effect of stress in the PN 50 groups such that less Fos was evident in the PN 50 brains after stressed conspecific interactions than in PN 50 brains after naïve conspecific interactions, and there was less Fos in the AI and DI in the PN 50 Stressed group compared to the PN 30 stressed group ($F_{STRESS*AGE}(1, 36) = 6.21$, $p = 0.017$; $F_{SUBREGION}(2, 72) = 7.90$, $p = 0.001$). **(C)** Mean Fos immunoreactivity (pooled across IC subregions) predicted time spent in social interaction. A linear regression analysis with Age and Stress conditions included as moderators indicated that insula Fos levels predict social interaction (p <0.001, reflecting both the age and stress state of the conspecific. * $p < 0.05$, ** $p < 0.01$.

3.3.3 Optogenetic inhibition of IC disrupts social affective preference behavior.

The foregoing experiments provided compelling evidence that IC was involved in SAP behavior, but together did not provide strong causal evidence that each rat's preference behavior depended on IC. Therefore, we next employed optogenetic inhibition of IC in a within-subjects design of the SAP test. To this end, rats were transduced with halorhodopsin (eNpHr3.0) under the CamKII promoter (AAV5-CamKIIa-eNpHR3.0-mCherry), which allowed reversible neuronal silencing (Fig. 3.3 A). Two weeks after virus infusion, rats underwent SAP tests in a 2 by 2 by 2 design with conspecific Age (juvenile or adult conspecific), conspecific Affect (naïve or stress) and Light (OFF or light ON) as within-subjects factors (Fig. 3.3 B). Green light (ON) or no light (OFF) was delivered to the insula continuously during the SAP test. A 3-way ANOVA ($F_{AGE*STRESS*LIGHT}(1, 19) = 41.31$, $p < 0.001$) revealed, as expected, the majority of rats in the OFF condition exhibited preference to interact with the stressed juvenile ($p < 0.001$, 9 of 12 experimental rats, Fig. 3.3 C) and avoided the stressed adult ($p < 0.001$, 12 of 14 experimental rats; Fig. 3.3 D). The 5 rats that did not exhibit the expected preference pattern in light OFF were analyzed separately (Fig. 3.7 B).

Pairwise comparisons in the juvenile OFF condition identified an increase in interaction of the stressed juvenile compared to naïve juvenile ($p < 0.001$), whereas in the adult OFF condition there was a significant decrease in interaction with the stressed adult conspecific compared to the naïve adult conspecific ($p = 0.01$). In the light ON condition, there were no significant differences in exploration times between naïve and stressed conspecifics in either age condition (PN 30 $p = 0.22$ and PN 50 $p = 0.054$). Social interaction times were converted to a preference and a 2-way ANOVA ($F_{AGE*LIGHT}(1, 19) = 23.53$, $p = 0.0001$) revealed that optogenetic silencing of IC eliminated the preference for interaction with the stressed juvenile, and blocked the pattern of avoidance of stressed adult conspecifics ($F_{AGE*LIGHT}(1, 19) = 23.53$, $p = 0.0001$). No effect of optical stimulation was observed in sham transduced rats. Thus, silencing the IC prevented the expression of social affective behaviors.

Importantly, optical treatment had no effect on rats with sham transfections when interacting with PN 30 conspecifics (Fig. 3.4 E), and light condition did not influence exploratory activity or general behavior in the SAP test (Fig. 3.4 E). After testing, rats were perfused and we verified that virus expression was found in bilateral IC. Viral transfer was validated in whole cell recordings of mCherry-positive neurons in acute slices containing IC. Application of green light (wavelength = 532nm, 10mW/mm^2) through the objective of the electrophysiology microscope induced robust hyperpolarizations which silenced spiking when provided during a train of evoked spikes, scale bar 50mV/250ms (Fig. 3.4 C & D).

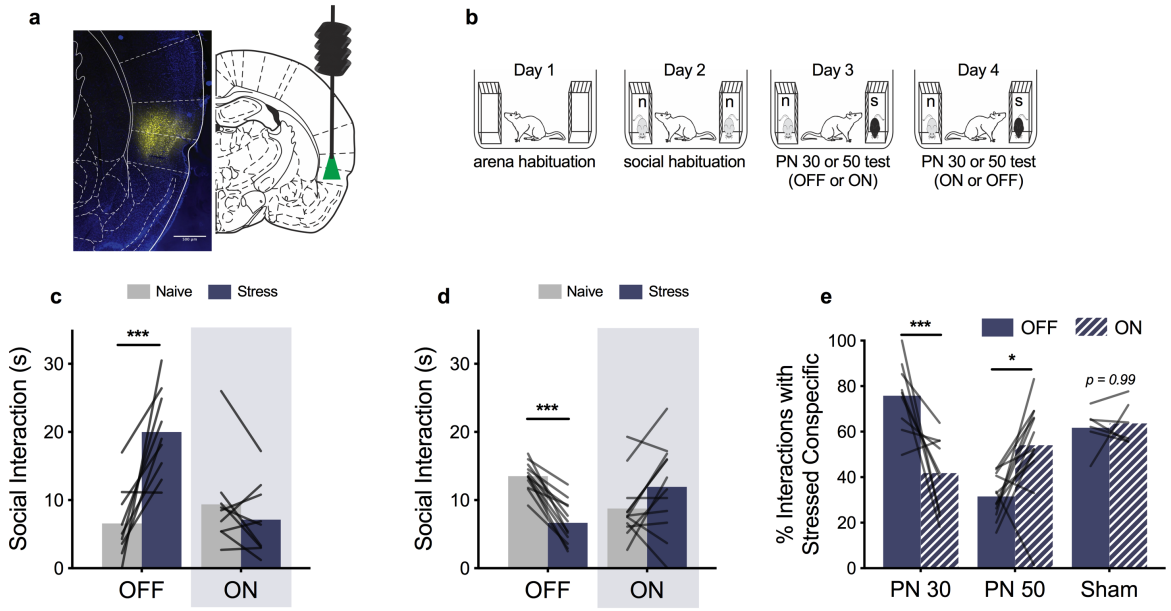


Figure 3.3 Optogenetic Inhibition of IC during SAP testing. (A) Native mCherry expression in the IC (false colored yellow) from a brain slice adjacent to one containing the cannula tract. (Scale bar = 500mm). Atlas diagram redrawn with permission from (Paxinos & Watson, 1998) **(B)** Diagram of SAP tests for optogenetic experiments. **(C)** Mean (n = 9) time spent interacting with PN 30 juvenile conspecifics or **(D)** PN 50 adult conspecifics (n = 12) on Days 3 and 4 of the SAP test. In the light OFF condition, the experimental adult spent significantly more time interacting with the stressed PN 30 conspecific but this pattern was abolished in the light ON condition ($F_{AGE*STRESS*LIGHT}(1, 19) = 41.31$, $p < 0.001$). In the light OFF condition, the experimental adult spent significantly less time interacting with the stressed PN 50 conspecific but this pattern was reversed in the light ON condition. **(E)** Data from C and D converted to percent preference for interaction with stressed conspecifics. Here a clear age by light interaction is apparent with optogenetic silencing of IC eliminating preference for interaction with the stressed juvenile, and blocking the pattern of avoidance of stressed adult conspecifics ($F_{AGE*LIGHT}(1, 19) = 23.53$, $p = 0.0001$). No effect of optical stimulation was observed in sham transduced rats. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

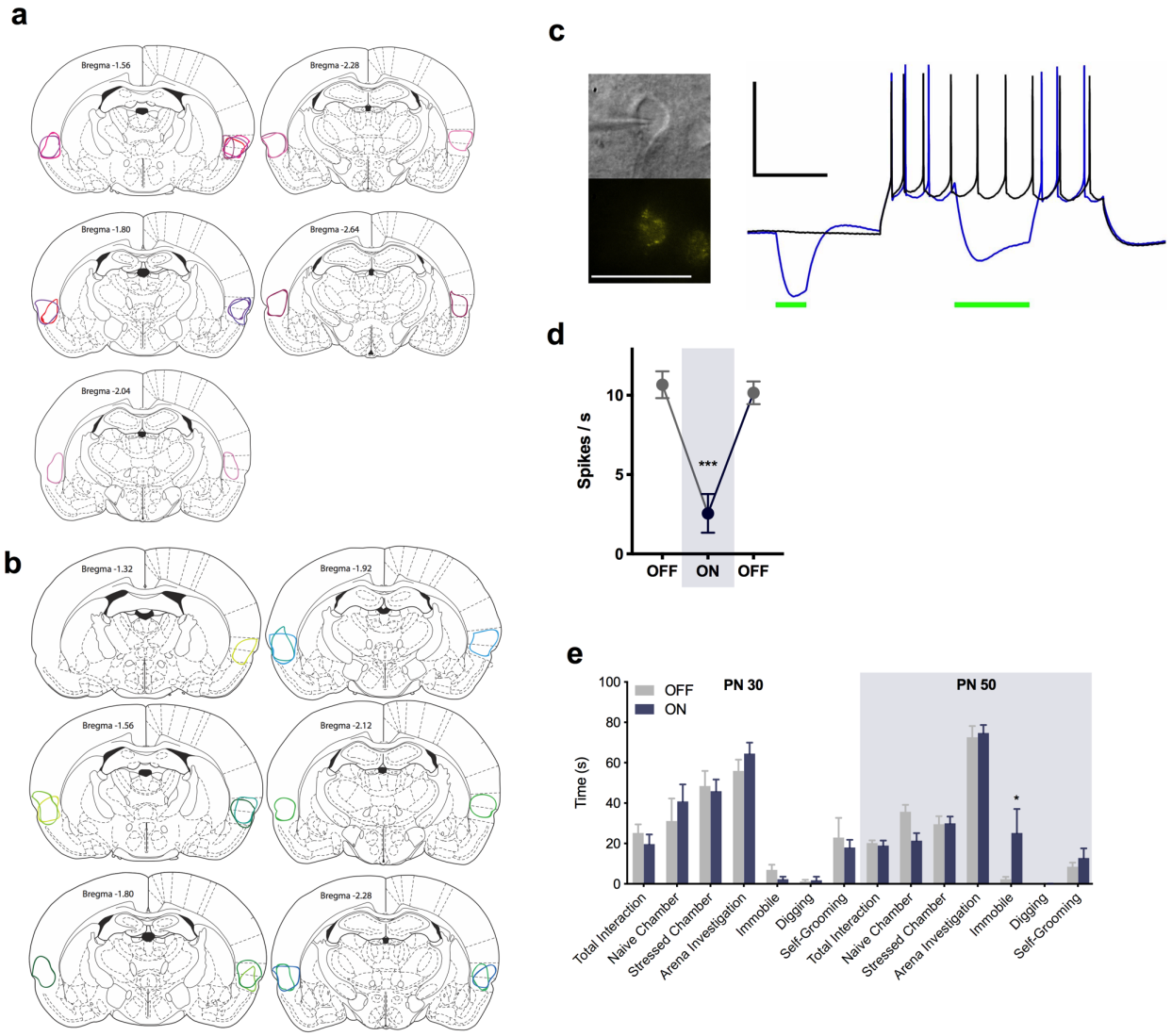


Figure 3.4 Verification of Halorhodopsin transductions and behavioral analysis in SAP testing. Placement of AAV5-CamKIIa-eNpHr3.0-mCherry transfections in experimental adult rats given SAP tests with **(a)** PN 30 juvenile conspecifics or **(b)** PN 50 conspecifics. Different colored traces correspond to individual replicates. In each case the tip of the fiber optic cannula was found within the IC and either directly above or within the area marked as containing mCherry (unamplified) fluorescence. Atlas drawings reproduced with permission from Paxinos & Watson (1998). **(c)** Viral transfer was validated in whole cell recordings of mCherry (above DIC image, below mCherry

false colored yellow. 40x, Scale bar = 40m) positive neurons in acute slices containing IC. **(d)** Mean (+/- SEM) spikes during sequence of OFF/ON/OFF application as in panel (C), n = 6 cells, one-way repeated measures ANOVA, ($F(2, 15) = 22.82$, $p < 0.0001$ with the spike rate in the ON condition significantly less than either off condition, ***$p < 0.0001$ (Sidak). **(e)** Mean (+SEM) time spent in different behaviors of adult conspecifics in optogenetic experiment (PN 30, n=9, PN 50, n=11). Behavior levels are equal between light ON and OFF conditions except for increased immobility in the PN 50 light ON condition, Age by Light by Behavior interaction, $F(4, 60) = 3.01$, $p = 0.025$. *$p = 0.001$ (Sidak). The increase in immobility is likely due to 2 of 11 rats that exhibited very high immobility (25-26s). *** $p < 0.001$

3.3.4 Oxytocin in the insular cortex is necessary for both approach and avoidance responses to stressed conspecifics.

As discussed, IC contains a dense distribution of oxytocin receptors relative to surrounding cortical areas (Dumais et al., 2013), and OT is widely implicated in myriad social behaviors. Therefore, we next determined whether insular cortex OTRs contribute to the prosocial or asocial behaviors in the SAP test. Experimental rats (n = 20) were implanted with bilateral cannula guides to IC. After recovery, the rats underwent the SAP procedure, with preference tests on days 3 and 4 in a repeated measures design. This allowed for a 2 by 2 by 2 experimental design with conspecific Age (PN 30 vs. PN 50) as a between-subjects factor, and with Drug (Vehicle or OTRa 20ng/side) and conspecific Stress (stress or naïve) as within-subjects factors (Fig. 3.5 A). The rats received microinjections of the oxytocin receptor antagonist (OTRa) 15 min before SAP tests on days 3 and 4, with drug order counter balanced. The conspecific stimuli were always unfamiliar and no effect of test order was apparent. Two subjects in the PN 50 condition with misplaced cannula were excluded (Fig. 3.7 A). Fig. 3.5 B shows the time spent interacting with the naïve and stressed conspecifics and Fig. 3.5 C shows the time spent interacting with the stressed conspecifics as a percent of total interactions. In

the vehicle condition, 7 of 10 experimental rats exhibited a preference for stressed over unstressed naïve juveniles and 7 of the 8 experimental rats exhibited a preference for unstressed naïve over stressed adults. Rats that did not exhibit preference in the vehicle condition were analyzed separately (Fig. 3.7 B). There was a significant Age by Drug by Affect interaction ($F_{AGE*DRUG*STRESS}(1, 12) = 31.84$, $p < 0.001$) with significantly greater time spent investigating the stressed PN 30 conspecific (vs. the naïve PN 30 conspecific, p <0.001) and less time investigating the stressed PN 50 conspecific (vs. the naïve PN 50 conspecific) in the vehicle conditions (p < 0.001) but more time interacting with the naïve PN 30 conspecific ($p = 0.06$, approaching significance in the opposite direction of vehicle behavior) and less time with the naïve PN 50 conspecific (p = 0.014) in the OTRa condition.

Percent preference scores (Fig. 3.5 C) revealed an Age by Drug interaction ($F_{AGE*DRUG}(1, 12) = 26.38$, $p < 0.001$) with opposing effects of OTRa in experimental rats' behavior towards PN 30 ($p = 0.011$) and PN 50 ($p = 0.002$) groups, respectively. Thus, blockade of IC OTRs prevented the age-dependent approach and avoidance behavior in response to stressed conspecifics in the SAP test.

A separate set of rats (N = 16) was surgically implanted with bilateral IC cannula as above, and after recovery, rats were randomly assigned to one-on-one interactions with either a stressed PN 30 juvenile or a stressed PN 50 adult conspecific (between-subjects) 15 min after vehicle or OTRa injections (within-subjects) in a 2 by 2 design; drug order was counter balanced by day as above (Fig. 3.5 D). One experimental rat interacting with adult conspecifics had a misplaced cannula and was excluded. ANOVA revealed a main effect of Age and Age by Drug interaction ($F_{AGE}(1, 13) = 28.66$, $p <$

0.001; $F_{AGE*DRUG}(1, 13) = 32.56$, $p < 0.001$). The main effect of age indicates more interaction time with the stressed juveniles than the stressed adults. OTRa microinjection reduced the time spent interacting with the stressed PN 30 conspecifics ($p < 0.001$) and increased the time interacting with stressed PN 50 conspecifics ($p = 0.009$) in the one-on-one tests (Fig. 3.5 E). In a separate cohort of animals that underwent one-on-one tests with PN 30 conspecifics after vehicle or OTRa injections in IC as above, we report that OTRa did not reduce overall levels of social exploration $t(15) = 1.61$, p = 0.13 (2-tailed). This supports that the effects of OTRa specifically mediated the preference for a given conspecific.

We next determined whether intra-insular OT administration was sufficient to increase or decrease experimental rat interaction with non-stressed PN 30 or PN 50 conspecifics, respectively. Bilateral insular cannula implants were made in 32 rats and after recovery each received 2 one-on-one social interaction tests (3 min duration) with unfamiliar naïve PN 30 or PN 50 conspecifics. A within-subjects design was used such that each rat received vehicle injections prior to one test and OT (250 pg/side; equivalent to 500nM) before the other. Injections were made 15 min before testing and drug/vehicle treatment order was counterbalanced. One rat from each age group did not receive injections and was removed from analysis. ANOVA revealed a significant interaction ($F_{AGE*DRUG}(1, 28) = 30.08$, $p < 0.0001$) and post hoc comparisons revealed that OT microinjection increased time spent interacting with the naïve PN 30 conspecific ($p < 0.001$) and decreased time with the naïve PN 50 conspecific ($p = 0.002$, Fig. 3.5 F). These findings suggest that the effect of OT in IC is dependent upon conspecific age.

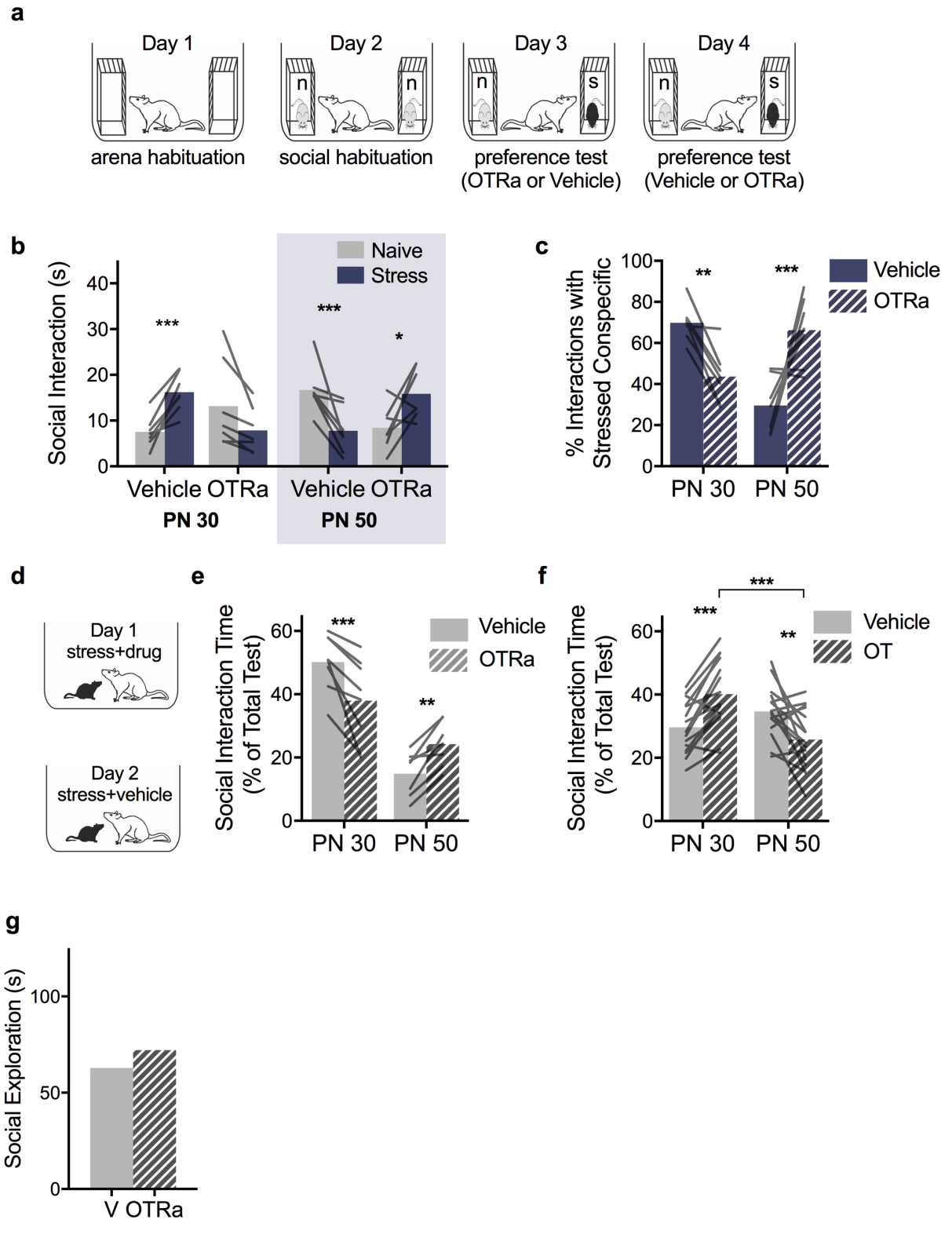

a
Day 1
Day 2
Day 3
Day 4
n
n
n
s
n
s
arena habituation
social habituation
preference test
(OTRa or Vehicle)
preference test
(Vehicle or OTRa)
b
Social Interaction (s)
Naive
Stress
***
***
*
Vehicle OTRa
Vehicle OTRa
PN 30
PN 50
c
% Interactions with
Stressed Conspecific
**
***
Vehicle
OTRa
PN 30
PN 50
d
Day 1
stress+drug
Day 2
stress+vehicle
e
Social Interaction Time
(% of Total Test)
***
**
Vehicle
OTRa
PN 30
PN 50
f
Social Interaction Time
(% of Total Test)
***
***
**
Vehicle
OT
PN 30
PN 50
g
Social Exploration (s)
V OTRa

Figure 3.5 Oxytocin in IC mediates SAP behavior. (A) Diagram of experimental design. **(B)** Mean (individual replicates) time spent exploring either PN 30 (n = 7) or PN 50 (n=7) conspecifics after bilateral intra-insula infusion of a selective OTR antagonist (OTRa, 20ng/side) in the SAP test. Vehicle treated experimental adult rats spent more time interacting with the stressed PN 30 juvenile conspecifics and less time with the stressed PN 50 adult conspecifics. These trends were blocked and reversed by infusion of OTRa ($F_{AGE*DRUG*STRESS}(1, 12) = 31.84$, $p < 0.001$). **(C)** Data in (B) expressed as percent preference for interaction with the stressed conspecific (Mean with individual replicates). OTRa significantly reduced preference for the stressed PN 30 while increasing time spent with the stressed PN 50 conspecific ($F_{AGE*DRUG}(1, 12) = 26.38$, $p < 0.001$). **(D)** Diagram of 1-on-1 social interaction tests with stressed conspecifics and pretreatment with either vehicle or OTRa. **(E)** Mean (with individual replicates, normalized as percent of 3 to 5 min long test, time spent interacting with the stressed conspecific in a 1-on-1 test; PN 30: $n = 8$, PN 50: $n = 7$). OTRa significantly reduced time interacting with the stressed PN 30 conspecific but increased time interacting with the stressed PN 50 conspecific ($F_{AGE}(1, 13) = 28.66$, $p < 0.001$; $F_{AGE*DRUG}(1, 13) = 32.56$, $p < 0.001$). **(F)** Mean (with individual replicates) time spent interacting with a naïve conspecific after intra insular cortex OT (250pg/side) or vehicle administration in a 1-on-1 social interaction (PN 30: $n = 15$; PN 50: $n = 15$). OT caused a significant increase in social interaction with naïve PN 30 juveniles but a significant decrease in interaction with naïve PN 50 adults ($F_{AGE*DRUG}(1, 28) = 30.08$, $p < 0.0001$). **(G)** Mean time spent exploring a naïve PN 30 conspecific during a 3 min one-on-one test after injections of vehicle of OTRa, order counterbalanced. There was no effect of OTRa on social interaction $t(15) = 1.61$, p = 0.13 (2-tailed). $^{**}p < 0.01$, $^{***}p < 0.001$.

3.3.5 Oxytocin alters the excitability of the insular cortex and requires Protein Kinase C

Considering that OT is a neuromodulator, the foregoing suggested the possibility that behavioral responses to stressed conspecifics depend upon modulation of intrinsic excitability in the IC by OT. Therefore, we predicted that interference with insular cortex PKC, a downstream target of OT intracellular signaling (Blume et al., 2008), would mimic the effect of OTRa. Rats received bilateral IC cannula implants and underwent SAP tests as above, following microinjections of either vehicle (10% DMSO in water) or the pan-PKC antagonist Gö 6983 (0.5uL/side 200nM). Eight of 10 rats exhibited preference for the stressed PN 30 conspecific after vehicle injection and 7 of 11 rats avoided the stressed PN 50 conspecific (Fig. 3.6 A). A 3-way ANOVA revealed a

significant interaction ($F_{STRESS*AGE*DRUG}(1, 13) = 63.75$, $p < 0.001$) in which Gö 6983 reversed the pattern observed under vehicle. In tests with PN 30 conspecifics, experimental rats spent more time interacting with the stressed than naïve conspecifics after vehicle injection ($p < 0.001$) and less time interacting with the stressed than naïve conspecific after Gö injection ($p = 0.009$). In tests with PN 50 conspecifics, the experimental rats spent less time interacting with the stressed conspecific in the vehicle condition ($p = 0.006$). Social exploration times were converted to a preference score for the stressed conspecific. Experimental rats displayed a significant reduction in preference for the stressed PN 30 conspecific after Gö 6983 ($p < 0.001$) whereas Gö 6983 increased the preference score for the stressed PN 50 conspecific (Fig. 3.6 B). These findings recapitulate the effects of OTRa on social affective preference behavior.

The foregoing suggested that exposure to stressed conspecifics triggers a neurobiological response that alters IC activity, and we next investigated the effect of OT application and Gö 6983 on synaptic efficacy. Input/output curves were conducted in acute IC slices on a perforated 60 channel multiple electrode array (Fig. 3.6 C & D). OT application induced a leftward shift (Fig. 3.6 E & G), with significantly larger fEPSP amplitude compared to baseline at stimuli from 2 to 5V with washout levels differing from OT beginning at 3V ($F_{STIMULUS}(10, 90) = 598.20$, $p < 0.0001$; $F_{DRUG}(2, 18) = 11.99$, $p < 0.001$; $F_{STIMULUS*DRUG}(20, 180) = 11.34$, $p < 0.0001$). In the aCSF control slices synaptic responses were stable across all phases of the experiment. In the presence of Gö 6983 (200nM), OT (1μM) did not alter fEPSP input/output curves (Fig. 3.6 F & G; $F_{STIMULUS}(10, 80) = 385.90$, $p < 0.0001$). Thus, OT augmented evoked excitatory synaptic transmission.

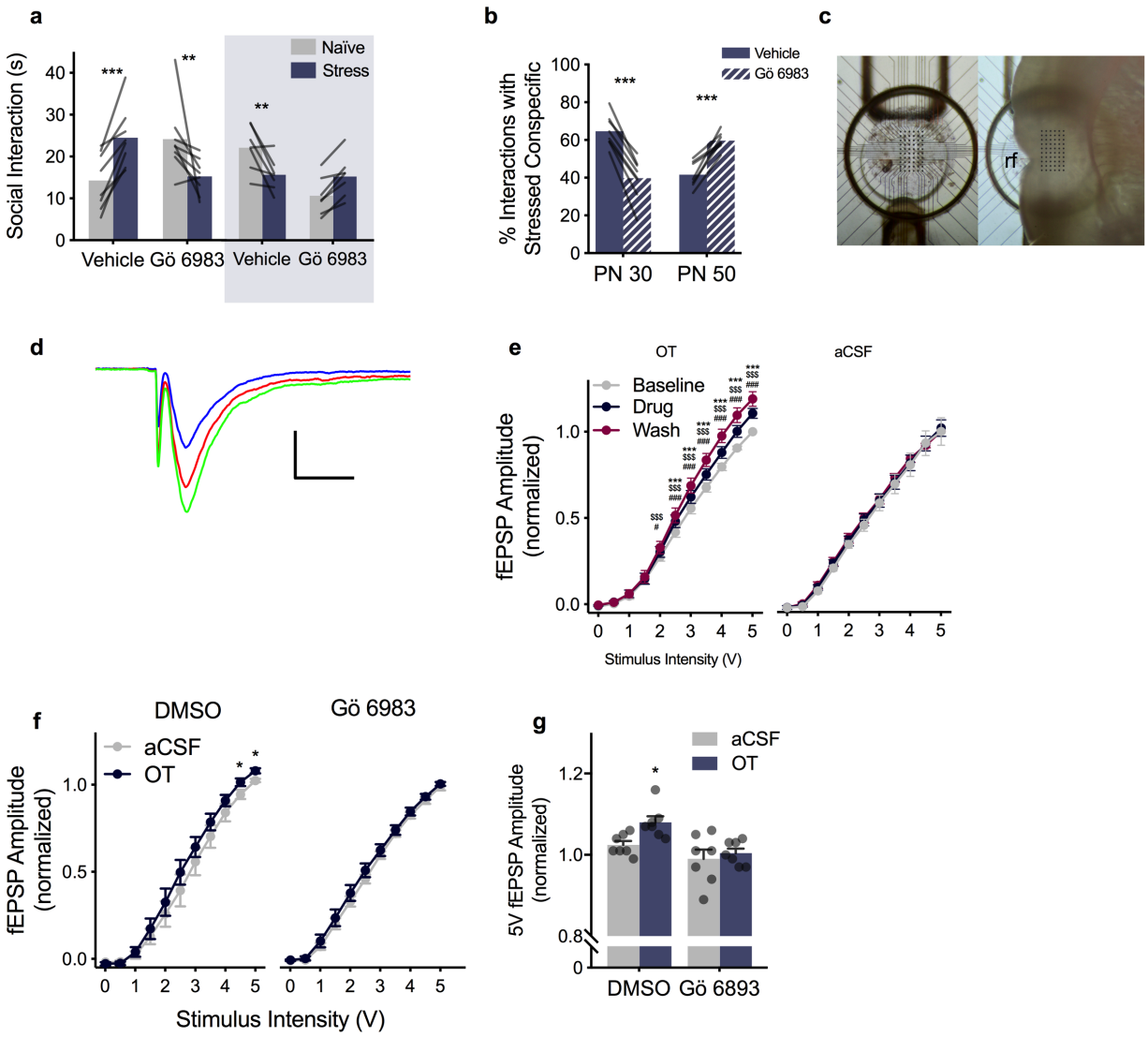


Figure 3.6 Oxytocin modulates excitatory synaptic transmission in the IC, which is prevented by pharmacological blockade of Protein Kinase C. **(A)** Mean (individual replicates) time spent exploring either PN 30 (n = 8) or PN 50 (n = 7) conspecifics after intra-insula infusion of Gö 6983 (0.5uL/side 200nM) or vehicle (10% DMSO in water) in the SAP test. Vehicle treated experimental adult rats spent more time interacting with the stressed PN 30 juvenile conspecifics and less time with the stressed PN 50 adult conspecifics. These trends were blocked and reversed by the PKC inhibitor ($F_{STRESS*AGE*DRUG}(1, 13) = 63.75$, $p < 0.001$). **(B)** Data in (A) expressed as percent preference for interaction with the stressed conspecific (Mean with individual replicates). Gö 6983 significantly reduced preference for the stressed PN 30 while increasing time spent with the stressed PN 50 conspecific ($F_{AGE*DRUG}(1, 13) = 141.10$, $p < 0.001$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (Sidak). **(C)** Top view of 60 channel perforated MEA (left) for acute extracellular recordings of IC. Right depicts location of IC slice during recording, rf = rhinal fissure. **(D)** Typical fEPSPs evoked by biphasic

extracellular stimulation at baseline (blue) during application of 500nM OT (red) and after washout (green). Scale bar 500mV/ms. **(E)** Input/output curves for fEPSPs (Mean +/- SEM, OT n = 10, aCSF n = 9 slices) normalized to the peak amplitude observed in response to 5V stimulation under baseline conditions. OT significantly increased EPSP amplitude beginning at 2V with further enhancement during the washout ($F_{STIMULUS}(10, 90) = 598.20$, $p < 0.0001$; $F_{DRUG}(2, 18) = 11.99$, $p < 0.001$; $F_{STIMULUS*DRUG}(20, 180) = 11.34$, $p < 0.0001$). Without application of OT, EPSPs remain stable across the duration of the experiment (aCSF: $F_{STIMULUS}(10, 80) = 385.90$, $p < 0.0001$). # $p < 0.05$ OT vs. Baseline, ### $p < 0.001$ OT vs. Baseline, $$$ $p < 0.001$ Wash vs. Baseline, *** $p < 0.001$ Wash vs. OT (Sidak). **(F)** Input/output curve for fEPSPs (Mean +/- S.E.M., n = 7 slices/condition) normalized to the peak amplitude observed in response to 5V stimulation under baseline conditions. OT (1μM) increased fEPSP at 4.5 and 5mV while no effect was observed in the presence of Gö 6983. *$F_{STIMULUS*DRUG}(10, 240) = 3.40$, $p < 0.001$, OT vs. aCSF at 4.5 and 5V, $ps < 0.031$ (Sidak). **(G)** Mean (+ S.E.M. and individual replicates) fEPSP amplitude from (G) at 5V ($F_{OT}(1, 24\text{-}) = 5.076$, $p = 0.034$). *OT-DMSO vs all other groups, $ps < 0.018$ (Fisher PLSD). *$p < 0.05$,**$p < 0.01$, *** $p < 0.001$.

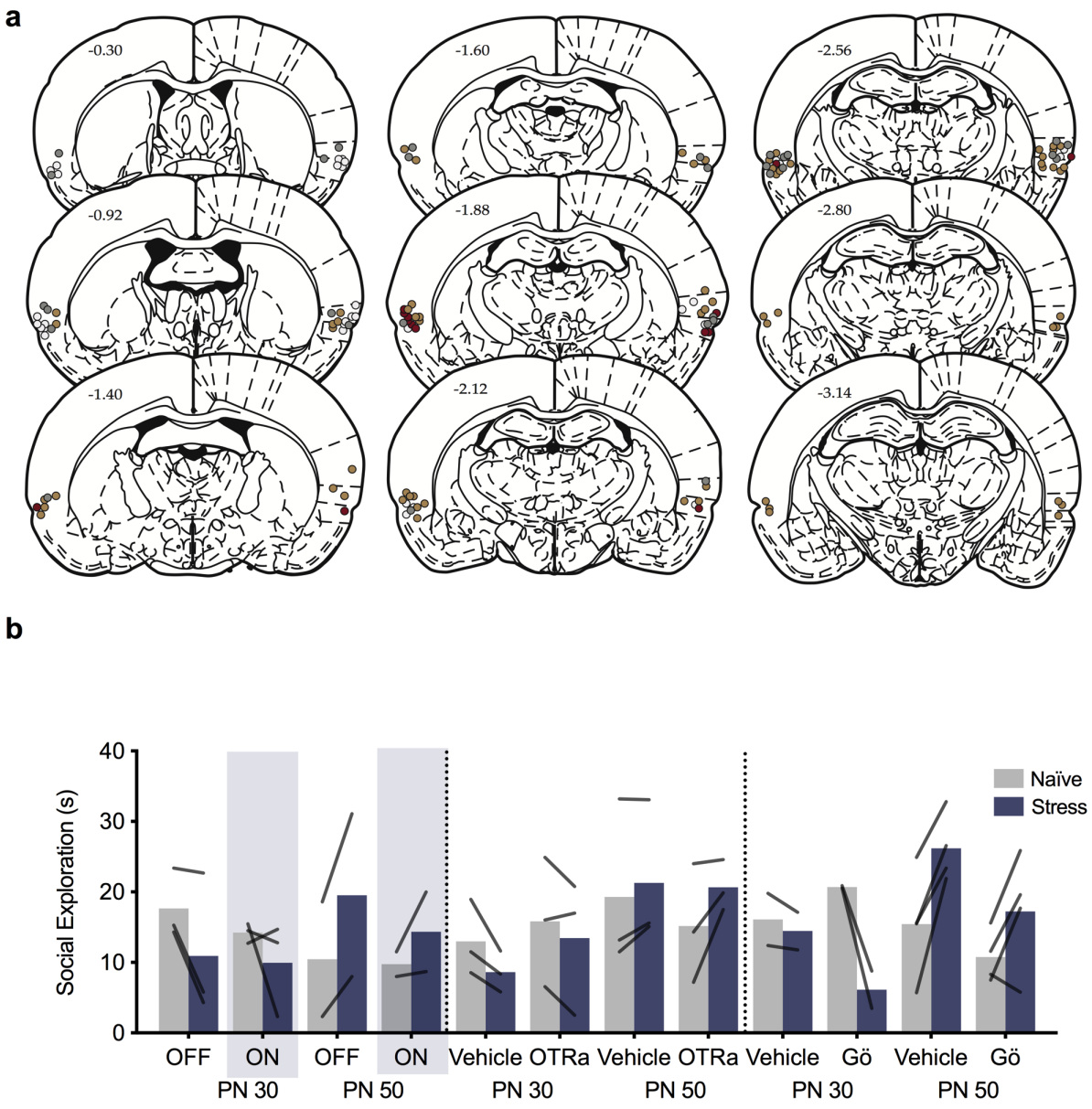


Figure 3.7 Placement of cannula tips for microinjection experiments and excluded subjects data. (A) Cannula tip placement within IC was verified in 40m fresh frozen sections stained with Cresyl Violet under light microscopy. Tip locations are indicated as compared to the atlas of Paxinos and Watson (1998) and images are reproduced with permission. Section distance from Bregma indicated in mm. Open/White Circles relate to Figure 3.5 B & C, Red circles relate to Figure 3.5 E and Gold circles to Figure 3.5 F, Grey circles related to Figure 3.6 A & B. **(B)** SAP test behavior from the rats excluded from mechanistic experiments because the baseline behavior (light OFF or vehicle condition) did not reflect the phenomena under study. LEFT: excluded from Figure 3.3C-D, CENTER: excluded from Figure 3.5B-C, RIGHT: excluded from Figure 3.6A-B.

Discussion

To summarize the behavioral studies, experimental adult rats preferred to interact with stressed juveniles, but avoided interaction with stressed adult conspecifics. Reversible inactivation of IC with muscimol blocked the experimental rats' preference to explore stressed juvenile conspecifics and naïve adult conspecific. This finding was corroborated with both optogenetic silencing of IC and pharmacological blockade of the OTR and PKC in IC, each of which interfered with experimental rats' responses to stressed conspecifics. Microinfusions of OT to IC was sufficient to reproduce the phenomena with naïve conspecifics. Exposure to stressed juvenile conspecifics elicited greater IC activation than social interactions with stressed adult conspecifics, which suggest IC has unique responses to conspecifics of different affective states. Further, the electrophysiology findings suggest that OT renders IC neurons more responsive to excitatory inputs via activation of PKC second messenger cascades. Together, these data suggest that exposure to a stressed conspecific evokes OT release within IC which, via modulation of IC output neuron excitability, orchestrates species-specific, age-dependent approach or avoidance behaviors. The findings provide new insight into the neural basis of elementary social affective processes and, consistent with human neuroimaging work, warrant consideration of how the insular cortex may contribute to social behaviors, possibly via interconnections with the group of brain structures that comprise the social decision-making network (O'Connell & Hofmann, 2011).

To approach or avoid a stressed conspecific may reflect 1) reflexive responses to social affective cues; perhaps adult and juveniles elicit different cues that convey different messages to the experimental adult rat, or 2) a social decision in which the

experimental rat determines the appropriate response after evaluating various factors like age and affect. While there is insufficient data to favor either possibility, how the experimental rat detects salient sensory information to either react or decide may depend on oxytocin in IC. In data not shown (Rogers-Carter et al., 2018b), OT altered several properties of IC neurons that are consistent with such a modulatory role, namely, reduction in AP amplitude, increase in R_{input}, increase in input/output relationships, reduction in sAHP and potentiation of evoked insular fEPSCs. Indeed, OT has been shown to increase spike output *in vivo* (Moaddab et al., 2015) and our data suggest an intrinsic mechanism that may underlie shifts in synaptic efficacy and excitatory/inhibitory balance found in cortical regions where OT is critical for myriad social behaviors (Marlin et al., 2015; Choe et al., 2015). Thus, one possibility is that OT may be necessary for, or facilitate more, IC neurons to fire in response to social affective cues, consistent with its known ability to increase salience in cortex (Choe et al., 2015). This idea is consistent with our Fos data in which stressed conspecifics elicited greater IC activity than naïve conspecifics. Further, conspecific age and affect may determine how much OT is released in IC and therefore drive either more or less IC activity depending on affective state. An interesting follow-up to explore if OT is necessary to observe different levels of Fos immunoreactivity would be to repeat the Fos experiment with a second cohort of animals, all of which receive microinfusions of an OT receptor antagonist before testing. If OT does in fact drive differential IC activity with the various conspecifics, blocking OT should prevent any different in Fos immunoreactive cells.

In this scenario, if social affective cues can increase insular OT and facilitate the amount of active IC neurons and the information they encode to efferent targets, this may be a mechanism by which baseline social behaviors responses (i.e. approach) are overridden in order to produce context-specific behaviors (i.e. avoidance). This can happen via the same of different outputs of IC, or via another possible explanation in which OT modulates the output of IC to different areas that promote either approach or avoidance. No experimental evidence yet supports this idea, but possibly insula efferents to the ventral striatum or prefrontal cortex promote interaction with stressed juveniles, whereas efferents to the basolateral amygdala promote avoidance in response to stressed adults as these are thought to be the proximal mediators of the rewarding, empathic, and emotional aspects of social behavior, respectively (Adolphs, 2009). Future work examining how oxytocin accomplishes modulation of cortical projections neurons is warranted to further interpret these findings.

The necessity of OTRs in the IC in this paradigm compliments an extensive literature that has explored OT as a modulator of social behaviors. However, the bulk of this work utilizes intranasal OT administration in humans and intracerebroventricular injections in animals, which do not provide anatomical specificity. Thus, further mechanistic exploration of OT in the IC is warranted and encouraged by advanced techniques, which are beginning to unveil region-and circuit-specific roles for OT. For example, OT is anxiolytic in the central amygdala where stimulation of an OT-ergic pathway reduced freezing to a conditioned fear cue (Knobloch et al., 2012).
OT appears to be anxiogenic, however, in the lateral septum where genetic overexpression of OTR enhanced stress-induced contextual fear conditioning following

social defeat (Guzmán et al., 2009). Circuit and region-specific roles for OT in social behaviors have also been identified using optogenetics. In (Choe et al., 2015) OT in the piriform cortex is necessary for both aversive and appetitive social learning and in (Oettl et al., 2016), optogenetic stimulation of OT in olfactory cortex increased social exploration and recognition via modulating the output tone of olfactory cortex. Regarding parenting behavior, activation of OTergic neurons in the left auditory cortex of female mice promoted retrieval of pups after enhancing pup call response (Marlin et al., 2015). Further, the effects of OT administration on social decision-making are sensitive to situational and interpersonal factors with OT sometimes producing prosocial effects, and at other times anti-social effects (Bartz et al., 2011), which were both evident after insular OT administration to IC in the SAP test. OT in the IC appears to modulate processing of social affective information providing yet another example of site and circuit specific functions of OT in organizing social behavior.

The interference of social affective behaviors by the OTR antagonist strongly suggests that OT acting in the IC is critical to this behavior. This finding prompts the question: where does the insular OT come from? One possible source is the periventricular nucleus of the hypothalamus (PVN), which is a known synthesis site of OT, projects to numerous forebrain regions, and is activated by social stimuli (Knobloch et al., 2012). Perhaps exposure to a stressed conspecific recruits OT to the IC by way of a novel OTergic circuit, which could modulate IC output. Several of our findings support the notion that the IC is sensitive to OT activity: OTR antagonism in the IC blocked social affective behavior, and administration of OT to the IC increased exploration with a juvenile conspecific and decreased exploration with an adult conspecific. PKC

antagonism abolished social affective behavior during the SAP test and it is possible that intracellular signal cascades modulate IC's sensitivity to social sensory inputs or modulate circuit activation. To this point, OT can activate both ERK1/2 (extracellular signal-regulated kinase 1/2) and PKC signaling, which drive numerous processes that underlie neuromodulation (Ayar et al., 2014; Blume et al., 2008; Van den Berg et al., 1999). If the output tone of the IC is indeed modulated by OT, this supports that this region is contributing to a larger social brain circuit.

The findings presented here underscore the clinical relevance of studying OT at the circuit level. The necessity of OT in this paradigm is consistent with a large literature implicating OT in social behaviors. In rodents, OT has been extensively attributed to pair bonding, social learning, approach and avoidance behaviors, and mother-infant bond formation (Choe et al., 2015; Harari-Dahan & Bernstein, 2014; Ross et al., 2009; Walum et al., 2012). There is a well-justified interest in the therapeutic power of OT considering abnormal levels of plasma OT are seen in populations with autism, schizophrenia, and Fragile X syndrome (Modahl et al., 1998) and these disorders are plagued with social abnormalities. Genetic polymorphisms in the OT receptor (OTR) are risk factors for autism spectrum disorder symptoms like social deficits (LoParo & Waldman, 2015) and antisocial behavior (Waller et al., 2016). Interestingly, administration of OT in humans can attenuate these social deficits (Auyeung et al., 2015; Guastella et al., 2015; Shin et al., 2015; Young & Barrett, 2015), increase sense of trust (Kosfeld et al., 2005), and decrease anxiety (Van den Berg et al., 1999) which is relevant considering that the anxiolytic mechanisms of OT are likely related to its prosocial effects. However, without an anatomical and mechanistic account of OT in the context of component social

behaviors the translatability of OT research intro treatment will be slowed (Insel, 2010; Radke & de Bruijn, 2015). Our results suggest that disruption of IC function may be key to pathophysiology in behaviors that depend upon social decisions regarding the emotions of others. The SAP test and the network model of IC input to the SDMN presented here provide a platform for further research into the neuroanatomical and physiological systems that integrate social information with decision-making and behavior.

Chapter 4: Insular Cortex Projections to the Nucleus Accumbens Core Mediate Social Approach to Stressed Juvenile Rats

The work presented in this chapter is currently under review:

Rogers-Carter, M.M., Djerdjaj, A., Gribbons, K.G., Varela, J., Christianson, J.P. (2019). Insular cortex projections to the nucleus accumbens core mediate social approach to stressed juvenile rats. Under Review.

4.1 Introduction

Social decision-making enables animals to respond to their social environments with flexible and context-appropriate behaviors (Insel & Fernald, 2004a; O'Connell & Hofmann, 2011). An important feature of social decision-making is that animals appraise the affective state of others to generate behavioral responses. Accordingly, a number of studies report that rodents demonstrate either prosocial or avoidance behaviors toward distressed others (reviewed by Meyza et al., 2017). These responses are modulated by situational factors, including familiarity, age, social rank, sex and prior experience (Burkett et al., 2016; Guzmán et al., 2009; Ishii et al., 2012; Kiyokawa et al., 2014; Langford et al., 2006; Li et al., 2014; Meyza et al., 2017; Rogers-Carter et al., 2018a; Rogers-Carter et al., 2018b), which suggest that socioemotional information is integrated with context and situational factors to inform behavioral actions. However, how areas in the brain that integrate affective cues, external stimuli and physiology can inform behavioral responses remains elusive.

Mechanistic studies have identified several structures in the social decision-making network (SDMN; O'Connell & Hofmann, 2012) that produce flexible behavioral responses during social interactions (Felix-Ortiz et al., 2016; Hong et al., 2014; Lee et al., 2014; Yang et al., 2017a; Yao et al., 2017). However, none of these regions are equipped to directly detect the socioemotional cues by which animals communicate their affective state, such as vocalizations and olfactory cues. One candidate region that may detect socioemotional cues is the insular cortex (IC), a site of multisensory integration (Gogolla et al., 2014; Gogolla, 2017; Rodgers et al., 2008) which a growing literature implicates in social cognition (Menon & Uddin, 2010). In addition to detecting

social cues, social decision-making utilizes reward valuation to determine behavioral responses, and socioemotional information is likely one factor that influences reward valuation and behavior during healthy and disordered social cognition (Kohls et al., 2012). The nucleus accumbens core (NAc) is implicated in reward (Wise, 2002) and IC provides a major cortical input to the NAc (Wright & Groenewegen, 1996). Thus, there is compelling evidence to investigate the IC → NAc projection as a possible pathway by which socially relevant sensory information reaches the reward system to inform social decisions.

We have reported a role for insular cortex in a social affective preference (SAP) test in which rats demonstrate age-specific responses to stressed others (Rogers-Carter et al., 2018b). Specifically, in the SAP test, experimental rats exhibit approach toward stressed juvenile rats, but avoid exploring stressed adult rats. Optogenetic silencing of IC pyramidal neurons abolished this pattern. While the role of NAc in control of behavior during interactions with stressed individuals is unknown, it is well established that the nucleus accumbens (NAc) is critical to a number of social behaviors. NAc mediates social recognition (Ploeger et al., 1991) and reward with juvenile (Trezza et al., 2011) and adult conspecifics (Dölen et al., 2013). Regarding emotional cues, NAc encodes ultrasonic vocalizations calls that convey stress (Willuhn et al., 2014) and shows enhanced dopaminergic transmission in subjects that observe a conspecific receive footshock (Wu et al., 1999), which suggests the NAc encodes the socioemotional cues present during interactions with stressed others.

In the SAP paradigm, we hypothesize that IC is integral to the binding of social and situational factors to inform social decisions which, via glutamatergic efferents to

nodes in the social decision making-network, can shape the pattern of circuit activity to favor approach or avoidant behavioral strategies. No prior mechanistic studies have explored the role of IC projection tracts in social behavior. Here we employed pharmacological and tract-specific manipulations to test if the IC → NAc projections are necessary for SAP behavior. We report that this tract is necessary and sufficient for approach toward stressed juvenile conspecifics, but not avoidance of stressed adult conspecifics.

4.2 Materials and Methods

4.2.1 Overview of approach

To determine the role of the IC → NAc tract in social affective behavior we performed a series of experiments. First, to test the role of the NAc we used reversible inactivation by infusing tetrodotoxin (TTX) directly to NAc before SAP tests with either post-natal day 30 (PN 30) juvenile or PN 50 adult conspecifics. Next, we used retrograde tracing and Fos immunohistochemistry to quantify activity in IC → NAc neurons in experimental rats who underwent social interactions with either stressed or naïve juveniles. The cell counting results suggested that activity in this tract increases during encounters with stressed juveniles in the posterior IC. To establish the necessity and sufficiency of this tract to the social affective preference behavior we chose to use a tract-specific chemogenetic approach (Jaramillo et al., 2018b). This required that we first demonstrate feasibility of chemogenetic manipulations in the SAP test. To this end we virally expressed the inhibitory hM4Di receptor (Roth, 2016) in the IC and achieved inhibition via systemic administration of clozapine-n-oxide (CNO), which blocked the

social preference for stressed juveniles and replicated our prior findings with pharmacological and optogenetic inhibition of IC (Rogers-Carter et al., 2018b). Following methods and considerations described by others (Mahler & Aston-Jones, 2018; Smith et al., 2016; Stachniak et al., 2014)
to gain tract-specific, chemogenetic control of the IC → NAc circuit, we introduced hM4Di or hM3Dq in the IC under the synapsin promoter to achieve receptor expression in axon terminals in the NAc. CNO was delivered via bilateral cannula to the NAc, so that excitation or inhibition was selective to terminals of neurons that originate in IC, prior to SAP or conventional social interaction testing. A number of control, verification and chemogenetic specificity experiments are also described to address important concerns that relate to the interpretation of chemogenetic experiments.

4.2.2 Subjects

Male Sprague-Dawley rats were purchased from Charles River Laboratories (Wilmington, MA) at either 250g, or postnatal (PN) day 22 or 42. All rats were acclimated to the vivarium in the Boston College Animal Care facility for a minimum of 7 days before surgery or behavioral testing. Subjects were housed in groups of 2 or 3, maintained on a 12h light/dark cycle, and provided food and water *ad libitum.* Behavioral testing occurred 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 *Guide for the Care and Use of Laboratory Animals.*

4.2.3 Social affective preference tests

As previously reported (Rogers-Carter et al., 2018b), the SAP test allows for the quantification of social exploration directed at two novel conspecifics, one of which is stressed via footshock and the other naïve to stress treatment. On day 1, experimental rats were acclimated to a plastic tub cage arena (50 x 40 x 20cm; L x W x H) containing beta chip bedding and a wire lid. On day 2, the experimental rats were presented two naïve conspecifics, either juvenile (PN 28-32) or adult (PN 50-54) pairs which were held in clear acrylic chambers (18 x 21 x 10cm; L x W x H). Conspecific chambers were comprised of acrylic rods spaced 1 cm apart center-to-center (Fig. 4.1 C) and placed at opposing ends of the cage for a 5 min trial. The chambers were designed to be large enough to permit the contained rat to move freely. On days 3 and 4, one conspecific was stressed via 2 brief footshocks immediately prior to each test (5s duration, 1mA, 60s inter-shock-interval; Precision Regulated Animal Shocker, Coulbourn Instruments, Whitehall, PA). SAP tests began when the chambers containing naïve and stressed conspecifics were placed in the test arena. A trained observer blind to treatment quantified the amount of social exploration initiated by the experimental rat which included: nose-to-nose contact, nose-to-body contact or reaching towards the conspecifics. Social exploration was quantified first during live testing and again from digital video recordings by a second observer who was blind to experimental conditions to establish inter-rater-reliability. The correlation between observers for the SAP trials included in the current experiments was $r_{35} = 0.922$, $R^2 = 0.849$, $p < 0.0001$.

4.2.4 One-on-one social interactions

This procedure is used to quantify social exploration when an experimental rat and a target conspecific were free to interact. On day 1, experimental rats were habituated to a standard plastic cage with beta chip bedding and wire lid for 1 h. On day 2, a juvenile or adult conspecific was introduced to the arena for 5 min and a trained observer quantified social interaction initiated by the experimental rat including pinning, sniffing and allogrooming. Exploration was quantified first during live testing and again by an observer blind to treatment from digital video recordings. The correlation between observers for the social interaction tests included in the current experiments was r_{12} = 0.997, R^2=0.993, $p < 0.0001$.

4.2.5 Cannula placements and virus microinjections

Under inhaled isoflurane (2-5% v/v in O_2), bilateral cannula (Plastics One) were implanted in the NAc (from Bregma: A/P +1.9mm, M/L +/- 1.8mm, D/V -6.5mm) and fixed in place with acrylic cement and stainless steel screws. For chemogenetic manipulations, 600nl of a viral vector containing either pAAV5-hSyn-hM4D(Gi)-mCherry (hM4Di; Addgene viral prep; Cat. No: 50475-AAV5; titer: $9x10^{12}$GC/mL), pAAV5-hSyn-hM3D(Gq)-mCherry (hM3Dq; Addgene viral prep; Cat. No: 50474-AAV5; titer: $4.8x10^{12}$GC/mL), or pAAV5-hSyn-mCherry (mCherry; Addgene viral prep; Cat. No: 114472), were microinjected bilaterally at 2 sites in the posterior insular cortex (from bregma: A/P –1.8mm and –1.6mm, M/L +/ – 6.5mm, D/V –6.9mm) at 100nl/minute and allowed 7 min for diffusion. These coordinates led to transduction within the posterior IC for consistency with our prior work and the results of the retrograde Fos counting

experiment. In tract-specific chemogenetic manipulations, bilateral NAc cannula were implanted during the same surgery as described above. This approach allowed tract-specific control by directly infusing the hM3Dq and hM4Di agonist CNO to the terminals of IC neurons in NAc (see pharmacological manipulations). For retrograde tracing, 300nl of Cholera Toxin B conjugated to AlexaFluor 488 (CTb^{488}, Thermo Fisher, Cat. No: C34775) were microinjected to the right hemisphere NAc as previously (Dong et al., 2017; Park et al., 2018) at a rate of 100nl/minute and allowed 4 min for diffusion. After surgery, rats were administered meloxicam (1mg/kg, Eloxiject, Henry Schein) and the antibiotic penicillin (12,000 Units, Combi-pen, Henry Schein) and allowed 2 to 3 weeks to recover before behavioral testing as described below.

4.2.6 Pharmacological manipulations

To reversibly inhibit NAc activity the voltage gated sodium channel blocker tetrodotoxin (TTX; Tocris Bioscience) was dissolved in sterile 0.9% saline to a concentration of 10μM. TTX or vehicle (sterile 0.9% saline) were infused bilaterally (0.5μl/side) to NAc 15 min prior to SAP testing (Christianson et al., 2011). For chemogenetic inhibition of IC cell bodies, experimental rats were weighed on the day of testing and given 0, 0.3 or 3mg/Kg CNO (Tocris Bioscience) dissolved in a vehicle of 10% DMSO and sterile 0.9% saline 45 min prior to SAP testing via i.p. injection. For tract-specific experiments, 1μM of CNO in a vehicle of 0.1% DMSO and sterile 0.9% saline was infused through the NAc guide cannula (0.5μl/side) 30 min prior to testing as in (Mahler et al., 2014).

4.2.7 Tissue collection and immunohistochemistry

Immediately after testing, rats from TTX experiments were overdosed with tribromoethanol (Fisher Scientific), decapitated, brains were dissected and flash frozen in 2-methylbutane (Fisher Scientific). All other rats were overdosed with tribromoethanol and perfused with cold 0.01M heparinized phosphate buffered saline (PBS) followed by 4% paraformaldehyde as in (Rogers-Carter et al., 2018b). Dissected brains were stored at 4°C in 4% paraformaldehyde for 24 h and transferred to 30% sucrose for 2 days. 40µm coronal sections were cut on a freezing cryostat (Leica). Alternating NAc sections were either directly mounted to gelatin subbed slides and stained with cresyl violet for cannula tip verification or stored in cryoprotectant as free-floating sections for immunostaining. IC sections were directly mounted to slides and coverslipped with Vectashield (Vector Labs) to confirm virus transduction. To visualize Fos (as in (Rogers-Carter et al., 2018b)) or mCherry fibers in NAc, floating sections were quenched for endogenous enzymes with 3% H_20_2 and blocked with 2% normal donkey serum (Jackson ImmunoResearch) in PBS-T (0.01% Triton-X 100) and then incubated overnight in rabbit anti-c-Fos primary antibody (1:5000; EMD millipore; Cat. No: ABE457; Lot: 2987437) or the rabbit anti-mCherry primary antibody (1:200; Invitrogen; Cat. No: PA5-34974; Lot: 114936) for 2 hr. After, sections were washed in PBS-T and incubated in biotinylated donkey-anti-rabbit secondary antibody (1:200; Jackson ImmunoResearch; Cat. No: 711-035-152) followed by the avidin-biotin complex kit (ABC Elite Kit, Vector Labs) and visualized with chromogen precipitate (NovaRed, Vector Labs). Stained sections were mounted on slides, dehydrated, cleared and coverslipped with Permount (Fisher Scientific). For Fos counting, tiled images of NAc tissue were

acquired using a Zeiss AxioCam HRc digital camera through a 10x objective (N.A. 0.45) and Fos immunoreactive cells were manually counted in ImageJ by an observer blind to treatment. For tissue containing CTb^{488}, floating sections were washed in PBS-T, blocked in 5% normal goat serum in PBS-T, and incubated overnight in rabbit-anti-c-Fos primary antibody as above at 4°C, washed, incubated in the Dylight 549 goat-anti-rabbit secondary antibody (1:500; Vector Labs; Cat. No: DI-1549), and floated on slides and coverslipped with Vectashield. For cell counting, green (CTb^{488}) and red (Fos) channel tiled images of NAc were acquired using a Hamamatsu digital camera through a 10x objective (N.A. 0.45) and CTb^{488} and Fos-positive co-labeled cells were manually counted in ImageJ by a trained observer blind to treatment. Cells were counted within a 1250μm^2 area in IC and from a 350 μm^2 area in NAc.

4.2.8 Electrophysiology

To verify the efficacy of hM4di and hM3Dq on neural activity whole cell recordings were made in 300μm thick coronal sections a subset of rats at the conclusion of the behavioral experiments. Slice preparation, internal and external solutions, patch-clamp electrodes and current-clamp experiments and data analysis were conducted as previously (Rogers-Carter et al., 2018b). Briefly, whole cell recordings were achieved in cells visually identified as mCherry positive. Intrinsic excitability and input/output curves were determined in 3 replicates in normal artificial cerebrospinal fluid and again after 10 min of bath application of CNO (10μM as in (Venniro et al., 2017). Efficacy of CNO in hM4Di was evinced by hyperpolarization of resting membrane potential and reduction in action potentials generated by depolarizing

current injection. Efficacy of CNO in hM3Dq was evinced by depolarization of resting membrane potential and increase in action potential frequency in response to depolarizing current injection. CNO had no effect on cells that did not express mCherry.

4.2.9 Data analysis

Experimental treatments were counterbalanced in order and repeated measures designs were used except when noted. Social exploration times during SAP tests were compared using a 2-way ANOVA with conspecific stress (naïve or stressed) and drug treatment (vehicle or drug) analyzed as within-subjects factors. Data were visually inspected for normality; all data are depicted in the figures. To control for individual variation in sociality, exploration times were converted to a preference score, which was computed as the percentage of total social exploration time directed toward the stressed conspecific and analyzed with t-test or 2-way ANOVA, depending on experimental design. T-tests were used to compare social exploration in rats sacrificed for post mortem CTb, Fos, and CTb+Fos counts which were analyzed with mixed model ANOVAs with insula ROI as a repeated measure and conspecific stress as a between subject factor. All analyses were conducted in Prism 8.0 (GraphPad). Significant main effects and interactions were followed with Sidak post hoc comparisons to control experiment-wise type 1 errors to $p < 0.05$. Sample sizes were chosen from previous work (Rogers-Carter et al., 2018a; Rogers-Carter et al., 2018b) and all data were included unless cannula implants or virus transduction missed the target site.

4.3 Summary of Experiments and Results

4.3.1 Pharmacological inactivation of NAc abolished preference for stressed PN 30, but not PN 50, conspecifics.

To test if NAc was necessary for SAP behavior, experimental rats were implanted with bilateral NAc cannula (Fig. 4.1 A & B), allowed 1 week of recovery, and underwent SAP testing with either PN 30 (n = 9) or PN 50 (n = 7) conspecifics. Experimental rats were tested after vehicle and drug injections on days 3 and 4 of testing and the amount of time the experimental rat explored each conspecific was recorded; treatment order was counterbalance (Fig. 4.1 C). For rats tested with PN 30 conspecifics, we report a significant Conspecific Stress X Drug interaction ($F(1,8) = 18.49$; $p = 0.003$). Following vehicle treatment, rats tested with PN 30 conspecifics showed a preference to explore the stressed conspecific over the naïve ($p = 0.006$), which was abolished following bilateral pharmacological inactivation of NAc ($p = 0.203$, Fig. 4.1 D). For rats tested with PN 50 conspecifics, a 2-way ANOVA ($F_{AGE*DRUG}(1,6) = 0.071$, $p = 0.799$) confirmed replication our previous findings (Rogers-Carter et al., 2018a; Rogers-Carter et al., 2018b) that experimental rats prefer to explore the naïve conspecific over the stressed following vehicle treatment ($p = 0.031$), which was also maintained after TTX treatment ($p = 0.020$; Fig. 4.1 E). To compare the effect of TTX in NAc between age experiments, social exploration times were converted to percent preference scores and shown in Fig. 4.1 F for comparison. Because these experiments were not conducted simultaneously, the effect of TTX within each age condition was assessed with independent t-tests. We observed that preference scores from experimental rats tested with PN 30, but not PN 50 conspecifics, are sensitive to TTX

treatment: preference scores from experimental rats tested with PN 30 conspecifics are significantly greater in vehicle trials than TTX trials ($t(8) = 6.967$, $p = 0.0001$). However, TTX did not alter preference scores in experimental rats tested with PN 50 conspecifics ($t(6) = 0.804$, $p = 0.452$). This reflects that avoidance of stressed PN 50 conspecifics is maintained after TTX treatment (Fig. 4.1 F). These analyses demonstrate that the NAc is necessary for experimental rats' preference to explore stressed PN 30 but did not influence interactions with either stressed or naïve PN 50, conspecifics.

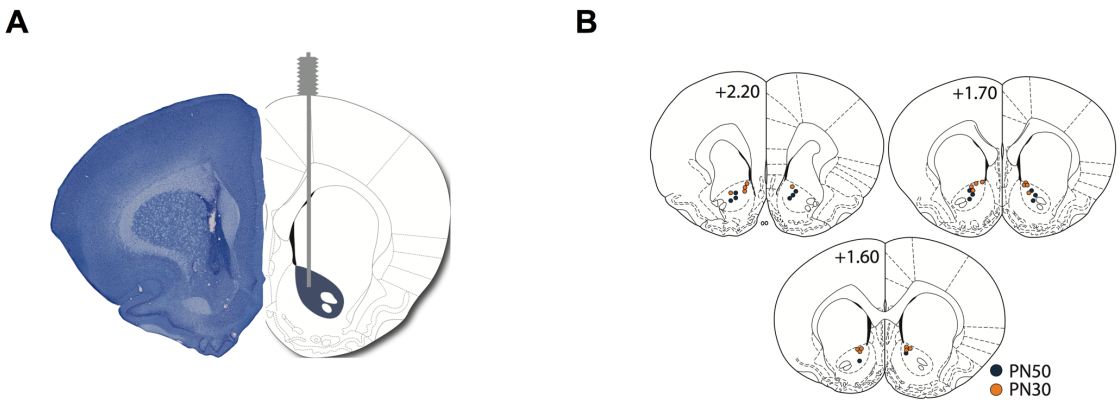

A
B
+2.20
+1.70
+1.60
PN50
PN30
C
Day 1
Day 2
Day 3
Day 4
n
n
n
s
n
s
arena habituation
social habituation
PN 30 or 50 test
(TTX or Vehicle)
PN 30 or 50 test
(TTX or Vehicle)
D
**
ns
Social Exploration (s)
with PN30 conspecifics
45
30
15
0
Naïve
Stressed
Vehicle
TTX
E
*
*
Soical Exploration (s)
with PN50 conspecifics
45
30
15
0
Naïve
Stressed
Vehicle
Drug
F
***
ns
% Preference for
Stressed Conspecific
100
80
60
40
20
0
Vehicle
TTX
PN30
PN50

Figure 4.1 Pharmacological blockade of NAc abolished the social affective preference for stressed PN 30, but not PN 50, conspecifics. **(A)** Representative image of a cannula tract in NAc (left) and corresponding rat brain atlas diagram (right). **(B)** Map of bilateral cannula tip placements in NAc from experimental rats tested with PN 30 (orange; n = 9) and PN 50 (blue; n = 7) conspecifics. **(C)** Diagram of SAP test procedure with naïve (n) and stressed (s) conspecifics. **(D)** Mean (with individual replicates) time spent exploring the naïve and stressed PN 30 conspecifics during the 5 min trial. A Conspecific Stress X Drug interaction was observed ($F_{STRESS*DRUG}(1,8)$ = 18.49. p = 0.003). Vehicle-treated rats prefered to explore stressed PN 30 conspecifics compared to naïve PN 30 conspecifics (p = 0.006), which was abolished via bilateral pharmacological infusion of tetrodotoxin (TTX; 100nM, 0.5µl/side) in NAc 15 min prior to testing (p = 0.203). **(E)** Mean (with individual replicates) time spent exploring the naïve and stressed PN 50 conspecifics during the 5 min trial ($F_{STRESS*DRUG}(1,6)$ = 0.071, p = 0.799). Both vehicle (p = 0.031) and TTX-treated (p = 0.020) rats prefered to explore naïve PN 50 conspecifics compared to the stressed PN 50 conspecifics. **(F)** Mean (with individual replicates) data from (D,E) shown as the percentage of total social exploration time that was spent investigating the stressed conspecific. Rats tested with PN 30 conspecifics show preference (as indicated by scores greater than 50%) for the stressed conspecific under vehicle treatment, which was significantly reduced after pharmacological inactivation of NAc with TTX (p = 0.0001). Rats tested with PN 50 conspecifics show a preference for naïve conspecifics, which was unaffected by TTX treatment (p = 0.452). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

4.3.2 Exposure to stressed PN 30 conspecifics activated IC neurons that project to NAc.

The NAc is a major target of IC afferents (Wright & Groenewegen, 1996) and the foregoing results suggest that these two structures are implicated in control of social responses to stressed juvenile conspecifics. Thus, we predicted that interaction with a stressed PN 30 conspecific would activate NAc projecting IC neurons. To test this hypothesis, 12 experimental rats received unilateral microinjections of the retrograde tracer CTb^{488} in the left hemisphere NAc (Fig. 4.2 A & B) as previously (Dong et al., 2017; Park et al., 2018) so that neurons that project from IC to NAc can be identified for cell counting (Fig. 4.2 E). 2 weeks after surgery, in a between-subjects design, 6 experimental rats underwent social interactions with naïve PN 30 conspecifics, and 6 underwent interactions with stressed PN 30 conspecifics (Fig. 4.2 C). As previously

reported (Rogers-Carter et al., 2018b), experimental rats displayed higher levels of social exploration with stressed PN 30 conspecifics compared to naïve PN 30 conspecifics ($t(10) = 3.281$, $p = 0.008$; Fig. 4.2 D). Rats were sacrificed 90 min after testing and tissue was processed and stained for fluorescent Fos expression. Tiled images of the IC ipsilateral to the NAc injection site were acquired at the following IC locations relative to Bregma: +2.52mm and 0mm (anterior IC), -0.24mm and -0.72mm (medial IC) and -1.72 and -2.04 (posterior IC) so that the number of Fos-positive, CTb^{488} -positive, and co-labeled neurons could be counted across the rostral-caudal extent of IC. All cells were counted in a field of veiw with a fixed area across all ROIs. Prior to conducting analysis, brains were screened for evidence of CTb^{488} deposits localized to the NAc (Fig. 4.2 B). 4 animals, 2 from each stress group, did not exhibit CTb^{488} in the NAc and so were excluded from all subsequent analysis. We were unable to obtain counts from one subject from each treatment group at the anterior IC and medial IC regions resulting in ns = 3 or 4 observations per group. All individual replicates are depicted in Fig. 4.2 F-H. To establish whether the resulting small sample size was sufficiently powered for our experimental questions, we used a 2-way ANOVA with ROI and Conspecific Stress as within-subjects fators to compare CTb^{488} expression across anterior, medial and posterior IC. It is established that the insula projections to NAc is most dense in the anterior IC, compared to the posterior IC (Park et al., 2018) and this trend was evident and supported by a significant main effect of ROI ($F_{REGION}(2,16) = 19.3$, $p < 0.0001$). Anterior IC had significantly more CTb^{488} -positive cells than medial or posterior IC (ps < 0.001, Fig. 4.2 F). Proceeding to the Fos analyses, a 2-way ANOVA revealed a main effect of ROI (F_{REGION} $(2,10) = 29.8$, $p < 0.0001$). More Fos-

positive cells were found in anterior IC, than medial or posterior IC (ps < 0.001, Fig. 4.2 G) and there were no significant differences in Fos between conspecific stress groups. Regarding CTb^{488} and Fos-positive co-labeled neurons, we report a main effect of Conspecific Stress ($F_{STRESS}(1,16) = 17.0$, $p = 0.0008$). While the trend of greater CTb^{488} and Fos-positive was evident across ROIs, the only significant post-hoc comparision was observed between navie and stressed conspecific groups in the posterior IC ($p = 0.015$, Fig. 4.2 H). In sum, exposure to stressed conspecifics led to greater activity in NAc-projecting IC neurons with the greatest differential activation observed in the posterior subregion of IC.

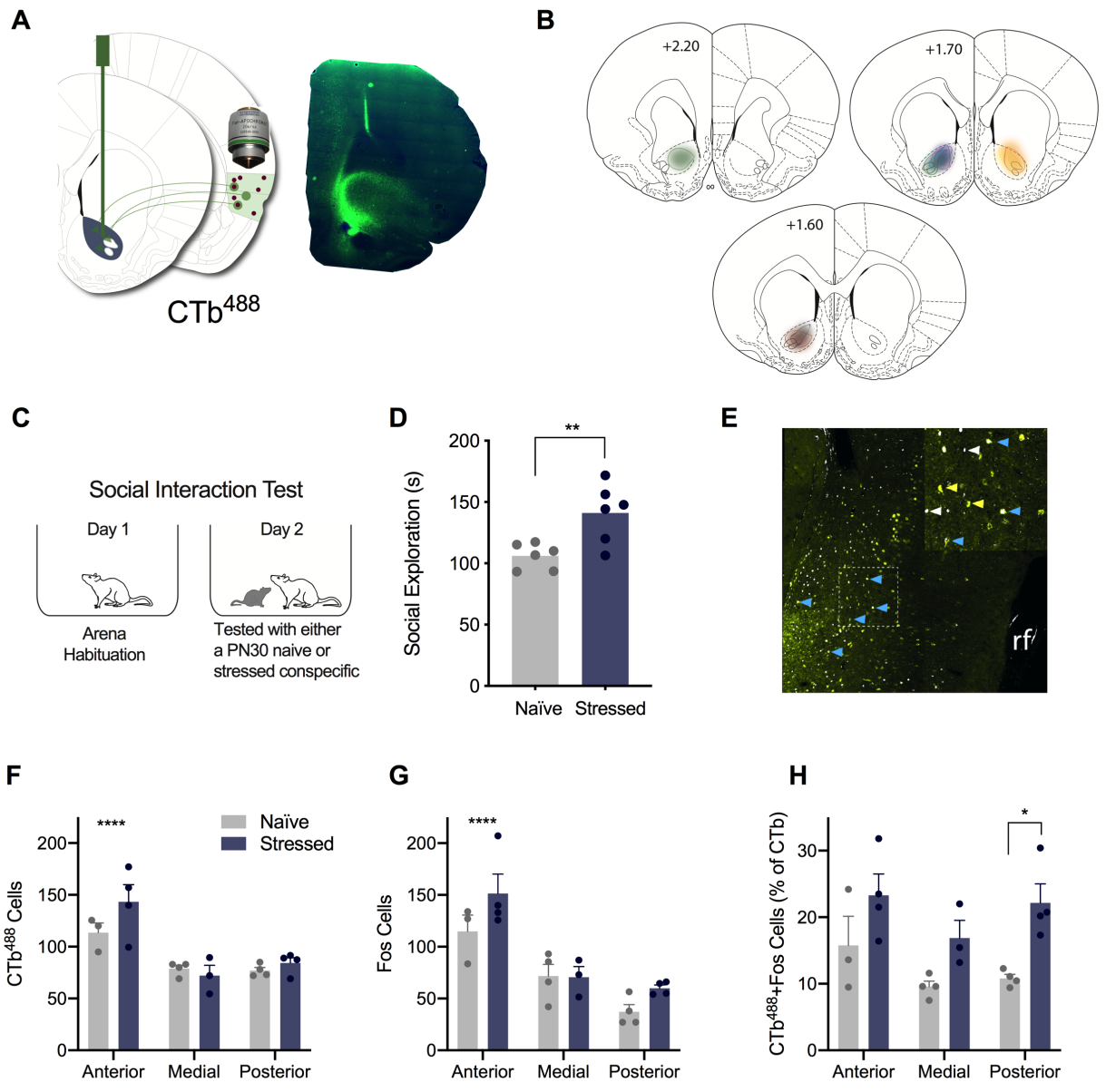


Figure 4.2 Exposure to stressed PN 30 conspecifics elicited greater Fos immunoreactivity in IC → NAc neurons than exposure to naïve PN 30 conspecifics. **(A)** Schematic depicting unilateral 300nl injections of the retrograde tracer CTb^{488} in the right hemisphere NAc (left) and representative image of CTb^{488} in NAc (right). **(B)** CTb^{488} expression in NAc: each experimental rat is represented in a different color. All injections were made to the right hemisphere NAc (here several experimental rats are presented on the left hemisphere (middle panel) for visualization purposes). **(C)** Diagram of social interaction test. 6 experimental rats explored naïve PN 30 conspecifics and 6 experimental rats explored stressed PN 30 conspecifics. **(D)** Mean (with individual values) social exploration time. Experimental rats presented stressed PN 30 conspecifics engaged in more social exploration than rats presented

naïve PN 30 conspecifics ($t_{(10)}$ = 3.281, p = 0.008). **(E)** CTb^{488} and Fos-positive neurons in IC. mage is false colored; white = Fos-positive neurons, yellow = CTb^{488}-positive, blue = co-labeled cells. rf = rhinal fissure. **(F)** Cell counts of CTb^{488}-positive cells in anterior, medial and posterior IC. More CTb^{488}-positive cells were observed in anterior IC than medial or posterior IC (ps < 0.0001). **(G)** Cell counts of Fos-positive cells in each IC region. More Fos-positive cells were observed in anterior IC than medial or posterior (ps < 0.0001). **(H)** Cell counts of colabeled CTb^{488}- and Fos-positive neurons. A main effect of Stress was observed (F_{STRESS} (1,16) = 17.0, p = 0.0008), and experimental rats who explored stressed PN 30 conspecifics expressed more Fos immunoreactivity in CTb^{488} -positive neurons in posterior IC (p = 0.015) compared to experimental rats who explored naïve PN 30 conspecifics. *p < 0.05, **p < 0.01, ****p < 0.0001.

4.3.3 Chemogenetic inhibition of IC blocked social affective preference behavior.

We previously reported (Rogers-Carter et al., 2018b) that the preference to explore stressed PN 30 and naïve PN 50 conspecifics was mediated by IC using an optogenetic approach. Here, an intersectional chemogenetic approach was preferred as the manipulation is less restrictive, well suited to control neural activity in social behavior, and has been used to investigate IC projections to NAc and central amygdala in different paradigms (Jaramillo et al., 2018a; Venniro et al., 2017). Therefore, we first sought to replicate our prior finding and establish feasibility of chemogenetic control of IC neurons by using the inhibitory chemogenetic hM4Di receptor expressed in neurons under control of the synapsin promoter. Experimental rats (n = 10) were bilaterally transduced with pAAV5-hSyn-hM4D(Gi)-mCherry in IC (Fig. 4.3 A & B). 2 weeks later, rats underwent the SAP test procedure with PN 30 conspecifics following injections of vehicle or CNO (3mg/kg; i.p.), order counterbalanced, 45 min before testing (Fig. 4.3 E). This produced a significant Conspecific Stress x Drug interaction ($F_{STRESS* DRUG TREATMENT}$(1,9) = 34.22, p = 0.0002). Vehicle-treated rats spent more time exploring stressed PN 30 conspecifics than naïve PN 30 conspecifics (p = 0.0001; Fig. 4.3 F).

This preference was abolished after inhibition of IC neurons as experimental rats tested with CNO did not show a preference for the naïve or stressed PN 30 conspecific ($p = 0.517$; Fig. 4.3 F). To interpret the effect of CNO in behavioral experiments a number of factors must be considered (MacLaren et al., 2016; Mahler & Aston-Jones, 2018; Manvich et al., 2018). First, a subset of rats expressing hM4Di in the IC were sacrificed for acute slice whole cell recordings as in (Rogers-Carter et al., 2018b). Following the methods described by (Venniro et al., 2017) bath application of 10μM CNO reliably hyperpolarized and reduced excitability of IC neurons visually identified as mCherry positive at the time of recording (Fig. 4.3 D). To confirm these results were not an effect of CNO alone, experimental rats without virus transduction (n = 8) underwent the SAP procedure after i.p. CNO injections at the following doses: 0mg/kg, 0.3mg/kg, and 3.0mg/kg, the latter two of which have been previously reported to effectively modulate neurons transduced with chemogenetic viruses (Roth, 2016). A 2-way ANOVA with Conspecific Stress and Dose as within-subjects factors revealed a main effect of Conspecific Stress ($F_{STRESS}(1,7) = 62.93$, $p = 0.0001$). Post-hoc comparisons further revealed that, at each dose, experimental rats spent more time exploring the stressed PN 30 conspecifics than the naïve (0mg/kg, $p = 0.0009$; 0.3mg/kg, $p = 0.0036$; 3.0mg/kg, $p = 0.0063$). These findings demonstrate that i.p. injections of CNO at 0.3 or 3.0mg/kg *per se* did not interfere with social affective preference behavior (Fig. 4.3 G & H). To demonstrate virus efficacy, a cohort of experimental rats (n = 9) was bilaterally transduced with the sham virus pAAV5-hSyn-mCherry in IC (Fig. 4.3 C), and two weeks later underwent SAP test after vehicle and 3.0mg/kg i.p. CNO injections (order counterbalanced). Rats spent more time exploring the stressed PN 30 conspecific after

both vehicle (p = 0.003) and CNO (p = 0.002) treatment (Fig. 4.3 G), which supports the main finding that IC inhibition achieved by chemogenetic inhibition blocked the social affective preference for the stressed PN 30 conspecific and establish feasibility for chemogenetic control of IC neurons during SAP testing. For side-by-side comparison of the effect of CNO in the sham and virus preparations, the data in Fig. 4.3 F & G were converted to % preference scores and presented side by side for comparison in Fig. 4.3 H. In no instance did administration of CNO alone ($F_{\text{DRUG TREATMENT}}(7,14) = 0.343$, p = 0.920), or vehicle or CNO administration to rats with the sham mCherry virus ($t(8) = 0.010$, p = 0.992) disrupt the baseline preference to explore the stressed PN 30 conspecific. In rats with transduced with hM4Di, administration of CNO attenuated the preference for the stressed conspecific ($t(9) = 5.654$, p = 0.0003).

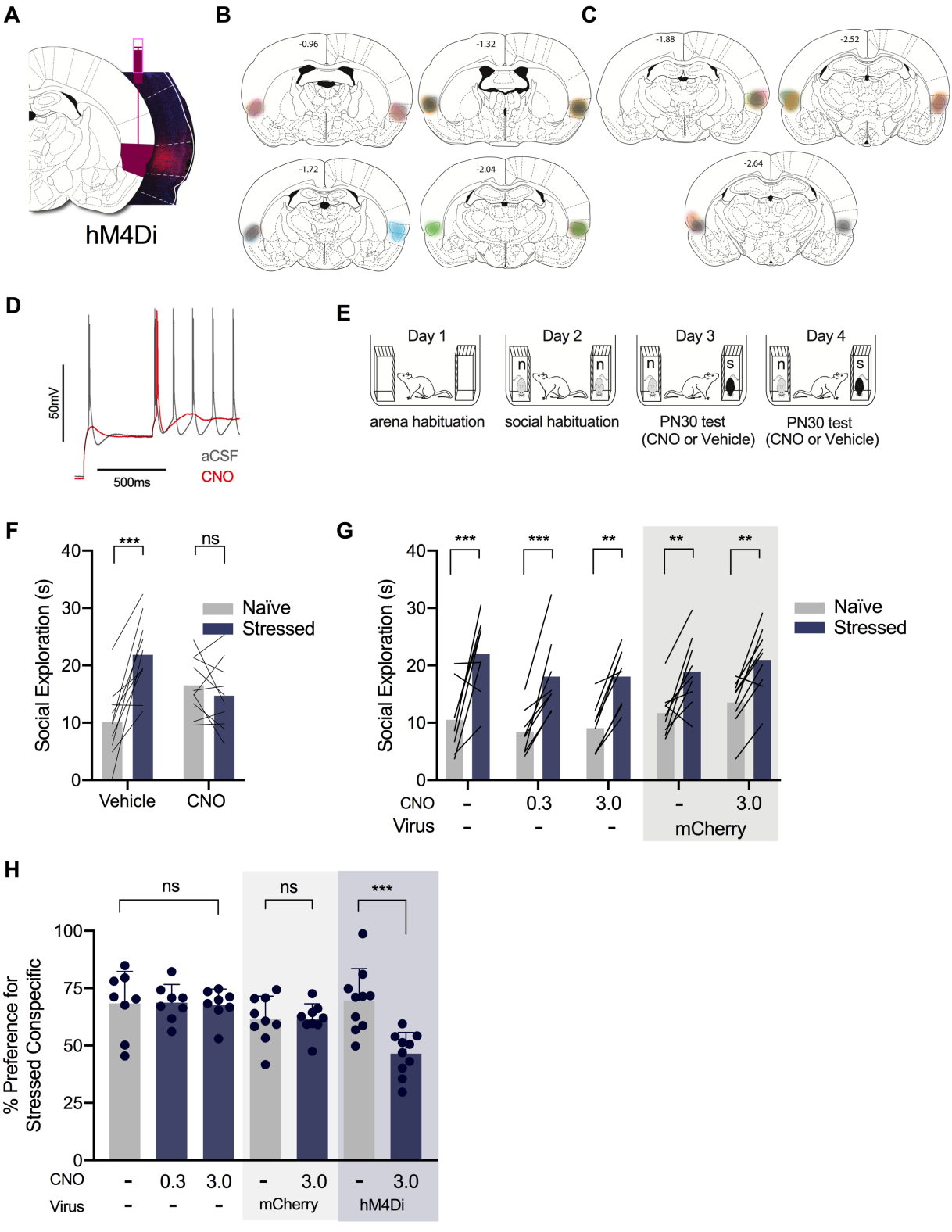

A
hM4Di
B
-0.96
-1.32
-1.72
-2.04
C
-1.88
-2.52
-2.64
D
50mV
500ms
aCSF
CNO
E
Day 1
Day 2
Day 3
Day 4
n
s
arena habituation
social habituation
PN30 test
(CNO or Vehicle)
PN30 test
(CNO or Vehicle)
F
***
ns
Social Exploration (s)
Naïve
Stressed
Vehicle
CNO
G
***
***
**
**
**
CNO
Virus
0.3
3.0
mCherry
H
ns
ns
***
% Preference for
Stressed Conspecific
hM4Di

Figure 4.3 Chemogenetic inhibition of IC blocked the social affective preference for stressed PN 30 conspecifics. **(A)** Representative image of pAAV5-hSyn-hM4D(Gi)-mCherry transduction in IC (right) and corresponding rat brain atlas diagram (left). **(B)** Map of bilateral IC pAAV5-hSyn-hM4D(Gi)-mCherry expression in experimental rats. Each subject is represented in a different color (n = 10); bold outlines represent the greatest intensity of mCherry expression and the corresponding faded overlay depicts the full extent of virus transduction. **(C)** Map of bilateral IC pAAV5-hSyn-mCherry (600nl) sham viral expression, n = 8. **(D),** In acute, whole-cell recordings, bath application of CNO hyperpolarized the resting membrane potential and reduced action potential output during depolarizing current injection of hM4Di-mCherry cells in the insular cortex. **(E)** Diagram of SAP test procedure. **(F)** Mean (with individual replicates) time spent exploring the naïve and stressed PN 30 conspecifics during the 5 min trial. A Conspecific Stress x Drug interaction was observed ($F_{\text{STRESS*DRUG TREAMTMENT}}(1,9)$ = 34.22, p = 0.0002. Vehicle-treated rats spent more time exploring the stressed PN 30 conspecifics than the naïve PN 30 (p = 0.0001), which was abolished in trials following injections of CNO (3mg/kg; i.p.) 45 min before testing (p = 0.517). **(G)** Control experiments: Experimental rats (n = 8) without viral transduction underwent the SAP test procedure 45 min after CNO injections at three doses: 0, 0.3 and 3.0mg/kg (i.p.). No dose of CNO disrupted the preference for the stressed PN 30 conspecific (0mg/kg, p = 0.0009; 0.3mg/kg, p = 0.0036mg/kg; 3mg/kg, p = 0.0063). Experimental rats transduced with a sham virus (n = 9) preferred to explore the stressed PN 30 conspecific under vehicle (p = 0.003) and 3mg/kg CNO (i.p.) (p = 0.002).**p < 0.01, ***p < 0.001. **(H)** Data from (f) and (g) converted to % preference for the stressed conspecific. The only condition in which CNO reduced the preference for the stressed PN 30 conspecific was in experimental rats transduced with hM4Di ($t(9)$ = 5.654, p = 0.0003). **p < 0.01, ***p < 0.001.

4.3.4 Chemogenetic inhibition of IC terminals in NAc blocked the preference for the stressed PN 30 conspecifics.

To test if the IC → NAc pathway is necessary for SAP behavior with PN 30 conspecifics, 10 experimental rats were transduced with the inhibitory hM4Di receptor via bilateral injection of pAAV5-hSyn-hM4D(Gi)-mCherry. Bilateral cannula were placed in NAc so that CNO could be directly administered to IC terminals (Fig. 4.4 A left). Each experimental rat included in the analysis was validated for virus expression in IC as in Fig. 4.4 A right; map of virus expression in Fig. 4.4 D, as well as for cannula tip placement in NAc (Fig. 4.4 B, map in Fig. 4.4 C), and terminal fiber expression in NAc

(Fig. 4.4 B, inset). Two experimental rats were excluded due to lack of virus expression. 3 weeks after testing, experimental rats underwent the SAP procedure with PN 30 conspecifics after microinjections of vehicle or CNO in NAc 15 min before testing (order counterbalanced; Fig. 4.4 E). A 2-way ANOVA of social exploration time revealed a main effect of Conspecific Stress (F_{STRESS} (1,9) = 16.28, p = 0.003); an interaction between Conspecific Stress and Drug approached significance ($F_{STRESS*DRUG\ TREATMENT}$(1,9) = 4.214, p = 0.070). In the vehicle condition, experimental rats preferred to explore the stressed PN 30 conspecific over the naïve (p = 0.023). In the CNO condition, experimental rats did not exhibit preference for either the naïve or stressed conspecific (p = 0.959; Fig 4.4 F). When social exploration was converted to percent preference scores (Fig. 4.4 G), after vehicle injections experimental rats showed scores that reflected more time spent with the stressed conspecific, which was reduced by CNO (t(9) = 2.089, p = 0.033). To rule out off-target effects of CNO, a separate cohort of experimental rats without any virus administration was bilaterally implanted with guide cannula in NAc and underwent SAP testing with PN 30 conspecifics. One trial followed microinjections of vehicle solution, and the other followed microinjections of CNO. A 2-way ANOVA revealed a main effect of Drug ($F_{DRUG\ TREATMENT}$(1,6) = 15.48, p = 0.008) and Conspecific Stress (F_{STRESS}(1,6) = 34.78, p = 0.001). Experimental rats preferred to explore the stressed PN 30 conspecific in both the vehicle (p = 0.003) and CNO (p = 0.031) conditions (Fig. 4.4 H), which supports that the effect of CNO observed in hM4Di expressing rats cannot be attributed to off target effects of CNO on NAc function. Together, these results indicate that IC projections to NAc are necessary to show a preference to explore the stressed PN 30 conspecific compared to the naïve PN 30.

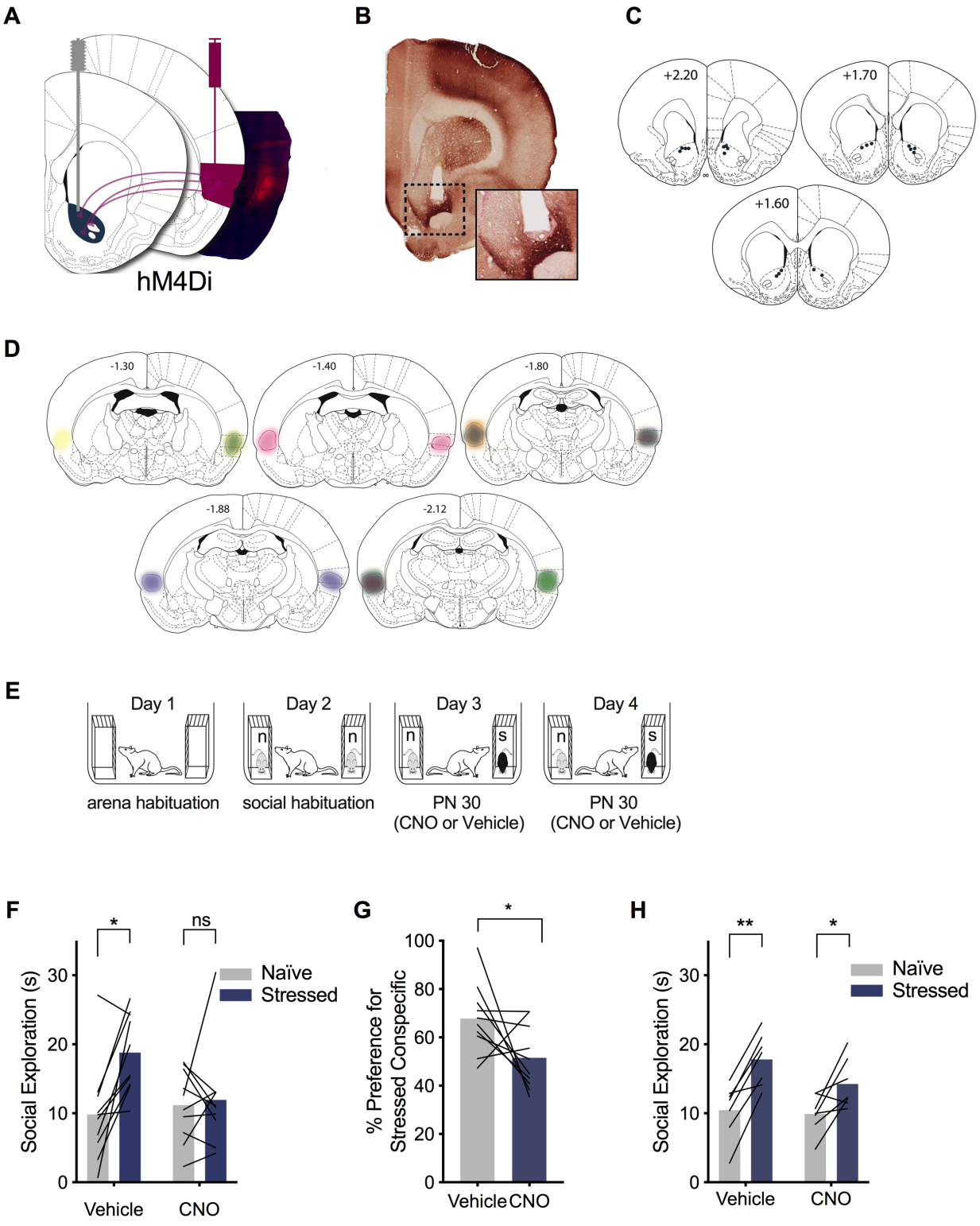


Figure 4.4 Chemogenetic inhibition of IC → NAc terminals blocked the social affective preference for stressed PN 30 conspecifics. **(A)** Schematic of IC neurons

transduced with pAAV5-hSyn-hM4D(Gi)-mCherry in IC and cannula implanted in NAc (left) and representative image of virus transduction (right). **(B)** Verification of cannula tip in NAc and terminal fiber expression (inset). **(C)** Schematic map of cannula tip placements in NAc. **(D)** Map of virus expression in IC (n = 10); bold outlines represent the region of maximum mCherry expression and the corresponding faded overlay depicts the full extent of virus transduction. **(E)** Diagram of SAP procedure. **(F)** Mean (with individual replicates, n = 10) time spent exploring the naïve and stressed PN 30 conspecifics during the 5 min trial ($F_{\text{STRESS*DRUG TREATMENT}}(1,9) = 4.214$, p = 0.070). Vehicle-treated rats spent more time exploring the stressed PN 30 conspecifics than the naïve PN 30 conspecifics (p = 0.023), which was abolished via 1μM CNO injections 45 min prior to testing through the guide cannula (p = 0.956). **(G)** Data from (D) presented as the percent preference for the stressed conspecific. Experimental rats show a preference to explore the stressed conspecific under vehicle treatment, which was significantly reduced after pharmacological inhibition of IC terminals in NAc in CNO ($t(9) = 2.908$, p = 0.033). **(H)** Experimental rats without virus transduction underwent the SAP test. Under vehicle treatment, rats preferred to explore the stressed PN 30 conspecifics (p = 0.003), which was maintained after microinfusion of 1μM CNO to the NAc 45 min prior to testing (0.031).

4.3.5 Chemogenetic activation of IC terminals in NAc increased exploration with PN 30, but not PN 50, conspecifics.

The results reviewed thus far suggest that during interactions with stressed juveniles IC projections to the NAc are selectively recruited and mediate the increase in social investigation directed toward the stressed targets. This set the stage to inquire whether this tract was sufficient to promote social exploration in the absence of social stress signals. We chose to again include PN 50 conspecifics here because, while prior experiments suggested this pathway was not necessary for interactions with PN 50 conspecifics, we did not have any prior data to rule out the possibility that the pathway was sufficient for interactions with PN 50. To test this possibility, we augmented IC terminals in NAc by introducing the Gq coupled hM3Dq receptor via bilateral viral administration of pAAV5-hSyn-hM3D(Gq)-mCherry in IC in 10 experimental rats. Cannula were placed bilaterally in NAc so that CNO could be directly applied to IC

terminals in NAc as above (Fig. 4.5 A). For each experimental rat included in the analysis, we confirmed the virus transduction via fluorescent imaging of the mCherry reporter (Fig. 4.5 A & F), the location of the cannula tips in NAc (Fig. 4.5 B & E), and the presence of terminal fibers expressing mCherry in NAc (Fig. 4.5 B, inset). Three rats were excluded due to misplaced cannula or lack of virus expression. Further, acute whole-cell patch clamp recording confirmed 10μM CNO reliably depolarized and inreased excitability of IC neurons visually identified as mCherry positive at the time of recording (Fig. 4.5 D). 3 weeks after testing, experimental rats received social exploration tests over 5 days consisting of a habituation day, and then 2 tests with naïve PN 30 conspecifics and 2 tests with naïve PN 50 conspecifics. One trial for each conspecific age pairing occured after vehicle treatment, and the other following infusion of CNO (0.5μl/side) to NAc 30 min before testing (Fig. 4.5 G). The order of conspecific age and order of drug treatment were counterbalanced which allowed for a within-subjects design. Social exploration was analyzed as a 2-way ANOVA with Conspecific Age and Drug as within-subjects factors ($F_{\text{AGE*DRUG TREATMENT}}(1,6) = 5.851$, $p = 0.052$). This analysis revealed main effects of Conspecific Age ($F_{\text{AGE}}(1,6) = 6.568$, $p = 0.043$) and Drug ($F_{\text{DRUG TREATMENT}}(1,6) = 12.80$, $p = 0.012$). Post-hoc comparisons show mean social exploration was greater with PN 30 conspecifics after CNO treatment than vehicle ($p = 0.030$), whereas mean social exploration with PN 50 conspecifics did not differ between CNO and vehicle treatment ($p = 0.998$) (Fig. 4.5 H). Lastly, a day after testing ended, experimental rats received bilateral microinjections of vehicle (n = 4) or CNO (n = 3) 2h before sacrifice. NAc sections were stained for Fos to test if CNO was sufficient to increase activation of NAc neurons. CNO-treated rats expressed greater Fos-

immunoreactivity compared to vehicle-treated rats ($t(5) = 2.727$, $p = 0.041$, Fig. 4.5 C), supporting the efficacy of chemogenetic stimulation of IC-NAc terminals. These results indicate that excitation of IC terminals in NAc via CNO was sufficient to increase exploration with PN 30, but not PN 50 conspecifics.

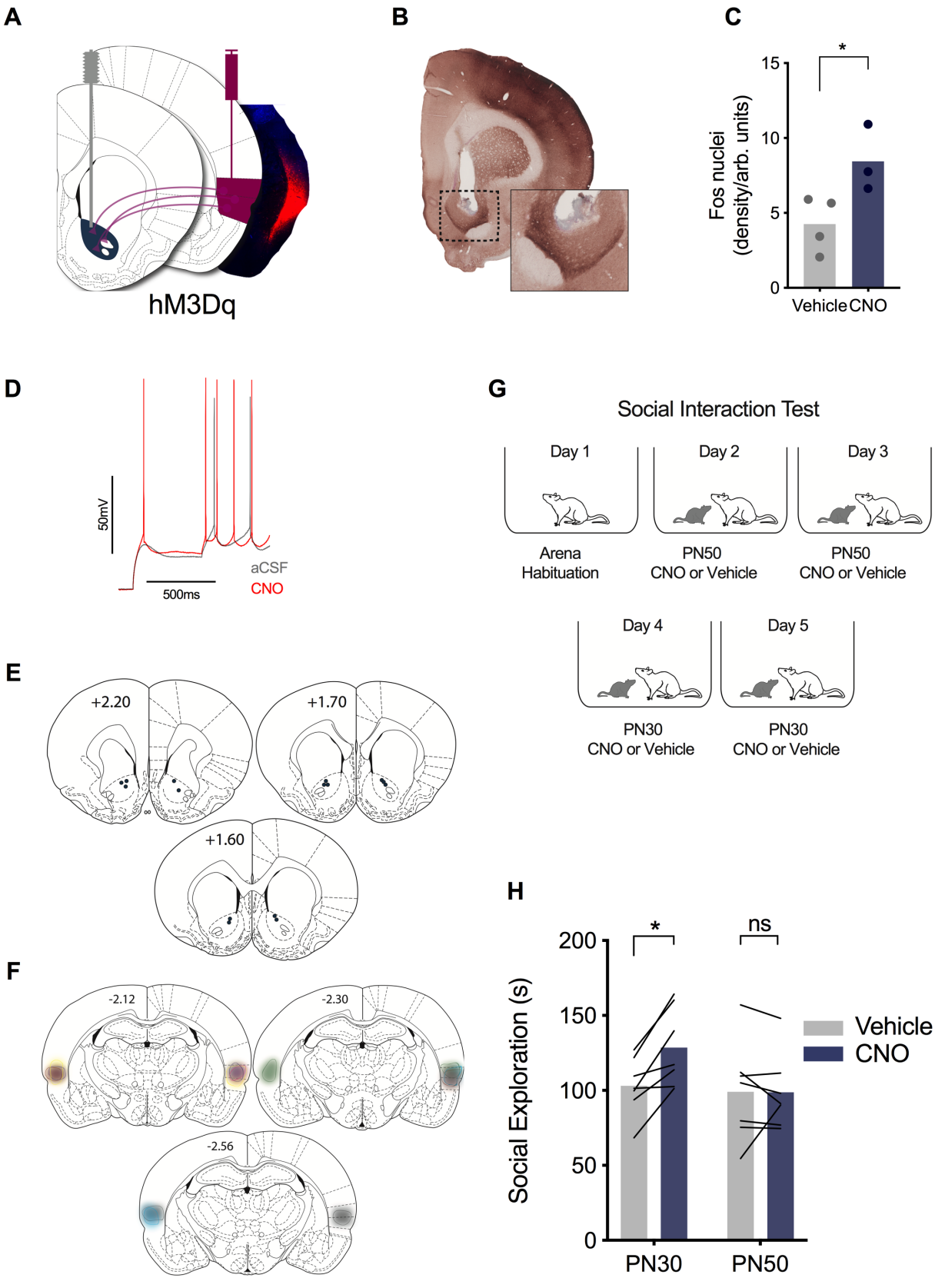

A
hM3Dq
B
C
Fos nuclei
(density/arb. units)
15
10
5
0
*
Vehicle
CNO
D
50mV
500ms
aCSF
CNO
E
+2.20
+1.70
+1.60
F
-2.12
-2.30
-2.56
G
Social Interaction Test
Day 1
Day 2
Day 3
Arena
Habituation
PN50
CNO or Vehicle
PN50
CNO or Vehicle
Day 4
Day 5
PN30
CNO or Vehicle
PN30
CNO or Vehicle
H
Social Exploration (s)
200
150
100
50
0
*
ns
PN30
PN50
Vehicle
CNO

Figure 4.5 Chemogenetic excitation of IC terminals in NAc increased social exploration with PN 30, but not PN 50, conspecifics. **(A)** Schematic of IC neurons transduced with pAAV5-hSyn-hM3D(Gq)-mCherry in IC and cannula implanted in NAc (left). Terminal excitation of mCherry-positive fibers was achieved via bilateral clozapine-n-oxide (CNO) microinjections to NAc (1μM) 45 min before testing. Representative image of virus transduction in IC (right). **(B)** Example of cannula tip placement in NAc and expression of terminal fibers in NAc (inset). **(C),** Greater NAc Fos immunoreactivity was observed in rats injected with CNO prior to perfusion, compared to rats treated with vehicle ($t(5) = 2.727$, $p = 0.041$). **(D)** In acute, whole-cell recordings CNO depolarized the resting membrane potential and increased the number of action potentials evoked by current injection in hM3Dq-mCherry positive neurons in the IC. **(E)** Map of cannula tip placements in NAc. **(F)** Map of bilateral IC pAAV5-hSyn-hM3D(Gq)-mCherry expression in experimental rats (right). Each subject is represented by a different color (n = 7). **(G)** Diagram of social interaction trials. **(H)** Mean (with individual replicates) time spent exploring naïve PN 30 and PN 50 conspecifics following vehicle and CNO treatment (order counterbalanced) Experimental rats spent more time exploring naïve PN 30 conspecifics following CNO injections than after vehicle treatment ($p = 0.030$), whereas social exploration with PN 50 conspecifics was unaffected by drug treatment ($p = 0.998$). $^{*}p < 0.05$.

4.4 Discussion

Here we investigated the role of the nucleus accumbens (NAc) and the projections from insular cortex to NAc in social affective behavior. In the social affective preference (SAP) test in which adult experimental rats demonstrate a preference to interact with stressed juvenile rats but avoid stressed adult rats (Rogers-Carter et al., 2018b), we observed age-specific effects of NAc and IC → NAc manipulations. First, pharmacological inactivation of the NAc prevented the expected preference for experimental rats to explore stressed PN 30 conspecifics, but did not alter the avoidance of stressed PN 50 conspecifics. Greater Fos immunoreactivity in NAc-projecting posterior IC neurons was evident in experimental rats following social interactions with stressed PN 30 conspecifics compared to naïve PN 30 conspecifics. In SAP tests, chemogenetic inhibition of this circuit blocked the preference for the stressed PN 30 conspecific. Accordingly, chemogenetic stimulation of IC terminals in NAc

increased exploration with PN 30, but not PN 50, conspecifics. These data suggest the IC → NAc pathway is involved in prosocial behaviors directed toward juveniles. In light of our existing understanding of social decision-making, we suggest that the IC serves an important role in integrating sensory information about social stimuli with nodes in the social decision-making network, such as NAc, to modulate situationally appropriate social behaviors.

The involvement of NAc in social affective behavior is consistent with evidence implicating NAc involvement in myriad social processes. NAc mediates social recognition (Ploeger et al., 1991), social reward (Dölen et al., 2013; Smith et al., 2018), mating and sexual behavior (Fujiwara & Chiba, 2018; Xiao & Becker, 1997), social novelty-seeking (Smith et al., 2017), and interactions with both juvenile (Trezza et al., 2011) and adult (Dölen et al., 2013; Van den Berg et al., 1999) conspecifics. Furthermore, NAc is a node of the social decision-making network (O'Connell & Hofmann, 2011) and can decipher aversive from appetitive stimuli (Xiu et al., 2014) to modulate activity patterns in other social decision-making network structures based on the valence of sensory information (Johnson et al., 2017). In the SAP paradigm, the preference to explore stressed juveniles may be mediated by the NAc because this region integrates the valence of conspecific affect with reward valuation (Hamel et al., 2017). Consistent with this thought, when NAc was pharmacologically inactivated with TTX, rats did not show a social preference for the stressed PN 30 conspecific and thus behaved as if each conspecific was equal in valence.

Like NAc, IC output neurons are necessary for the social affective preference for the stressed juvenile (Rogers-Carter et al., 2018b). IC is a site of multisensory

integration (Gogolla et al., 2014; Gogolla, 2017; Rodgers et al., 2008) and is reciprocally connected to the extended amygdala (Shi & Cassell, 1998) which may underlie its known role in human emotional recognition
(Adolphs et al., 2003; Gu et al., 2013; Terasawa et al., 2015a).

While the exact mechanism by which rodents convey emotion or affect is unknown, in the SAP test we reported that there are changes to several overt conspecific behaviors (e.g. self-grooming) and to the pattern of ultrasonic vocalizations which together could convey important features of conspecific stress and age (Rogers-Carter et al., 2018b). IC is anatomically situated to encode these features and relay this to the social decision making network. NAc is major target of IC efferents, and consistent with the hypothesis that IC projections to NAc mediate approach toward the stressed juvenile, we observed greater Fos IC → NAc neurons after interactions with stressed juveniles with the greatest difference observed in the posterior IC. A limitation of the current experiment is small sample size; this experiment may have been underpowered to detect smaller effects of conspecific stress on IC → NAc neurons in the anterior IC, which tend to be greater after interactions with stressed juveniles. Future studies are needed to directly address whether these projections are involved in social approach behavior. Further, using both inhibitory and excitatory tract-specific chemogenetic manipulations of IC → NAc terminals, we find that this tract is both necessary for the preference for the stressed juvenile, and sufficient to increase overall social exploration directed toward juvenile conspecifics. Together these findings demonstrate the modulatory role of IC → NAc projections on social approach in a preference paradigm.

Rats, like most mammals, require parental support to develop and survive (Lamers et al., 1986), and the same mechanisms that have evolved to enable animals to provide parental care may also underlie more complex social abilities (Marsh, 2018; Scheiber et al., 2017). Because these social actions are necessary for species survival, they are likely reinforced by the reward system in order to promote their occurrence (O'Connell & Hofmann, 2011). Although, we cannot be sure that approach to stressed juveniles is a prosocial reponse, increased NAc activity is associated with many prosocial behaviors. Optogenetic excitation of ventral tegmental neurons that project to the nucleus accumbens promoted mating behavior (Beny-Shefer et al., 2017) and microinfusion of a dopamine reuptake inhibitor increased social play in rats (Manduca et al., 2016). Similarly, oxytocin in NAc increases social approach (Steinman et al., 2018) and is important for forming pair bonds (Insel & Shapiro, 1992; Walum et al., 2012; Walum & Young, 2018). Our findings that chemogenetic manipulation of IC terminals in NAc relate to adult-to-juvenile behaviors adds to these NAc-dependent prosocial behaviors, and suggest an anatomical tract for socioemotional information to be integrated in social decision-making.

While the primary goal of this study was to investigate neural substrates of social behavior, this work adds to several studies which seek to identify functions of the IC using intersectional behavioral neuroscience methods. Recent reports in drug administration models show excitaiton of IC projections to central amygdala promote relapse of methamphetmaine use (Venniro et al., 2017) and IC projections to the bed nucleus of the stria terminalis mediate negative affect during alcohol abstinence

(Centanni et al., 2019). Similarily, silencing of the IC projection to NAc decreased alcohol self-administration (Jaramillo et al., 2018b) and increased sensitivity to alcohol (Jaramillo et al., 2018a), and IC projections to the central amydala drive avoidance of an avertisve tastant (Schiff et al., 2018). While more work will add clarification, an emergent theme is that IC mediates both appetitive and aversive behavioral responses to external stimuli via different projections. IC contributes to both social approach and avoidance (Rogers-Carter et al., 2018b)
the latter of which may be mediated IC projections to bed nucleus or central amygdala, which are associated with many correlates of negative affect such as avoidance.

The current findings parallel a number of important features of social behavior that are apparent in humans, namely that social stress cues modulate rodent social interactions in ways that mirror adult preference to lend help to juveniles while showing less empathy toward other adults (Batson & Powell, 2003). This parallel may facilitate translation of the current findings to people. Abnormalities in social processes like emotion recognition are symptoms of several psychiatric conditions including autism spectrum disorders (Griffiths et al., 2017; Lozier et al., 2014; Sato et al., 2017; Tracy et al., 2011) and it is important to note that functional neuroimaging studies of individuals with autism find reduced insular activation to emotional stimuli (Di Martino et al., 2009; Hall et al., 2013; Morita et al., 2012), reduced striatal activation to social rewards (Schmitz et al., 2008; Scott-Van Zeeland et al., 2010; Supekar et al., 2018),
and reduced functional connectivity between insular regions and ventral striatum (Fuccillo, 2016). The current experimental results provide important mechanistic support for this axis as a key to normative social cognition. Future research might take

advantage of the SAP phenomenon to identify the tracts that mediate the avoidance of stressed adults and determine how genetic and experimental risk factors for autism spectrum disorders influence the IC and its targets in the social decision making network.

Chapter 5: Discussion

5.1 Summary of Findings

The goals of this dissertation were to 1) develop a paradigm in which rodents display social affective behaviors, 2) test if social affective preferences depended upon the insular cortex, and 3) determine if the insular cortex communicates with the social decision-making network to produce such social affective behavior. We report that conspecific age and stress experience are important determinants of an experimental rat's social behavior in the social affective preference test: both male and female adult rats will prefer to explore stressed juvenile conspecifics, but avoid stress adults. Recordings of ultrasonic vocalizations from naïve and stressed conspecifics suggest these preferences may result from detecting auditory cues that convey stress. Further, female experimental rats will approach stressed adult conspecifics with which they are familiar, whereas males will avoid stressed adults regardless of familiarity. These findings suggest the SAP test is sensitive at least some of the variables that are believed to shape of social decision-making because social responses to stressed conspecifics depended on age and prior relationship. Regarding goal 2, pharmacological and optogenetic inhibition of the insular cortex and its oxytocin receptors blocked both the preference to explore stressed juveniles and avoid stressed adults, and OT increased excitability in the insular cortex *in vitro.* Together, these results indicate OT acting in the insular cortex may enable rats to detect and respond to social affective cues. Lastly, insular cortex projections to the nucleus accumbens core, an important node in the social decision-making network, were necessary for the preference to explore stressed juveniles, but not necessary for social interactions with adult conspecifics. This finding demonstrates insular cortex communicates with the

SDMN to orchestrate rodent social affective behaviors. More broadly, this work identifies a mechanism by which sensory information influences social behavior, which helps to explain why the insular cortex has been implicated across a wide range of human social cognitions (Adolfi et al., 2017; Lockwood, 2016).

I will discuss the implications and limitations of these results on our understanding of the neural mechanisms by which rodents detect and respond to social affect, with focus on several big-picture questions about this work that remain unanswered. The SAP test has not yet been used by other research groups, nor has the insular cortex been widely studied in other rodent social behavior paradigms (but see (Cho et al., 2017) and so there is ample room to discuss the implications of this work. This discussion will address the possible ways by which affect is communicated by the conspecific and perceived by the experimental rat in the SAP test, speculate on the exact role the insular cortex may fulfill in this behavior, and if oxytocin changes how the insula detects social affect to drive behavioral responses. Further, I will speculate why the accumbens is only necessary for juvenile-directed behaviors, and propose how insula connectivity with the accumbens may fit into a larger circuit that controls SAP behavior. Lastly, I will discuss the clinical applications of this work to expand our understanding of social affect in psychiatric disorders from the lens of the insular cortex.

5.2 What socioemotional stimuli are used to convey affect in the SAP test?

What exactly an experimental rat detects in the stressed conspecific is unknown. One assumption made about our SAP test paradigm is that the stressed conspecific communicates its affective state via a constellation of expressions and is therefore

distinguishable from the naïve conspecific. In Chapter 2 I presented findings that stressed conspecifics emit distinct ultrasonic vocalizations than naïve conspecifics. Both PN 30 and PN 50 stressed conspecifics emitted fewer “rising” and “trill” type ultrasonic vocalizations than their naïve counterparts. These are considered appetitive vocalizations (Brudzynski, 2013) and so it is possible that, during social interactions, conspecifics emit a baseline level of rising and trill calls, which the stress manipulation in the SAP test reduces. In this case, a reduction in such calls from a juvenile conspecific may signal danger or harm, which prompts an experimental adult rat to approach. A reduction in such calls from a PN 50 conspecific, however, may reflect that social approach is unwelcomed, and therefore the experimental rat does not engage in exploration. In this interpretation, both PN 30 and PN 50 conspecifics communicate a change in their affective state through the same expressions, but how the change is perceived by the adult conspecific varies by conspecific age. Alternatively, age-specific preferences may be the product of PN 50 and PN 30 conspecifics emitting unique calls. We report that PN 50 conspecifics emitted 22 kHz aversive (Brudzynski, 2013) vocalizations whereas PN 30 conspecifics did not. Further, stressed PN 50 conspecifics emitted more aversive calls than naïve PN 50 conspecifics and so these comparisons may explain both the age-specific and PN 50 affect-specific preference behavior. Avoidance of the stressed PN 50 conspecific may be a direct response to antisocial vocalizations that are unique to adult conspecifics.

A limitation of our approach is that ultrasonic vocalizations had to be recorded from one-on-one social exploration test, rather than during SAP testing where both a naïve and stressed conspecific is present. This is because it is difficult to discern which

rodent emitted which call, and this problem is exacerbated in the SAP test where three animals are present. Thus, we cannot assume the calls recorded from this experiment generalize to the SAP test.

Our assessment of affect communication in the SAP test is also incomplete in regards to non-vocal sensory modalities. Olfactory cues and chemosignals are widely implicated in rodent social behaviors (Cheal & Sprott, 1971). However, these compounds are difficult to identify and isolate with certainty, which hinders conducting causal experiments to implicate olfactory communication in social affective preference behavior. Despite this limitation, the known literature about olfactory cues allows for speculation regarding possible ways that odors could underlie age- and affect-specific preferences. Approach and avoidance are both linked to olfactory cues; odors that convey sickness, stress or infection have been reported to drive avoidance behavior (Arakawa et al., 2011; Hamasato et al., 2017; Kavaliers & Choleris, 2018), whereas appetitive olfactory cues may elicit approach behavior (Ryan et al., 2008). This begets the question, do stressed rats emit different cues than naïve rats in the SAP test, which is reflected in the experimental rat's avoidance or approach behavior, respectively? To this end Kiyokawa (2017) reported that stressed rats emitted alarm pheromones that activate anxiety responses in conspecifics, whereas non-stressed rats emitted appetitive olfactory cues. While this logic explains well the behavior with PN 50 conspecifics, it does not explain why experimental rats approach stressed juveniles. This raises several questions: do juveniles emit alarm pheromones? If so, are they aversive in nature like those emitted by adults, or is there something about the type of odor from juveniles or its perceived meaning that elicits approach? The answers remain

unknown, but like a combination age- and affect-specific odors account for SAP behavior.

A compelling approach to determine if social affective behavior depends on odor communication would be to test the necessity of well-known olfactory processing brain regions, and we have found correlation evidence in support of this idea. In experiment 3.3.2, rats underwent one-on-one social interactions with either a naïve juvenile, a stressed juvenile, a naïve adult or a stressed adult conspecific, after which tissue was collected and Fos immunoreactivity across 29 ROIs that were previously implicated in social or emotional processes was analyzed using graph theory. Highly correlated patterns of activity emerged in 2 modules (data not shown but see (Rogers-Carter et al., 2018b) which were composed primarily of structures found in the social decision-making network or the structures associated with emotion, such as the nuclei of the extended amygdala and prefrontal cortex. Several structures, including piriform cortex and medial amygdala, were found in both of these modules with a high degree of participation in both networks, suggesting they may integrate social and emotional processes. The piriform cortex and medial amygdala are both key olfactory processing areas known to mediate social buffering of fear (Kiyokawa et al., 2012) and are modulated by OT during social interactions (Oettl & Kelsch, 2017), suggesting these structures may be sensitive to social olfactory cues. The medial amygdala and piriform cortex are reciprocally connected with each other, as well as with the insular cortex (Pardo-Bellver et al., 2012). These findings and anatomical connections warrant investigation of the role of 1) olfactory communication in the SAP test and 2) insular connectivity with odor-processing areas. A mechanistic approach to determine if olfaction is necessary for the

SAP test could include a series of tract-specific manipulations in which olfactory inputs to insula from the olfactory bulb, piriform cortex, and medial amygdala, as well as pathways between these structures, are inactivated prior to SAP testing to determine if 1) olfactory cues drive preference behavior and 2) if the insular cortex responds to such olfactory information.

5.3 Why is the insular cortex necessary for social affective behavior?

While the optogenetic and pharmacological methods used in Chapter 3 effectively silenced the insular cortex and demonstrated its necessary for social affective behavior, these techniques alone are insufficient to unveil the cognitive computations that occur within IC to shape social affective behaviors. It may be that the insula has a specific empathic role with which it can detect and compute the value of social affective cues, or it may be that the insula acts broadly as a salience detector to relay salient information to other brain structures to generate behavioral responses to the most relevant environmental stimuli. Both views could explain how experimental rats decipher a stressed conspecific from a naïve via IC activity. In the empathy view, the IC may evaluate, or even emulate, the affective state of another animal. This could produce a “shared” affect between the two animals, and it may be that a stressed rat produces a different shared affective state than the naïve. The experimental rat can then determine which conspecific to approach as a product of evaluating its own affective state. If this were true, future experiments could hypothesize that a neural correlate of the empathy view is feature detection, whereby unique ensembles of IC neurons are active during exposure to specific conspecifics features (e.g. age, stress,

and sex), which then project to various structures in the SDMN to drive specific patterns of activity that correspond to certain social behaviors (Newman, 1999). For example, an ensemble of neurons that detect stress may drive rat's default response to avoid a stressed conspecific via basolateral amygdala inputs to the prelimbic cortex (Burgos-Robles et al., 2017), whereas a separate ensemble in IC that detects "juvenile" features may inhibit basolateral amygdala output in thus circuit and thus give way to approach behavior.

In the salience view, IC may have a more general function in cognitive control (Menon & Uddin, 2010). IC may continually detect incoming sensory information as one component of the brain's default network, a basal state of intrinsic brain activity observed in the absence of meaningful stimuli (Raichle, 2015), and in the presence of a salient cue in the external environment switch cognitive control from this default state to one driven by the executive control network. This allows one to coordinate behavioral and motor responses to the cue (Eisenreich et al., 2017). Contextualized in the SAP test, experimental rats may have some inherent behavioral reaction to explore conspecifics, but specific sensory cues that convey affect or age may engage executive control circuitry that overrides this baseline behavior in order to produce a context-appropriate response. In this case, stress or age are cues that can override the baseline response. A neural correlate supporting this explanation would be evidence of coincidence detection; if an animal would always "approach" in a social context, there would be no need to factor any other information in this encounter. However, animals have flexible responses to their social environments and can thus account for the various factors present in a given interaction (i.e. age, stress). This could be reflected in

neural responses to converging inputs that each communicate specific external factors; neurons could increase or modulate their firing and temporal responses to convergent information in ways that produce distinguishable neural responses from those produced by each input individually. This alteration in signal strength could convey socially-relevant information to SMN and influence patterns of activity to shift behavioral responses toward social approach or avoidance.

An important distinction between these two views pertains to the loci where the decisions to approach or avoid a conspecific are made: in the empathy view, IC selects the behavioral response as a product of comparing the shared affect with a naïve versus a stressed conspecific, whereas in the salience view, the IC increases the value of social affective information across the social decision-making network, so that downstream regions can use such information to select a specific response; importantly no empirical evidence is available to untangle these possibilities. A limitation to the work presented in this dissertation is that, while there is compelling causal evidence that IC is necessary for social affective behaviors, we do not provide any empirical test that the specific role of IC is to detect social affective cues. Though, to this point, we do not observe a reduction in overall social exploration during the SAP test following insular cortex inactivation, which suggests the role of IC is specific to affective responses rather than social exploration *per se*. This supports the idea of the salience view because the animal is still able to perform its baseline response to a social encounter: explore. What the animal loses is the capacity to direct their exploration to one conspecific over the other and thus behaves as if there is no salient cue to respond to. However, it remains elusive if IC is acting in a uniquely social role, or rather serves a broader capacity to

inform behavioral responses to salient sensory information. Current findings from human neuroimaging implicate IC as a salience detector for pain and temperature (Peltz et al., 2011). Thus, an interesting first step would be to determine in rodents if IC is also necessary to distinguish salient non-social stimuli.

5.4 Does oxytocin increase the salience of sensory information?

The effects of oxytocin on social behaviors range from antisocial to prosocial and depend on many internal and external factors. To account for such divergent effects of oxytocin, (Shamay-Tsoory & Abu-Akel, 2016) proposed a hypothesis in which oxytocin increases the salience of social information by modulating one's attention to relevant cues in the external environment to contextualized them with other factors to generate context-appropriate responses. Consistent with this thought are a number of reports that intranasal administration of oxytocin attenuated social cognitive deficits in autism, possibly by increasing attention to social cues (Dadds et al., 2014; Geng et al., 2018; Gordon et al., 2016; Guastella et al., 2010; Keech et al., 2018; Parker et al., 2017; Clark-Elford et al., 2014; Domes et al., 2013; Ellenbogen et al., 2012; Hubble et al., 2017). If the insular cortex receives converging social affective cues, does oxytocin function to increase their salience?

While there are no accounts of the physiological effects of oxytocin on insular cortex neurons beyond the data presented in Chapter 3, prior work conducted in sensory cortical areas allow for speculation regarding the mechanisms by which oxytocin can rapidly increase the salience of sensory cues at the level of local circuits

(Marlin & Froemke, 2017). In olfactory cortex, OT was found necessary for forming associations between odor and social experiences, suggesting OT increased the salience of social odor cues (Choe et al., 2015). Similarly, OT facilitated accurate odor coding; by increasing the firing rate of olfactory cortex neurons that synapse onto inhibitory interneurons, lateral inhibition onto excitatory principal cells increased the signal-to-noise ratio of responses by decreasing noise from excitatory activity in the microcircuit (Oettl et al., 2016). In hippocampus, administration of an OT agonist increased the signal-to-noise ration by exciting fast-spike inhibitory interneurons, which in turn dampened excitation and sharpen the timing of spike responses (Owen et al., 2013). Interestingly, OT can decrease inhibitory post-synaptic currents, which shift the excitatory/inhibitory balance in auditory cortex so that pup vocalizations more rapidly elicit maternal responses from lactating mice (Marlin et al., 2015). These findings highlight the ability of OT to augment local circuits and better detect and encode sensory cues, likely via inhibitory interneurons. Though no existing studies have tested the neuromodulatory effects of OT on insular cortex interneurons, (Rodríguez-García & Miranda, 2016) report that GABAergic activation in IC is sensitive to neuromodulation during the presentation of a novel taste stimulus. This justifies further exploration of modulation of inhibitory responses in IC during sensory processing.

Either in addition to, or instead of modulating local circuity, OT may increase the salience of a sensory stimulus at single neuron level by modulating dendritic propagation and integration of sensory inputs in IC neurons. While this idea has yet to be explored in the context of OT in any brain region, it is plausible that OT functions like other neuromodulators that alter the various aspects of dendritic integration, including

the amplitude of postsynaptic currents, as well as spatial and temporal summation across the dendritic arbor (Magee, 2000). Consistent with this idea, OT increased the amplitude and spatial summation of excitatory post-synaptic potentials to facilitate action potential generation in mollusc neurons (Dyatlov, 1991) and facilitated long-term potentiation in the medial prefrontal cortex (Ninan, 2011). However, the majority of studies that account for OT effects on dendritic activity do so from a developmental lens in which OT induces long-lasting changes in dendritic morphology (Ripamonti et al., 2017). Such a change would not account for the rapid increase in the salience of social sensory cues that may occur during the SAP test. To this point, the data in Chapter 3 demonstrate that OT increased local field excitability in the insular cortex, and findings from whole-cell recordings in (Rogers-Carter et al., 2018b) show OT increased intrinsic excitability, suggesting OT has fast-acting effects on excitability. With the techniques used here we cannot determine if increased excitability reflects the sum of increased excitability of each insular cortex pyramidal cell, or rather is a consequence of OT modulating local microcircuits so that dampened inhibition from interneurons results in increased pyramidal cell activity. In either case, increased excitability of insular cortex neurons could contribute to either increased salience, by allowing IC projection neurons to drive stronger changes in SDMN activity patterns via robust changes in their neural responses to social information to the SDMN, or increased empathy by being more sensitive to social affective cues and thus more easily mimicking the affective state in the experimental animal.

5.5 Does the nucleus accumbens determine reward in the social affective preference test?

Many social behaviors are thought to be naturally rewarding (Krach et al., 2010) to reinforce social actions that promote species survival (O'Connell & Hofmann, 2011) and accordingly, the nucleus accumbens is widely implicated in social (Vanderschuren et al., 2016) and motivated behaviors (Kalivas & Nakamura, 1999). Accumbens is likely involved in such behaviors because it can determine the valence of incoming sensory information (Johnson et al., 2017) to compute reward valuation and generate corresponding behavioral responses (Hamel et al., 2017). But how do valence and reward, as determined by the accumbens, relate to social affective behavior? One possible explanation is that an experimental rat appraises the sensory cues emitted by each conspecific in the SAP test, and then engages in more social exploration with whichever conspecific is determined to be more rewarding. In support of this idea, human neuroimaging revealed that nucleus accumbens reactivity positively correlated with amount of money gained in a monetary incentive game (Carter et al., 2009). Thus, while the rat may be inherently motived to explore both conspecifics, one "wins out" as a result of their social affective cues driving a higher reward valuation, and accumbens activity, than the other conspecific. Another possibility is that the experimental rat perceives one conspecific as positive and the other as negative in terms of their reward value, and "avoidance" of the conspecific with the negative valence is merely a consequence of approaching the conspecific with the positive affect and presumably higher reward value, and vice versa. In support of this, the accumbens can determine the valence of sensory cues and generate behavioral responses accordingly; positive

and negative stimuli have been found to activate distinct populations of neurons in the accumbens (Xiu et al., 2014), which elicit opposing behavioral responses via distinct dopamine receptor subtype-expressing neurons in the accumbens (Kravitz et al., 2012). By this mechanism, social affective cues could engage distinct neural subpopulations that underlie either approach or avoidance.

While these ideas begin to speculate how affect and valance could influence reward valuation, our results indicate that accumbens activity is likely sensitive to other situational factors. Regarding age, we find that interactions with juveniles require the accumbens, whereas interactions with adults do not. This may because rats are inherently more motivated to explore juvenile conspecifics than adults, possibly as a product of evolutionarily-adaptive circuitry that promotes tending to the needs of younger animals. Accordingly, we report that experimental rats spend more time exploring naïve juvenile conspecifics than naïve adults (Fig. 2.4) which supports the idea that age drives the intrinsic motivation to engage in social exploration. Another possibility, however, is that juveniles are perceived as more salient than adult conspecifics, which can more robustly influence behavioral responses. In fact, human neuroimaging indicates that the salience of a sensory stimulus is one factor determines its reward value (Cooper & Knutson, 2008). Thus, it is possible that the insular cortex is more easily excited by sensory cues from juvenile than from adults, which results in greater accumbens activation via the insula → accumbens pathway. Perhaps adults are simply not salient enough i to activate the insula or accumbens, and so loss-of-function experiments do not produce behavioral effects. In any case, the mechanisms by which situational factors like age and familiarity can influence salience and subsequent

valuation of salience sensory cues is necessary to explain the role of the nucleus accumbens in social affective behaviors.

5.6 Where does the insular cortex fit in the context of a larger network-level model of social decision-making?

Despite not knowing the exact role of IC in social affective behavior, we can speculate how increased insula activity may function as part of a broader circuit that allows sensory cues to inform social behaviors responses. While the exact nodes of this circuit and how they communicate to produce socioemotional responses is not fully understood, the following hypothetical circuit is inspired from a combination of mechanistic rodent experiments and human neuroimaging studies that have explored various circuits that underlie social decision-making: during encounters like the social affective preference test, social cues that convey the affect of another individual are first detected by thalamic nuclei (Cheung et al., 2006; Cservenák et al., 2017), and then relayed to unimodal sensory cortical regions (Nummenmaa et al., 2008) and the hypothalamus (Cservenák et al., 2017; Sterley et al., 2018) which begin to process each sensory cue (Brecht & Freiwald, 2012), and initiate physiological responses to the stimuli (Hoke et al., 2005), respectively. Next, unimodal sensory inputs converge in posterior insula (see intro for complete discussion) where they are further processed to produce a more complex sensory representation of a stimulus (Ackermann & Riecker, 2004), such as a cohesive understanding of a conspecific's age, sex, affect, and other features. That unimodal areas project to higher-order sensory regions, however, is hardly specific to social situations and thus begets the question, how could IC be

uniquely excited by social stimuli? The answer may lie in fact that social affective cues can excite neurons in the paraventricular nucleus (PVN) of the hypothalamus (Sterley et al., 2018) which synthesize oxytocin (Bargmann & Scharrer, 1951) and project to posterior insula (Knobloch et al., 2012). Thus, the PVN, when potentiated by social affective stimuli, may increase the excitability of insula to incoming sensory cues via its oxytocinergic projections. Whether this increased excitability is because oxytocin causes otherwise unresponsive insula neurons to fire in response to sensory cues, or if oxytocin shifts the output of the IC as a whole to favor a different efferent target is unknown, but either would allow IC to influence activity patterns in the social decision-making network, including the nucleus accumbens, to generate either approach or avoidance. While this pathway is purely speculative, these mechanisms could enable insula to generate context-appropriate social responses to the affective states of others.

5.7 Relevance to clinical research

The work presented in this dissertation not only informs our understanding of the neural circuitry underlying rodent social decision-making, but also has implications for clinical populations. Numerous psychiatric disorders like autism and schizophrenia are characterized by deficits in emotion recognition (Pepper et al., 2018; Singh et al., 2015), and human neuroimaging has reported impaired insular cortex functional organization and connectivity (Caria & de Falco, 2015; Wylie & Tregellas, 2010; Yamada et al., 2016) as well as reduced reactivity to emotional stimuli (Rosenblau et al., 2017), in autism and schizophrenia. Our findings provide causal support for the correlation findings in human neuroimaging experiments that impaired emotion recognition correlates with aberrant IC

activity. Furthermore, oxytocin administration can relieve deficits in social cognition in psychiatric patients (Guastella et al., 2010; Ota et al., 2018; Shilling & Feifel, 2016) and has garnered enthusiasm as a potential therapeutic for social impairments (Young & Barrett, 2015). Thus, our findings that oxytocin is necessary to decipher a naïve from stressed conspecific warrants more research to determine how oxytocin can attenuate deficits in social cognition. Lastly, despite the notion that social reward and motivation processes are disrupted in autism (Dölen, 2015) the nucleus accumbens and other reward areas remain largely unstudied in human research. This may be because fMRI imaging techniques do not offer the necessary resolution to image deeper brain structures implicated in in social cognition and, therefore, are biased to assign causation to superficial cortical structures. It is at the nexus of cortex and SMDN structures that the work discussed in this dissertation is informative; here we begin to bridge the gap between the systems well-studied in human neuroimaging research, and the deeper brain areas that are typically the focus of rodent and other non-human social behaviors. Models that seek to account for the neural basis of social decision making in humans may now consider the IC as a central hub where emotional processes are integrated with the elementary circuits that are the proximate mediators of social behaviors.

5.8 Conclusion

This work tested the necessity of the insular cortex in generating specific responses to social affective stimuli. The findings presented here are the first to implicate the insula in any rodent social behavior, a role we believe is a consequence of the unique sensory integrative properties of posterior insula. Furthermore, the need to

accurately detect the socioemotional cues of others in order to generate appropriate social responses has been previously discussed (Insel & Fernald, 2004b; Newman, 1999), but a direct pathway by which sensory information is communicated to social brain structures had not yet been identified. While the work presented here does not fully describe this process, it provides a conceptual foundation to inspire future studies that aim to determine the mechanisms of sensory detection in social interactions.

References

Ackermann, H., & Riecker, A. (2004). The contribution of the insula to motor aspects of speech production: a review and a hypothesis. *Brain Lang*, *89*(2), 320-328. doi:10.1016/S0093-934X(03)00347-X

Adolfi, F., Couto, B., Richter, F., Decety, J., Lopez, J., Sigman, M., Manes, F., & Ibáñez, A. (2017). Convergence of interoception, emotion, and social cognition: A twofold fMRI meta-analysis and lesion approach. *Cortex*, *88*, 124-142. doi:10.1016/j.cortex.2016.12.019

Adolphs, R. (2001). The neurobiology of social cognition. *Curr Opin Neurobiol*, *11*(2), 231-239. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11301245

Adolphs, R. (2009). The social brain: neural basis of social knowledge. *Annu Rev Psychol*, *60*, 693-716. doi:10.1146/annurev.psych.60.110707.163514

Adolphs, R., Tranel, D., & Damasio, A. R. (2003). Dissociable neural systems for recognizing emotions. *Brain Cogn*, *52*(1), 61-69. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12812805

Akhondzadeh, S., & Stone, T. W. (1995). Induction of a novel form of hippocampal long-term depression by muscimol: involvement of GABAA but not glutamate receptors. *Br J Pharmacol*, *115*(3), 527-533. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7582468

Alexander, W. H., & Brown, J. W. (2011). Medial prefrontal cortex as an action-outcome predictor. *Nat Neurosci*, *14*(10), 1338-1344. doi:10.1038/nn.2921

Amodio, D. M., & Frith, C. D. (2006). Meeting of minds: the medial frontal cortex and social cognition. *Nat Rev Neurosci*, *7*(4), 268-277. doi:10.1038/nrn1884

Andics, A., McQueen, J. M., Petersson, K. M., Gál, V., Rudas, G., & Vidnyánszky, Z. (2010). Neural mechanisms for voice recognition. *Neuroimage*, *52*(4), 1528-1540. doi:10.1016/j.neuroimage.2010.05.048

Angoa-Pérez, M., & Kuhn, D. M. (2015). Neuroanatomical dichotomy of sexual behaviors in rodents: a special emphasis on brain serotonin. *Behav Pharmacol*, *26*(6), 595-606. doi:10.1097/FBP.0000000000000157

Arakawa, H., Arakawa, K., Blandino, P., & Deak, T. (2011). The role of neuroinflammation in the release of aversive odor cues from footshock-stressed rats: Implications for the neural mechanism of alarm pheromone. *Psychoneuroendocrinology*, *36*(4), 557-568. doi:10.1016/j.psyneuen.2010.09.001

Arakawa, H., Blanchard, D. C., Arakawa, K., Dunlap, C., & Blanchard, R. J. (2008). Scent marking behavior as an odorant communication in mice. *Neurosci Biobehav Rev*, *32*(7), 1236-1248. doi:10.1016/j.neubiorev.2008.05.012

Arakawa, H., Suzuki, A., Zhao, S., Tsytsarev, V., Lo, F. S., Hayashi, Y., Itohara, S., Iwasato, T., & Erzurumlu, R. S. (2014). Thalamic NMDA receptor function is necessary for patterning of the thalamocortical somatosensory map and for sensorimotor behaviors. *J Neurosci*, *34*(36), 12001-12014. doi:10.1523/JNEUROSCI.1663-14.2014

Aransay, A., Rodríguez-López, C., García-Amado, M., Clascá, F., & Prensa, L. (2015). Long-range projection neurons of the mouse ventral tegmental area: a single-cell axon tracing analysis. *Front Neuroanat*, *9*, 59. doi:10.3389/fnana.2015.00059

Argue, K. J., & McCarthy, M. M. (2015). Characterization of juvenile play in rats: importance of sex of self and sex of partner. *Biol Sex Differ*, *6*, 16. doi:10.1186/s13293-015-0034-x

Auyeung, B., Lombardo, M. V., Heinrichs, M., Chakrabarti, B., Sule, A., Deakin, J. B., Bethlehem, R. A., Dickens, L., Mooney, N., Sipple, J. A., Thiemann, P., & Baron-Cohen, S. (2015). Oxytocin increases eye contact during a real-time, naturalistic social interaction in males with and without autism. *Transl Psychiatry*, *5*, e507. doi:10.1038/tp.2014.146

Ayar, A., Ozcan, M., Alcin, E., Serhatlioglu, I., Ozcan, S., Kutlu, S., & Kelestimur, H. (2014). Oxytocin activates calcium signaling in rat sensory neurons through a protein kinase C-dependent mechanism. *J Physiol Biochem*, *70*(1), 43-48. doi:10.1007/s13105-013-0278-z

Azzi, J. C., Sirigu, A., & Duhamel, J. R. (2012). Modulation of value representation by social context in the primate orbitofrontal cortex. *Proc Natl Acad Sci U S A*, *109*(6), 2126-2131. doi:10.1073/pnas.1111715109

Babineau, B. A., Bliss-Moreau, E., Machado, C. J., Toscano, J. E., Mason, W. A., & Amaral, D. G. (2011). Context-specific social behavior is altered by orbitofrontal cortex lesions in adult rhesus macaques. *Neuroscience*, *179*, 80-93. doi:10.1016/j.neuroscience.2011.01.019

Báez-Mendoza, R., Harris, C. J., & Schultz, W. (2013). Activity of striatal neurons reflects social action and own reward. *Proc Natl Acad Sci U S A*, *110*(41), 16634-16639. doi:10.1073/pnas.1211342110

Bagaev, V., & Aleksandrov, V. (2006). Visceral-related area in the rat insular cortex. *Auton Neurosci*, *125*(1-2), 16-21. doi:10.1016/j.autneu.2006.01.006

Bales, K. L., Mason, W. A., Catana, C., Cherry, S. R., & Mendoza, S. P. (2007). Neural correlates of pair-bonding in a monogamous primate. *Brain Res*, *1184*, 245-253. doi:10.1016/j.brainres.2007.09.087

Barat, E., Wirth, S., & Duhamel, J. R. (2018). Face cells in orbitofrontal cortex represent social categories. *Proc Natl Acad Sci U S A*, *115*(47), E11158-E11167. doi:10.1073/pnas.1806165115

BARGMANN, W., & SCHARRER, E. (1951). The site of origin of the hormones of the posterior pituitary. *Am Sci*, *39*(2), 255-259. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24540858

Barnabi, F., & Cechetto, D. F. (2001). Neurotransmitters in the thalamus relaying visceral input to the insular cortex in the rat. *Am J Physiol Regul Integr Comp Physiol*, *281*(5), R1665-74. doi:10.1152/ajpregu.2001.281.5.R1665

Bartz, J. A., Zaki, J., Bolger, N., & Ochsner, K. N. (2011). Social effects of oxytocin in humans: context and person matter. *Trends Cogn Sci*, *15*(7), 301-309. doi:10.1016/j.tics.2011.05.002

Batson, C. D., & Powell, A. A. (2003). *Altruism and Prosocial Behavior*. John Wiley & Sons. Retrieved from https://doi/org/10.1002/0471264385.wei0519

Beach, F. A. (1942). Analysis of the stimuli adequate to elicit mating behavior in the sexually inexperienced rat. *Journal of Comparative Neurology*, *33*(2), 163-207. Retrieved from

Beach, F. A., & Jaynes, J. (1956). Studies of maternal retrieving in rats. Iii. Sensory cues involved in the lactating female's response to her young. *Behavior*, *10*(1). Retrieved from

Bekoff, M. (1972). The development of social interaction, play, and metacommunication in mammals: an ethological perspective. *The Quarterly Review of Biology*, *47*(4), 412-434. Retrieved from

Ben-Ami Bartal, I., Rodgers, D. A., Bernardez Sarria, M. S., Decety, J., & Mason, P. (2014). Pro-social behavior in rats is modulated by social experience. *Elife*, *3*, e01385. doi:10.7554/eLife.01385

Beny-Shefer, Y., Zilkha, N., Lavi-Avnon, Y., Bezalel, N., Rogachev, I., Brandis, A., Dayan, M., & Kimchi, T. (2017). Nucleus Accumbens Dopamine Signaling Regulates Sexual Preference for Females in Male Mice. *Cell Rep*, *21*(11), 3079-3088. doi:10.1016/j.celrep.2017.11.062

Bernhardt, B. C., & Singer, T. (2012). The neural basis of empathy. *Annu Rev Neurosci*, *35*, 1-23. doi:10.1146/annurev-neuro-062111-150536

Beyeler, A., Chang, C. J., Silvestre, M., Lévêque, C., Namburi, P., Wildes, C. P., & Tye, K. M. (2018). Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. *Cell Rep*, *22*(4), 905-918. doi:10.1016/j.celrep.2017.12.097

Bird, C. W., Barto, D., Magcalas, C. M., Rodriguez, C. I., Donaldson, T., Davies, S., Savage, D. D., & Hamilton, D. A. (2017). Ifenprodil infusion in agranular insular cortex alters social behavior and vocalizations in rats exposed to moderate levels of ethanol during prenatal development. *Behav Brain Res*, *320*, 1-11. doi:10.1016/j.bbr.2016.11.036

Blume, A., Bosch, O. J., Miklos, S., Torner, L., Wales, L., Waldherr, M., & Neumann, I. D. (2008). Oxytocin reduces anxiety via ERK1/2 activation: local effect within the rat hypothalamic paraventricular nucleus. *Eur J Neurosci*, *27*(8), 1947-1956. doi:10.1111/j.1460-9568.2008.06184.x

Bobrov, E., Wolfe, J., Rao, R. P., & Brecht, M. (2014). The representation of social facial touch in rat barrel cortex. *Curr Biol*, *24*(1), 109-115. doi:10.1016/j.cub.2013.11.049

Bolding, K. A., & Franks, K. M. (2017). Complementary codes for odor identity and intensity in olfactory cortex. *Elife*, *6*. doi:10.7554/eLife.22630

Borelli, K. G., Blanchard, D. C., Javier, L. K., Defensor, E. B., Brandão, M. L., & Blanchard, R. J. (2009). Neural correlates of scent marking behavior in C57BL/6J mice: detection and recognition of a social stimulus. *Neuroscience*, *162*(4), 914-923. doi:10.1016/j.neuroscience.2009.05.047

Botvinick, M., Jha, A. P., Bylsma, L. M., Fabian, S. A., Solomon, P. E., & Prkachin, K. M. (2005). Viewing facial expressions of pain engages cortical areas involved in the direct experience of pain. *Neuroimage*, *25*(1), 312-319. doi:10.1016/j.neuroimage.2004.11.043

Boucher, O., Rouleau, I., Lassonde, M., Lepore, F., Bouthillier, A., & Nguyen, D. K. (2015). Social information processing following resection of the insular cortex. *Neuropsychologia*, *71*, 1-10. doi:10.1016/j.neuropsychologia.2015.03.008

Bouton, M. E., & Bolles, R. C. (1979). Role of conditioned contextual stimuli in reinstatement of extinguished fear. *J Exp Psychol Anim Behav Process*, *5*(4), 368-378. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/528893

Brecht, M., & Freiwald, W. A. (2012). The many facets of facial interactions in mammals. *Curr Opin Neurobiol*, *22*(2), 259-266. doi:10.1016/j.conb.2011.12.003

Britt, J. P., Benaliouad, F., McDevitt, R. A., Stuber, G. D., Wise, R. A., & Bonci, A. (2012). Synaptic and behavioral profile of multiple glutamatergic inputs to the nucleus accumbens. *Neuron*, *76*(4), 790-803. doi:10.1016/j.neuron.2012.09.040

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol*, *338*(2), 255-278. doi:10.1002/cne.903380209

Bromberg-Martin, E. S., Matsumoto, M., & Hikosaka, O. (2010). Dopamine in motivational control: rewarding, aversive, and alerting. *Neuron*, *68*(5), 815-834. doi:10.1016/j.neuron.2010.11.022

Brudzynski, S. M. (2013). Ethotransmission: communication of emotional states through ultrasonic vocalization in rats. *Curr Opin Neurobiol*, *23*(3), 310-317. doi:10.1016/j.conb.2013.01.014

Budinger, E., Laszcz, A., Lison, H., Scheich, H., & Ohl, F. W. (2008). Non-sensory cortical and subcortical connections of the primary auditory cortex in Mongolian gerbils: bottom-up and top-down processing of neuronal information via field AI. *Brain Res*, *1220*, 2-32. doi:10.1016/j.brainres.2007.07.084

Burgdorf, J., Kroes, R. A., Moskal, J. R., Pfaus, J. G., Brudzynski, S. M., & Panksepp, J. (2008). Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. *J Comp Psychol*, *122*(4), 357-367. doi:10.1037/a0012889

Burgos-Robles, A., Kimchi, E. Y., Izadmehr, E. M., Porzenheim, M. J., Ramos-Guasp, W. A., Nieh, E. H., Felix-Ortiz, A. C., Namburi, P., Leppla, C. A., Presbrey, K. N., Anandalingam, K. K., Pagan-Rivera, P. A., Anahtar, M., Beyeler, A., & Tye, K. M. (2017). Amygdala inputs to prefrontal cortex guide behavior amid conflicting cues of reward and punishment. *Nat Neurosci*, *20*(6), 824-835. doi:10.1038/nn.4553

Burke, C. J., Kisko, T. M., Swiftwolfe, H., Pellis, S. M., & Euston, D. R. (2017). Specific 50-kHz vocalizations are tightly linked to particular types of behavior in juvenile rats anticipating play. *PLoS One*, *12*(5), e0175841. doi:10.1371/journal.pone.0175841

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science*, *351*(6271), 375-378. doi:10.1126/science.aac4785

Cabanac, M. (2002). What is emotion. *Behav Processes*, *60*(2), 69-83. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12426062

Cádiz-Moretti, B., Abellán-Álvaro, M., Pardo-Bellver, C., Martínez-García, F., & Lanuza, E. (2016). Afferent and Efferent Connections of the Cortex-Amygdala Transition Zone in Mice. *Front Neuroanat*, *10*, 125. doi:10.3389/fnana.2016.00125

Calder, A. J., Keane, J., Manes, F., Antoun, N., & Young, A. W. (2000). Impaired recognition and experience of disgust following brain injury. *Nat Neurosci*, *3*(11), 1077-1078. doi:10.1038/80586

Caldú, X., & Dreher, J. C. (2007). Hormonal and genetic influences on processing reward and social information. *Ann N Y Acad Sci*, *1118*, 43-73. doi:10.1196/annals.1412.007

Campanella, F., Shallice, T., Ius, T., Fabbro, F., & Skrap, M. (2014). Impact of brain tumour location on emotion and personality: a voxel-based lesion-symptom mapping study on mentalization processes. *Brain*, *137*(Pt 9), 2532-2545. doi:10.1093/brain/awu183

Cao, W. Y., Xu, Y., Luo, Y. W., Zhong, X. L., Duan, J., Wang, X. Q., Hu, Z. L., Li, F., Dai, R. P., Luo, X. G., Zhang, J. Y., & Li, C. Q. (2013). Activation of ERK1/2 is required for normal response of isosexual social interactions in male rats. *Brain Res*, *1538*, 51-60. doi:10.1016/j.brainres.2013.08.046

Caria, A., & de Falco, S. (2015). Anterior insular cortex regulation in autism spectrum disorders. *Front Behav Neurosci*, *9*, 38. doi:10.3389/fnbeh.2015.00038

Carruthers, I. M., Natan, R. G., & Geffen, M. N. (2013). Encoding of ultrasonic vocalizations in the auditory cortex. *J Neurophysiol*, *109*(7), 1912-1927. doi:10.1152/jn.00483.2012

Cavalcante, L. E. S., Zinn, C. G., Schmidt, S. D., Saenger, B. F., Ferreira, F. F., Furini, C. R. G., Myskiw, J. C., & Izquierdo, I. (2017). Modulation of the storage of social recognition memory by neurotransmitter systems in the insular cortex. *Behav Brain Res*, *334*, 129-134. doi:10.1016/j.bbr.2017.07.044

Cechetto, D. F., & Chen, S. J. (1990). Subcortical sites mediating sympathetic responses from insular cortex in rats. *Am J Physiol*, *258*(1 Pt 2), R245-55. doi:10.1152/ajpregu.1990.258.1.R245

Cechetto, D. F., & Saper, C. B. (1987). Evidence for a viscerotopic sensory representation in the cortex and thalamus in the rat. *J Comp Neurol*, *262*(1), 27-45. doi:10.1002/cne.902620104

Centanni, S. W., Morris, B. D., Luchsinger, J. R., Bedse, G., Fetterly, T. L., Patel, S., & Winder, D. G. (2019). Endocannabinoid control of the insular-bed nucleus of the stria terminalis circuit regulates negative affective behavior associated with alcohol abstinence. *Neuropsychopharmacology*, *44*(3), 526-537. doi:10.1038/s41386-018-0257-8

Charles Lawrence, R., Cale Bonner, H., Newsom, R. J., & Kelly, S. J. (2008). Effects of alcohol exposure during development on play behavior and c-Fos expression in response to play behavior. *Behav Brain Res*, *188*(1), 209-218. doi:10.1016/j.bbr.2007.10.028

Cheal, M. L., & Sprott, R. L. (1971). Social olfaction: a review of the role of olfaction in a variety of animal behaviors. *Psychol Rep*, *29*(1), 195-243. doi:10.2466/pr0.1971.29.1.195

Chelnokova, O., Laeng, B., Eikemo, M., Riegels, J., Løseth, G., Maurud, H., Willoch, F., & Leknes, S. (2014). Rewards of beauty: the opioid system mediates social motivation in humans. *Mol Psychiatry*, *19*(7), 746-747. doi:10.1038/mp.2014.1

Chen, C., Omiya, Y., & Yang, S. (2015). Dissociating contributions of ventral and dorsal striatum to reward learning. *J Neurophysiol*, *114*(3), 1364-1366. doi:10.1152/jn.00873.2014

Chen, H., Sharp, B. M., Matta, S. G., & Wu, Q. (2011). Social interaction promotes nicotine self-administration with olfactogustatory cues in adolescent rats. *Neuropsychopharmacology*, *36*(13), 2629-2638. doi:10.1038/npp.2011.149

Chen, J. (2018). Empathy for Distress in Humans and Rodents. *Neurosci Bull*, *34*(1), 216-236. doi:10.1007/s12264-017-0135-0

Chen, Q., Panksepp, J. B., & Lahvis, G. P. (2009). Empathy is moderated by genetic background in mice. *PLoS One*, *4*(2), e4387. doi:10.1371/journal.pone.0004387

Cheung, C. C., Lee, T. M., Yip, J. T., King, K. E., & Li, L. S. (2006). The differential effects of thalamus and basal ganglia on facial emotion recognition. *Brain Cogn*, *61*(3), 262-268. doi:10.1016/j.bandc.2006.01.008

Cho, H., Kim, C. H., Knight, E. Q., Oh, H. W., Park, B., Kim, D. G., & Park, H. J. (2017). Changes in brain metabolic connectivity underlie autistic-like social deficits in a rat model of autism spectrum disorder. *Sci Rep*, *7*(1), 13213. doi:10.1038/s41598-017-13642-3

Choe, H. K., Reed, M. D., Benavidez, N., Montgomery, D., Soares, N., Yim, Y. S., & Choi, G. B. (2015). Oxytocin Mediates Entrainment of Sensory Stimuli to Social Cues of Opposing Valence. *Neuron*, *87*(1), 152-163. doi:10.1016/j.neuron.2015.06.022

Christianson, J. P., Benison, A. M., Jennings, J., Sandsmark, E. K., Amat, J., Kaufman, R. D., Baratta, M. V., Paul, E. D., Campeau, S., Watkins, L. R., Barth, D. S., & Maier, S. F. (2008). The sensory insular cortex mediates the stress-buffering effects of safety signals but not behavioral control. *J Neurosci*, *28*(50), 13703-13711. doi:10.1523/JNEUROSCI.4270-08.2008

Christianson, J. P., Jennings, J. H., Ragole, T., Flyer, J. G., Benison, A. M., Barth, D. S., Watkins, L. R., & Maier, S. F. (2011). Safety signals mitigate the consequences of uncontrollable stress via a circuit involving the sensory insular cortex and bed nucleus of the stria terminalis. *Biol Psychiatry*, *70*(5), 458-464. doi:10.1016/j.biopsych.2011.04.004

Clark-Elford, R., Nathan, P. J., Auyeung, B., Mogg, K., Bradley, B. P., Sule, A., Müller, U., Dudas, R. B., Sahakian, B. J., & Baron-Cohen, S. (2014). Effects of oxytocin on attention to emotional faces in healthy volunteers and highly socially anxious males. *Int J Neuropsychopharmacol*, *18*(2). doi:10.1093/ijnp/pyu012

Clark, L., Bechara, A., Damasio, H., Aitken, M. R., Sahakian, B. J., & Robbins, T. W. (2008). Differential effects of insular and ventromedial prefrontal cortex lesions on risky decision-making. *Brain*, *131*(Pt 5), 1311-1322. doi:10.1093/brain/awn066

Cohen, L., Rothschild, G., & Mizrahi, A. (2011). Multisensory integration of natural odors and sounds in the auditory cortex. *Neuron*, *72*(2), 357-369. doi:10.1016/j.neuron.2011.08.019

Connor, J. R., & Diamond, M. C. (1982). A comparison of dendritic spine number and type on pyramidal neurons of the visual cortex of old adult rats from social or isolated environments. *J Comp Neurol*, *210*(1), 99-106. doi:10.1002/cne.902100111

Consiglio, A. R., Borsoi, A., Pereira, G. A., & Lucion, A. B. (2005). Effects of oxytocin microinjected into the central amygdaloid nucleus and bed nucleus of stria terminalis on maternal aggressive behavior in rats. *Physiol Behav*, *85*(3), 354-362. doi:10.1016/j.physbeh.2005.05.002

Coolen, L. M., & Wood, R. I. (1998). Bidirectional connections of the medial amygdaloid nucleus in the Syrian hamster brain: simultaneous anterograde and retrograde tract tracing. *J Comp Neurol*, *399*(2), 189-209. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9721903

Cooper, J. C., & Knutson, B. (2008). Valence and salience contribute to nucleus accumbens activation. *Neuroimage*, *39*(1), 538-547. doi:10.1016/j.neuroimage.2007.08.009

Coria-Avila, G. A., Ouimet, A. J., Pacheco, P., Manzo, J., & Pfaus, J. G. (2005). Olfactory conditioned partner preference in the female rat. *Behav Neurosci*, *119*(3), 716-725. doi:10.1037/0735-7044.119.3.716

Corradi-Dell'Acqua, C., Hofstetter, C., & Vuilleumier, P. (2011). Felt and seen pain evoke the same local patterns of cortical activity in insular and cingulate cortex. *J Neurosci*, *31*(49), 17996-18006. doi:10.1523/JNEUROSCI.2686-11.2011

Creutz, L. M., & Kritzer, M. F. (2004). Mesostriatal and mesolimbic projections of midbrain neurons immunoreactive for estrogen receptor beta or androgen receptors in rats. *J Comp Neurol*, *476*(4), 348-362. doi:10.1002/cne.20229

Critchley, H. D., & Harrison, N. A. (2013). Visceral influences on brain and behavior. *Neuron*, *77*(4), 624-638. doi:10.1016/j.neuron.2013.02.008

Croy, I., Drechsler, E., Hamilton, P., Hummel, T., & Olausson, H. (2016). Olfactory modulation of affective touch processing - A neurophysiological investigation. *Neuroimage*, *135*, 135-141. doi:10.1016/j.neuroimage.2016.04.046

Cservenák, M., Keller, D., Kis, V., Fazekas, E. A., Öllös, H., Lékó, A. H., Szabó, É. R., Renner, É., Usdin, T. B., Palkovits, M., & Dobolyi, Á. (2017). A Thalamo-Hypothalamic Pathway That Activates Oxytocin Neurons in Social Contexts in Female Rats. *Endocrinology*, *158*(2), 335-348. doi:10.1210/en.2016-1645

Dadds, M. R., MacDonald, E., Cauchi, A., Williams, K., Levy, F., & Brennan, J. (2014). Nasal oxytocin for social deficits in childhood autism: a randomized controlled trial. *J Autism Dev Disord*, *44*(3), 521-531. doi:10.1007/s10803-013-1899-3

Darwin, C. (1976). *The expression of the emotions in man and animals* (London: New York).

De La Rosa-Prieto, C., De Moya-Pinilla, M., Saiz-Sanchez, D., Ubeda-Banon, I., Arzate, D. M., Flores-Cuadrado, A., Liberia, T., Crespo, C., & Martinez-Marcos, A. (2015). Olfactory and cortical projections to bulbar and hippocampal adult-born neurons. *Front Neuroanat*, *9*, 4. doi:10.3389/fnana.2015.00004

de Waal, F. B. M., & Preston, S. D. (2017). Mammalian empathy: behavioural manifestations and neural basis. *Nat Rev Neurosci*, *18*(8), 498-509. doi:10.1038/nrn.2017.72

Defensor, E. B., Corley, M. J., Blanchard, R. J., & Blanchard, D. C. (2012). Facial expressions of mice in aggressive and fearful contexts. *Physiol Behav*, *107*(5), 680-685. doi:10.1016/j.physbeh.2012.03.024

Di Martino, A., Shehzad, Z., Kelly, C., Roy, A. K., Gee, D. G., Uddin, L. Q., Gotimer, K., Klein, D. F., Castellanos, F. X., & Milham, M. P. (2009). Relationship between cingulo-insular functional connectivity and autistic traits in neurotypical adults. *Am J Psychiatry*, *166*(8), 891-899. doi:10.1176/appi.ajp.2009.08121894

Di Simplicio, M., & Harmer, C. J. (2016). Oxytocin and emotion processing. *J Psychopharmacol*, *30*(11), 1156-1159. doi:10.1177/0269881116641872

Dinopoulos, A., & Parnavelas, J. G. (1991). The development of ventral tegmental area (VTA) projections to the visual cortex of the rat. *Neurosci Lett*, *134*(1), 12-16. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1815143

Dölen, G. (2015). Autism: Oxytocin, serotonin, and social reward. *Soc Neurosci*, *10*(5), 450-465. doi:10.1080/17470919.2015.1087875

Dölen, G., Darvishzadeh, A., Huang, K. W., & Malenka, R. C. (2013). Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. *Nature*, *501*(7466), 179-184. doi:10.1038/nature12518

Domes, G., Sibold, M., Schulze, L., Lischke, A., Herpertz, S. C., & Heinrichs, M. (2013). Intranasal oxytocin increases covert attention to positive social cues. *Psychol Med*, *43*(8), 1747-1753. doi:10.1017/S0033291712002565

Dong, H. W., & Swanson, L. W. (2004). Projections from bed nuclei of the stria terminalis, posterior division: implications for cerebral hemisphere regulation of defensive and reproductive behaviors. *J Comp Neurol*, *471*(4), 396-433. doi:10.1002/cne.20002

Dong, H. W., & Swanson, L. W. (2006). Projections from bed nuclei of the stria terminalis, dorsomedial nucleus: implications for cerebral hemisphere integration of neuroendocrine, autonomic, and drinking responses. *J Comp Neurol*, *494*(1), 75-107. doi:10.1002/cne.20790

Dong, X., Li, S., & Kirouac, G. J. (2017). Collateralization of projections from the paraventricular nucleus of the thalamus to the nucleus accumbens, bed nucleus of the stria terminalis, and central nucleus of the amygdala. *Brain Struct Funct*, *222*(9), 3927-3943. doi:10.1007/s00429-017-1445-8

Duhamel, J. R. (2002). Multisensory integration in cortex: shedding light on prickly issues. *Neuron*, *34*(4), 493-495. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12062031

Dumais, K. M., Bredewold, R., Mayer, T. E., & Veenema, A. H. (2013). Sex differences in oxytocin receptor binding in forebrain regions: correlations with social interest in brain region- and sex- specific ways. *Horm Behav*, *64*(4), 693-701. doi:10.1016/j.yhbeh.2013.08.012

Dyatlov, V. A. (1991). Influence of oxytocin on integration of postsynaptic potentials in molluscan neurons. *Comp Biochem Physiol C*, *98*(2-3), 271-274. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1712690

Edwards, J., Jackson, H. J., & Pattison, P. E. (2002). Emotion recognition via facial expression and affective prosody in schizophrenia: a methodological review. *Clin Psychol Rev*, *22*(6), 789-832. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12214327

Eisenreich, B. R., Akaishi, R., & Hayden, B. Y. (2017). Control without Controllers: Toward a Distributed Neuroscience of Executive Control. *J Cogn Neurosci*, *29*(10), 1684-1698. doi:10.1162/jocn_a_01139

Ellenbogen, M. A. (2018). Oxytocin and Facial Emotion Recognition. *Curr Top Behav Neurosci*, *35*, 349-374. doi:10.1007/7854_2017_20

Ellenbogen, M. A., Linnen, A. M., Grumet, R., Cardoso, C., & Joober, R. (2012). The acute effects of intranasal oxytocin on automatic and effortful attentional shifting to emotional faces. *Psychophysiology*, *49*(1), 128-137. doi:10.1111/j.1469-8986.2011.01278.x

Eres, R., Decety, J., Louis, W. R., & Molenberghs, P. (2015). Individual differences in local gray matter density are associated with differences in affective and cognitive empathy. *Neuroimage*, *117*, 305-310. doi:10.1016/j.neuroimage.2015.05.038

Erzurumlu, R. S., & Gaspar, P. (2012). Development and critical period plasticity of the barrel cortex. *Eur J Neurosci*, *35*(10), 1540-1553. doi:10.1111/j.1460-9568.2012.08075.x

Evans, S. L., Dal Monte, O., Noble, P., & Averbeck, B. B. (2014). Intranasal oxytocin effects on social cognition: a critique. *Brain Res*, *1580*, 69-77. doi:10.1016/j.brainres.2013.11.008

Faget, L., Osakada, F., Duan, J., Ressler, R., Johnson, A. B., Proudfoot, J. A., Yoo, J. H., Callaway, E. M., & Hnasko, T. S. (2016). Afferent Inputs to Neurotransmitter-

Defined Cell Types in the Ventral Tegmental Area. *Cell Rep*, *15*(12), 2796-2808. doi:10.1016/j.celrep.2016.05.057

Fan, Y., Duncan, N. W., de Greck, M., & Northoff, G. (2011). Is there a core neural network in empathy? An fMRI based quantitative meta-analysis. *Neuroscience & Biobehavioral Reviews*, *35*(3), 903-911. Retrieved from https://www.georgnorthoff.com/s/Is-there-a-core-neural-network-in-empathy.pdf

Faser, K. A., Poucet, B., Partlow, G., & Herrmann, T. (1991). Role of the medial and lateral septum in a variable goal spatial problem solving task. *Physiol Behav*, *50*(4), 739-744. Retrieved from

Felix-Ortiz, A. C., Burgos-Robles, A., Bhagat, N. D., Leppla, C. A., & Tye, K. M. (2016). Bidirectional modulation of anxiety-related and social behaviors by amygdala projections to the medial prefrontal cortex. *Neuroscience*, *321*, 197-209. doi:10.1016/j.neuroscience.2015.07.041

Finlayson, K., Lampe, J. F., Hintze, S., Würbel, H., & Melotti, L. (2016). Facial Indicators of Positive Emotions in Rats. *PLoS One*, *11*, e0166446. doi:10.1371/journal.pone.0166446

Fleming, A. S., & Rosenblatt, J. S. (1974). Olfactory regulation of maternal behavior in rats. II. Effects of peripherally induced anosmia and lesions of the lateral olfactory tract in pup-induced virgins. *J Comp Physiol Psychol*, *86*(2), 233-246. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4590492

Friedel, S., Whittle, S. L., Vijayakumar, N., Simmons, J. G., Byrne, M. L., Schwartz, O. S., & Allen, N. B. (2015). Dispositional mindfulness is predicted by structural development of the insula during late adolescence. *Dev Cogn Neurosci*, *14*, 62-70. doi:10.1016/j.dcn.2015.07.001

Fuccillo, M. V. (2016). Striatal Circuits as a Common Node for Autism Pathophysiology. *Front Neurosci*, *10*, 27. doi:10.3389/fnins.2016.00027

Fujita, S., Adachi, K., Koshikawa, N., & Kobayashi, M. (2010). Spatiotemporal dynamics of excitation in rat insular cortex: intrinsic corticocortical circuit regulates caudal-rostro excitatory propagation from the insular to frontal cortex. *Neuroscience*, *165*(1), 278-292. doi:10.1016/j.neuroscience.2009.09.073

Fujiwara, M., & Chiba, A. (2018). Sexual odor preference and dopamine release in the nucleus accumbens by estrous olfactory cues in sexually naïve and experienced male rats. *Physiol Behav*, *185*, 95-102. doi:10.1016/j.physbeh.2017.12.029

Furtak, S. C., Wei, S. M., Agster, K. L., & Burwell, R. D. (2007). Functional neuroanatomy of the parahippocampal region in the rat: the perirhinal and postrhinal cortices. *Hippocampus*, *17*(9), 709-722. doi:10.1002/hipo.20314

Galef, B. G., Kennett, D. J., & Stein, M. (1985). Demonstrator influence on observer diet preference: Effects of simple exposure and the presence of a demonstrator. *Animal Learning & Behavior*, *13*(1), 25-30. Retrieved from

Galef, B. G., & Wigmore, S. W. (1983). Transfer of information concerning distant foods: A laboratory investigation of the 'information-centre' hypothesis. *Anim Behav*, *31*(3), 748-758. Retrieved from

Gallo, M., Roldan, G., & Bures, J. (1992). Differential involvement of gustatory insular cortex and amygdala in the acquisition and retrieval of conditioned taste aversion in rats. *Behav Brain Res*, *52*(1), 91-97. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1335264

García, R., Simón, M. J., & Puerto, A. (2013). Conditioned place preference induced by electrical stimulation of the insular cortex: effects of naloxone. *Exp Brain Res*, *226*(2), 165-174. doi:10.1007/s00221-013-3422-7

Geng, Y., Zhao, W., Zhou, F., Ma, X., Yao, S., Hurlemann, R., Becker, B., & Kendrick, K. M. (2018). Oxytocin Enhancement of Emotional Empathy: Generalization Across Cultures and Effects on Amygdala Activity. *Front Neurosci*, *12*, 512. doi:10.3389/fnins.2018.00512

Gil, M., Nguyen, N. T., McDonald, M., & Albers, H. E. (2013). Social reward: interactions with social status, social communication, aggression, and associated neural activation in the ventral tegmental area. *Eur J Neurosci*, *38*(2), 2308-2318. doi:10.1111/ejn.12216

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiol Rev*, *81*(2), 629-683. doi:10.1152/physrev.2001.81.2.629

Gogolla, N. (2017). The insular cortex. *Curr Biol*, *27*(12), R580-R586. doi:10.1016/j.cub.2017.05.010

Gogolla, N., Takesian, A. E., Feng, G., Fagiolini, M., & Hensch, T. K. (2014). Sensory integration in mouse insular cortex reflects GABA circuit maturation. *Neuron*, *83*(4), 894-905. doi:10.1016/j.neuron.2014.06.033

Gordon, I., Jack, A., Pretzsch, C. M., Vander Wyk, B., Leckman, J. F., Feldman, R., & Pelphrey, K. A. (2016). Intranasal Oxytocin Enhances Connectivity in the Neural Circuitry Supporting Social Motivation and Social Perception in Children with Autism. *Sci Rep*, *6*, 35054. doi:10.1038/srep35054

Gordon, N. S., Kollack-Walker, S., Akil, H., & Panksepp, J. (2002). Expression of c-fos gene activation during rough and tumble play in juvenile rats. *Brain Res Bull*, *57*(5), 651-659. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11927369

Green, J. T. (1969). Altruistic behavior in the albino rat. *Psychonomic Society*, *14*(1), 47-48. Retrieved from

Green, S. A., Hernandez, L., Bookheimer, S. Y., & Dapretto, M. (2016). Salience Network Connectivity in Autism Is Related to Brain and Behavioral Markers of Sensory Overresponsivity. *J Am Acad Child Adolesc Psychiatry*, *55*(7), 618-626.e1. doi:10.1016/j.jaac.2016.04.013

Griffiths, S., Jarrold, C., Penton-Voak, I. S., Woods, A. T., Skinner, A. L., & Munafò, M. R. (2017). Impaired Recognition of Basic Emotions from Facial Expressions in Young People with Autism Spectrum Disorder: Assessing the Importance of Expression Intensity. *J Autism Dev Disord*. doi:10.1007/s10803-017-3091-7

Groenewegen, H. J., Berendse, H. W., & Haber, S. N. (1993). Organization of the output of the ventral striatopallidal system in the rat: ventral pallidal efferents. *Neuroscience*, *57*(1), 113-142. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8278047

Gschwendt, M., Dieterich, S., Rennecke, J., Kittstein, W., Mueller, H. J., & Johannes, F. J. (1996). Inhibition of protein kinase C mu by various inhibitors. Differentiation from protein kinase c isoenzymes. *FEBS Lett*, *392*(2), 77-80. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8772178

Gu, X., Hof, P. R., Friston, K. J., & Fan, J. (2013). Anterior insular cortex and emotional awareness. *J Comp Neurol*, *521*(15), 3371-3388. doi:10.1002/cne.23368

Guastella, A. J., Einfeld, S. L., Gray, K. M., Rinehart, N. J., Tonge, B. J., Lambert, T. J., & Hickie, I. B. (2010). Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. *Biol Psychiatry*, *67*(7), 692-694. doi:10.1016/j.biopsych.2009.09.020

Guastella, A. J., Graustella, A. J., & MacLeod, C. (2012). A critical review of the influence of oxytocin nasal spray on social cognition in humans: evidence and future directions. *Horm Behav*, *61*(3), 410-418. doi:10.1016/j.yhbeh.2012.01.002

Guastella, A. J., Ward, P. B., Hickie, I. B., Shahrestani, S., Hodge, M. A., Scott, E. M., & Langdon, R. (2015). A single dose of oxytocin nasal spray improves higher-order social cognition in schizophrenia. *Schizophr Res*, *168*(3), 628-633. doi:10.1016/j.schres.2015.06.005

Guldin, W. O., & Markowitsch, H. J. (1983). Cortical and thalamic afferent connections of the insular and adjacent cortex of the rat. *J Comp Neurol*, *215*(2), 135-153. doi:10.1002/cne.902150203

Gulia, K. K., Kumar, V. M., & Mallick, H. N. (2002). Role of the lateral septal noradrenergic system in the elaboration of male sexual behavior in rats. *Pharmacol Biochem Behav*, *72*(4), 817-823. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12062571

Guzmán, Y. F., Tronson, N. C., Guedea, A., Huh, K. H., Gao, C., & Radulovic, J. (2009). Social modeling of conditioned fear in mice by non-fearful conspecifics. *Behav Brain Res*, *201*(1), 173-178. doi:10.1016/j.bbr.2009.02.024

Hall, S. S., Jiang, H., Reiss, A. L., & Greicius, M. D. (2013). Identifying large-scale brain networks in fragile X syndrome. *JAMA Psychiatry*, *70*(11), 1215-1223. doi:10.1001/jamapsychiatry.2013.247

Hamasato, E. K., Lovelock, D., Palermo-Neto, J., & Deak, T. (2017). Assessment of social behavior directed toward sick partners and its relation to central cytokine expression in rats. *Physiol Behav*, *182*, 128-136. doi:10.1016/j.physbeh.2017.10.011

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*(1). doi:10.1523/ENEURO.0330-16.2017

Hammerschmidt, K., Radyushkin, K., Ehrenreich, H., & Fischer, J. (2012). The structure and usage of female and male mouse ultrasonic vocalizations reveal only minor differences. *PLoS One*, *7*, e41133. doi:10.1371/journal.pone.0041133

Hanamori, T., Kunitake, T., Kato, K., & Kannan, H. (1998). Neurons in the posterior insular cortex are responsive to gustatory stimulation of the pharyngolarynx, baroreceptor and chemoreceptor stimulation, and tail pinch in rats. *Brain Res*, *785*(1), 97-106. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9526057

Harari-Dahan, O., & Bernstein, A. (2014). A general approach-avoidance hypothesis of oxytocin: accounting for social and non-social effects of oxytocin. *Neurosci Biobehav Rev*, *47*, 506-519. doi:10.1016/j.neubiorev.2014.10.007

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D. C., & Cullinan, W. E. (2003). Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. *Front Neuroendocrinol*, *24*(3), 151-180. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14596810

Herrero, M. T., Insausti, R., & Gonzalo, L. M. (1991). Cortically projecting cells in the periaqueductal gray matter of the rat. A retrograde fluorescent tracer study. *Brain Res*, *543*(2), 201-212. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2059832

Higa, K. K., Young, J. W., Ji, B., Nichols, D. E., Geyer, M. A., & Zhou, X. (2017). Striatal dopamine D1 receptor suppression impairs reward-associative learning. *Behav Brain Res*, *323*, 100-110. doi:10.1016/j.bbr.2017.01.041

Hiser, J., & Koenigs, M. (2018). The Multifaceted Role of the Ventromedial Prefrontal Cortex in Emotion, Decision Making, Social Cognition, and Psychopathology. *Biol Psychiatry*, *83*(8), 638-647. doi:10.1016/j.biopsych.2017.10.030

Hoebel, B. G., Avena, N. M., & Rada, P. (2007). Accumbens dopamine-acetylcholine balance in approach and avoidance. *Curr Opin Pharmacol*, *7*(6), 617-627. doi:10.1016/j.coph.2007.10.014

Hoke, K. L., Ryan, M. J., & Wilczynski, W. (2005). Social cues shift functional connectivity in the hypothalamus. *Proc Natl Acad Sci U S A*, *102*(30), 10712-10717. doi:10.1073/pnas.0502361102

Hong, W., Kim, D. W., & Anderson, D. J. (2014). Antagonistic control of social versus repetitive self-grooming behaviors by separable amygdala neuronal subsets. *Cell*, *158*(6), 1348-1361. doi:10.1016/j.cell.2014.07.049

Hubble, K., Daughters, K., Manstead, A. S. R., Rees, A., Thapar, A., & van Goozen, S. H. M. (2017). Oxytocin increases attention to the eyes and selectively enhances self-reported affective empathy for fear. *Neuropsychologia*, *106*, 350-357. doi:10.1016/j.neuropsychologia.2017.10.019

Hurtado, M. M., & Puerto, A. (2018). Tolerance to rewarding brain electrical stimulation: Differential effects of contingent and non-contingent activation of parabrachial complex and lateral hypothalamus. *Behav Brain Res*, *336*, 15-21. doi:10.1016/j.bbr.2017.08.030

Ihnat, R., White, N. R., & Barfield, R. J. (1995). Pup's broadband vocalizations and maternal behavior in the rat. *Behav Processes*, *33*(3), 257-271. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/24896999

Illig, K. R., & Wilson, D. A. (2009). *Olfactory Cortex: Comparative Anatomy*. Academic Press.

Insel, T. R. (2010). The challenge of translation in social neuroscience: a review of oxytocin, vasopressin, and affiliative behavior. *Neuron*, *65*(6), 768-779. doi:10.1016/j.neuron.2010.03.005

Insel, T. R., & Fernald, R. D. (2004a). How the brain processes social information: searching for the social brain. *Annu Rev Neurosci*, *27*, 697-722. doi:10.1146/annurev.neuro.27.070203.144148

Insel, T. R., & Fernald, R. D. (2004b). How the brain processes social information: searching for the social brain. *Annu Rev Neurosci*, *27*, 697-722. doi:10.1146/annurev.neuro.27.070203.144148

Insel, T. R., & Shapiro, L. E. (1992). Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. *Proc Natl Acad Sci U S A*, *89*(13), 5981-5985. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1321430

Ishii, A., Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2016). Social buffering ameliorates conditioned fear responses in female rats. *Horm Behav*, *81*, 53-58. doi:10.1016/j.yhbeh.2016.03.003

Ishii, H., Ohara, S., Tobler, P. N., Tsutsui, K., & Iijima, T. (2012). Inactivating anterior insular cortex reduces risk taking. *J Neurosci*, *32*(45), 16031-16039. doi:10.1523/JNEUROSCI.2278-12.2012

Issa, J. B., Haeffele, B. D., Young, E. D., & Yue, D. T. (2017). Multiscale mapping of frequency sweep rate in mouse auditory cortex. *Hear Res*, *344*, 207-222. doi:10.1016/j.heares.2016.11.018

Jaramillo, A. A., Randall, P. A., 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. doi:10.1016/j.neuropharm.2017.11.035

Jaramillo, A. A., Van Voorhies, K., Randall, P. A., & Besheer, J. (2018b). Silencing the insular-striatal circuit decreases alcohol self-administration and increases sensitivity to alcohol. *Behav Brain Res*, *348*, 74-81. doi:10.1016/j.bbr.2018.04.007

Jeon, D., Kim, S., Chetana, M., Jo, D., Ruley, H. E., 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*(4), 482-488. doi:10.1038/nn.2504

Jo, S., & Jung, M. W. (2016). Differential coding of uncertain reward in rat insular and orbitofrontal cortex. *Sci Rep*, *6*, 24085. doi:10.1038/srep24085

Johnson, D. M., Illig, K. R., Behan, M., & Haberly, L. B. (2000). New features of connectivity in piriform cortex visualized by intracellular injection of pyramidal cells suggest that “primary” olfactory cortex functions like “association” cortex in other sensory systems. *J Neurosci*, *20*(18), 6974-6982. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10995842

Johnson, Z. V., Walum, H., Xiao, Y., Riefkohl, P. C., & Young, L. J. (2017). Oxytocin receptors modulate a social salience neural network in male prairie voles. *Horm Behav*, *87*, 16-24. doi:10.1016/j.yhbeh.2016.10.009

Johnston, R. E. (2003). Chemical Communication in Rodents: from Pheromones to Individual Recognition. *84*, *4*, 1141-1162. Retrieved from

Jones, C. E., Riha, P. D., Gore, A. C., & Monfils, M. H. (2014). Social transmission of Pavlovian fear: fear-conditioning by-proxy in related female rats. *Anim Cogn*, *17*(3), 827-834. doi:10.1007/s10071-013-0711-2

Kalin, N. H., Shelton, S. E., & Davidson, R. J. (2007). Role of the primate orbitofrontal cortex in mediating anxious temperament. *Biol Psychiatry*, *62*(10), 1134-1139. doi:10.1016/j.biopsych.2007.04.004

Kalivas, P. W., & Nakamura, M. (1999). Neural systems for behavioral activation and reward. *Curr Opin Neurobiol*, *9*(2), 223-227. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10322190

Kavaliers, M., & Choleris, E. (2018). The role of social cognition in parasite and pathogen avoidance. *Philos Trans R Soc Lond B Biol Sci*, *373*(1751). doi:10.1098/rstb.2017.0206

Kavaliers, M., Colwell, D. D., & Choleris, E. (2001). NMDA-mediated social learning of fear-induced conditioned analgesia to biting flies. *Neuroreport*, *12*(4), 663-667. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11277559

Kayser, C., & Logothetis, N. K. (2007). Do early sensory cortices integrate cross-modal information. *Brain Struct Funct*, *212*(2), 121-132. doi:10.1007/s00429-007-0154-0

Keech, B., Crowe, S., & Hocking, D. R. (2018). Intranasal oxytocin, social cognition and neurodevelopmental disorders: A meta-analysis. *Psychoneuroendocrinology*, *87*, 9-19. doi:10.1016/j.psyneuen.2017.09.022

Kim, E. J., Kim, E. S., Covey, E., & Kim, J. J. (2010). Social transmission of fear in rats: the role of 22-kHz ultrasonic distress vocalization. *PLoS One*, *5*(12), e15077. doi:10.1371/journal.pone.0015077

Kimura, A., Imbe, H., & Donishi, T. (2010). Efferent connections of an auditory area in the caudal insular cortex of the rat: anatomical nodes for cortical streams of auditory processing and cross-modal sensory interactions. *Neuroscience*, *166*(4), 1140-1157. doi:10.1016/j.neuroscience.2010.01.032

Kiyokawa, Y. (2017). Social Odors: Alarm Pheromones and Social Buffering. *Curr Top Behav Neurosci*, *30*, 47-65. doi:10.1007/7854_2015_406

Kiyokawa, Y., & Hennessy, M. B. (2018). Comparative studies of social buffering: A consideration of approaches, terminology, and pitfalls. *Neurosci Biobehav Rev*, *86*, 131-141. doi:10.1016/j.neubiorev.2017.12.005

Kiyokawa, Y., Honda, A., Takeuchi, Y., & Mori, Y. (2014). A familiar conspecific is more effective than an unfamiliar conspecific for social buffering of conditioned fear responses in male rats. *Behav Brain Res*, *267*, 189-193. doi:10.1016/j.bbr.2014.03.043

Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2007). Two types of social buffering differentially mitigate conditioned fear responses. *Eur J Neurosci*, *26*(12), 3606-3613. doi:10.1111/j.1460-9568.2007.05969.x

Kiyokawa, Y., Wakabayashi, Y., Takeuchi, Y., & Mori, Y. (2012). The neural pathway underlying social buffering of conditioned fear responses in male rats. *Eur J Neurosci*, *36*(10), 3429-3437. doi:10.1111/j.1460-9568.2012.08257.x

Knapska, E., Mikosz, M., Werka, T., & Maren, S. (2010). Social modulation of learning in rats. *Learn Mem*, *17*(1), 35-42. doi:10.1101/lm.1670910

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*(10), 3858-3862. doi:10.1073/pnas.0511302103

Knobloch, H. S., Charlet, A., Hoffmann, L. C., Eliava, M., Khrulev, S., Cetin, A. H., Osten, P., Schwarz, M. K., Seeburg, P. H., Stoop, R., & Grinevich, V. (2012). Evoked axonal oxytocin release in the central amygdala attenuates fear response. *Neuron*, *73*(3), 553-566. doi:10.1016/j.neuron.2011.11.030

Kober, H., Barrett, L. F., Joseph, J., Bliss-Moreau, E., Lindquist, K., & Wager, T. D. (2008). Functional grouping and cortical-subcortical interactions in emotion: a meta-analysis of neuroimaging studies. *Neuroimage*, *42*(2), 998-1031. doi:10.1016/j.neuroimage.2008.03.059

Kohls, G., Chevallier, C., Troiani, V., & Schultz, R. T. (2012). Social 'wanting' dysfunction in autism: neurobiological underpinnings and treatment implications. *J Neurodev Disord*, *4*(1), 10. doi:10.1186/1866-1955-4-10

Kosfeld, M., Heinrichs, M., Zak, P. J., Fischbacher, U., & Fehr, E. (2005). Oxytocin increases trust in humans. *Nature*, *435*(7042), 673-676. doi:10.1038/nature03701

Krach, S., Paulus, F. M., Bodden, M., & Kircher, T. (2010). The rewarding nature of social interactions. *Front Behav Neurosci*, *4*, 22. doi:10.3389/fnbeh.2010.00022

Kravitz, A. V., Tye, L. D., & Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nat Neurosci*, *15*(6), 816-818. doi:10.1038/nn.3100

Krushel, L. A., & van der Kooy, D. (1988). Visceral cortex: integration of the mucosal senses with limbic information in the rat agranular insular cortex. *J Comp Neurol*, *270*(1), 39-54, 62. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2453537

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

Lacey, E. A., Alexander, R. D., Braude, S., Sherman, P. W., & Jarvis, J. U. M. (1991). An ethogram for the naked mole-rat: Nonvocal behaviors. In *The Biology of the Naked Mole-rat*. Princeton, N.J.: Princeton University Press.

Lamers, W. H., Mooren, P. G., Griep, H., Endert, E., Degenhart, H. J., & Charles, R. (1986). Hormones in perinatal rat and spiny mouse: relation to altricial and precocial timing of birth. *Am J Physiol*, *251*(1 Pt 1), E78-85. doi:10.1152/ajpendo.1986.251.1.E78

Lamm, C., & Singer, T. (2010). The role of anterior insular cortex in social emotions. *Brain Struct Funct*, *214*(5-6), 579-591. doi:10.1007/s00429-010-0251-3

Langford, D. J., Crager, S. E., Shehzad, Z., Smith, S. B., Sotocinal, S. G., Levenstadt, J. S., Chanda, M. L., Levitin, D. J., & Mogil, J. S. (2006). Social modulation of pain as evidence for empathy in mice. *Science*, *312*(5782), 1967-1970. doi:10.1126/science.1128322

Langford, D. J., Tuttle, A. H., Brown, K., Deschenes, S., Fischer, D. B., Mutso, A., Root, K. C., Sotocinal, S. G., Stern, M. A., Mogil, J. S., & Sternberg, W. F. (2010). Social approach to pain in laboratory mice. *Soc Neurosci*, *5*(2), 163-170. doi:10.1080/17470910903216609

Lecorps, B., & Féron, C. (2015). Correlates between ear postures and emotional reactivity in a wild type mouse species. *Behav Processes*, *120*, 25-29. doi:10.1016/j.beproc.2015.08.002

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annu Rev Neurosci*, *23*, 155-184. doi:10.1146/annurev.neuro.23.1.155

LeDoux, J. E., Farb, C. R., & Romanski, L. M. (1991). Overlapping projections to the amygdala and striatum from auditory processing areas of the thalamus and cortex. *Neurosci Lett*, *134*(1), 139-144. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1815147

Lee, C. C. (2013). Thalamic and cortical pathways supporting auditory processing. *Brain Lang*, *126*(1), 22-28. doi:10.1016/j.bandl.2012.05.004

Lee, E., Rhim, I., Lee, J. W., Ghim, J. W., Lee, S., Kim, E., & Jung, M. W. (2016). Enhanced Neuronal Activity in the Medial Prefrontal Cortex during Social Approach Behavior. *J Neurosci*, *36*(26), 6926-6936. doi:10.1523/JNEUROSCI.0307-16.2016

Lee, H., Kim, D. W., Remedios, R., Anthony, T. E., Chang, A., Madisen, L., Zeng, H., & Anderson, D. J. (2014). Scalable control of mounting and attack by Esr1+ neurons in the ventromedial hypothalamus. *Nature*, *509*(7502), 627-632. doi:10.1038/nature13169

Lehman, M. N., Winans, S. S., & Powers, J. B. (1980). Medial nucleus of the amygdala mediates chemosensory control of male hamster sexual behavior. *Science*, *210*(4469), 557-560. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7423209

Lenschow, C., & Brecht, M. (2018). Physiological and Anatomical Outputs of Rat Genital Cortex. *Cereb Cortex*, *28*(4), 1472-1486. doi:10.1093/cercor/bhx359

Li, H., & Crair, M. C. (2011). How do barrels form in somatosensory cortex. *Ann N Y Acad Sci*, *1225*, 119-129. doi:10.1111/j.1749-6632.2011.06024.x

Li, Z., Lu, Y. F., Li, C. L., Wang, Y., Sun, W., He, T., Chen, X. F., Wang, X. L., & Chen, J. (2014). Social interaction with a cagemate in pain facilitates subsequent spinal nociception via activation of the medial prefrontal cortex in rats. *Pain*, *155*(7), 1253-1261. doi:10.1016/j.pain.2014.03.019

Liberles, S. D. (2014). Mammalian pheromones. *Annu Rev Physiol*, *76*, 151-175. doi:10.1146/annurev-physiol-021113-170334

Lichtenberg, N. T., Lee, B., Kashtelyan, V., Chappa, B. S., Girma, H. T., Green, E. A., Kantor, S., Lagowala, D. A., Myers, M. A., Potemri, D., Pecukonis, M. G., Tesfay, R. T., Walters, M. S., Zhao, A. C., Blair, R. J. R., Cheer, J. F., & Roesch, M. R. (2018). Rat behavior and dopamine release are modulated by conspecific distress. *Elife*, *7*. doi:10.7554/eLife.38090

Lindvall, O., & Stenevi, U. (1978). Dopamine and noradrenaline neurons projecting to the septal area in the rat. *Cell Tissue Res*, *190*(3), 383-407. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/688339

Linke, R., & Schwegler, H. (2000). Convergent and complementary projections of the caudal paralaminar thalamic nuclei to rat temporal and insular cortex. *Cereb Cortex*, *10*(8), 753-771. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10920048

Lockwood, P. L. (2016). The anatomy of empathy: Vicarious experience and disorders of social cognition. *Behav Brain Res*, *311*, 255-266. doi:10.1016/j.bbr.2016.05.048

LoParo, D., & Waldman, I. D. (2015). The oxytocin receptor gene (OXTR) is associated with autism spectrum disorder: a meta-analysis. *Mol Psychiatry*, *20*(5), 640-646. doi:10.1038/mp.2014.77

Lozier, L. M., Vanmeter, J. W., & Marsh, A. A. (2014). Impairments in facial affect recognition associated with autism spectrum disorders: a meta-analysis. *Dev Psychopathol*, *26*(4 Pt 1), 933-945. doi:10.1017/S0954579414000479

Lucas, M. V., Anderson, L. C., Bolling, D. Z., Pelphrey, K. A., & Kaiser, M. D. (2015). Dissociating the Neural Correlates of Experiencing and Imagining Affective Touch. *Cereb Cortex*, *25*(9), 2623-2630. doi:10.1093/cercor/bhu061

Lupfer, G., Frieman, J., & Coonfield, D. (2003). Social transmission of flavor preferences in two species of hamsters (Mesocricetus auratus and Phodopus campbelli). *J Comp Psychol*, *117*(4), 449-455. doi:10.1037/0735-7036.117.4.449

Ma, L. Q., Ning, L., Wang, Z., & Wang, Y. W. (2016). Visual and noxious electrical stimulus-evoked membrane-potential responses in anterior cingulate cortical neurons. *Mol Brain*, *9*(1), 82. doi:10.1186/s13041-016-0262-y

Machado, C. J., & Bachevalier, J. (2007). Measuring reward assessment in a semi-naturalistic context: the effects of selective amygdala, orbital frontal or hippocampal lesions. *Neuroscience*, *148*(3), 599-611. doi:10.1016/j.neuroscience.2007.06.035

MacLaren, D. A., Browne, R. W., Shaw, J. K., Krishnan Radhakrishnan, S., Khare, P., España, R. A., & Clark, S. D. (2016). Clozapine N-Oxide Administration Produces Behavioral Effects in Long-Evans Rats: Implications for Designing DREADD Experiments. *eNeuro*, *3*(5). doi:10.1523/ENEURO.0219-16.2016

Maejima, S., Ohishi, N., Yamaguchi, S., & Tsukahara, S. (2015). A neural connection between the central part of the medial preoptic nucleus and the bed nucleus of the stria terminalis to regulate sexual behavior in male rats. *Neurosci Lett*, *606*, 66-71. doi:10.1016/j.neulet.2015.08.047

Magee, J. C. (2000). Dendritic integration of excitatory synaptic input. *Nat Rev Neurosci*, *1*(3), 181-190. doi:10.1038/35044552

Mahler, S. V., & Aston-Jones, G. (2018). CNO Evil? Considerations for the Use of DREADDs in Behavioral Neuroscience. *Neuropsychopharmacology*, *43*(5), 934-936. doi:10.1038/npp.2017.299

Mahler, S. V., Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., Wilson, S. P., Deisseroth, K., Woodward, J. J., & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nat Neurosci*, *17*(4), 577-585. doi:10.1038/nn.3664

Maier, J. X., Blankenship, M. L., Li, J. X., & Katz, D. B. (2015). A Multisensory Network for Olfactory Processing. *Curr Biol*, *25*(20), 2642-2650. doi:10.1016/j.cub.2015.08.060

Mainardi, M., Landi, S., Gianfranceschi, L., Baldini, S., De Pasquale, R., Berardi, N., Maffei, L., & Caleo, M. (2010). Environmental enrichment potentiates thalamocortical transmission and plasticity in the adult rat visual cortex. *J Neurosci Res*, *88*(14), 3048-3059. doi:10.1002/jnr.22461

Manduca, A., Servadio, M., Damsteegt, R., Campolongo, P., Vanderschuren, L. J., & Trezza, V. (2016). Dopaminergic Neurotransmission in the Nucleus Accumbens Modulates Social Play Behavior in Rats. *Neuropsychopharmacology*, *41*(9), 2215-2223. doi:10.1038/npp.2016.22

Manvich, D. F., Webster, K. A., Foster, S. L., Farrell, M. S., Ritchie, J. C., Porter, J. H., & Weinshenker, D. (2018). The DREADD agonist clozapine N-oxide (CNO) is

reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. *Sci Rep*, *8*(1), 3840. doi:10.1038/s41598-018-22116-z

Marlin, B. J., & Froemke, R. C. (2017). Oxytocin modulation of neural circuits for social behavior. *Dev Neurobiol*, *77*(2), 169-189. doi:10.1002/dneu.22452

Marlin, B. J., Mitre, M., D'amour, J. A., Chao, M. V., & Froemke, R. C. (2015). Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature*, *520*(7548), 499-504. doi:10.1038/nature14402

Marsh, A. A. (2018). The Caring Continuum: Evolved Hormonal and Proximal Mechanisms Explain Prosocial and Antisocial Extremes. *Annu Rev Psychol*. doi:10.1146/annurev-psych-010418-103010

Martin-Cortecero, J., & Nuñez, A. (2016). Sensory responses in the medial prefrontal cortex of anesthetized rats. Implications for sensory processing. *Neuroscience*, *339*, 109-123. doi:10.1016/j.neuroscience.2016.09.045

Mason, M., Magee, J. C., & Fiske, S. T. (2014). Neural substrates of social status inference: roles of medial prefrontal cortex and superior temporal sulcus. *J Cogn Neurosci*, *26*(5), 1131-1140. doi:10.1162/jocn_a_00553

Matsumoto, Y. K., & Okanoya, K. (2016). Phase-Specific Vocalizations of Male Mice at the Initial Encounter during the Courtship Sequence. *PLoS One*, *11*(2), e0147102. doi:10.1371/journal.pone.0147102

Mazzola, L., Lopez, C., Faillenot, I., Chouchou, F., Mauguière, F., & Isnard, J. (2014). Vestibular responses to direct stimulation of the human insular cortex. *Ann Neurol*, *76*(4), 609-619. doi:10.1002/ana.24252

Menon, V., & Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct*, *214*(5-6), 655-667. doi:10.1007/s00429-010-0262-0

Meyza, K., Nikolaev, T., Kondrakiewicz, K., Blanchard, D. C., Blanchard, R. J., & Knapska, E. (2015). Neuronal correlates of asocial behavior in a BTBR T (+)

Itpr3(tf)/J mouse model of autism. *Front Behav Neurosci*, *9*, 199. doi:10.3389/fnbeh.2015.00199

Meyza, K. Z., Bartal, I. B., Monfils, M. H., Panksepp, J. B., & Knapska, E. (2017). The roots of empathy: Through the lens of rodent models. *Neurosci Biobehav Rev*, *76*(Pt B), 216-234. doi:10.1016/j.neubiorev.2016.10.028

Mikosz, M., Nowak, A., Werka, T., & Knapska, E. (2015). Sex differences in social modulation of learning in rats. *Sci Rep*, *5*, 18114. doi:10.1038/srep18114

Mitre, M., Marlin, B. J., Schiavo, J. K., Morina, E., Norden, S. E., Hackett, T. A., Aoki, C. J., Chao, M. V., & Froemke, R. C. (2016). A Distributed Network for Social Cognition Enriched for Oxytocin Receptors. *J Neurosci*, *36*(8), 2517-2535. doi:10.1523/JNEUROSCI.2409-15.2016

Mittal, A., Gupta, M., Lamarre, Y., Jahagirdar, B., & Gupta, K. (2016). Quantification of pain in sickle mice using facial expressions and body measurements. *Blood Cells Mol Dis*, *57*, 58-66. doi:10.1016/j.bcmd.2015.12.006

Miyazaki, T., Takase, K., Nakajima, W., Tada, H., Ohya, D., Sano, A., Goto, T., Hirase, H., Malinow, R., & Takahashi, T. (2012). Disrupted cortical function underlies behavior dysfunction due to social isolation. *J Clin Invest*, *122*(7), 2690-2701. doi:10.1172/JCI63060

Mizoguchi, H., Katahira, K., Inutsuka, A., Fukumoto, K., Nakamura, A., Wang, T., Nagai, T., Sato, J., Sawada, M., Ohira, H., Yamanaka, A., & Yamada, K. (2015). Insular neural system controls decision-making in healthy and methamphetamine-treated rats. *Proc Natl Acad Sci U S A*, *112*(29), E3930-9. doi:10.1073/pnas.1418014112

Mizumori, S. J. (2013). Context prediction analysis and episodic memory. *Front Behav Neurosci*, *7*, 132. doi:10.3389/fnbeh.2013.00132

Moaddab, M., Hyland, B. I., & Brown, C. H. (2015). Oxytocin excites nucleus accumbens shell neurons in vivo. *Mol Cell Neurosci*, *68*, 323-330. doi:10.1016/j.mcn.2015.08.013

Modahl, C., Green, L., Fein, D., Morris, M., Waterhouse, L., Feinstein, C., & Levin, H. (1998). Plasma oxytocin levels in autistic children. *Biol Psychiatry*, *43*(4), 270-277. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9513736

Mogenson, G. J., & Yang, C. R. (1991). The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. *Adv Exp Med Biol*, *295*, 267-290. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1776572

Molapour, T., Golkar, A., Navarrete, C. D., Haaker, J., & Olsson, A. (2015). Neural correlates of biased social fear learning and interaction in an intergroup context. *Neuroimage*, *121*, 171-183. doi:10.1016/j.neuroimage.2015.07.015

Morita, T., Kosaka, H., Saito, D. N., Ishitobi, M., Munesue, T., Itakura, S., Omori, M., Okazawa, H., Wada, Y., & Sadato, N. (2012). Emotional responses associated with self-face processing in individuals with autism spectrum disorders: an fMRI study. *Soc Neurosci*, *7*(3), 223-239. doi:10.1080/17470919.2011.598945

Moyaho, A., Rivas-Zamudio, X., Ugarte, A., Eguibar, J. R., & Valencia, J. (2015). Smell facilitates auditory contagious yawning in stranger rats. *Anim Cogn*, *18*(1), 279-290. doi:10.1007/s10071-014-0798-0

Nakamura, K., Ishii, A., Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2016). The strain of an accompanying conspecific affects the efficacy of social buffering in male rats. *Horm Behav*, *82*, 72-77. doi:10.1016/j.yhbeh.2016.05.003

Neafsey, E. J., Hurley-Gius, K. M., & Arvanitis, D. (1986). The topographical organization of neurons in the rat medial frontal, insular and olfactory cortex projecting to the solitary nucleus, olfactory bulb, periaqueductal gray and superior colliculus. *Brain Res*, *377*(2), 261-270. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/3730872

Neunuebel, J. P., Taylor, A. L., Arthur, B. J., & Egnor, S. E. (2015). Female mice ultrasonically interact with males during courtship displays. *Elife*, *4*. doi:10.7554/eLife.06203

Newman, D. B., Hilleary, S. K., & Ginsberg, C. Y. (1989). Nuclear terminations of corticoreticular fiber systems in rats. *Brain Behav Evol*, *34*(4), 223-64. Retrieved from

Newman, S. W. (1999). The medial extended amygdala in male reproductive behavior. A node in the mammalian social behavior network. *Ann N Y Acad Sci*, *877*, 242-257. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10415653

Ninan, I. (2011). Oxytocin suppresses basal glutamatergic transmission but facilitates activity-dependent synaptic potentiation in the medial prefrontal cortex. *J Neurochem*, *119*(2), 324-331. doi:10.1111/j.1471-4159.2011.07430.x

Nummenmaa, L., Hirvonen, J., Parkkola, R., & Hietanen, J. K. (2008). Is emotional contagion special? An fMRI study on neural systems for affective and cognitive empathy. *Neuroimage*, *43*(3), 571-580. doi:10.1016/j.neuroimage.2008.08.014

O'Connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. *J Comp Neurol*, *519*(18), 3599-3639. doi:10.1002/cne.22735

O'Connell, L. A., & Hofmann, H. A. (2012). Evolution of a vertebrate social decision-making network. *Science*, *336*(6085), 1154-1157. doi:10.1126/science.1218889

Oettl, L. L., & Kelsch, W. (2017). Oxytocin and Olfaction. *Curr Top Behav Neurosci*. doi:10.1007/7854_2017_8

Oettl, L. L., Ravi, N., Schneider, M., Scheller, M. F., Schneider, P., Mitre, M., da Silva Gouveia, M., Froemke, R. C., Chao, M. V., Young, W. S., Meyer-Lindenberg, A., Grinevich, V., Shusterman, R., & Kelsch, W. (2016). Oxytocin Enhances Social Recognition by Modulating Cortical Control of Early Olfactory Processing. *Neuron*, *90*(3), 609-621. doi:10.1016/j.neuron.2016.03.033

Okuyama, T. (2018). Social memory engram in the hippocampus. *Neurosci Res*, *129*, 17-23. doi:10.1016/j.neures.2017.05.007

Ota, M., Yoshida, S., Nakata, M., Yada, T., & Kunugi, H. (2018). The effects of adjunctive intranasal oxytocin in patients with schizophrenia. *Postgrad Med*, *130*(1), 122-128. doi:10.1080/00325481.2018.1398592

Otto, T., & Eichenbaum, H. (1992). Complementary roles of the orbital prefrontal cortex and the perirhinal-entorhinal cortices in an odor-guided delayed-nonmatching-to-sample task. *Behav Neurosci*, *106*(5), 762-775. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1445656

Ouda, L., Jílek, M., & Syka, J. (2016). Expression of c-Fos in rat auditory and limbic systems following 22-kHz calls. *Behav Brain Res*, *308*, 196-204. doi:10.1016/j.bbr.2016.04.030

Owen, S. F., Tuncdemir, S. N., Bader, P. L., Tirko, N. N., Fishell, G., & Tsien, R. W. (2013). Oxytocin enhances hippocampal spike transmission by modulating fast-spiking interneurons. *Nature*, *500*(7463), 458-462. doi:10.1038/nature12330

Papagno, C., Pisoni, A., Mattavelli, G., Casarotti, A., Comi, A., Fumagalli, F., Vernice, M., Fava, E., Riva, M., & Bello, L. (2016). Specific disgust processing in the left insula: New evidence from direct electrical stimulation. *Neuropsychologia*, *84*, 29-35. doi:10.1016/j.neuropsychologia.2016.01.036

Pardo-Bellver, C., Cádiz-Moretti, B., Novejarque, A., Martínez-García, F., & Lanuza, E. (2012). Differential efferent projections of the anterior, posteroventral, and posterodorsal subdivisions of the medial amygdala in mice. *Front Neuroanat*, *6*, 33. doi:10.3389/fnana.2012.00033

Paredes-Ramos, P., McCarthy, M. M., Bowers, J. M., Miquel, M., Manzo, J., & Coria-Avila, G. A. (2014). Brain activation by an olfactory stimulus paired with juvenile play in female rats. *Physiol Behav*, *133*, 39-44. doi:10.1016/j.physbeh.2014.05.008

Park, J. Y., Park, S. Y., Kwon, H., Song, Y., Yun, B., Lee, Y., Cho, Y., Joo, A., & Han, P. L. (2018). A Group of Descending Glutamatergic Neurons Activated by Stress in Corticolimbic Regions Project to the Nucleus Accumbens. *Exp Neurobiol*, *27*(5), 387-396. doi:10.5607/en.2018.27.5.387

Parker, K. J., Oztan, O., Libove, R. A., Sumiyoshi, R. D., Jackson, L. P., Karhson, D. S., Summers, J. E., Hinman, K. E., Motonaga, K. S., Phillips, J. M., Carson, D. S., Garner, J. P., & Hardan, A. Y. (2017). Intranasal oxytocin treatment for social deficits and biomarkers of response in children with autism. *Proc Natl Acad Sci U S A*, *114*(30), 8119-8124. doi:10.1073/pnas.1705521114

Partan, S., & Marler, P. (1999). Communication goes multimodal. *Science*, *283*(5406), 1272-1273. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10084931

Pasch, B., George, A. S., Hamlin, H. J., Guillette, L. J., & Phelps, S. M. (2011). Androgens modulate song effort and aggression in Neotropical singing mice. *Horm Behav*, *59*(1), 90-97. doi:10.1016/j.yhbeh.2010.10.011

Pattij, T., Schetters, D., & Schoffelmeer, A. N. (2014). Dopaminergic modulation of impulsive decision making in the rat insular cortex. *Behav Brain Res*, *270*, 118-124. doi:10.1016/j.bbr.2014.05.010

Paxinos, G., Watson, C. R., & Emson, P. C. (1980). AChE-stained horizontal sections of the rat brain in stereotaxic coordinates. *J Neurosci Methods*, *3*(2), 129-149. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6110810

Peltz, E., Seifert, F., DeCol, R., Dörfler, A., Schwab, S., & Maihöfner, C. (2011). Functional connectivity of the human insular cortex during noxious and innocuous thermal stimulation. *Neuroimage*, *54*(2), 1324-1335. doi:10.1016/j.neuroimage.2010.09.012

Peñagarikano, O., Lázaro, M. T., Lu, X. H., Gordon, A., Dong, H., Lam, H. A., Peles, E., Maidment, N. T., Murphy, N. P., Yang, X. W., Golshani, P., & Geschwind, D. H. (2015). Exogenous and evoked oxytocin restores social behavior in the Cntnap2 mouse model of autism. *Sci Transl Med*, *7*(271), 271ra8. doi:10.1126/scitranslmed.3010257

Pepper, K. L., Demetriou, E. A., Park, S. H., Song, Y. C., Hickie, I. B., Cacciotti-Saija, C., Langdon, R., Piguet, O., Kumfor, F., Thomas, E. E., & Guastella, A. J. (2018). Autism, early psychosis, and social anxiety disorder: understanding the role of social cognition and its relationship to disability in young adults with disorders characterized by social impairments. *Transl Psychiatry*, *8*(1), 233. doi:10.1038/s41398-018-0282-8

Petrulis, A. (2013). Chemosignals and hormones in the neural control of mammalian sexual behavior. *Front Neuroendocrinol*, *34*(4), 255-267. doi:10.1016/j.yfrne.2013.07.007

Phan, K. L., Wager, T., Taylor, S. F., & Liberzon, I. (2002). Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*, *16*(2), 331-348. doi:10.1006/nimg.2002.1087

Phillips, M. L., Young, A. W., Senior, C., Brammer, M., Andrew, C., Calder, A. J., Bullmore, E. T., Perrett, D. I., Rowland, D., Williams, S. C., Gray, J. A., & David, A. S. (1997). A specific neural substrate for perceiving facial expressions of disgust. *Nature*, *389*(6650), 495-498. doi:10.1038/39051

Ploeger, G. E., Willemen, A. P., & Cools, A. R. (1991). Role of the nucleus accumbens in social memory in rats. *Brain Res Bull*, *26*(1), 23-27. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/1673081

Porges, S. W. (2001). The polyvagal theory: phylogenetic substrates of a social nervous system. *Int J Psychophysiol*, *42*(2), 123-146. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/11587772

Portfors, C. V., & Perkel, D. J. (2014). The role of ultrasonic vocalizations in mouse communication. *Curr Opin Neurobiol*, *28*, 115-120. doi:10.1016/j.conb.2014.07.002

Prather, J. F. (2013). Auditory signal processing in communication: perception and performance of vocal sounds. *Hear Res*, *305*, 144-155. doi:10.1016/j.heares.2013.06.007

Puglia, M. H., Lillard, T. S., Morris, J. P., & Connelly, J. J. (2015). Epigenetic modification of the oxytocin receptor gene influences the perception of anger and fear in the human brain. *Proc Natl Acad Sci U S A*, *112*(11), 3308-3313. doi:10.1073/pnas.1422096112

Pultorak, J. D., Kelm-Nelson, C. A., Holt, L. R., Blue, K. V., Ciucci, M. R., & Johnson, A. M. (2016). Decreased approach behavior and nucleus accumbens immediate early gene expression in response to Parkinsonian ultrasonic vocalizations in rats. *Soc Neurosci*, *11*(4), 365-379. doi:10.1080/17470919.2015.1086434

Radke, S., & de Bruijn, E. R. (2015). Does oxytocin affect mind-reading? A replication study. *Psychoneuroendocrinology*, *60*, 75-81. doi:10.1016/j.psyneuen.2015.06.006

Ramos, L., Hicks, C., Caminer, A., Goodwin, J., & McGregor, I. S. (2015). Oxytocin and MDMA ('Ecstasy') enhance social reward in rats. *Psychopharmacology (Berl)*, *232*(14), 2631-2641. doi:10.1007/s00213-015-3899-9

Ranote, S., Elliott, R., Abel, K. M., Mitchell, R., Deakin, J. F., & Appleby, L. (2004). The neural basis of maternal responsiveness to infants: an fMRI study. *Neuroreport*, *15*(11), 1825-1829. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/15257156

Rao, R. P., Mielke, F., Bobrov, E., & Brecht, M. (2014). Vocalization-whisking coordination and multisensory integration of social signals in rat auditory cortex. *Elife*, *3*. doi:10.7554/eLife.03185

Reep, R. L., & Winans, S. S. (1982). Afferent connections of dorsal and ventral agranular insular cortex in the hamster Mesocricetus auratus. *Neuroscience*, *7*(5), 1265-1288. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7110587

Rendon, N. M., Keesom, S. M., Amadi, C., Hurley, L. M., & Demas, G. E. (2015). Vocalizations convey sex, seasonal phenotype, and aggression in a seasonal mammal. *Physiol Behav*, *152*(Pt A), 143-150. doi:10.1016/j.physbeh.2015.09.014

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

RICE, G. E., & GAINER, P. (1962). "Altruism" in the albino rat. *J Comp Physiol Psychol*, *55*, 123-125. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14491896

Ripamonti, S., Ambrozkiewicz, M. C., Guzzi, F., Gravati, M., Biella, G., Bormuth, I., Hammer, M., Tuffy, L. P., Sigler, A., Kawabe, H., Nishimori, K., Toselli, M., Brose, N., Parenti, M., & Rhee, J. (2017). Transient oxytocin signaling primes the development and function of excitatory hippocampal neurons. *Elife*, *6*. doi:10.7554/eLife.22466

Rodgers, K. M., Benison, A. M., Klein, A., & Barth, D. S. (2008). Auditory, somatosensory, and multisensory insular cortex in the rat. *Cereb Cortex*, *18*(12), 2941-2951. doi:10.1093/cercor/bhn054

Rodríguez-García, G., & Miranda, M. I. (2016). Opposing Roles of Cholinergic and GABAergic Activity in the Insular Cortex and Nucleus Basalis Magnocellularis during Novel Recognition and Familiar Taste Memory Retrieval. *J Neurosci*, *36*(6), 1879-1889. doi:10.1523/JNEUROSCI.2340-15.2016

Roeling, T. A., Veening, J. G., Kruk, M. R., Peters, J. P., Vermelis, M. E., & Nieuwenhuys, R. (1994). Efferent connections of the hypothalamic "aggression area" in the rat. *Neuroscience*, *59*(4), 1001-1024. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8058117

Rogers-Carter, M. M., Djerdjaj, A., Culp, A. R., Elbaz, J. A., & Christianson, J. P. (2018a). Familiarity modulates social approach toward stressed conspecifics in female rats. *PLoS One*, *13*(10), e0200971. doi:10.1371/journal.pone.0200971

Rogers-Carter, M. M., Varela, J. A., Gribbons, K. B., Pierce, A. F., McGoey, M. T., Ritchey, M., & Christianson, J. P. (2018b). Insular cortex mediates approach and avoidance responses to social affective stimuli. *Nat Neurosci*, *21*(3), 404-414. doi:10.1038/s41593-018-0071-y

Romanski, L. M., & LeDoux, J. E. (1993). Information cascade from primary auditory cortex to the amygdala: corticocortical and corticoamygdaloid projections of temporal cortex in the rat. *Cereb Cortex*, *3*(6), 515-532. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7511012

Root, D. H., Estrin, D. J., & Morales, M. (2018). Aversion or Salience Signaling by Ventral Tegmental Area Glutamate Neurons. *iScience*, *2*, 51-62. doi:10.1016/j.isci.2018.03.008

Rosenblau, G., Kliemann, D., Dziobek, I., & Heekeren, H. R. (2017). Emotional prosody processing in autism spectrum disorder. *Soc Cogn Affect Neurosci*, *12*(2), 224-239. doi:10.1093/scan/nsw118

Ross, H. E., Cole, C. D., Smith, Y., Neumann, I. D., Landgraf, R., Murphy, A. Z., & Young, L. J. (2009). Characterization of the oxytocin system regulating affiliative behavior in female prairie voles. *Neuroscience*, *162*(4), 892-903. doi:10.1016/j.neuroscience.2009.05.055

Rossier, J., & Schenk, F. (2003). Olfactory and/or visual cues for spatial navigation through ontogeny: olfactory cues enable the use of visual cues. *Behav Neurosci*, *117*(3), 412-425. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12802871

Roth, B. L. (2016). DREADDs for Neuroscientists. *Neuron*, *89*(4), 683-694. doi:10.1016/j.neuron.2016.01.040

Russell, J. A. (2003). Core affect and the psychological construction of emotion. *Psychol Rev*, *110*(1), 145-172. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12529060

Ryan, B. C., Young, N. B., Moy, S. S., & Crawley, J. N. (2008). Olfactory cues are sufficient to elicit social approach behaviors but not social transmission of food preference in C57BL/6J mice. *Behav Brain Res*, *193*(2), 235-242. doi:10.1016/j.bbr.2008.06.002

Sadananda, M., Wöhr, M., & Schwarting, R. K. (2008). Playback of 22-kHz and 50-kHz ultrasonic vocalizations induces differential c-fos expression in rat brain. *Neurosci Lett*, *435*(1), 17-23. doi:10.1016/j.neulet.2008.02.002

Saitovitch, A., Bargiacchi, A., Chabane, N., Brunelle, F., Samson, Y., Boddaert, N., & Zilbovicius, M. (2012). Social cognition and the superior temporal sulcus: implications in autism. *Rev Neurol (Paris)*, *168*(10), 762-770. doi:10.1016/j.neurol.2012.07.017

Sakuma, Y. (2008). Neural substrates for sexual preference and motivation in the female and male rat. *Ann N Y Acad Sci*, *1129*, 55-60. doi:10.1196/annals.1417.009

Samuelsen, C. L., & Fontanini, A. (2017). Processing of Intraoral Olfactory and Gustatory Signals in the Gustatory Cortex of Awake Rats. *J Neurosci*, *37*(2), 244-257. doi:10.1523/JNEUROSCI.1926-16.2016

Sarna, J. R., Dyck, R. H., & Whishaw, I. Q. (2000). The Dalila effect: C57BL6 mice barber whiskers by plucking. *Behav Brain Res*, *108*(1), 39-45. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10680755

Sato, W., Sawada, R., Uono, S., Yoshimura, S., Kochiyama, T., Kubota, Y., Sakihama, M., & Toichi, M. (2017). Impaired detection of happy facial expressions in autism. *Sci Rep*, *7*(1), 13340. doi:10.1038/s41598-017-11900-y

Scheiber, I. B. R., Weiß, B. M., Kingma, S. A., & Komdeur, J. (2017). The importance of the altricial - precocial spectrum for social complexity in mammals and birds - a review. *Front Zool*, *14*, 3. doi:10.1186/s12983-016-0185-6

Schiff, H. C., Bouhuis, A. L., Yu, K., Penzo, M. A., Li, H., He, M., & Li, B. (2018). An Insula-Central Amygdala Circuit for Guiding Tastant-Reinforced Choice Behavior. *J Neurosci*, *38*(6), 1418-1429. doi:10.1523/JNEUROSCI.1773-17.2017

Schmitz, N., Rubia, K., van Amelsvoort, T., Daly, E., Smith, A., & Murphy, D. G. (2008). Neural correlates of reward in autism. *Br J Psychiatry*, *192*(1), 19-24. doi:10.1192/bjp.bp.107.036921

Schoenbaum, G., Setlow, B., & Ramus, S. J. (2003). A systems approach to orbitofrontal cortex function: recordings in rat orbitofrontal cortex reveal interactions with different learning systems. *Behav Brain Res*, *146*(1-2), 19-29. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/14643456

Scott-Van Zeeland, A. A., Dapretto, M., Ghahremani, D. G., Poldrack, R. A., & Bookheimer, S. Y. (2010). Reward processing in autism. *Autism Res*, *3*(2), 53-67. doi:10.1002/aur.122

Sforazzini, F., Bertero, A., Dodero, L., David, G., Galbusera, A., Scattoni, M. L., Pasqualetti, M., & Gozzi, A. (2016). Altered functional connectivity networks in acallosal and socially impaired BTBR mice. *Brain Struct Funct*, *221*(2), 941-954. doi:10.1007/s00429-014-0948-9

Shamay-Tsoory, S. G. (2011). The neural bases for empathy. *Neuroscientist*, *17*(1), 18-24. doi:10.1177/1073858410379268

Shamay-Tsoory, S. G., & Abu-Akel, A. (2016). The Social Salience Hypothesis of Oxytocin. *Biol Psychiatry*, *79*(3), 194-202. doi:10.1016/j.biopsych.2015.07.020

Shi, C. J., & Cassell, M. D. (1998). Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J Comp Neurol*, *399*(4), 440-468. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9741477

Shilling, P. D., & Feifel, D. (2016). Potential of Oxytocin in the Treatment of Schizophrenia. *CNS Drugs*, *30*(3), 193-208. doi:10.1007/s40263-016-0315-x

Shin, J. W., Geerling, J. C., & Loewy, A. D. (2008). Inputs to the ventrolateral bed nucleus of the stria terminalis. *J Comp Neurol*, *511*(5), 628-657. doi:10.1002/cne.21870

Shin, N. Y., Park, H. Y., Jung, W. H., Park, J. W., Yun, J. Y., Jang, J. H., Kim, S. N., Han, H. J., Kim, S. Y., Kang, D. H., & Kwon, J. S. (2015). Effects of Oxytocin on Neural Response to Facial Expressions in Patients with Schizophrenia. *Neuropsychopharmacology*, *40*(9), 2286. doi:10.1038/npp.2015.78

Shinder, M. E., & Newlands, S. D. (2014). Sensory convergence in the parieto-insular vestibular cortex. *J Neurophysiol*, *111*(12), 2445-2464. doi:10.1152/jn.00731.2013

Siegel, A., & Tassoni, J. P. (1971). Differential efferent projections of the lateral and medial septal nuclei to the hippocampus in the cat. *Brain Behav Evol*, *4*(3), 201-219. doi:10.1159/000125434

Siemann, J. K., Muller, C. L., Bamberger, G., Allison, J. D., Veenstra-VanderWeele, J., & Wallace, M. T. (2014). A novel behavioral paradigm to assess multisensory processing in mice. *Front Behav Neurosci*, *8*, 456. doi:10.3389/fnbeh.2014.00456

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*(3), 609-618. doi:10.1007/s10071-013-0692-1

Simerly, R. B., & Swanson, L. W. (1986). The organization of neural inputs to the medial preoptic nucleus of the rat. *J Comp Neurol*, *246*(3), 312-342. Retrieved from

Singer, T., Critchley, H. D., & Preuschoff, K. (2009). A common role of insula in feelings, empathy and uncertainty. *Trends Cogn Sci*, *13*(8), 334-340. doi:10.1016/j.tics.2009.05.001

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. *Science*, *303*(5661), 1157-1162. doi:10.1126/science.1093535

Singer, T., Seymour, B., O'Doherty, J. P., Stephan, K. E., Dolan, R. J., & Frith, C. D. (2006). Empathic neural responses are modulated by the perceived fairness of others. *Nature*, *439*(7075), 466-469. doi:10.1038/nature04271

Singewald, G. M., Rjabokon, A., Singewald, N., & Ebner, K. (2011). The modulatory role of the lateral septum on neuroendocrine and behavioral stress responses. *Neuropsychopharmacology*, *36*(4), 793-804. doi:10.1038/npp.2010.213

Singh, S., Modi, S., Goyal, S., Kaur, P., Singh, N., Bhatia, T., Deshpande, S. N., & Khushu, S. (2015). Functional and structural abnormalities associated with empathy in patients with schizophrenia: An fMRI and VBM study. *J Biosci*, *40*(2), 355-364. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25963262

Skuse, D. H., & Gallagher, L. (2009). Dopaminergic-neuropeptide interactions in the social brain. *Trends Cogn Sci*, *13*(1), 27-35. doi:10.1016/j.tics.2008.09.007

Smith, C. J. W., Mogavero, J. N., Tulimieri, M. T., & Veenema, A. H. (2017). Involvement of the oxytocin system in the nucleus accumbens in the regulation of juvenile social novelty-seeking behavior. *Horm Behav*, *93*, 94-98. doi:10.1016/j.yhbeh.2017.05.005

Smith, C. J. W., Wilkins, K. B., Li, S., Tulimieri, M. T., & Veenema, A. H. (2018). Nucleus accumbens mu opioid receptors regulate context-specific social preferences in the juvenile rat. *Psychoneuroendocrinology*, *89*, 59-68. doi:10.1016/j.psyneuen.2017.12.017

Smith, K. S., Bucci, D. J., Luikart, B. W., & Mahler, S. V. (2016). DREADDS: Use and application in behavioral neuroscience. *Behav Neurosci*, *130*(2), 137-155. doi:10.1037/bne0000135

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2009). Ventral pallidum roles in reward and motivation. *Behav Brain Res*, *196*(2), 155-167. doi:10.1016/j.bbr.2008.09.038

Smotherman, W. P., Bell, R. W., Starzec, J., Elias, J., & Zachman, T. A. (1974). Maternal responses to infant vocalizations and olfactory cues in rats and mice. *Behav Biol*, *12*(1), 55-66. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4429513

Sotocinal, S. G., Sorge, R. E., Zaloum, A., Tuttle, A. H., Martin, L. J., Wieskopf, J. S., Mapplebeck, J. C., Wei, P., Zhan, S., Zhang, S., McDougall, J. J., King, O. D., & Mogil, J. S. (2011). The Rat Grimace Scale: a partially automated method for quantifying pain in the laboratory rat via facial expressions. *Mol Pain*, *7*, 55. doi:10.1186/1744-8069-7-55

Soumiya, H., Godai, A., Araiso, H., Mori, S., Furukawa, S., & Fukumitsu, H. (2016). Neonatal Whisker Trimming Impairs Fear/Anxiety-Related Emotional Systems of the Amygdala and Social Behaviors in Adult Mice. *PLoS One*, *11*(6), e0158583. doi:10.1371/journal.pone.0158583

Spear, L. P. (2015). Adolescent alcohol exposure: Are there separable vulnerable periods within adolescence. *Physiol Behav*, *148*, 122-130. doi:10.1016/j.physbeh.2015.01.027

Stachniak, T. J., Ghosh, A., & Sternson, S. M. (2014). Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus→midbrain pathway for feeding behavior. *Neuron*, *82*(4), 797-808. doi:10.1016/j.neuron.2014.04.008

Stanzani, S., & Russo, A. (1980). [Afferent connections in the ventromedial hypothalamus of rats demonstrated by retrograde transport of horseradish peroxidase]. *Boll Soc Ital Biol Sper*, *56*(17), 1721-1725. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/6161623

Staub, E., & Baer, R. S. (1974). Stimulus characteristics of a sufferer and difficulty of escape as determinants of helping. *Journal of Personality and Social Psychology*, *30*(2), 279-284. Retrieved from

Stein, B. E., Meredith, M. A., Huneycutt, W. S., & McDade, L. (1989). Behavioral Indices of Multisensory Integration: Orientation to Visual Cues is Affected by Auditory Stimuli. *J Cogn Neurosci*, *1*, 12-24. doi:10.1162/jocn.1989.1.1.12

Stein, B. E., Stanford, T. R., & Rowland, B. A. (2014). Development of multisensory integration from the perspective of the individual neuron. *Nat Rev Neurosci*, *15*(8), 520-535. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/25158358

Steinman, M. Q., Duque-Wilckens, N., & Trainor, B. C. (2018). Complementary Neural Circuits for Divergent Effects of Oxytocin: Social Approach Versus Social Anxiety. *Biol Psychiatry*. doi:10.1016/j.biopsych.2018.10.008

Sterley, T. L., Baimoukhametova, D., Füzesi, T., Zurek, A. A., Daviu, N., Rasiah, N. P., Rosenegger, D., & Bains, J. S. (2018). Social transmission and buffering of synaptic changes after stress. *Nat Neurosci*, *21*(3), 393-403. doi:10.1038/s41593-017-0044-6

Stern, J. M. (1990). Multisensory regulation of maternal behavior and masculine sexual behavior: a revised view. *Neurosci Biobehav Rev*, *14*(2), 183-200. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/2190118

Stowers, L., Cameron, P., & Keller, J. A. (2013). Ominous odors: olfactory control of instinctive fear and aggression in mice. *Curr Opin Neurobiol*, *23*(3), 339-345. doi:10.1016/j.conb.2013.01.007

Strigo, I. A., & Craig, A. D. (2016). Interoception, homeostatic emotions and sympathovagal balance. *Philos Trans R Soc Lond B Biol Sci*, *371*(1708). doi:10.1098/rstb.2016.0010

Sueur, C., & Pelé, M. (2016). Social network and decision-making in primates: a report on Franco-Japanese research collaborations. *Primates*, *57*(3), 327-332. doi:10.1007/s10329-015-0505-z

Sullivan, R. M., Landers, M. S., Flemming, J., Vaught, C., Young, T. A., & Jonathan Polan, H. (2003). Characterizing the functional significance of the neonatal rat vibrissae prior to the onset of whisking. *Somatosens Mot Res*, *20*(2), 157-162. doi:10.1080/0899022031000105190

Sun, W., Tan, Z., Mensh, B. D., & Ji, N. (2016). Thalamus provides layer 4 of primary visual cortex with orientation- and direction-tuned inputs. *Nat Neurosci*, *19*(2), 308-315. doi:10.1038/nn.4196

Supekar, K., Kochalka, J., Schaer, M., Wakeman, H., Qin, S., Padmanabhan, A., & Menon, V. (2018). Deficits in mesolimbic reward pathway underlie social interaction impairments in children with autism. *Brain*, *141*(9), 2795-2805. doi:10.1093/brain/awy191

Tai, A. X., & Kromer, L. F. (2014). Corticofugal projections from medial primary somatosensory cortex avoid EphA7-expressing neurons in striatum and thalamus. *Neuroscience*, *274*, 409-418. doi:10.1016/j.neuroscience.2014.05.039

Takahashi, Y., Kiyokawa, Y., Kodama, Y., Arata, S., Takeuchi, Y., & Mori, Y. (2013). Olfactory signals mediate social buffering of conditioned fear responses in male rats. *Behav Brain Res*, *240*, 46-51. doi:10.1016/j.bbr.2012.11.017

Tasaka, G. I., Guenthner, C. J., Shalev, A., Gilday, O., Luo, L., & Mizrahi, A. (2018). Genetic tagging of active neurons in auditory cortex reveals maternal plasticity of coding ultrasonic vocalizations. *Nat Commun*, *9*(1), 871. doi:10.1038/s41467-018-03183-2

Terasawa, Y., Kurosaki, Y., Ibata, Y., Moriguchi, Y., & Umeda, S. (2015a). Attenuated sensitivity to the emotions of others by insular lesion. *Front Psychol*, *6*, 1314. doi:10.3389/fpsyg.2015.01314

Terasawa, Y., Kurosaki, Y., Ibata, Y., Moriguchi, Y., & Umeda, S. (2015b). Attenuated sensitivity to the emotions of others by insular lesion. *Front Psychol*, *6*, 1314. doi:10.3389/fpsyg.2015.01314

Timmermann, M., Jeung, H., Schmitt, R., Boll, S., Freitag, C. M., Bertsch, K., & Herpertz, S. C. (2017). Oxytocin improves facial emotion recognition in young adults with antisocial personality disorder. *Psychoneuroendocrinology*, *85*, 158-164. doi:10.1016/j.psyneuen.2017.07.483

Tracy, J. L., Robins, R. W., Schriber, R. A., & Solomon, M. (2011). Is emotion recognition impaired in individuals with autism spectrum disorders. *J Autism Dev Disord*, *41*(1), 102-109. doi:10.1007/s10803-010-1030-y

Tremblay, S., Sharika, K. M., & Platt, M. L. (2017). Social Decision-Making and the Brain: A Comparative Perspective. *Trends Cogn Sci*, *21*(4), 265-276. doi:10.1016/j.tics.2017.01.007

Trezza, V., Damsteegt, R., Achterberg, E. J., & Vanderschuren, L. J. (2011). Nucleus accumbens μ-opioid receptors mediate social reward. *J Neurosci*, *31*(17), 6362-6370. doi:10.1523/JNEUROSCI.5492-10.2011

Tye, K. M. (2012). Glutamate inputs to the nucleus accumbens: does source matter. *Neuron*, *76*(4), 671-673. doi:10.1016/j.neuron.2012.11.008

Tye, K. M., Prakash, R., Kim, S. Y., Fenno, L. E., Grosenick, L., Zarabi, H., Thompson, K. R., Gradinaru, V., Ramakrishnan, C., & Deisseroth, K. (2011). Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature*, *471*(7338), 358-362. doi:10.1038/nature09820

Uddin, L. Q., Davies, M. S., Scott, A. A., Zaidel, E., Bookheimer, S. Y., Iacoboni, M., & Dapretto, M. (2008). Neural basis of self and other representation in autism: an FMRI study of self-face recognition. *PLoS One*, *3*(10), e3526. doi:10.1371/journal.pone.0003526

Valenta, J. G., & Rigby, M. K. (1968). Discrimination of the odor of stressed rats. *Science*, *161*(3841), 599-601. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/5663306

Valtcheva, S., & Froemke, R. C. (2018). Neuromodulation of maternal circuits by oxytocin. *Cell Tissue Res*. doi:10.1007/s00441-018-2883-1

Van den Berg, C. L., Pijlman, F. T., Koning, H. A., Diergaarde, L., Van Ree, J. M., & Spruijt, B. M. (1999). Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. *Behav Brain Res*, *106*(1-2), 133-142. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10595429

Vanderschuren, L. J., Achterberg, E. J., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neurosci Biobehav Rev*, *70*, 86-105. doi:10.1016/j.neubiorev.2016.07.025

Vann, S. D., Aggleton, J. P., & Maguire, E. A. (2009). What does the retrosplenial cortex do? *Nat Rev Neurosci*, *10*(11), 792-802. Retrieved from

Varela, J. A., Wang, J., Christianson, J. P., Maier, S. F., & Cooper, D. C. (2012). Control over stress, but not stress per se increases prefrontal cortical pyramidal neuron excitability. *J Neurosci*, *32*(37), 12848-12853. doi:10.1523/JNEUROSCI.2669-12.2012

Venniro, M., Caprioli, D., Zhang, M., Whitaker, L. R., Zhang, S., Warren, B. L., Cifani, C., Marchant, N. J., Yizhar, O., Bossert, J. M., Chiamulera, C., Morales, M., & Shaham, Y. (2017). The anterior insular cortex —> central amygdala glutamatergic pathway is critical to relpase after contingency management. *Neuron*, *96*(2), 414-427. Retrieved from

Vetere, G., Kenney, J. W., Tran, L. M., Xia, F., Steadman, P. E., Parkinson, J., Josselyn, S. A., & Frankland, P. W. (2017). Chemogenetic Interrogation of a Brain-wide Fear Memory Network in Mice. *Neuron*, *94*(2), 363-374.e4. doi:10.1016/j.neuron.2017.03.037

Viaene, A. N., Petrof, I., & Sherman, S. M. (2011). Properties of the thalamic projection from the posterior medial nucleus to primary and secondary somatosensory cortices in the mouse. *Proc Natl Acad Sci U S A*, *108*(44), 18156-18161. doi:10.1073/pnas.1114828108

Volkmar, F. R., & Greenough, W. T. (1972). Rearing complexity affects branching of dendrites in the visual cortex of the rat. *Science*, *176*(4042), 1445-1447. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/5033647

Vreeburg, J. T., & Ooms, M. P. (1985). Induction of ear wiggling in the estrous female rat by gonadectomized rats treated with androgens and estrogens. *Horm Behav*, *19*(3), 231-236. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/4054849

Waller, R., Corral-Frías, N. S., Vannucci, B., Bogdan, R., Knodt, A. R., Hariri, A. R., & Hyde, L. W. (2016). An oxytocin receptor polymorphism predicts amygdala

reactivity and antisocial behavior in men. *Soc Cogn Affect Neurosci*, *11*(8), 1218-1226. doi:10.1093/scan/nsw042

Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., Pedersen, N. L., Anckarsäter, H., Larsson, H., & Westberg, L. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biol Psychiatry*, *71*(5), 419-426. doi:10.1016/j.biopsych.2011.09.002

Walum, H., & Young, L. J. (2018). The neural mechanisms and circuitry of the pair bond. *Nat Rev Neurosci*, *19*(11), 643-654. doi:10.1038/s41583-018-0072-6

Wang, F., Zhu, J., Zhu, H., Zhang, Q., Lin, Z., & Hu, H. (2011). Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science*, *334*(6056), 693-697. doi:10.1126/science.1209951

Weiss, V. G., Hofford, R. S., Yates, J. R., Jennings, F. C., & Bardo, M. T. (2015). Sex differences in monoamines following amphetamine and social reward in adolescent rats. *Exp Clin Psychopharmacol*, *23*(4), 197-205. doi:10.1037/pha0000026

Weller, K. L., & Smith, D. A. (1982). Afferent connections to the bed nucleus of the stria terminalis. *Brain Res*, *232*(2), 255-270. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/7188024

Willuhn, I., Tose, A., Wanat, M. J., Hart, A. S., Hollon, N. G., Phillips, P. E., Schwarting, R. K., & Wöhr, M. (2014). Phasic dopamine release in the nucleus accumbens in response to pro-social 50 kHz ultrasonic vocalizations in rats. *J Neurosci*, *34*(32), 10616-10623. doi:10.1523/JNEUROSCI.1060-14.2014

Wilson, C. J. (2014). The sensory striatum. *Neuron*, *83*(5), 999-1001. doi:10.1016/j.neuron.2014.08.025

Wilson, E. O. (1992). The effects of complex social life on evolution and biodiversity. *Oikos*, *63*(1), 13-18. Retrieved from

Wise, R. A. (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron*, *36*(2), 229-240. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12383779

Wolfe, J., Mende, C., & Brecht, M. (2011). Social facial touch in rats. *Behav Neurosci*, *125*(6), 900-910. doi:10.1037/a0026165

Wong, L. C., Wang, L., D'Amour, J. A., Yumita, T., Chen, G., Yamaguchi, T., Chang, B. C., Bernstein, H., You, X., Feng, J. E., Froemke, R. C., & Lin, D. (2016). Effective Modulation of Male Aggression through Lateral Septum to Medial Hypothalamus Projection. *Curr Biol*, *26*(5), 593-604. doi:10.1016/j.cub.2015.12.065

Wright, C. I., & Groenewegen, H. J. (1996). Patterns of overlap and segregation between insular cortical, intermediodorsal thalamic and basal amygdaloid afferents in the nucleus accumbens of the rat. *Neuroscience*, *73*(2), 359-373. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/8783254

Wu, Y. L., Yoshida, M., Emoto, H., & Tanaka, M. (1999). Psychological stress selectively increases extracellular dopamine in the 'shell', but not in the 'core' of the rat nucleus accumbens: a novel dual-needle probe simultaneous microdialysis study. *Neurosci Lett*, *275*(1), 69-72. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/10554987

Wylie, K. P., & Tregellas, J. R. (2010). The role of the insula in schizophrenia. *Schizophr Res*, *123*(2-3), 93-104. doi:10.1016/j.schres.2010.08.027

Xiao, L., & Becker, J. B. (1997). Hormonal activation of the striatum and the nucleus accumbens modulates paced mating behavior in the female rat. *Horm Behav*, *32*(2), 114-124. doi:10.1006/hbeh.1997.1412

Xiu, J., Zhang, Q., Zhou, T., Zhou, T. T., Chen, Y., & Hu, H. (2014). Visualizing an emotional valence map in the limbic forebrain by TAI-FISH. *Nat Neurosci*, *17*(11), 1552-1559. doi:10.1038/nn.3813

Yamada, T., Itahashi, T., Nakamura, M., Watanabe, H., Kuroda, M., Ohta, H., Kanai, C., Kato, N., & Hashimoto, R. I. (2016). Altered functional organization within the insular cortex in adult males with high-functioning autism spectrum disorder: evidence from connectivity-based parcellation. *Mol Autism*, *7*, 41. doi:10.1186/s13229-016-0106-8

Yang, L., Li, P., Mao, H., Wang, H., Shu, C., Bliksted, V., & Zhou, Y. (2017a). Theory of mind deficits partly mediate impaired social decision-making in schizophrenia. *BMC Psychiatry*, *17*(1), 168. doi:10.1186/s12888-017-1313-3

Yang, T., Yang, C. F., Chizari, M. D., Maheswaranathan, N., Burke, K. J., Borius, M., Inoue, S., Chiang, M. C., Bender, K. J., Ganguli, S., & Shah, N. M. (2017b). Social Control of Hypothalamus-Mediated Male Aggression. *Neuron*, *95*(4), 955-970.e4. doi:10.1016/j.neuron.2017.06.046

Yao, S., Bergan, J., Lanjuin, A., & Dulac, C. (2017). Oxytocin signaling in the medial amygdala is required for sex discrimination of social cues. *Elife*, *6*. doi:10.7554/eLife.31373

Yokoyama, C., & Sasaki, K. (1999). Regional expressions of Fos-like immunoreactivity in rat cerebral cortex after stress; restraint and intraperitoneal lipopolysaccharide. *Brain Res*, *816*(2), 267-275. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/9878776

Young, L. J., & Barrett, C. E. (2015). Neuroscience. Can oxytocin treat autism. *Science*, *347*(6224), 825-826. doi:10.1126/science.aaa8120

Zhang, D., Yang, S., Yang, C., Jin, G., & Zhen, X. (2008). Estrogen regulates responses of dopamine neurons in the ventral tegmental area to cocaine. *Psychopharmacology (Berl)*, *199*(4), 625-635. doi:10.1007/s00213-008-1188-6