ORBITOFRONTAL CORTEX AND SOCIAL PROCESSING IN RODENT MODELS

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DEDICATION

To my Mom, who is the best listener in the world, and to my Dad who, through countless viewings of Star Wars, late nights on the telescope, and trips to mountains and oceans, taught me to ask questions, seek meaning, and strive for great things.

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Katharine DiAnn Andrews

ORBITOFRONTAL CORTEX AND SOCIAL PROCESSING IN RODENT MODELS

Social processing is the reception, interpretation, and reciprocation of social information and is critical for mental health. The neural structures, circuits, and substrates regulating these complex mechanisms are not well understood. Social processing in the form of social safety learning, as measured by a rat model of social familiarity-induced anxiolysis (SoFiA), was impaired following mild blast traumatic brain injury (mbTBI). Initial findings indicated that mbTBI altered resting state network activity in the orbitofrontal cortex (OFC) and was associated with accumulation of neurotoxin marker, acrolein, in lateral prefrontal cortex (PFC) (including OFC), indicating OFC as a brain region of interest that may contribute to social processing. Measuring GABA and Glutamate-related gene expression in OFC of mbTBI or sham-exposed rat brain revealed specific elevations of metabotropic glutamate receptor type 1 and 5 (mGluR1/5) expression in mbTBI but not sham OFC. Exposure-naïve rats intracranially injected with mGluR1/5 agonist demonstrated attenuated SoFiA, and this coincided with an impairment of social recognition (SR) behavior. Additionally, inactivation of OFC by local intracranial injection of $GABA_A$ agonist, muscimol, impaired two different measures of SR in which two conspecifics, or members of the same species, one novel and one familiar, were presented and required discrimination. Novelty seeking, decision-making, memory, and gregariousness were tested in isolation to determine OFC contributions to these specific behavioral contributions to SR test performance. OFC inactivation did not impair novelty seeking, non-social decision-making, or non-social memory as measured by novel object recognition (NOR) test, or gregariousness or social decision-making as measure by social

preference (SP) test. When measuring SR behavior via consecutive presentation of two different conspecifics, OFC inactivation did not impact SR. Therefore, OFC is not directly responsible for social recognition, but rather the discrimination or ability to act upon discrimination of two simultaneously present conspecifics. These data suggest a novel role for OFC in high order processing or execution of action based on social information.

Xiao-Ming Xu, PhD, Chair

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LIST OF ABBREVIATIONS

ACC	Anterior cingulate cortex
AIC	Agranular insular cortex
ANOVA	Analysis of variance
BLA	Basolateral amygdala
BLC	Bright light challenge
CRF	Corticotrophin releasing factor
DHPG	Dihydroxyphenylglycine
dmPFC	Dorsomedial prefrontal cortex
fMRI	Functional magnetic resonance imaging
GABA	ɣ-Aminobutyric Acid
Grm	Glutamate metabotropic receptor
IL	Infralimbic cortex
LSD	Least significant difference
mbTBI	Mild blast traumatic brain injury
MD	Mediodorsal nucleus of the thalamus
mGluR	Metabotropic glutamate receptor
mPFC	Medial prefrontal cortex
MRI	Magnetic resonance imaging
NOR	Novel object recognition
OF	Open field
OFC	Orbitofrontal cortex
OMPFC	Orbital and medial prefrontal cortex

PFC	Prefrontal cortex
PL	Prelimbic cortex
PTSD	Post-Traumatic Stress Disorder
RM	Repeated measures
SD	Standard deviation
SI	Social interaction
SI-hab	Social interaction habituation
SoFiA	Social familiarity-induced anxiolysis
SP	Social preference
SR	Social recognition
TLDA	Taqman Low Density Array
TS	Tail suspension
vmPFC	Ventromedial prefrontal cortex

Chapter One. Introduction

Social support and health

The benefit derived from social interactions, henceforth referred to as social support, is critical for both physical and mental health (for review, see (Ditzen & Heinrichs, 2014)). Social support reduces mortality risk (Eng, Rimm, Fitzmaurice, & Kawachi, 2002; Holt-Lunstad, Smith, & Layton, 2010), and studies have repeatedly demonstrated positive effects of social support on cardiovascular, neuroendocrine, and immune health (for review, see (Uchino, 2006; Uchino, Cacioppo, & Kiecolt-Glaser, 1996). Perhaps more intuitively, social support positively influences mental health, specifically improving depression treatment outcome (G. C. Carter et al., 2012; J. Wang, Mann, Lloyd-Evans, Ma, & Johnson, 2018), protecting against negative mental health effects of partner violence (Coker et al., 2002), mediating progression of anxiety and depression symptoms (H. J. Dour et al., 2014), and facilitating posttraumatic growth (Cao et al., 2018; K. M. Han et al., 2018). Mechanisms of the interaction between social support and mental and physical health have been explored in detail in (Cohen, 1988; Thoits, 2011), though much remains unknown (Uchino, Bowen, Carlisle, & Birmingham, 2012).

Given the high lifetime prevalence (near 50%) of mental illness in the adult American population, and that the most common class of mental illness is anxiety disorder (Kessler, Chiu, Demler, Merikangas, & Walters, 2005), it is important to understand the interaction of social support with anxiety. Anxiety and social support are inversely related, regardless of the source of anxiety (Davaridolatabadi & Abdeyazdan, 2016; Ghorbani Saeedian et al., 2014; B. Han et al., 2014; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003), suggesting that social support may be anxiety reducing, or anxiolytic. It is important

to note that not all social interactions are supportive and some may even be anxiogenic in and of themselves; however this project focuses on positive social interactions that have the capacity to be anxiolytic.

Perceived social support influences anxiety, and high levels of perceived social support increase positive outcomes of psychotherapy compared to low perception of social support (H. J. Dour et al., 2014; Hawkley & Cacioppo, 2010; Levy, Ellison, Scott, & Bernecker, 2011; M. Price, Gros, Strachan, Ruggiero, & Acierno, 2013). In clinically anxious children, proximity of a caregiver reduced anxiety and activation of the hypothalamus's response to stressful stimuli (Conner et al., 2012). In addition, patients with Post-Traumatic Stress Disorder (PTSD) benefit most from therapies that are exposure-based interpersonal or group therapies, and success of these treatments is dependent on perceptions of social support (M. Price et al., 2013).

On the other end of the spectrum, social isolation is associated with increased mental health disorder, including higher risk of generalized anxiety disorder (Chou, Liang, & Sareen, 2011). Even a stranger offering social support can reduce emotional and physical arousal in response to verbal confrontation (Gerin, Pieper, Levy, & Pickering, 1992) or performing a complex task (Thorsteinsson, James, & Gregg, 1998).

Social familiarity directs social support-driven anxiolysis

Every person exists within a complex framework of social connections, and a fundamental descriptor of these connections is familiarity. The level of intimacy between strangers, acquaintances, friends, family, or partners establishes the types of interactions

that occur within the relationship. Because of the importance of familiarity on social behavior, familiarity must be evaluated as an important variable in clinical science.

One review by Thoits emphasizes that the distinction of familiarity is not often explored in literature relating social support and health. Per Thoits, a person's social network can be divided into primary and secondary groups of people. Primary group members are usually family and close friends, while secondary group members involve less personal relationships, such as those found through work or community activities (e.g., religious organizations). Thoits argues that these two groups of people provide fundamentally different benefits to the individual. For example, primary group members may support an individual through mechanisms such as validating the individual's need for care without being able to specifically relate to the individual's source of stress, while secondary group members may lack the intimacy to offer meaningful care yet exist as a wide enough network that someone likely can serve as a supporter who has undergone a similar stress as that of the individual (Thoits, 2011). The ability to discern and discriminate levels of familiarity in social relationships would therefore be critical to maximizing the benefit that can be gained from a varied social network.

Empirical studies confirm the importance of distinguishing levels of familiarity when assessing the benefits of social support. First, social support is greater from a known partner than a stranger. In one study, self-reported pain from shock was decreased when viewing pictures of a partner but not when viewing a stranger or object (N. I. Eisenberger et al., 2011). Support from a partner, but not stranger, attenuated fear acquisition (Hornstein & Eisenberger, 2017) and enhanced fear extinction (Hornstein, Haltom, Shirole, & Eisenberger, 2017). In a separate study, handholding with a partner, but not stranger,

reduced subjective distress to shock and decreased activity in a neural network of threat assessment. Surprisingly, type of partner (spouse, cohabitating partner, dating partner, or platonic friend) did not affect threat response, though the authors indicate this finding may be a false negative (J. A. Coan et al., 2017).

Furthermore, studies have demonstrated that the degree and character of familiarity of the social partner modulates the benefits of social support. In one study, conditioned fear response was significantly attenuated when paired with a positive social support figure (defined as someone who provides the most social support on a daily basis, suggesting high familiarity) compared to a neutral social figure (a professor for a class the participant was currently enrolled in, suggesting less familiarity than a social support figure but greater familiarity than a stranger) (Hornstein, Fanselow, & Eisenberger, 2016). PTSD patients perceive greater social support from peers with anxiety compared to staff, suggesting PTSD patients receive higher anxiolytic quality from peers with shared anxiety (Chinman et al., 2014; Hundt, Robinson, Arney, Stanley, & Cully, 2015).

Still, the relationship between social support and familiarity is complicated. In one study, salivary cortisol level was used as a marker of stress induced by public speaking. Men had significantly greater reduction in cortisol levels when supported by female partners than strangers or without support, while women actually showed elevated cortisol levels when supported by partners (Kirschbaum, Klauber, Filipp, & Hellhammer, 1995). Such conflicting results highlight the complexity of the relationship between social familiarity and the benefit of social support and suggest sex may be an important variable when considering the effects of social support.

Taken together, these data begin to suggest the creation of a social familiarity continuum, reflecting the relationship between the degree of familiarity and the capacity of anxiolysis possible. At one end of this spectrum, isolation would be regarded as offering no anxiolytic capacity, followed by strangers offering some anxiolytic capacity but less than acquaintances, followed by friends, then family, significant others, and ultimately, a therapist (Majumdar, Lungwitz, Andrews, Chambers, & Truitt, 2018). An argument can be made that a therapist exists at the uppermost end of this spectrum. The patient-therapeutic alliance is a core contributor to the effectiveness of psychotherapy (for review, see (Ardito & Rabellino, 2011)). The anxiolytic capacity of social support may be highest in interpersonal or group psychotherapies, where the goal is directed toward specifically learning to reduce anxiety (Jacoby & Abramowitz, 2016; Vervliet, Craske, & Hermans, 2013).

Social familiarity-induced anxiolysis (SoFiA)

Social safety learning

Safety learning is learning to associate external stimuli as cues or signals with the nonoccurrence of adverse events; this in turn leads to a reduction in fear and/or anxiety behavior and is considered critical for mental health (Christianson et al., 2012; Kong, Monje, Hirsch, & Pollak, 2014). Social safety learning is when the external cue or signal is social in nature, for example the presence of a friend. The use of social support to buffer stress or induce anxiolysis falls under the concept of social safety learning. The acquisition of safety learning is enhanced by the presence of a social support figure (Hornstein & Eisenberger, 2017; Muscatell, Eisenberger, Dutcher, Cole, & Bower, 2016). One example

of social safety learning is vicarious extinction learning, in which an observer examines another person demonstrating fearlessness to a fearful stimulus and in turn has reduced fear acquisition (Golkar, Selbing, Flygare, Ohman, & Olsson, 2013). Such vicarious extinction learning ameliorates fear responses to a greater extent and can last longer than nonsocial safety learning (Golkar & Olsson, 2016; Golkar, Selbing, Flygare, Ohman, & Olsson, 2013; Golkar, Tjaden, & Kindt, 2017).

Preclinical modeling of social safety learning

Understanding the detailed neurocircuitry of complex behaviors such as social safety learning is greatly aided by preclinical animal modeling. Though animal modeling cannot fully capture the complexity of human thought and behavior, it facilitates systematic investigation of otherwise very tangled neuropsychiatric pathways. Preclinical animal models have successfully recapitulated social safety learning. For example, rats that undergo extinction learning in the presence of a conspecific (another member of the same species) demonstrate enhanced extinction learning (Brill-Maoz & Maroun, 2016; Mikami, Kiyokawa, Takeuchi, & Mori, 2016). This phenomenon is observed in mice as well (Colnaghi et al., 2016). And similarly to findings in humans regarding the influence of social familiarity, familiar conspecifics provide greater reduction in conditioned fear responses than unfamiliar conspecifics (Kiyokawa, Honda, Takeuchi, & Mori, 2014).

These studies successfully demonstrate that rodents can experience the benefit of social support offered by the presence of a conspecific, however in each of these studies rodents are learning safety towards a fear cue generated by fear conditioning. Our lab wished to examine the role of social support on anxiety, rather than fear, using an innate

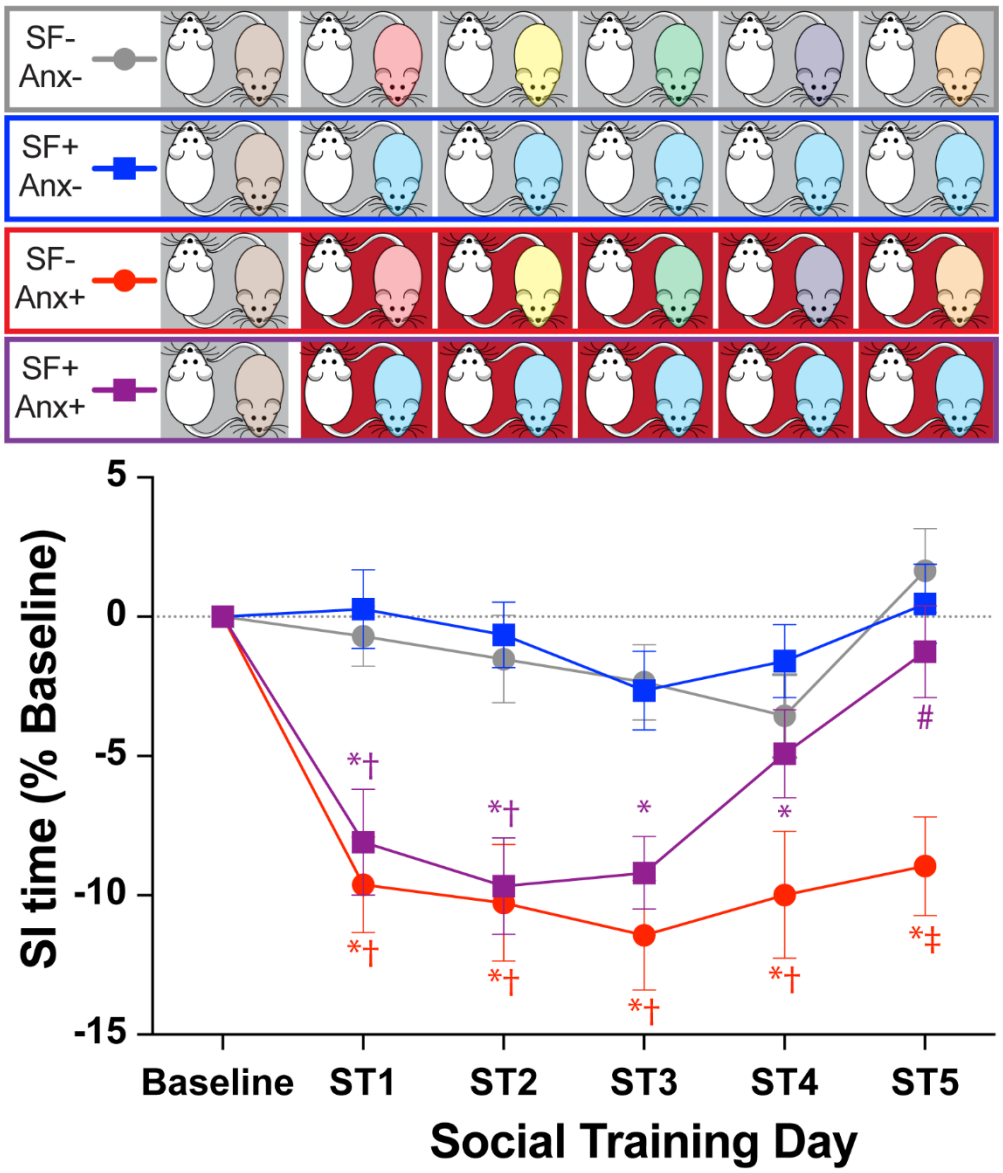
anxiogenic stimulus rather than a learned fear cue. Therefore, a model for social familiarity-induced anxiolysis (SoFiA) was developed for rats.

SoFiA paradigm for rats

The SoFiA paradigm for rats was developed previously by our lab (Lungwitz et al., 2014; Truitt et al., 2007). The paradigm models social safety learning as social support-mediated reduction in anxiety-like behavior. In SoFiA, age- and weight-matched male rats are paired together for 5 minutes in a traditional Social Interaction (SI) test, which is a validated measure of anxiety-like behavior in rodents (File, 1984). In the SI test, two rats, one designated an experimental rat and the other its social partner, are allowed to freely interact within an open field (OF) arena. In SoFiA, this 5-minute SI test is performed under an anxiogenic stimulus, the bright light challenge (BLC), in which rats are first habituated to dim red lighting then a bright light is switched on at the very start of the 5-minute SI testing session and remains on for the duration of the SI test. In SoFiA, this 5-minute SI test is repeated once per day for 5-6 days, termed the SI-habituation paradigm (SI-hab), in which the experimental rat receives the same partner each day. SoFiA is acquired when the experimental rat shows a reduction in anxiety-like behavior to the BLC. SoFiA acquisition is dependent on the presence of the BLC and the experimental rat receiving the same partner each day. Rats who receive the same partner every day in dim red lighting conditions, or rats who receive a novel partner each day in BLC do not demonstrate anxiolysis [(Figure 1.1) (Majumdar et al., 2018)]. SoFiA is not a result of habituation to the BLC, as rats do not readily habituate to the BLC within a 5-6 day period of time (Lungwitz et al., 2014).

Figure 1.1. Acquisition of SoFiA is dependent on a familiar conspecific and anxiogenic stimuli during social training sessions

(Top) Schematic representation of conspecific and anxiety-like conditions for each group employed during testing. Here, the white rat represents the experimental rat and the colored rats represent a social partner (or conspecific) for each SI session. Each color represents a different conspecific; repeated colors (e.g., light blue) represent the same conspecific being used for each of the sessions ST1 through ST5, indicating SF+, whereas a different color rat on each ST day represents the absence of SF-. Gray background represents absence of (Anx-) while red background represents presence of anxiety as induced by BLC (Anx+) during each SI session. A baseline SI session conducted under SF- and Anx- conditions preceded ST sessions. **(Bottom)** Presented are the changes in SI time (mean ± standard error of the mean), in seconds, from baseline for each group expressed as a percentage. The SI-hab protocol was used to determine effects of SF and Anx during ST sessions on the acquisition and expression of anxiolysis. Here, anxiolysis is defined as a significant increase in SI time compared to the first ST session. This resulted in main effects of Anx (three-way analysis of variance, $F_{1,5}$=102, P<0.0001), SF ($F_{1,5}$=7.282, P=0.0072) and day ($F_{5,5}$=10.85, P<0.0001) as well as Anx X day ($F_{5,5}$=5.169, P=0.0001) and Anx X SF ($F_{1,5}$=4.217, P=0.0405) interactions. Here, rats in the Anx- groups had no significant changes in SI times, regardless of SF condition, while rats in the Anx+ groups had significant reductions in SI time compared to baseline during ST days (1-5 for SF-/Anx+ and 1-4 for SF+/Anx+). Furthermore, when paired with the same conspecific (SF+), rats under Anx+ conditions developed a reduction in anxiety-like behavior over multiple ST sessions; rats in this group significantly increased SI time by the 5th ST day compared to the first. *Significantly different from baseline (Dunnett's, p≤0.0039); # significantly different from ST day 1 (Dunnett's, p<0.0001); † significantly different from Anx- groups (Tukey's p≤0.046); ‡ significantly different from all other groups (Tukey's p≤0.043) (n=22). Anx, anxiety stimulus; SF, social familiarity; SI, social interaction; SI-hab, social interaction-habituation; ST, social training.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Neural correlates of SoFiA

Elucidating the neural correlates of SoFiA is an ongoing priority in our lab. Thus far, two regions have been demonstrated instrumental for SoFiA: infralimbic cortex (IL) and basolateral amygdala (BLA). Inactivation of IL, via intracranial injection of the $GABA_A$ agonist muscimol, impairs expression of SoFiA behavior, suggesting IL is a key regulatory region of SoFiA (Lungwitz et al., 2014) This is congruent with the role of ventromedial prefrontal cortex (vmPFC) in safety learning generally (Christianson et al., 2012; Harrison et al., 2017), and specifically in cases of vicarious extinction learning (Golkar, Haaker, Selbing, & Olsson, 2016).

In addition, cytotoxic lesioning of GABAergic interneurons in BLA impairs SoFiA when SoFiA is generated by priming with stress-peptide corticotrophin releasing factor (CRF) receptor agonist urocortin 1 rather than BLC (Truitt et al., 2007). This suggests a role for BLA in SoFiA, as expected given the amygdala's robustly understood role in anxiety, as well as potential substrates of social safety learning in GABAergic signaling.

Project outline

The present project was aimed to further investigate the neural mechanisms and substrates of social processing, particularly social safety learning. One known disruptor of social processing in humans is traumatic brain injury (TBI) (S. McDonald & Flanagan, 2004; J. M. Spikman, M. E. Timmerman, M. V. Milders, W. S. Veenstra, & J. van der Naalt, 2012). In rats, a mild blast TBI (mbTBI) was found to impair SoFiA, offering a window of opportunity to gain insight into the possible brain regions contributing to SoFiA behavior. In Chapter Two, the OFC is identified as a region of interest that may be

important for SoFiA. When trying to identify how OFC contributes to SoFiA, we observed that OFC is important for social recognition (SR) behavior, which is the ability to discriminate novel versus familiar conspecifics. In Chapter Three, the role of OFC in SR behavior is characterized. OFC is deduced to be a region likely important for processing social familiarity. We hypothesize that OFC-mediated regulation of social familiarity makes OFC a key contributor to social safety learning and the benefits of social support on mental health.

Chapter Two. Changes in orbitofrontal cortex alter social familiarity-induced anxiolysis

Introduction

At least 3.2 million people in the United States are living with a disability related to traumatic brain injury (TBI) (Prevention, 2015). Social and emotional dysfunction after TBI are highly detrimental to daily life and societal re-entry (Morton & Wehman, 1995; Jacoba M Spikman, Marieke E Timmerman, Maarten V Milders, Wencke S Veenstra, & Joukje van der Naalt, 2012). Disruption of social and emotional behaviors is common after TBI (May et al., 2017; McCarthy et al., 2006). Furthermore, poor social and emotional health is related to poor functional outcome after TBI, including the inability to return to work (Struchen et al., 2008). Specifically, TBI patients can exhibit deficits in interpersonal relationships (J. L. Ponsford et al., 2014; Pugh et al., 2018) perceived social support (McCarthy et al., 2006), and emotion recognition (May et al., 2017). It is well established that healthy social behavior is correlated with overall mental health (reviewed in (Kawachi & Berkman, 2001)), and psychiatric diagnosis after TBI is correlated with poorer social and emotional health (Draper, Ponsford, & Schonberger, 2007). Congruently, TBI patients are diagnosed with psychiatric disorders at a higher rate than the general population, (reviewed in (J. Ponsford, Alway, & Gould, 2018)). These diagnoses are most often anxiety and mood disorders (Alway, Gould, Johnston, McKenzie, & Ponsford, 2016), and psychiatric symptoms can persist years after injury (Alway et al., 2016; J. L. Ponsford et al., 2014).

Despite the important role social and emotional factors play in the psychiatric health of TBI patients, little is understood about how TBI disrupts the cognitive integration

of social and emotional behaviors. Given the critical role social influences serve in overall mental health, disruption of social processing by TBI may contribute toward long-term psychiatric symptoms after TBI. Identifying the neural substrates of social processing and its deficit after TBI is critical for improving our understanding of the relationship between social processing and psychiatric disorders, as well as treatment of TBI-induced psychiatric disorders.

Preclinical modeling of social processes allows systematic investigation of the neural circuitry of social behaviors; however, it is challenging to isolate social deficit in an animal model of TBI. Often, TBI induces a variety of motor, cognitive, and emotion-like deficits which are difficult to correlate with specific injury physiology. In the present study we examined the effect of a selective mild blast TBI (mbTBI) on social safety learning, as measured by the SoFiA paradigm for rats. SoFiA requires the coordination of emotion-like (anxiety) and social signals, and therefore is a measure of social behavior.

Chapter objectives

In this chapter, an established model of mbTBI is used to explore the neural underpinnings of SoFiA. This model was found to elicit selective SoFiA deficit, allowing systematic investigation of the neural structures potentially responsible for SoFiA. Based on prior studies of SoFiA, we hypothesized that the acquired SoFiA deficit resulted from mbTBI-induced damage to either IL or BLA.

Methods

Note on Authorship

The data presented in this chapter reflect the effort of multiple people and will be published as a multi-author manuscript, however for clarity and consistency the entire project is presented in this thesis. The author of this thesis, Katharine Andrews, wrote the final text of the manuscript, performed all of the drug studies, and analyzed and interpreted all data except where noted (urinalysis and resting state network analysis). Specific contributions of the co-authors will be noted where appropriate. The following individuals were all responsible, in some capacity, for performance, analysis, and/or interpretation of data included in this chapter: Nicholas S. Race, Elizabeth A. Lungwitz, Sasha M. Vega Alvarez, Timothy R. Warner, Glen Acosta, Jiayue Cao, Kun-han Lu, Zhongming Liu, Amy D. Dietrich, Sreeparna Majumdar, Anantha Shekhar, William Truitt, and Riyi Shi.

Animals

Male 350-450g Sprague-Dawley rats (Envigo/Harlan Laboratories, Indianapolis, IN) were used in all experiments. Female rats were excluded from study due to the need for 6+ consecutive behavioral testing days to measure SoFiA. During this many consecutive days, females will have at least one day of proestrous and sexual receptivity, which is known to alter female social behavior towards other female conspecifics and confounds measures of anxiety-like behavior (Koss, Gehlert, & Shekhar, 2004). Rats were individually housed on a 12-hour light/dark cycle with *ad libitum* food and water. Rats were handled multiple times before behavioral testing. Procedures were conducted using

protocols approved by the Purdue University IACUC (Protocol #1111000280) or Indiana University School of Medicine IACUC (Protocol #11113).

Blast Exposure

Blast exposures (performed by N.R.) were performed as described previously (Walls et al., 2015). Briefly, animals were anesthetized with a ketamine/xylazine cocktail (80mg/kg and 10mg/kg, respectively) and secured in an open-ended shock tube blast apparatus with body protection and head fixation. The blast shock wave was generated by using compressed nitrogen to burst a Mylar membrane resulting in a blast overpressure magnitude of 150 kPa (side-on) with a 1.5 ms overpressure duration, striking the animal's head in a dorsoventral orientation. Sham rats received identical treatment including anesthesia, head fixation, and exposure to blast noise, but not the injurious shock wave.

Experiment 1. Behavior outcomes following mbTBI

Urine Collection and Analysis

Urine collection was conducted in rats for 2 days prior to mbTBI or sham exposure and daily on post-exposure days 1-4. Collection sessions were 4 hours in a free-roaming metabolic cage with water *ad libitum*. Urine 3-hydroxypropylmercapturic acid (3-HPMA) was quantified (performed by N.R., S.A., and G.A.) as in prior publications (C.-H. Chen et al., 2013; Eckert, Drexler, & Goen, 2010). Briefly, solid phase extraction prepared urine for elution and subsequent liquid chromatography with tandem mass spectrometry (LC/MS/MS). 3-HPMA levels were normalized to urine creatinine levels (performed by

N.R.) to account for variable urine water content (Yan, Byrd, Brown, & Borgerding, 2010; Zheng et al., 2013).

Open Field (OF) Test

Seven days following mbTBI or sham exposure, Open Field (OF) testing (performed by E.L.) was conducted to evaluate gross motor and non-social anxiety-like behaviors. Rats were placed in a black Plexiglas open top box with Length x Width x Height dimensions 91.44cm x 91.44cm x 30.48cm, for 5 min under dim red lighting. Video was recorded from above and analyzed with ANY-maze software (Stoelting Co. Wood Dale, IL). Time spent in each zone of the apparatus (outer, middle, and center), total distance traveled, and maximum and average speeds were quantified.

Rotarod

The rotarod test (performed by N.R., E.L., and S.A.) for motor coordination and activity was conducted following OF testing on day 7 after sham/blast exposure. Rats were placed on a wheel rotating at speed increasing from 3-30RPM over 3 min. After training (3 consecutive 60+ sec runs), the test was performed 3 times per rat. Session end criteria were the rat falling or remaining stationary for one complete wheel revolution.

Novel Object Recognition (NOR) Test

Novel Object Recognition (NOR) Test (performed by E.L.) was performed 7 days following sham/blast exposure. Rats were placed in an OF apparatus for 5 min with two identical objects secured to the floor. The rat was returned to its home cage for 10 min,

followed by a second 5 min test in the OF apparatus with one familiar object from the previous test and one novel object. The amount of time a rat spent interacting with each object in the second test was measured via ANY-maze software.

Social Interaction (SI) Test

SI testing (performed by E.L.), a validated test for anxiety-like behavior (File, 1984), was performed as described previously (Lungwitz et al., 2014). Briefly, rats were taken in their home cages to a dimly red-lit staging area outside the behavior room for 30 min prior to testing. The experimental rat and an age/weight/sex-matched conspecific partner were simultaneously placed into the OF apparatus for 5 min. Tests were video-recorded from above and scored by an observer blinded to treatment using ODlog (Macropod Software). The amount of time the experimental rat initiated non-aggressive physical contact or investigation became the SI time. Partner-initiated contact was not scored; partner SI and anxiety state do not affect experimental rat SI time (Lungwitz et al., 2014; Truitt et al., 2007).

Bright Light Challenge (BLC)

The Bright Light Challenge (BLC) procedure (performed by E.L.) has been previously described in detail (Lungwitz et al., 2014). With animals in the OF apparatus, the BLC was initiated by abruptly switching from dim red lighting to bright white lighting, which remained on throughout the 5 min testing session.

SI-Habituation (SI-Hab) Training to Measure Social Familiarity-induced Anxiolysis (SoFiA)

24 (cohort 1) or 48 (cohort 2) hours after baseline SI testing, SoFiA acquisition was measured through the SI-habituation training (SI-hab) paradigm (performed by E.L., T.W., N.R., and S.A.) as described previously (Lungwitz et al., 2014). Briefly, SI tests were performed under BLC conditions while pairing the experimental rat with the same conspecific for 6 consecutive daily SI sessions.

Two-Zone Social Recognition (SR) Test

The 2 min two-zone social recognition (SR) test was conducted (performed by E.L.) in a bi-partitioned OF apparatus. Two inserts (horizontal bars) were placed inside, enabling containment of conspecifics in opposing corners with an experimental rat freely moving in the center. One novel and one familiar conspecific were assigned to the corner enclosures in a counterbalanced fashion. Testing was performed under BLC conditions after blast/sham exposure and under dim red lighting after intracranial injection. The amount of time the test rat spent in the familiar or novel conspecific zone was quantified. Zones extended from the partitions to the diagonal midline of the OF box. The familiar conspecific was the same partner used for SI-habituation training. SR testing occurred the day following the final SI-habituating training day.

Tail Suspension (TS) Test

Tail Suspension (TS) was performed (performed by N.R., E.L., and S.A.) similar to a previously described protocol (Chermat, Thierry, Mico, Steru, & Simon, 1985). TS

consists of wrapping rats' tails in protective cloth tape, then suspending the tail via duct tape from a horizontal bar 4ft above ground. Rats were suspended from the bar throughout the 5 min test. Time spent immobile was quantified by ANY-maze software.

Experiment 2. Localizing neurotrauma following mbTBI exposure: seed-based resting state fMRI

T2-weighted Magnetic Resonance Imaging

T2-weighted anatomical magnetic resonance imaging (MRI) data were acquired (performed by N.R., J.C., K.L., and Z.L.) on a 7 Tesla scanner (Bruker Biosystems) with Paravision 6.0.1 software. Probe (RF RES 300 1H 112/086 QSN TO AD, Bruker Biosystems), gradient coil (BA-GA12SHP BC 70/30), and surface coil (RF SUC 300 1H R BR QSN RO AD, Bruker Biosystems) were consistent for all acquisitions. Anesthesia was initiated with 4% isoflurane in air (SomnoSuite Low-Flow Anesthesia System, Kent Scientific). Rats were moved to the scanner and secured in a MRI-compatible stereotaxic apparatus (custom 3D-printed). Nosecone isoflurane was continuously administered (0.1 – 0.5%) alongside dexmedetomidine sedation (subcutaneous 0.03 mg/kg bolus then continuous 0.03 mg/kg/h infusion), preserving cortical networks as previously described (Lu et al., 2012; Peeters, Tindemans, De Schutter, & Van der Linden, 2001; Weber, Ramos-Cabrer, Wiedermann, van Camp, & Hoehn, 2006). Respiration rate (30-40 breaths/minute) and body temperature (36-37 ºC) were monitored and maintained via minor anesthetic and warming surface adjustments. Scanner was tuned and matched; a localizer scan was run with B0 adjustment. A rapid low-resolution T2 scan enabled whole-brain visualization and

ellipsoid mapshim incorporation. The ellipsoid manually adjusted to encompass only brain tissue while excluding skin, muscle, and skull.

High-resolution T2-weighted images were acquired after the localizer and low-resolution scan, with a field of view (FOV) of 36 mm x 18 mm x 27 mm (x = lateral, y = dorsoventral, z = rostrocaudal). The FOV was composed of 240 x 120 x 90 voxels of size 0.15 mm x 0.15 mm x 0.3 mm in interleaved coronal slices acquired dorsal to ventral with an FOV saturation pulse used on the ventral aspect of the brain. Parameters were effective echo time (TE) 11.34 ms, repetition time (TR) 9979 ms, flip angle (FA) 90º, RARE factor eight, six averages, and one repetition. Fat suppression was enabled.

Resting-State Functional Magnetic Resonance Imaging

Resting-State functional magnetic resonance imaging (rs-fMRI) was performed (performed by N.R., J.C., K.L., and Z.L.) on rats at pre-blast, 24 hours post-injury, and 1-week post-injury. Equipment and anesthesia were consistent with procedures above. After anatomical scan acquisitions above, 6 sequential rs-fMRI scans were performed on each animal at each time point. Each acquisition consisted of a 600 repetition 2-D single-shot gradient echoplanar imaging sequence (Repetition time = 1 s, Echo time = 15 ms, Flip angle = 55º, slice thickness 1mm, in-plane resolution 0.5x0.5 mm^2).

After removal of the first 10 volumes, structural images were non-linearly registered first to each individual animal's T2 structural images, then to the Waxholm Space Atlas anatomical template (Papp, Leergaard, Calabrese, Johnson, & Bjaalie, 2014) with segmentation into cortical surfaces according to WSH atlas parcellations, consistent with prior reports (Glasser et al., 2013). Also in accordance with published methods, the

fMRI images underwent slice-time correction (*slicetimer*), motion correction (*3dvolreg* for inter-volume motion, *retroicor* for respiratory/cardiac activity), and echoplanar imaging distortion correction with normalization to Waxholm Space Atlas space and co-registration across subjects (*flirt* and *fnirt*) using Analysis of Functional Neuroimages software and custom Matlab scripts (Glasser et al., 2013). Detrending was performed by regressing out a 2nd-order polynomial function and bandpass filtering according to heart and respiration rate recordings. We also subtracted the mean, standardized signal variance, and performed spatial smoothing with a 3-D Gaussian kernel (0.5mm full width at half maximum) to minimize spurious correlations between neighboring voxels. Population-averaged seed-based correlation analysis was performed with custom Matlab scripts using the WHS parcellation corresponding to the vmPFC as the seed.

Experiment 3. Contributions of glutamatergic signaling in OFC to mbTBI-induced social processing impairment

Taqman® Low Density Array Gene Expression Analysis

15 days after blast/sham exposure, rats were sacrificed (performed by N.R.), and brains processed for RT-PCR as previously described [(performed by K.A. and A.D.) (Truitt et al., 2015)]. Briefly, OFC were dissected from frontal cortex sections (300 μm thick) and placed in lysis buffer. RNA was extracted from punches and converted to cDNA. cDNA was transferred into Taqman® Low Density Array (TLDA) microarrays consisting of 96 primers for endogenous control genes and genes related to GABA and Glutamate receptors (performed by K.A. and A.D.). Expression of all GABA and glutamate genes was normalized to endogenous control genes (analyzed by W.T.).

Stereotaxic Surgery and Microinjections

Isoflurane-anesthetized rats were implanted (performed by K.A.) with bilateral guide cannulae (Plastics One) at +3.2mm anteroposterior, ±2 mm mediolateral, and -4.8 mm dorsoventral to bregma (Paxinos & Watson, 2004), then fitted with dummy cannulae and protective cap and given at least 4 days to recover during which buprenorphine (0.03 mg/kg) was administered subcutaneously every 12 hours for a total of 4 injections for pain management. At the time of intracranial injection, the protective cap and dummy cannulae were removed and a bilateral injector cannula was inserted, extending 1mm beyond the guide cannula. Injectors were connected via PE20 tubing to 10 µL syringes and administered (performed by K.A.) 50 µM *(S)*-3,5-Dihydroxyphenylglycine (DHPG) (Tocris) or 0.9% saline vehicle of a total volume of 0.5 µL per side at a rate of 0.25 µL/min. Injectors were held in place for 1 min following injection to prevent drug backflow. Then, caps and dummy cannulae were replaced and the rat returned to home cage. At 30 minutes following microinjection with DHPG, rats underwent behavior testing. Microinjections were given daily prior to SI-hab training (performed by K.A. and E.L.).

Injection Site Confirmation

Rats were anesthetized with isoflurane then injected (performed by E.L.) via guide cannulae with 0.5 µL per side of diluted Normal Donkey Serum (Abcam) in 0.9% saline at 0.25 µL/min, followed by transcardial perfusion (performed by K.A.). Perfused brains were stained (performed by K.A.) with 1:500 goat anti-donkey secondary antibody (Fisher) to help qualitatively visualize area of injections. Injection sites were verified under light microscope.

Statistics

All data were analyzed as described in the main text below using one-way or repeated measures ANOVA and two-sample unpaired t-tests as appropriate in Prism 6.0 Software (La Jolla, CA); all data are presented as mean±SEM. To evaluate the statistical significance of changes in rs-fMRI correlations, the Fisher's r-to-z transform was applied to all images followed by a voxel-wise paired t-test. All significance levels were set at $p < 0.05$.

Results

Experiment 1: Behavioral outcomes following mbTBI

Experiment 1.1. Blast exposure resulted in mbTBI

The protocol and timeline used for Experiment 1 is summarized in Figure 2.1a. Two days prior and four days following the sham or blast exposure, urine was collected from both groups of rats and analyzed for 3-HPMA, a stable glutathione-reduced metabolite of acrolein, which is a known marker of oxidative stress and neurotrauma (Abdul-Muneer et al., 2013; Cho, Sajja, Vandevord, & Lee, 2013; Readnower et al., 2010; Walls et al., 2015). Blast exposure significantly increased urine 3-HPMA levels (Two-way repeated measures ANOVA, exposure main effect $F_{5,55}$=15.54, P=0.0023 & exposure X day interaction $F_{5,55}$=2.65, P=0.0322), compared to pre-blast levels on post exposure day 1 and compared to levels in sham rats on days 1-3 (Dunnett's, p=0.0094 and Fisher's LSD (Least Significant Difference) p≤0.0132, respectively; Figure 2.1b). One week following exposure, blast rats displayed no motor deficits compared to sham rats in rotarod or open field (OF) tests (Figure 2.1c, 2.1d). In addition to a lack of obvious motor impairment, blast rats also did not display any changes in anxiety-like behavior compared to sham rats in OF test as measured by time spent in outer, middle, or center zones (Figure 2.1e). Cohort 2 rats displayed equivalent motor and anxiety-like behaviors in OF as Cohort 1 (Figure 2.2a, 2.2b). In the novel object recognition test, Cohort 2 rats demonstrated an increase in time spent with the novel object (2-way ANOVA, main effect of object $F_{1,32}$ = 8.117, P=0.0076, Figure 2.1f). Blast rats, but not sham, spent significantly more time with the novel object than the familiar object, suggesting memory was intact following blast exposure

(Bonferroni's, p=0.0082). Collectively these data suggest blast exposure induced a mild blast TBI (mbTBI).

Experiment 1.2. mbTBI resulted in selective deficits in social processing

Starting at 9 days following sham or blast exposure, anxiety-like behavior was assessed via social interaction (SI) test and social processing was assessed via SI habituation training (SI-hab). Sham and blast rats had equal anxiety-like responses at baseline and to the initial anxiogenic challenge (SI-hab day 1), but their anxiety-like behavioral response to social familiarity differed across SI-hab (Two-way repeated measures ANOVA exposure X day interaction $F_{6,66}$=5.281, P=0.0002) (Figure 2.3a). Specifically, all rats had similar SI times at baseline which were significantly reduced in response to the BLC on SI-hab day 1, compared to baseline (Tukey's, p≤0.031). SI time reduction was unaffected by social familiarity in blast rats as SI times remained significantly lower than baseline across all SI-hab days (Tukey's, p≤0.0059). Contrarily, SI time in sham rats increased with social familiarity, where SI times on SI-hab days 4-6 were no longer reduced compared to their baseline and were significantly increased compared to SI-hab day 1, (Dunnett's, p≤0.0051) and compared to blast rats (Bonferroni's, p≤0.0355). Thus, sham rats acquired SoFiA and blast rats failed to acquire SoFiA. SoFiA deficit was replicated in a second cohort of blast rats (Figure 2.4). SoFiA acquisition values (calculated as difference in SI time between last and first SI-hab sessions) had a significant inverse correlation with the change in urine 3-HPMA levels 24 hours after injury; greater increases in urine 3-HPMA corresponded to lower SoFiA acquisition values (Pearson r=-0.682, p=0.0102, Figure 2.3b).

At 24 hrs following the last SI session, blast and sham rats were assessed for their ability to remember the familiar conspecific using a social recognition (SR) test (Figure 2.3c). Both sham and blast rats differentiated a familiar conspecific from a novel conspecific by spending significantly different amounts of time with the novel compared to the familiar conspecific (2-way ANOVA, exposure X zone interaction $F_{1,22}$=9.924, P=0.0046), indicative of intact social memory (Figure 2.3d). However, while sham rats spent more time with the novel conspecific (expected rodent behavior (Engelmann, Wotjak, & Landgraf, 1995)), blast rats demonstrated equivalently greater time spent with the familiar conspecific (Figure 2.3e). In a second cohort of rats, sham and blast rats displayed equivalent immobility time in the Tail Suspension test 24 hrs after the last SI-hab session (Figure 2.3f), further supporting selective social processing deficit following mbTBI exposure.

Experiment 2. mbTBI incited acute seed-based resting state fMRI alterations

Rats were subjected to resting state functional magnetic resonance imaging (rs-fMRI) at pre-injury, 24 hours post-injury, and 1-week post-injury time points (n=3; 6 repetitions/subject/time point). T2-weighted anatomical images collected in parallel did not demonstrate gross abnormalities on qualitative inspection (Figure 2.5). For rs-fMRI analysis, the vmPFC and amygdala were used as seed regions due to their known involvement in SoFiA (Lungwitz et al., 2014; Truitt et al., 2007) and their lack of blast-induced oxidative stress (Garcia-Gonzalez et al., 2018).

The network containing the vmPFC, amygdala, and lateral PFC remained intact (Figure 2.6a-f). Significant increases in correlated functional activity were observed within

these regions of interest after blast exposure compared to pre-injury imaging (voxel-wise paired t-test, (Figure 2.6e) t=3.94-15.96, p=0.005-4.75x10^{-8}, (Figure 2.6f) t=4.46-11.81, p=0.005-5.72x10^{-6}). Many voxel-wise increases in correlated functional connectivity were transient, returning to baseline levels at 1-week post-injury. However, region-wide trends of increased correlated functional activity observed between the amygdala and lateral PFC at 24 hours (Dunnett's, p=0.0527) and were significant (Dunnett's, p=0.0049) at 1-week post-injury (Figure 2.6d, nested 1-way ANOVA, $F_{2,6}$=12.09, P=0.0079).

Experiment 3. Contributions of glutamatergic signaling in OFC to mbTBI-induced social processing impairment

Experiment 3.1. Expression of GABA- and Glutamate-related genes in lateral PFC following mbTBI vs. sham exposure

Expression levels of GABA- and Glutamate-related genes were measured in OFC of blast vs. sham exposed rats (n=6/group) using a custom designed TaqMan Low Density Array (described in (Truitt et al., 2015)). Ten days after sham or mbTBI exposure, rats were sacrificed and tissue processed for RT-PCR, then assayed for expression of 87 GABA- and Glutamate-related genes (Table 1). Relative expression of only 2 of the 87 genes assayed were significantly different between groups (Figure 2.7). These 2 genes, Grm1 and Grm5, encode metabotropic glutamate receptors 1 and 5 (mGluR1/5). Expression of both Grm1 and Grm5 was significantly elevated in blast rats relative to sham (t-test, p=0.0298 and p=0.0403 respectively).

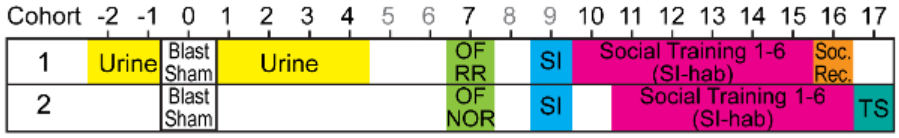
Experiment 3.2. Injection of mGluR1/5 selective agonist, DHPG, into OFC of uninjured rats partially recapitulated mbTBI-induced social processing deficit

To determine if mGluR1/5 in OFC are involved in SoFiA acquisition, exposure-naïve rats (no sham or blast) received bilateral intracranial injections of selective mGluR1/5 agonist, dihydroxyphenylglycine (DHPG) or saline vehicle (n=5/group) into OFC, 30 min prior to SI-hab days 1-5. DHPG injection attenuated SoFiA acquisition compared to vehicle injection (Two-way RM ANOVA drug X day interaction $F_{5,40}$=2.681, P=0.0351, Figure 2.8a). Here, vehicle-injected rats acquired SoFiA with SI times significantly greater than SI-hab day 1 on social training days 3-5 (Dunnett's, p≤0.0069), while DHPG-injected rats only had a transient increase in SI time compared to social training day 1 on day 4 (Dunnett's, p=0.0277). To determine if DHPG injection into the OFC also recapitulated blast-induced aberrant social recognition response, these rats underwent SR testing following injections of DHPG or vehicle into the OFC. Rats injected with DHPG demonstrated social memory deficit, while vehicle-injected rats demonstrated intact social memory (Two-way ANOVA drug X zone interaction $F_{1,16}$ = 10.37, P=0.0054) (Figure 2.8b). Vehicle-injected rats spent significantly more time near the novel conspecific (Fisher's LSD, p=0.0169) while DPHG-injected rats spent equal time near novel and familiar conspecifics, with a trend towards increased time spent with the familiar conspecific. Injection sites were confirmed post-mortem to be within OFC between anteroposterior coordinates +4.20mm and +3.00mm relative to bregma (Figure 2.8c).

Figure 2.1. Blast exposure resulted in mbTBI

(a) Experiment 1 timeline. **(b)** Blast exposure (indicated by dotted vertical line) increased levels of a marker of neurotrauma, urine 3-HPMA (Two-way repeated measures ANOVA, exposure main effect $F_{5,55}$=15.54, P=0.0023 and exposure X day interaction $F_{5,55}$=2.65, P=0.0322); blast rats urine 3-HPMA (black circles) was increased on post-injury day 1 compared to pre-injury day 1 (*Dunnett's, p=0.0094) and compared to sham rat 3-HPMA (white circles) on post-injury days 1-3 (†Fisher's LSD, p≤0.0132) Neither blast nor sham rats demonstrated motor deficits, as measured by **(c)** rotarod (*different than Trial 1, Tukey's, p=0.0120) or **(d)** open field test (distance traveled and speed, respectively). **(e)** Compared to sham rats, blast rats did not demonstrate different anxiety-like behavior under baseline conditions. **(f)** Blast rats demonstrated intact novel object recognition (NOR) (*Bonferroni's, p = 0.0082) n=6-9 sham; n=7-9 blast. Data collected and analyzed by N.R., G.A., S.A., and E.L.

(a)



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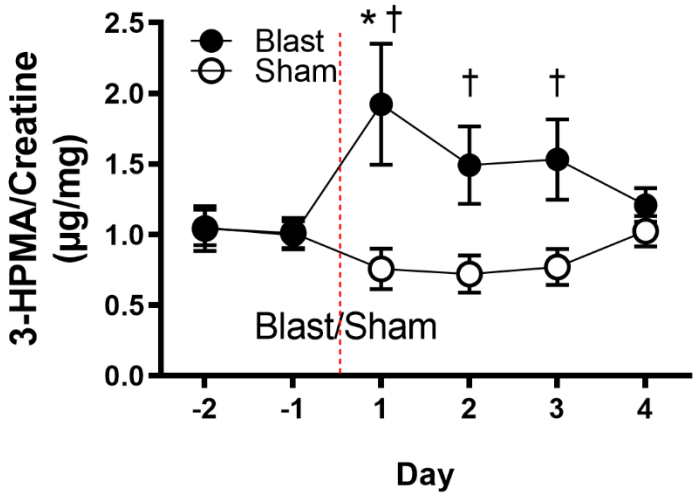
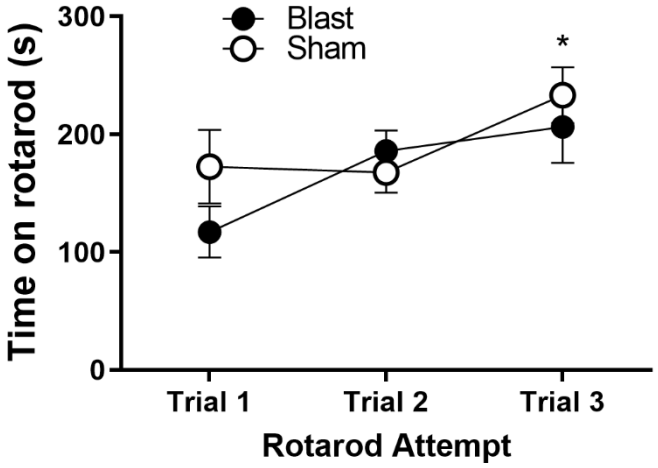
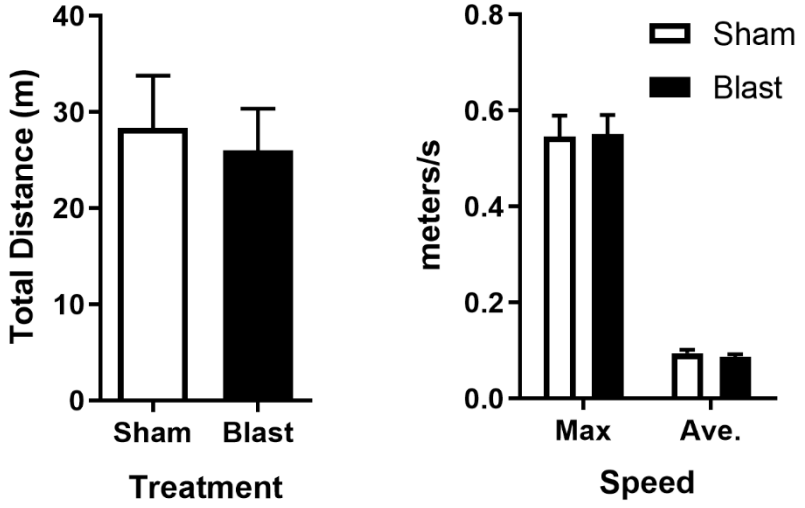


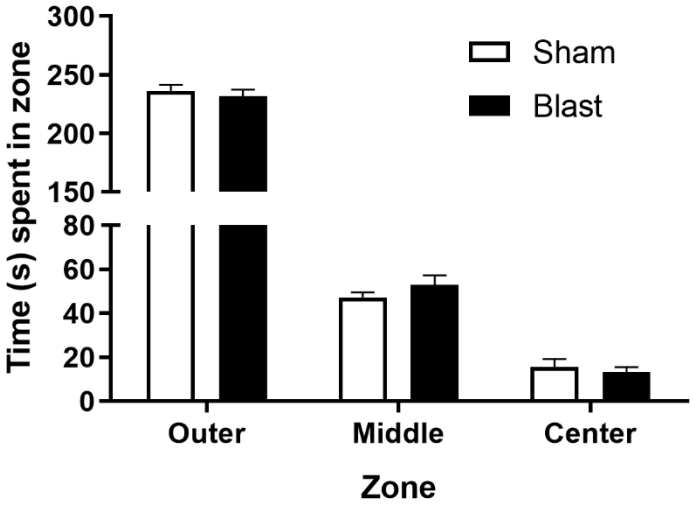
(c)


Blast
Sham
*
300
200
100
0
Time on rotarod (s)
Trial 1
Trial 2
Trial 3
Rotarod Attempt

(d)


40
30
20
10
0
Total Distance (m)
Sham
Blast
Treatment
0.8
0.6
0.4
0.2
0.0
meters/s
Sham
Blast
Max
Ave.
Speed

(e)


300
250
200
150
80
60
40
20
0
Time (s) spent in zone
Sham
Blast
Outer
Middle
Center
Zone

(f)

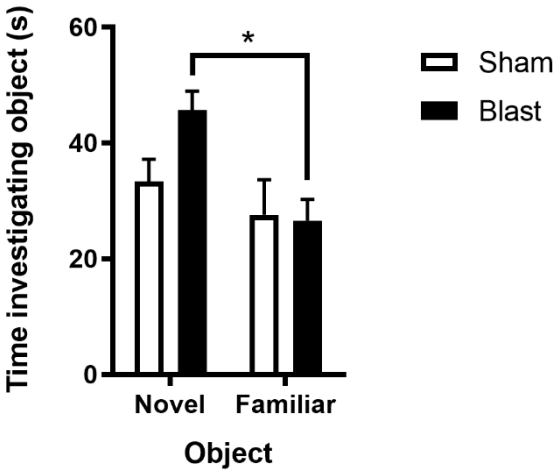
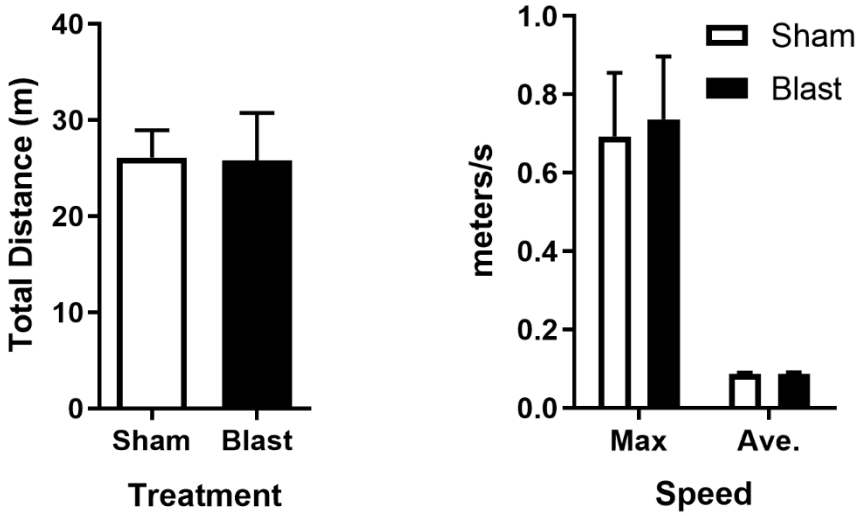

60
40
20
0
Time investigating object (s)
*
Sham
Blast
Novel
Familiar
Object

Figure 2.2. Second cohort of blast and sham rats replicated motor and anxiety-like behaviors of first cohort

A second cohort of blast and sham rats (n=9/group) demonstrated comparable **(a)** motor (distance traveled and speed, respectively) and **(b)** anxiety-like behavior in the OF test as cohort 1 rats; mbTBI exposure did not induce changes in motor or anxiety-like behavior compared to sham exposure. Data collected and analyzed by N.R. and E.L.

(a)



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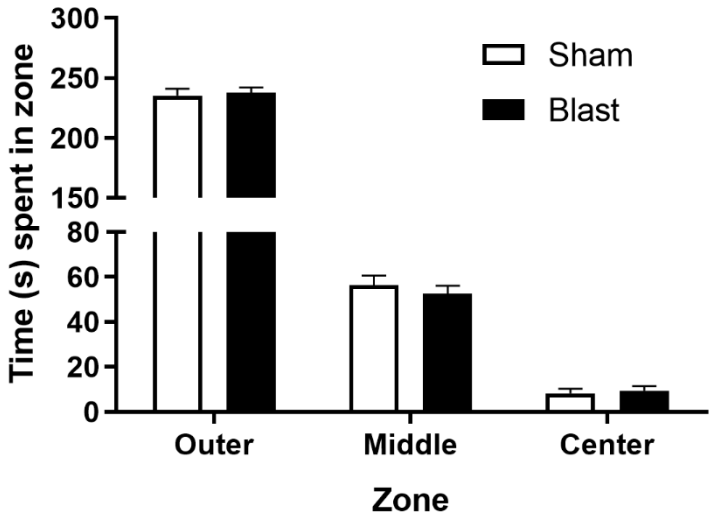
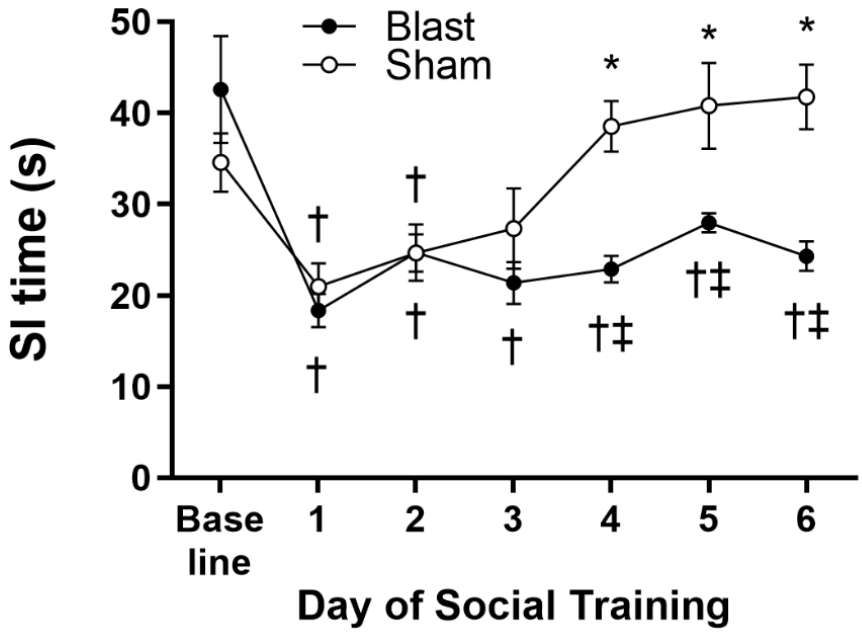


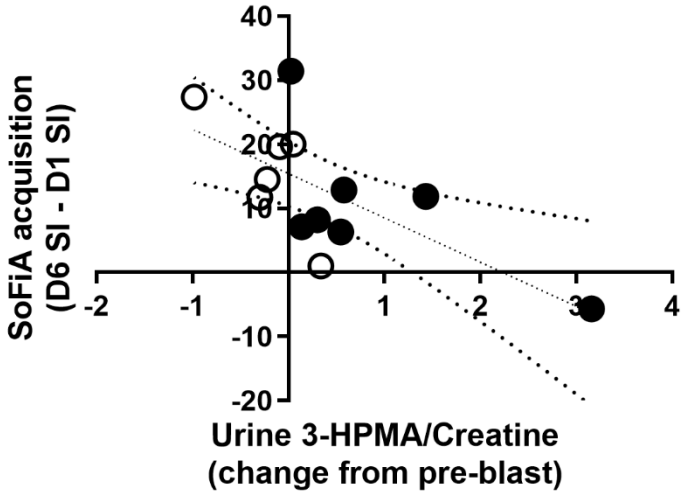
Figure 2.3. mbTBI resulted in selective deficits in social processing

(a) Blast rats (black circles) demonstrated SoFiA deficit compared to sham rats (white circles) (Two-way repeated measures ANOVA exposure X day interaction $F_{6,66}$=5.281, P=0.0002). Blast and sham rats demonstrated comparable baseline SI times which were reduced in response to bright light challenge (BLC) on SI-hab day 1 (†Tukey's, p≤0.031). However, blast rats' SI time was lower than baseline SI time across all SI-hab days (†Tukey's, p≤0.0059), while sham rats' SI time increased to levels comparable to baseline and greater than SI-hab day 1 (*Dunnett's, p≤0.0051) and blast rats SI time (‡Bonferroni's, p≤0.0355) on SI-hab days 4-6. **(b)** SoFiA acquisition value (difference in SI time between last and first SI-hab days) inversely correlated with change in urine 3-HPMA levels between pre-injury and post-injury day 1 (Pearson r=-0.682, p=0.0102) (white circles, sham; black circles, blast). **(c)** Schematic of social recognition (SR) test **(d)** In SR test, blast and sham rats distinguished a novel and familiar conspecific (2-way ANOVA, exposure X zone interaction $F_{1,22}$=9.924, P=0.0046; *Fisher's LSD, p≤0.0445). **(e)** Blast and sham rats demonstrated equivalent absolute difference in time spent between conspecifics **(f)** Blast and sham rats demonstrate equivalent time immobile in tail suspension (TS) test. n=6-9 sham; n=7-9 blast. Data collected and analyzed by E.L., T.W., N.R., S.A., and K.A.

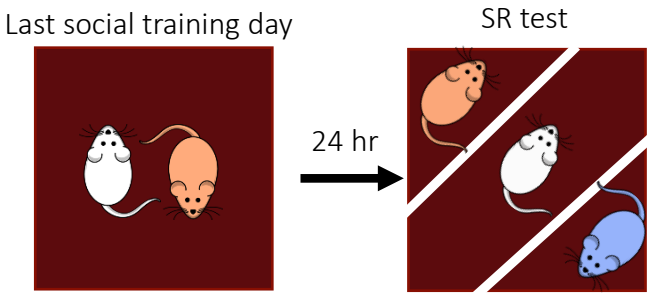
(a)



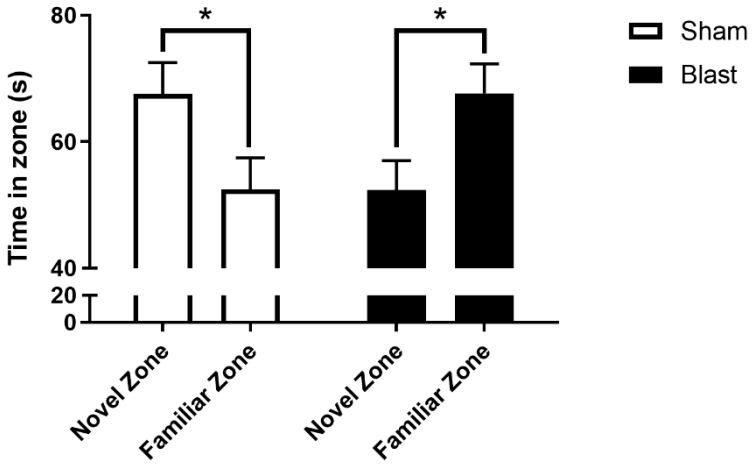
(b)


40
30
20
10
-10
-20
-2
-1
1
2
3
4
SoFiA acquisition
(D6 SI - D1 SI)
Urine 3-HPMA/Creatine
(change from pre-blast)

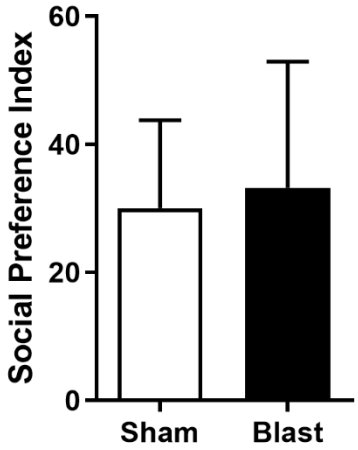
(c)


Last social training day
SR test
24 hr

(d)


80
60
40
20
0
Time in zone (s)
*
*
Sham
Blast
Novel Zone
Familiar Zone
Novel Zone
Familiar Zone

(e)


Social Preference Index
60
40
20
0
Sham
Blast

(f)

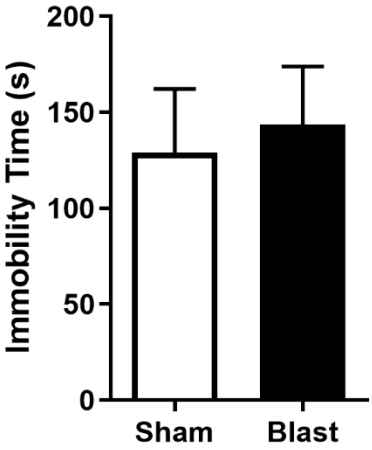

Immobility Time (s)
200
150
100
50
0
Sham
Blast

Figure 2.4. Second cohort of blast and sham rats replicated social processing deficit after mbTBI

A second cohort of blast and sham rats (n=9/group) demonstrated comparable SoFiA deficit as cohort 1 rats, replicating SoFiA deficit after mbTBI exposure but not sham exposure (2-way repeated measures ANOVA main effect of Day $F_{6,96}$=18.30, P<0.0001, and a day X group interaction $F_{6,96}$=4.625, P=0.0004). Blast rat SI time remained lower than baseline SI time across all SI-hab days, (†Tukey's, p<0.0001 for each day). Sham rats, but not blast rats, acquired SoFiA as demonstrated by an increase in SI time on days 3 – 6 compared to the first SI-hab day (‡Dunnett's, p≤0.0461). Data collected and analyzed by E.L., T.W., N.R., and S.A.

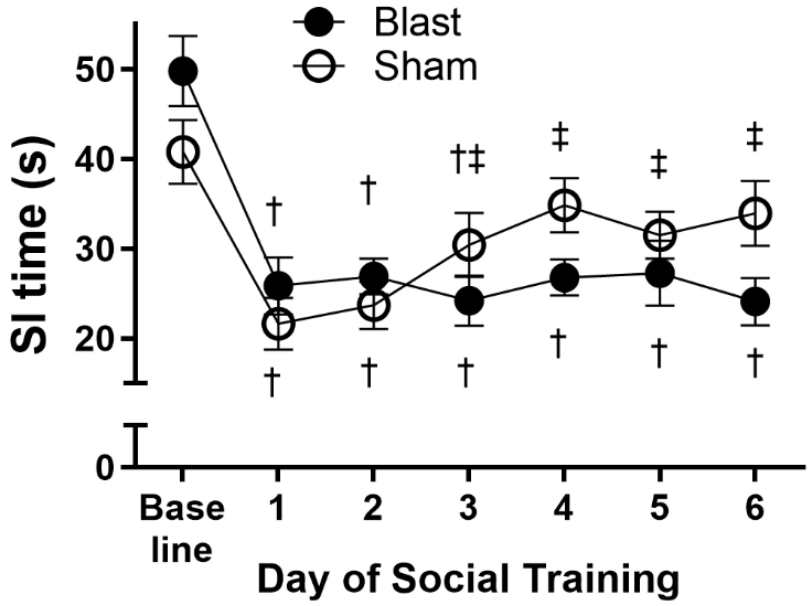


Figure 2.5. T2-weighted MRI scans do not demonstrate post-mbTBI abnormalities

T2-weighted imaging at post-injury day 1 and 1-week post-injury (n=3) did not demonstrate any obvious anatomical abnormalities or lesions on qualitative inspection compared to pre-injury images. Pictured is a transverse section of one animal illustrating major white matter tracts and the ventricular system. Data collected and analyzed by N.R., J.C., K.L., and Z.L.

Figure 2.6. Localizing neurotrauma following mbTBI: seed-based resting state fMRI

We assessed rs-fMRI connectivity using the vmPFC as the seed region at **(a)** pre-blast, **(b)** post-injury day 1, and **(c)** 1-week post-injury in the same animals (n=3; 6 repetitions/animal/time point). No major changes in gross network architecture were observed **(a-c)**. Some increased connectivity was observed between network member regions, but all regions observed at pre-blast imaging remained part of the functional network at both post-injury time points. **(d)** Increases in region-wise (aggregate of all voxels in each region) correlated functional activity (nested 1-way ANOVA, mean±SD) between the amygdala and prefrontal cortical regions including the vmPFC ($F_{2,6}$=1.241, P=0.3541), dorsomedial PFC [dmPFC ($F_{2,6}$=1.413, P=0.3142)], and lateral PFC [OFC+AIC; "LatPFC", ($F_{2,6}$=12.09, P=0.0079)] were observed at both post-injury time points. 24-hour post-injury region-wise analysis demonstrated non-significant trends of increased correlated functional activity in all regions (Dunnett's: LatPFC p=0.0527, vmPFC p=0.3138, dmPFC p=0.2725). At 1-week post-injury, lateral PFC-amygdala correlated functional activity was significantly increased (Dunnett's, p=0.0049), while the remaining tracts did not differ significantly from pre-injury levels (Dunnett's: vmPFC p=0.4024, dmPFC p=0.3911). Interestingly, at both **(e)** 24 hours post-injury (voxel-wise paired t-test, t=3.94-15.96, p=0.005-4.75x10^{-8}) and **(f)** 1-week post-injury (voxel wise paired t-test, t=4.46-11.81, p=0.005-5.72x10^{-6}), rs-fMRI correlated functional activity assessed via intra-regional, voxel-wise analysis demonstrated subregional variation with significant differences at both post-injury time points in all regions of interest. In **(e, f)**, all colored voxels have p≤0.005. Color bar at left applies to panels **(a-c)**. Data collected and analyzed by N.R., J.C., K.L. and Z.L.

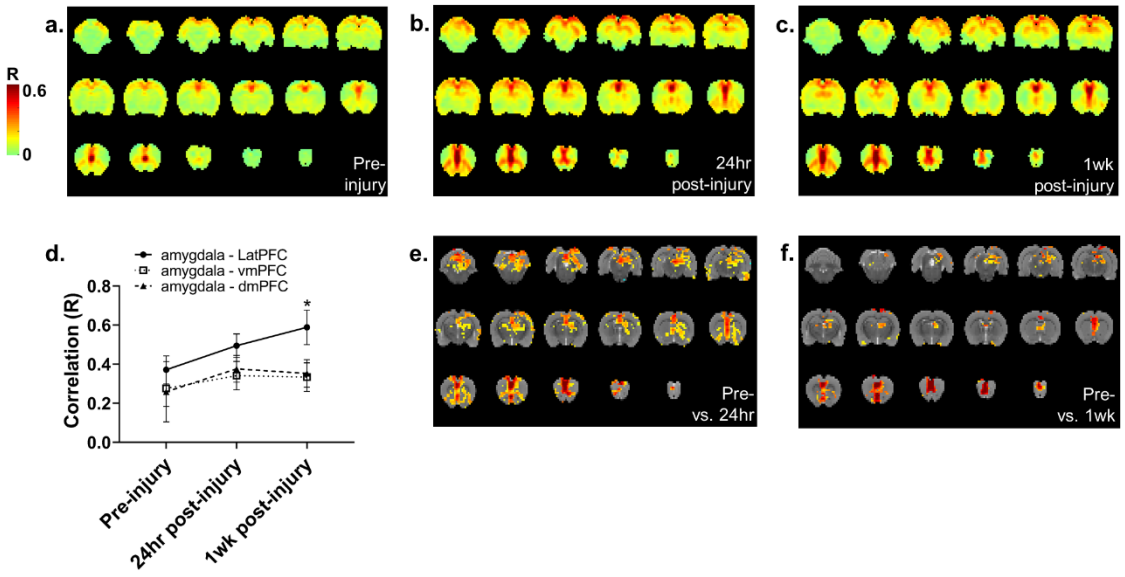


Table 1. GABA and Glutamate-related gene expression assay panel

OFC from rats exposed to mbTBI or sham was assayed for expression of 87 genes related to GABA or glutamate. 12 house-keeping genes were used as relative controls. The table presents the panel used. Data collected and analyzed by K.A., A.D., N.R., and W.T.

	Inhibitory aa related genes					Glutamate related genes							
rec. & subunits	Gabra1	Gabra2	Gabra3	Gabra4	Gabra5	Gria1	Gria2	Gria3	Gria4		Grid1	Grid2	rec. & subunits
	Gabra6	Gabrb1	Gabrb2	Gabrb3	Gabrd	Grin1	Grin2a	Grin2b	Grin2c	Grin2d	Grin3a	Grin3b	
	Gabrg1	Gabrg2	Gabrg3	Gabre	Gabrq	Grina	Grik1	Grik2	Grik3	Grik4	Grik5	Grm1	
	Gabbr1	Gabbr2	Gabrr3			Grm2	Grm3	Grm4	Grm5	Grm6	Grm7	Grm8	
	Glra1	Glra2	Glra3	Glra4	Glrb	Cacng1	Cacng2	Cacng3	Cacng4	Cacng5	Cacng6	Cacng7	receptor trafficking
rec. reg	Gabarap	Gabarapl2	Atf4	Dbi		Cacng8	Homer1	Homer2	Homer3	Grip1	Grip2	Gripap1	
Enz.	Gad1	Gad2	Abat			Slc1a1	Slc1a2	Slc1a3	Slc1a4	Slc1a6			transport
transport	Slc6a1	Slc6a11	Slc6a12	Slc6a13	Slc32a1	Slc17a6	Slc17a7	Slc17a8	Slc7a11				

Figure 2.7. Expression of GABA- and Glutamate-related genes in lateral PFC following mbTBI vs. sham exposure

Blast rat orbitofrontal cortex (OFC) demonstrated relative elevated expression of Grm 1 and Grm 5, genes encoding metabotropic glutamate receptors (mGluRs) 1 and 5, respectively, compared to sham rat OFC (t-test, p=0.0298 and p=0.0403, respectively). n=6 sham; n=6 blast. Data collected and analyzed by K.A., A.D., N.R., and W.T.

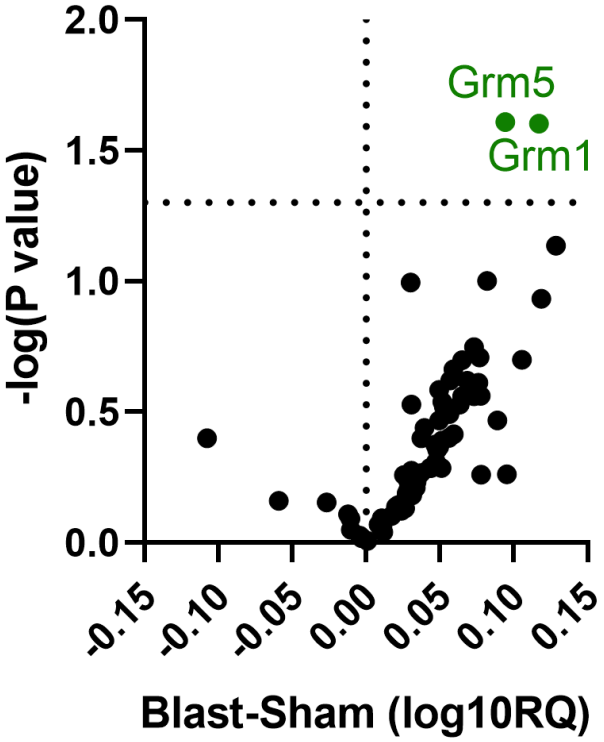
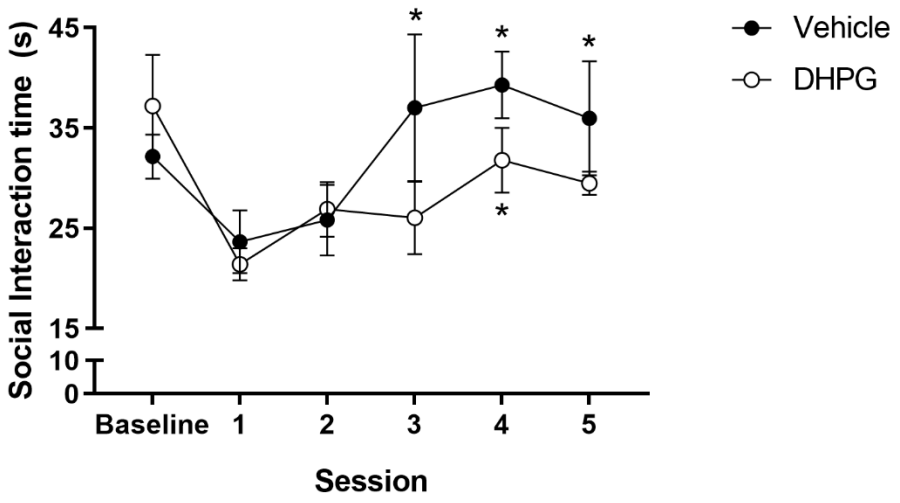


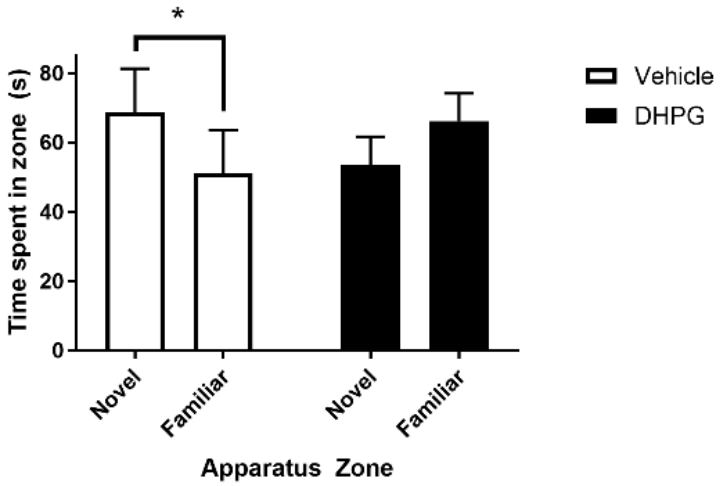
Figure 2.8. Injection of DHPG into OFC of uninjured rats partially recapitulated mbTBI-induced social processing deficit

(a) Rats injected with mGluR1/5 agonist, DHPG (black circles), into OFC, demonstrated attenuated SoFiA compared to rats injected with vehicle (white circles) (Two-way repeated measures ANOVA drug X day interaction $F_{5,40}$ = 2.681, P=0.0351). Vehicle rats had increased SI time on SI-hab sessions 3-5 compared to SI-hab session 1 (*Dunnett's, p≤0.0069). DHPG rats had only a transient increase in SI time on SI-hab session 4 compared to SI-hab session 1 (*Dunnett's, p=0.0277). **(b)** In a SR test, DHPG rats were unable to distinguish a novel and familiar conspecific, while vehicle rats demonstrated greater time spent with the novel conspecific (Fisher's LSD, p=0.0169). **(c)** Injection sites for all rats (white circles, sham; black circles, blast). Injections were located at the approximate locations shown between anteroposterior coordinates +4.20mm and +3.00mm relative to bregma. n=5 vehicle; n=5 DHPG. Data collected and analyzed by K.A. and E.L.

(a)



(b)


*
Time spent in zone (s)
0
20
40
60
80
Novel
Familiar
Novel
Familiar
Apparatus Zone
Vehicle
DHPG

(c)

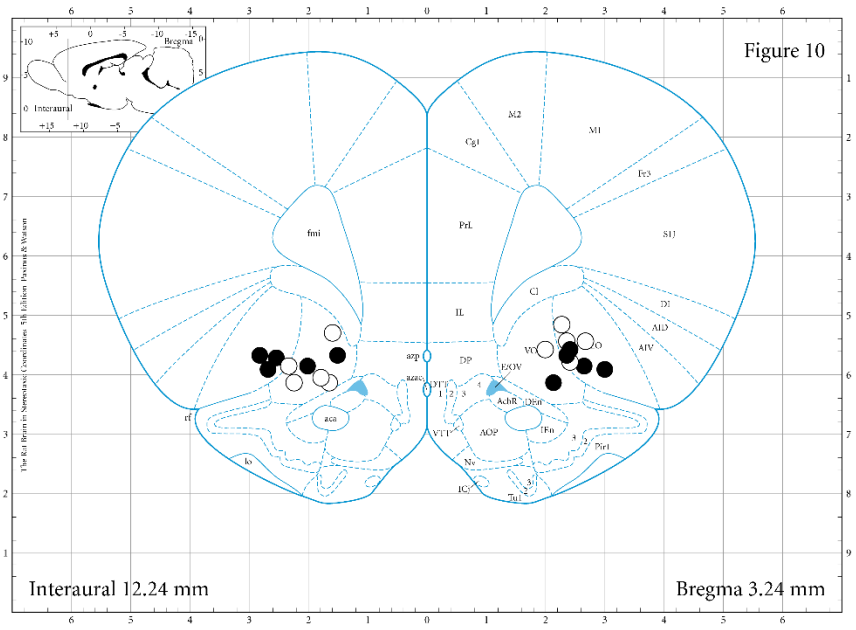


Figure 10
Interaural 12.24 mm
Bregma 3.24 mm

Discussion

Blast exposure induced mild TBI and selective social processing deficits

Blast resulted in neurotrauma, measured by transient presence of elevated 3-HPMA levels in urine. 3-HPMA is a stable metabolite of acrolein (Zheng et al., 2013), and elevation of acrolein within neural tissue is associated with neurotrauma and oxidative stress (R. Shi, Rickett, & Sun, 2011). Oxidative stress elevations in the brain are reported in rodent mbTBI (Cho et al., 2013; Du et al., 2013; Ewert et al., 2012; Readnower et al., 2010; Sajja, Hubbard, & VandeVord, 2015), and have been independently associated with numerous neuropsychiatric disorders (for review, see (Ng, Berk, Dean, & Bush, 2008)). Elevated acrolein levels in CNS following neurotrauma lead to increased 3-HPMA in urine, thus making 3-HPMA a viable biomarker for neurotrauma. In this study, blast was classified as a mild injury due to the lack of motor impairment at 7 days post-injury, and lack of gross abnormalities on structural fMRI scan, which is consistent with mbTBI in previous rodent literature (Rubovitch et al., 2011) and human imaging (*Blast Injuries: Fact Sheets for Professionals*, 2013), respectively. Importantly, 3-HPMA levels in urine at 24 hours post-injury inversely correlated with SoFiA acquisition during days 9-15 post-injury. This correlation is supported by independent evidence that intrinsic antioxidant capacity can predict neurofunctional recovery after TBI (Lin et al., 2014; Shohami, Beit-Yannai, Horowitz, & Kohen, 1997; H.-C. Wang et al., 2016). 3-HPMA may serve as an early biomarker to detect later-onset social disruption after TBI.

Blast exposure resulted in a change in social familiarity-dependent learning. Blast rats failed to acquire SoFiA, defined as the ability to reduce anxiety-like behavior via repeated presence of a familiar conspecific under anxiogenic conditions. In comparison,

sham rats acquired SoFiA comparably to rats in previous studies (Lungwitz et al., 2014; Truitt et al., 2007). Additionally, in the SR test, blast rats showed no deficit in their ability to differentiate novel and familiar conspecifics, however they did demonstrate an aberrant response by spending more time near the familiar conspecific rather than the novel conspecific, contrary to what is considered typical rodent behavior (Engelmann et al., 1995; van der Kooij & Sandi, 2012), which the sham rats displayed. These aberrant responses to social familiarity-dependent learning appear to be selective deficits in social processing, as mbTBI did not affect anxiety-like behavior in baseline or anxiogenic conditions, depression-like behavior, typical novelty seeking of an inanimate object in the novel object recognition test, nor ability to form a social memory. Collectively these results suggest that mbTBI alters the way in which social cues like familiarity are processed, rather than a global emotional or cognitive deficit.

The SoFiA model may measure the positive effect of social support on mental health (Majumdar et al., 2018). Using social cues to reduce anxiety-like behavior is a form of safety learning and is consistent with the positive role social support plays in overall mental health (for review, see (Kawachi & Berkman, 2001)). Reduced perception of social support has been reported by blast-injured veterans even when objective measures did not identify reduced social contact (Orff et al., 2016). Furthermore, social support has been reported as an independent variable impacting overall life satisfaction and recovery after TBI (Seidl et al., 2015) and is positively correlated with improved therapeutic outcomes for patients with anxiety, depression, and PTSD (Gebra Cuyún Carter et al., 2012; Halina J Dour et al., 2014; Southwick, Vythilingam, & Charney, 2005), which are common diagnoses among blast TBI patients. It is possible that subtle deficits in social processing

are associated with mbTBI, as observed in the current study, and may precede development of, or prevent recovery from, mental illness with standard treatments. In support of this, establishing the therapeutic alliance, which relies heavily on social support to be efficacious (James A Coan, Schaefer, & Davidson, 2006; Naomi I Eisenberger et al., 2011; Martin, Garske, & Davis, 2000), is challenging with TBI patients (Judd & Wilson, 2005). The important role of social processing in the rehabilitation process warrants further exploration.

Putative loci of mbTBI deficits

The vmPFC and BLA are pivotal for SoFiA acquisition and expression (Lungwitz et al., 2014; Truitt et al., 2007). Surprisingly, these structures do not demonstrate oxidative stress elevations (Garcia-Gonzalez et al., 2018), or altered connectivity following mbTBI. However, current findings suggest that the lateral PFC is a plausible neural correlate for mbTBI-induced social processing deficits. The lateral PFC, consisting of OFC and agranular insular cortex (AIC), is associated with elevated acrolein-lysine levels, bilaterally, post-blast (Garcia-Gonzalez et al., 2018). mbTBI also altered resting state functional connectivity between the lateral PFC, vmPFC, and amygdala. This resting state network was paradoxically strengthened rather than weakened after mbTBI. Strengthening of this network after blast may result from changes in glutamatergic and/or GABAergic signaling, as these are primary regulators of excitatory/inhibitory tone. Within lateral PFC, specifically OFC, GABA-related gene expression was unaltered following mbTBI, while selective increased expression of excitatory class I metabotropic glutamate receptors (mGluRs) genes Grm 1 and Grm5 occurred. Class I mGluRs contribute to TBI

pathophysiology (Lyeth, Gong, Shields, Muizelaar, & Berman, 2001), are upregulated in the presence of oxidative stress, and protect against accumulation of oxidative stress mediators (Sagara & Schubert, 1998). We hypothesize that blast exposure increases acrolein/oxidative stress within the lateral PFC, which increases expression of Grm1 and 5, driving excitatory signaling in a lateral PFC, vmPFC, and BLA network that disrupts social processing.

Supporting this hypothesis, we demonstrate that increasing activity of mGluR1/5 receptors in lateral PFC (OFC) disrupts social processing in rats. Exposure-naïve rats injected with mGluR1/5 agonist into OFC daily prior to SI-hab attenuated SoFiA acquisition, suggesting mGluR1/5 agonism in OFC is sufficient to recapitulate mbTBI-induced social processing impairment. Previous literature shows that injury to the lateral PFC results in altered social behavior in both rats and humans (Beer, John, Scabini, & Knight, 2006; Cicerone & Tanenbaum, 1997; Bryan Kolb & Nonneman, 1974; Edmund T Rolls, J Hornak, D Wade, & J McGrath, 1994; Varney & Menefee, 1993). Studies show that OFC functions to assign value to external stimuli and is therefore critical for decision-making; in addition, OFC is necessary for behavioral flexibility, particularly reversal learning and extinction learning (Bachevalier & Loveland, 2006; Cousens & Otto, 2003; Christopher J Machado & Jocelyne Bachevalier, 2006; Schoenbaum & Roesch, 2005; Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003; Schoenbaum, Setlow, & Ramus, 2003; Schoenbaum, Setlow, Saddoris, & Gallagher, 2003; Sul, Kim, Huh, Lee, & Jung, 2010; Winstanley, Theobald, Cardinal, & Robbins, 2004; Zelinski, Hong, Tyndall, Halsall, & McDonald, 2010). OFC may serve as a site of social valuation and its disruption may impair the use of social cues to learn safety. Class I mGluRs are well understood to

regulate learning and memory (for review, see (Mukherjee & Manahan-Vaughan, 2013)) and could be contributing to the learning mechanism behind SoFiA.

It is important to note that in the current study mGluR1/5 agonism is not an exact replication of the Grm 1 and Grm 5 upregulation found in blast rats, and applying the agonist did not completely mirror mbTBI deficits in social processing. Rats receiving the mGluR1/5 agonists into the OFC demonstrated an attenuation of SoFiA and impaired social memory in the SR test, while blast rats completely failed to acquire SoFiA and demonstrated an altered conspecific preference in the SR test. Additionally, other brain regions are likely to contribute to the full spectrum of mbTBI-induced social deficits. The AIC shares bilateral connectivity with OFC, vmPFC, and amygdala (A. McDonald, Mascagni, & Guo, 1996; A. J. McDonald & Jackson, 1987; Moraga-Amaro & Stehberg, 2012; Öngür & Price, 2000; C. J. Shi & Cassell, 1998a, 1998b) and is involved in social and emotional behaviors (Lamm & Singer, 2010; Mutschler et al., 2009). Previously observed bilateral acute post-injury oxidative stress elevations occur within a region containing OFC and AIC (Garcia-Gonzalez et al., 2018) and could thus impact central perception and modulation of anxiety states (AIC), emotion- or social-mediated decision making (OFC), and safety learning. Additionally, a brain region containing ventral hippocampus (bilaterally) has elevated acrolein levels after blast (Garcia-Gonzalez et al., 2018) and the ventral hippocampus is known to impact social behaviors via connections with BLA (Felix-Ortiz & Tye, 2014). Further investigations are necessary to better understand the mechanisms of mbTBI-induced social impairments.

In summary, we report the emergence of a selective social processing impairment in rats following a single mbTBI in the absence of major cognitive or affective

confounders. Deficit severity inversely correlated with urine oxidative stress measurements of 3-HPMA and was associated with functional connectivity alterations in the lateral PFC. Furthermore, mbTBI resulted in elevated expression of mGluR1/5 in OFC and selective mGluR1/5 agonist injected into OFC of exposure-naïve animals recapitulated mbTBI-induced social impairment. This mbTBI serves as a unique model to explore social processing circuitry, at the level of both brain region and neuronal substrate.

Chapter Three. Characterization of the role of OFC in social processing

Introduction

The ability to interpret and respond to social safety signals requires several intact cognitive processes working together simultaneously. One of these processes is the ability to form the memory of and recall a previously observed (now familiar) signal to facilitate learned association between the signal and safety outcome. This learned association then drives future behavioral outcomes or actions. In Chapter Two, the OFC was identified as a putative brain region contributing to the use of social safety cues to reduce anxiety in rats. In addition, driving excitatory signaling in rat OFC disrupted social recognition (SR) behavior, measured as the ability to discriminate between a novel and familiar conspecific. These findings suggest a role for OFC in social processing, which has little previous investigation in rats. Specifically, the role of OFC in social recognition (i.e., social memory), valuation and interpretation of social cues, and the ability to make social decisions may be potential pathways by which OFC contributes to the regulation of social safety learning.

The OFC

OFC homology across species

Significant controversy over the existence of OFC and for that matter, prefrontal cortex (PFC), in rodents still exists (Carlen, 2017; Laubach, Amarante, Swanson, & White, 2018; Preuss, 1995; Uylings, Groenewegen, & Kolb, 2003). Traditionally, the PFC has been defined as a collection of brain regions which have direct projections from the mediodorsal nucleus of the thalamus (MD) (Rose & Woolsey, 1948). Despite notable

differences including a lack of granular layer in rodent PFC compared to primate PFC as well as seemingly complete omission of a dorsolateral prefrontal area in rodent PFC compared to primate PFC, the basic definition of Rose and Woolsey suggests that rodent PFC does exist and shares similar connectivity as primate PFC (Carlen, 2017; Preuss, 1995; Uylings et al., 2003). Furthermore, studies of rodent and primate PFC-directed behaviors suggest "class-common behaviors", or behaviors consistent across species, even when structural homology is lacking (Carlen, 2017; Uylings et al., 2003) .

The OFC is located on the ventral surface of the frontal lobe in primates and consists of layers of granular, lightly granular, and agranular tissue (J. L. Price, 2007; Wallis, 2011). Rodent OFC is smaller and represents only approximately the caudal third of non-human primate OFC, and even less of human OFC; furthermore, the rodent OFC, like the rest of rodent PFC, is solely agranular (J. L. Price, 2007). Despite this, there is a general consensus that the similar arrangement and connectivity of OFC across humans, non-human primates, and rodents allow this region to be compared across species (J. L. Price, 2007; Wallis, 2011). An in-depth look at the homology of OFC structure, connectivity, and function across species can be found in (Wallis, 2011) and (J. L. Price, 2007).

OFC neural circuitry

In-depth studies of OFC cortico-cortical connectivity frequently examine the orbital and medial PFC as a unified (albeit heterogeneous) region termed OMPFC (Öngür & Price, 2000; J. L. Price, 2007). Both primate and rodent OMPFC can be separated into lateral (or orbital) and medial networks, which are distinguishable by anatomical location,

connectivity, and proposed function (Öngür & Price, 2000; J. L. Price, 2007; Wallis, 2011). While the lateral/orbital network receives largely sensory input and projects to multisensory areas, the medial network is an output center for visceromotor systems from hypothalamus and brainstem (Öngür & Price, 2000).

Most pertinent to this project, rodent OFC connects with hippocampus (Jay & Witter, 1991), parahippocampal regions (Kondo & Witter, 2014), limbic regions such as amygdala (Hoover & Vertes, 2011), and other prefrontal regions such as infralimbic (IL) and prelimbic (PL) cortices (Vertes, 2004).

OFC function

The OFC is a complex, multifunctional high order cortical region implicated broadly across cognitive, emotional, and social processes. One of the most established roles for OFC is encoding value as a means to guide behavioral outcomes and decision-making (for review, see (Wallis, 2011)). In this role, OFC in part guides association learning (Schoenbaum, Chiba, & Gallagher, 1998; Schoenbaum, Saddoris, & Stalnaker, 2007), including Pavlovian-type learning (Chudasama & Robbins, 2003; Takahashi et al., 2009). OFC is particularly important for reversal learning, in which a previously associated cue-reward pairing changes outcomes, and OFC lesions impair this behavioral flexibility (Chudasama & Robbins, 2003). OFC lesions also impair extinction learning of conditioned fear responses and result in overgeneralization of fear (Zelinski et al., 2010).

Therefore, it is plausible that OFC also contributes to safety learning, which is very similar to extinction learning, and direct evidence of this has recently emerged (Sarlitto, Foilb, & Christianson, 2018). Sarlitto's paper revealed that, after learning to associate

conditioned stimuli with fear versus safety, inactivation of ventrolateral OFC via the $GABA_A$ agonist, muscimol, impaired ability to discriminate between safety and fear cues during recall. The authors conclude that OFC plays an essential role in behavioral flexibility, as mentioned previously, and therefore in part guides behavioral outcomes (Sarlitto et al., 2018). Furthermore, there is evidence that OFC encodes assessment of risk and associates this with value (Jo & Jung, 2016), and OFC lesion leads to altered risky decision-making (Clark et al., 2008). The relationship between OFC-directed valuation, learning, decision-making, and behavioral outcome regulation with social processing is not well understood as these studies did not examine social paradigms.

OFC has previously been implicated in memory processes, particularly memory retrieval (Farovik et al., 2015; Milad et al., 2007). This is consistent with findings that OFC inhibition specifically impairs fear versus safety discrimination recall but not fear versus safety discrimination learning (Sarlitto et al., 2018). The role of OFC specifically in social memory is not well understood. One study measuring social recognition after OFC lesions in rodents found no effect on social recognition (Rudebeck et al., 2007).

Chapter objectives

Chapter Two identified that, when injected into the OFC, glutamatergic agonist, DHPG, destabilized SR when measured via the simultaneous presentation of two conspecifics, one novel and one familiar. However, while overactivation of OFC demonstrates that OFC is sufficient for regulating SR, it does not determine if OFC is necessary for SR. To determine if OFC plays a critical role in SR, we turned toward inactivating OFC rather than overacting it. We hypothesized that OFC was pivotal for SR

and that inactivation of OFC via the $GABA_A$ agonist muscimol would impair SR. Furthermore, due to this regulation of SR, the OFC would therefore be critical for higher order social processes such as social safety learning.

Methods

Animals

All experiments were performed using adult (300-450g) male Sprague-Dawley rats. Females were excluded from analysis for two reasons: first, all preliminary data from Chapter Two was based in male rats. Second, as in Chapter Two, female social and anxiety-like behavior can be altered by phases of proestrous and sexual receptivity (Koss et al., 2004). Rats were housed individually on a 12-hour light/dark cycle and were given food and water *ad libitum*. Rats were handled by the experimenter multiple times prior to each experiment. Rats were habituated to each testing apparatus for 5 minutes at least once prior to all behavioral experiments. All procedures were approved by the Indiana University School of Medicine Institutional Animal Care and Use Committee (IACUC) (Protocol #11113).

Surgical implantation of cannulae.

Methods used here were similar to those used in Chapter Two. Briefly, isoflurane-anesthetized rats were implanted with bilateral guide cannulae (Plastics One) at +3.2mm anteroposterior, +/- 2 mm mediolateral, and -4.8 mm dorsoventral to bregma (Paxinos & Watson, 2004), then fitted with dummy cannulae and protective cap and given at least 4 days to recover during which buprenorphine (0.03 mg/kg every 12 hours, 4 injections total) or carprofen (5 mg/kg every 24 hours, 3 injections total) was administered subcutaneously for pain management.

Intracranial Muscimol Injection

For injections, the protective caps and dummy cannulae were removed and injector cannulae were inserted. The injector cannulae protruded from the tip of the guide cannulae by 1mm. 0.9 mM muscimol (Sigma-Aldrich) was injected 10 minutes prior to each day of behavior testing at a rate of 0.1 µL/minute for a total injection volume of 0.1uL/side of brain. This dose of muscimol has been demonstrated previously to inhibit neuronal activity in prefrontal areas (Kuniishi et al., 2016; Van Kerkhof, Damsteegt, Trezza, Voorn, & Vanderschuren, 2013). All injections were followed by a 1-minute post-injection period in which the injector was held in place to ensure no back-flow of drug. Following injection, the dummy cannulae and protective caps were replaced and the animal returned to home cage. Muscimol was administered daily 10 minutes prior to the start of behavior testing.

Social Recognition (SR) Test

Two-Zone SR Test

Methods used here were similar to those used in Chapter Two. Briefly, two inserts (horizontal bars) were placed inside an OF apparatus, enabling containment of conspecifics in opposing corners with an experimental rat freely moving in the center. One novel and one familiar conspecific were assigned to the corner enclosures in a counterbalanced fashion. The familiar partner was familiarized to the experimental rat during a 10-minute familiarization session in which the experimental rat and conspecific could freely interact within a OF apparatus. The amount of time the test rat spent in the familiar or novel conspecific zone was quantified. Zones extended from the partitions to the diagonal midline of the OF box.

Three-Zone SR Test

Experimental rats received three familiarization sessions with the same conspecific for 5 min each separated by 15 min intervals. Familiarization sessions occurred in a neutral, clean housing cage. At 25 mins following the end of the final familiarization session, rats were placed in the middle chamber of a 3-room sociability chamber (Maze Engineers, Boston, MA) for 5 mins. In the 3-room sociability chamber, one room contained a novel conspecific confined within a centralized carousel cage, and the other room contained a familiar conspecific within a centralized carousel cage. Location of the novel and familiar conspecifics was counterbalanced. Time the experimental rat's head spent in each of the 3 rooms was scored.

One-Zone SR Test

Experimental rats were placed in the OF apparatus simultaneously with a novel conspecific for 5 mins, followed by a 25 min home cage rest. Then, experimental rats were again placed in the OF apparatus simultaneously with either the same conspecific as used in the previous social interaction session or a novel conspecific. Time the experimental rat spent interacting with the conspecific was manually scored for the first and second sessions. Previous literature demonstrates that rats with intact social recognition will display a decrease in social interaction time in the second session compared to the first session when given the same conspecific both sessions (Rudebeck et al., 2007).

Novel object recognition (NOR) Test

Rats were habituated to an OF apparatus three times across three consecutive days. On testing day, rats were placed in the OF apparatus for 5 min with two identical objects secured to the floor. The rat was returned to its home cage for 30 min, followed by a second 5 min test in the OF apparatus with one familiar object from the previous test and one novel object. The time the experimental rat spent interacting with each object, familiar versus novel, was manually scored for the first 2 mins of testing.

Social Preference (SP) Test

Experimental rats were placed in the bipartioned OF apparatus, as described in detail in Chapter Two Methods. Behind one set of bars was an empty area and behind the other set of bars was a novel conspecific. The rat was allowed to freely interact with either the empty area or novel conspecific for 10 minutes. Time spent in either half of the apparatus was scored via ANY-MAZE.

SI Test

Methods used here were similar to those in Chapter Two. Briefly, rats were taken in their home cages to a dimly red-lit staging area outside the behavior room at least 30 min prior to testing. The experimental rat and an age/weight/sex-matched conspecific were simultaneously placed into the OF apparatus for 5 min. The total amount of time the experimental rat initiated non-aggressive physical contact or investigation was scored. Partner-initiated contact was not scored.

BLC

Methods used here were similar to those in Chapter Two. Briefly, with animals in the OF apparatus, the BLC was initiated by abruptly switching from dim red lighting to bright white lighting, which remained on throughout the testing session.

Injection site confirmation

One cohort of rats was processed by transcardial perfusion followed by immunohistochemical analysis of normal donkey serum injected through the guide cannulae, identically to the procedure described in Chapter Two. All other rats were sacrificed and brains fresh frozen in isopentane. Brains were sliced on a cryostat at 50 μm thickness and counterstained with Neutral Red. Injection sites were localized using bright field microscopy. For a rat to be included in data analysis, the injection must have been located at least unilaterally within or bordering the OFC. Rats with significant lesions at injection site or injections located bilaterally outside OFC were excluded.

Software and Statistics

All data were analyzed as described in the main text below using one-way or RM ANOVA and two-sample unpaired t-tests as appropriate in Prism 7.0 or 8.0 Software (La Jolla, CA); all data are presented as mean±SEM. All significance levels were set at $p<0.05$.

Results

OFC inhibition attenuates SR: two-zone and three-zone tests

Rats receiving bilateral injection of $GABA_A$ agonist, muscimol, or saline vehicle, into OFC underwent a two-zone (Figure 3.1a) or three-zone Social Recognition (SR) test (Figure 3.2a). Rats were habituated to the apparatus prior to the test to reduce novelty exploration. In each SR test, experimental rats were free to spend time either in a zone with a novel conspecific or zone with a familiar conspecific (in the three-zone SR test, a third middle zone with no conspecific was also available to explore); location assignment of novel and familiar conspecifics was counterbalanced. In both two- and three-zone SR tests, rats receiving vehicle but not muscimol spent greater time in the zone with the novel conspecific than the familiar conspecific (Two-zone test: 2-way ANOVA drug x zone interaction $F_{1,8}$=7.558, P=0.0251, n=3/group; Three-zone test: 2-way ANOVA drug x zone interaction $F_{1,16}$=6.929, P=0.0181; main effect of zone $F_{1,16}$=12.21, P=0.0030, n=5/group) (Figure 3.1b, 3.2c). Specifically, in the two-zone test, rats receiving vehicle spent significantly greater time with the novel conspecific compared to the familiar conspecific (Fishers LSD, p=0.0297), while rats receiving muscimol had no significant difference in time spent between conspecifics. In the three-zone test, rats receiving vehicle spent significantly greater time with the novel conspecific compared to familiar conspecific (Sidak's, p=0.0010), whereas rats receiving muscimol spent comparable amounts of time between conspecifics. A representative heat map of the testing apparatus for a vehicle- and muscimol-treated rat each are shown in Figure 3.2b. In the three-zone test, vehicle and muscimol-treated rats did not differ in time spent in the middle zone. In the two-zone test, rats traveled comparable distances during the test, whereas in the three-zone test,

muscimol-treated rats traveled less distance than vehicle-treated rats (two-tailed t-test, p=0.0092; Figure 3.1c, 3.2d).

OFC inhibition does not impair NOR

Experimental rats receiving either bilateral muscimol or bilateral vehicle injection into OFC were measured in a test for novel object recognition (NOR) (Figure 3.3a). NOR test measures the ability to discriminate between two inanimate objects, and typical rat behavior is spending a greater amount of time investigating a novel object compared to a familiar object (Antunes & Biala, 2012). All experimental rats regardless of treatment group demonstrated greater time spent interacting with the novel object compared to the familiar object (2-way ANOVA main effect of object $F_{1,22}$=4.998, P=0.0358, n=6 vehicle, n=7 muscimol) (Figure 3.3b). Object location assignment was counterbalanced. Vehicle- and muscimol-treated rats traveled comparable distances during testing (Figure 3.3c)

OFC inhibition does not impair SP

Experimental rats receiving either bilateral muscimol or bilateral vehicle injection into OFC were measured for social preference [(SP) (Figure 3.4a)]. SP is a validated measure of sociability, or gregariousness, in rodents (Moy et al., 2004) in which rats may freely explore a zone containing a conspecific (social zone) or an empty zone (non-social zone); typical rat behavior is spending greater time in the social zone. Zone assignment was counterbalanced. All experimental rats regardless of treatment group demonstrated greater time spent in the social zone of the testing apparatus compared to the empty zone (2-way ANOVA main effect of zone $F_{1,42}$=143.1, P<0.0001, n=12 vehicle, n=13 muscimol)

(Figure 3.4b). In post-hoc analysis, both vehicle and muscimol groups demonstrated significantly greater time spent in the social zone than the non-social zone (Sidak's, $p<0.0001$ for both groups). Vehicle- and muscimol-treated rats traveled comparable distances during the test (Figure 3.4c).

OFC inhibition does not change baseline anxiety-like behavior or anxiogenic response to BLC

Experimental rats receiving either bilateral muscimol or bilateral vehicle intracranial injection into OFC were measured in a SI test under dim red light conditions for baseline anxiety-like behavior. Muscimol- and vehicle-treated rats did not differ in baseline anxiety-like behavior, as their SI times were comparable (two-tailed t-test, $p=0.7472$) (Figure 3.5a). In addition, muscimol- and vehicle-treated rats were measured in a SI test under anxiogenic BLC conditions, to measure anxiogenic response. Again, muscimol- and vehicle-treated rats did not differ in anxiogenic response to BLC, as their SI times under BLC were comparable (two-tailed t-test, $p=0.1938$) (Figure 3.5b).

OFC inhibition does not impair SR in a one-zone test

Based on previous literature, lesion of OFC does not impair social recognition in a "one-zone" version of the SR paradigm In this paradigm, rats are assigned a conspecific and allowed to freely interact for 5 min in an open arena (T1 session). Then, following a period of time, rats are returned to the arena with either the same (now familiar) or a novel conspecific and again allowed to freely interact for 5 min (T2 session) (Figure 3.6a). Previous data has shown that regardless of OFC lesion, rats during will spend less time

interacting with a familiar conspecific during the T2 session than the T1 session. Rats receiving another novel conspecific for the T2 session will spend comparable amounts of time interacting during both T1 and T2 sessions (Rudebeck et al., 2007).

To determine if bilateral injection of muscimol into OFC would produce similar results to OFC lesion, a similar protocol was performed in this study. Neither vehicle- or muscimol-treated rats demonstrated impaired social recognition as measured via the one-zone SR test (2-way RM ANOVA main effect of session $F_{1,5}$= 28.39, P=0.0031, n=3 vehicle, n=4 muscimol), suggesting OFC inhibition by muscimol and OFC lesion produce similar results in the one-zone SR test. Specifically, regardless of treatment assignment, experimental rats spent less time interacting with a familiar conspecific during the T2 session compared to the T1 session (Figure 3.6b). On post-hoc analysis, both vehicle- and muscimol-treated rats demonstrated greater SI time in the T1 session than T2 session (Sidak's, p=0.0227 and p=0.0297 for vehicle and muscimol groups, respectively). Furthermore, muscimol-injected rats spent comparable time interacting with a novel conspecific during the T2 session as with a novel conspecific during the T1 session (Figure 3.6c).

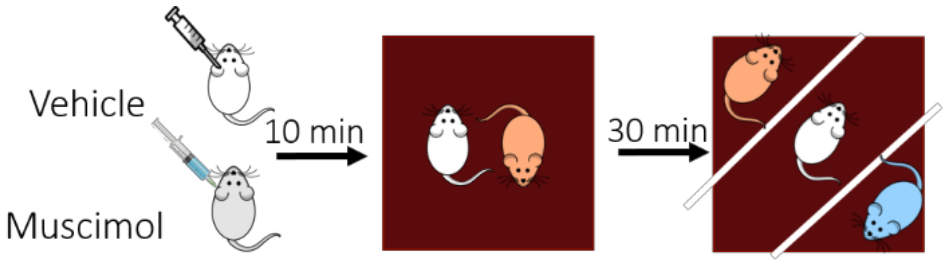
Injection Site Confirmation

All injection sites were confirmed post-mortem (Figure 3.7) to lie at least unilaterally within or bordering OFC between +2.76 mm anteroposterior and +4.68mm anteroposterior to bregma. Three rats were removed from analysis due to identification of significant unilateral or bilateral post-mortem lesion at the injection site. Five rats were removed from analysis due to identification of injection sites bilaterally outside the OFC.

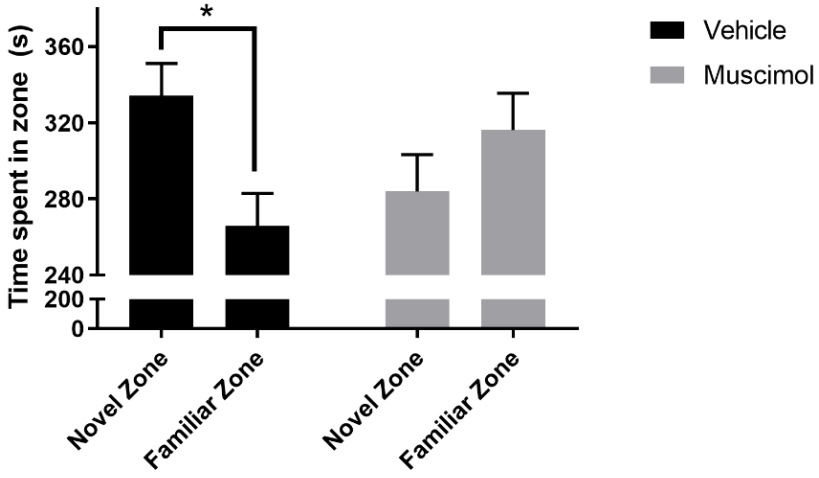
Figure 3.1. OFC inhibition impaired SR in two-zone SR test

(a) Two-zone SR test schematic. **(b)** OFC inhibition via bilateral muscimol intracranial injection impaired SR using a two-zone SR test compared to vehicle injection (2-way ANOVA drug x zone interaction $F_{1,8}$=7.558, P=0.0251). Vehicle-treated rats (black bar) spent significantly greater time in the novel conspecific zone (*Fishers LSD, p=0.0297), while muscimol-treated rats (gray bar) spent comparable amount of time in the novel and familiar conspecific zones. **(c)** Distance traveled during test per group. n=3/group.

(a)



(b)



(c)

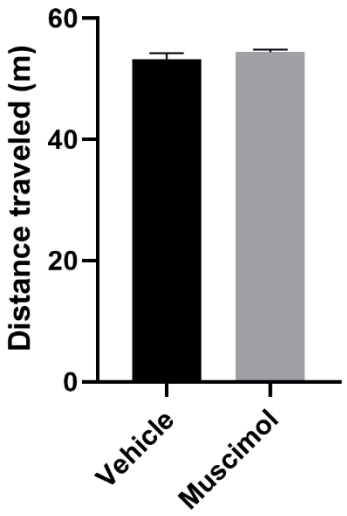
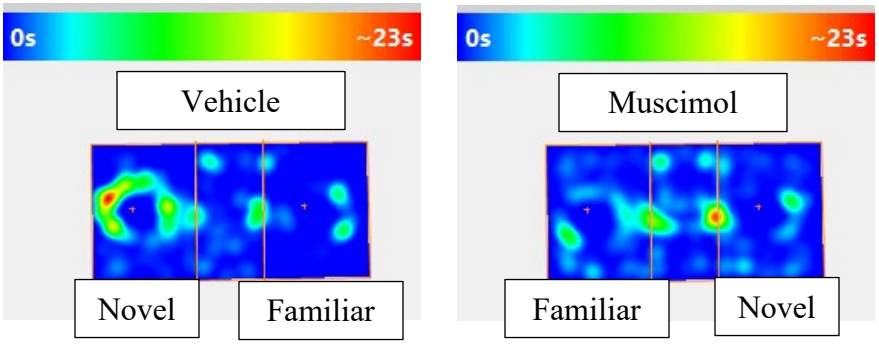


Figure 3.2. OFC inhibition impaired SR in three-zone SR test

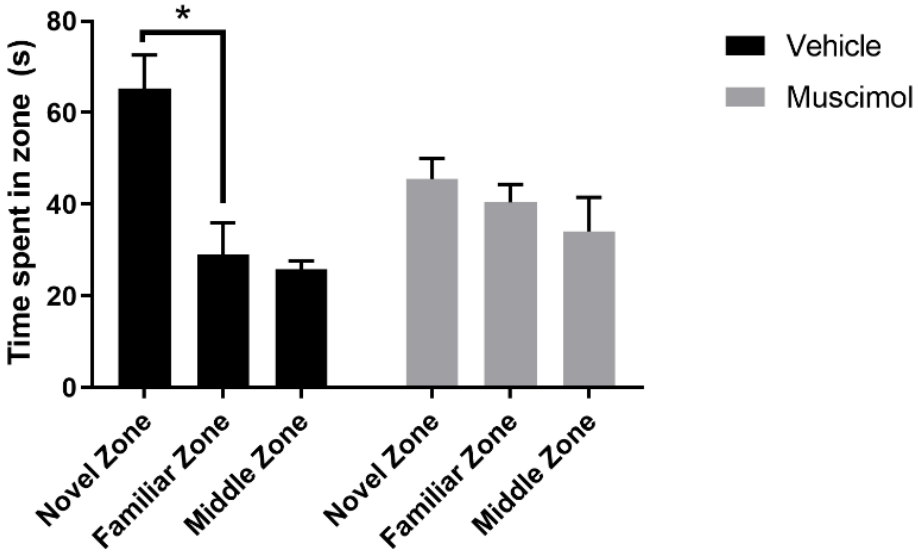
(a) Three-zone SR test schematic. White dotted lines around rats represent carousel cages enclosing conspecific partners during testing. **(b)** Representative heat maps of location of rat's head during testing; heat map shows 5 mins of testing. **(c)** Results from the two-zone SR test were replicated using a three-zone SR test. OFC inhibition via bilateral muscimol intracranial injection impaired SR compared to vehicle injection (2-way ANOVA interaction $F_{1,16}$=6.929, P=0.0181; main effect of zone $F_{1,16}$=12.21, P=0.0030). Vehicle-treated rats (black bar) spent significantly greater time in the novel conspecific zone than the familiar conspecific zone (*Sidak's, p=0.0010), while muscimol-treated rats again spent comparable amount of time in the novel and familiar conspecific zones. Vehicle- and muscimol-treated rats did not differ on time spent in the middle zone (two-tailed t-test, p=0.2674). **(d)** Distance traveled during test per group. Muscimol-treated rats traveled less distance than vehicle-treated rats (two-tailed t-test, p=0.0092). n=5/group.

(a)

(b)



(c)


Time spent in zone (s)
80
60
40
20
0
*
Novel Zone
Familiar Zone
Middle Zone
Novel Zone
Familiar Zone
Middle Zone
Vehicle
Muscimol

(d)

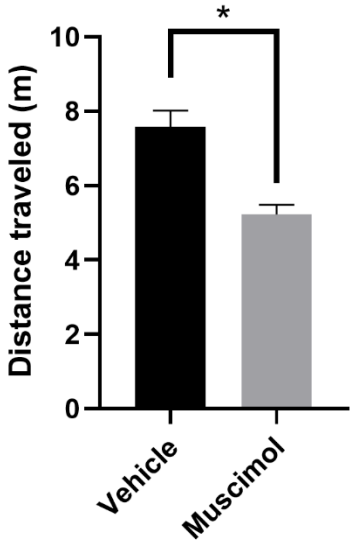
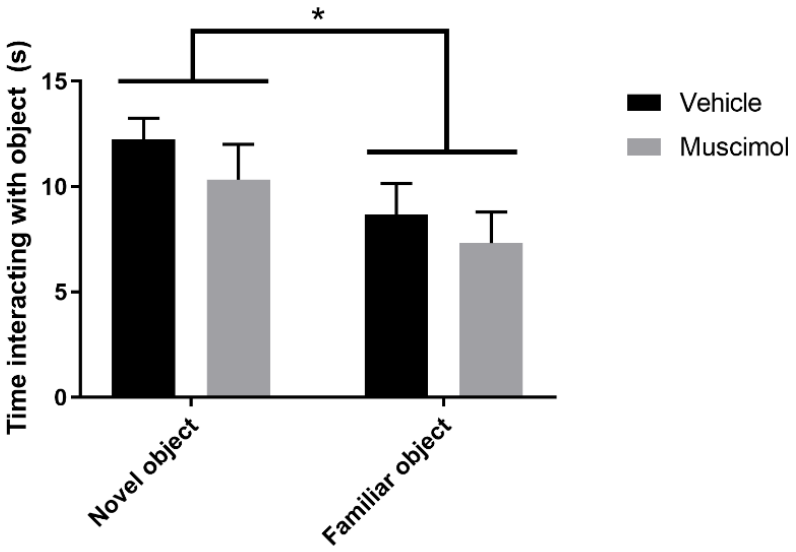

Distance traveled (m)
10
8
6
4
2
0
*
Vehicle
Muscimol

Figure 3.3. OFC inhibition did not impair NOR

(a) NOR test schematic. **(b)** Both vehicle- (black bar) and muscimol-treated (gray bar) rats spent greater time interacting with the novel object compared to the familiar object (*2-way ANOVA main effect of object $F_{1,22}$=4.998, P=0.0358). n= 6 vehicle, 7 muscimol.

(a)

(b)



(c)

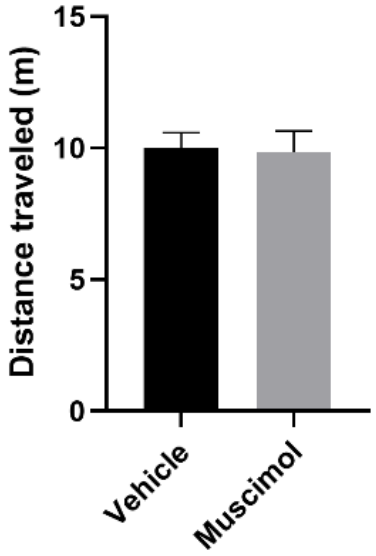
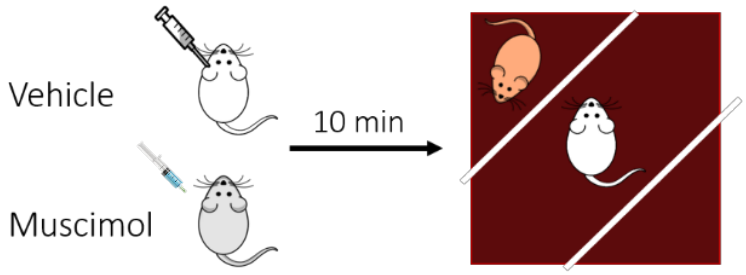


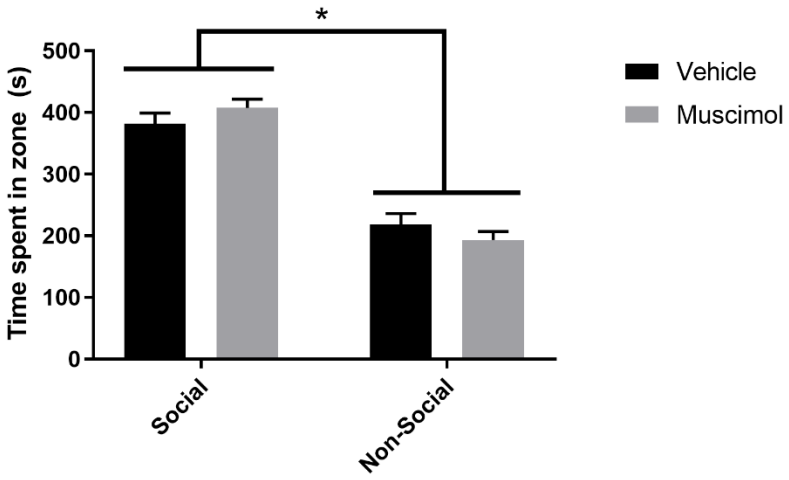
Figure 3.4. OFC inhibition did not impair SP

(a) SP test schematic. **(b)** Both vehicle- (black bar) and muscimol-treated (gray bar) rats spent greater time in the zone with a conspecific (social zone) compared to the empty zone (non-social zone) (2-way ANOVA main effect of zone $F_{1,42}$=143.1, P<0.0001). **(c)** Distance traveled during test per group. n=11 vehicle, 12 muscimol.

(a)



(b)



(c)

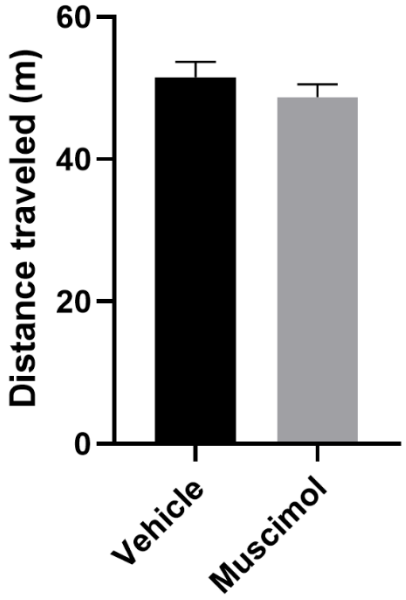


Figure 3.5. OFC inhibition did not alter baseline anxiety-like behavior or anxiogenic response to BLC

Rats receiving vehicle or muscimol intracranial injection did not differ in anxiogenic response to the BLC, measured by the SI test (2 way RM ANOVA main effect of session $F_{1,10}$=58.20, P<0.0001). n=6/group.

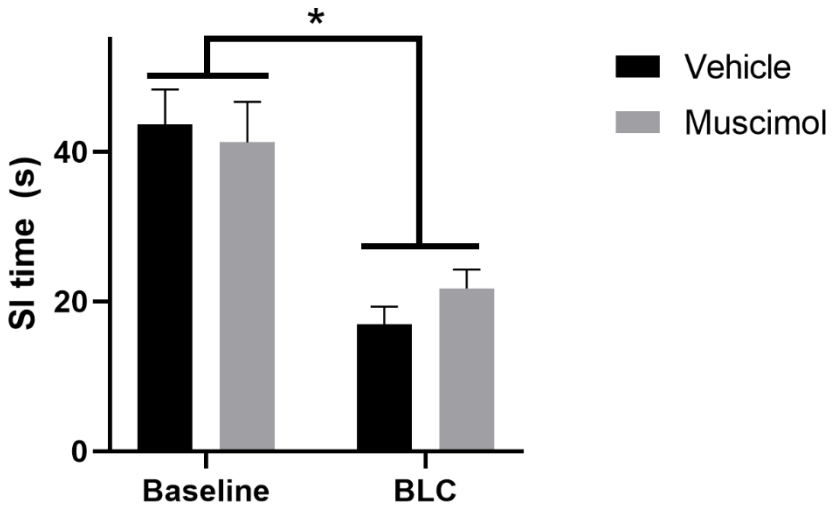
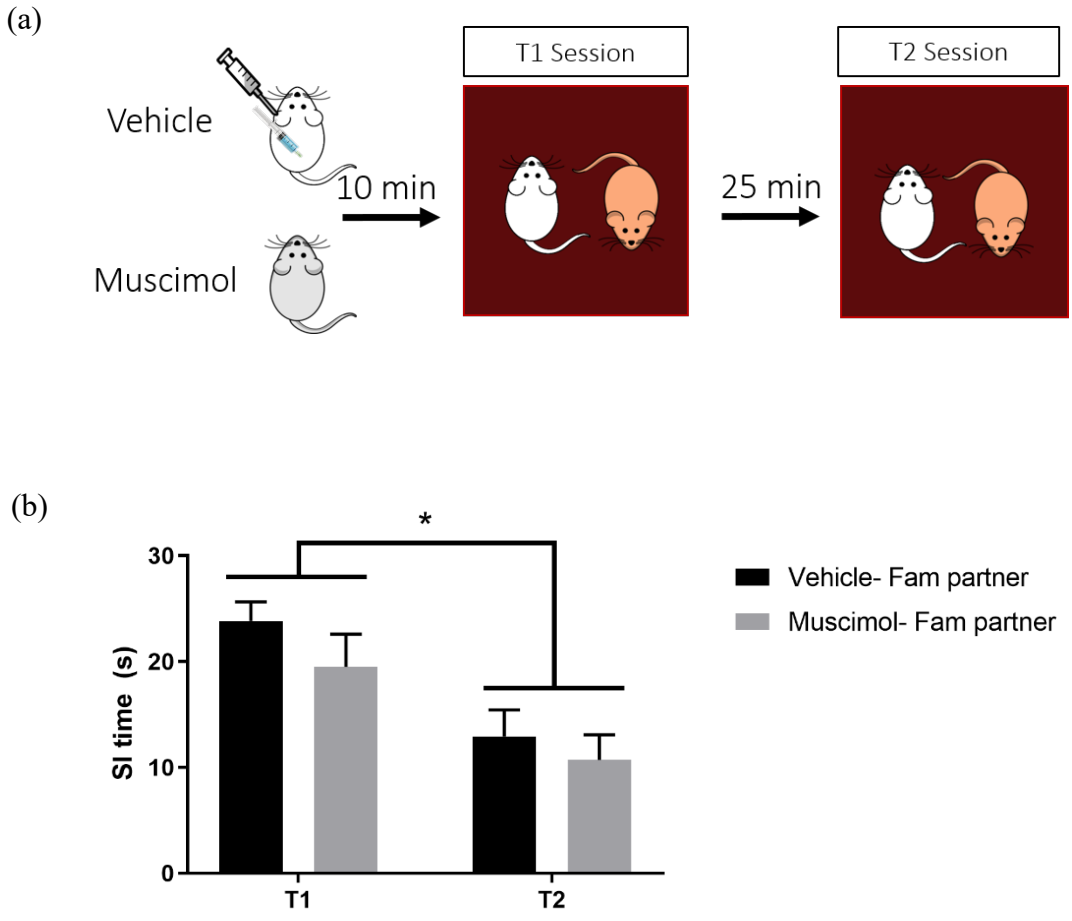


Figure 3.6. OFC inhibition did not impair SR in one-zone SR test

(a) One-zone SR test schematic. **(b)** Both vehicle- (black bar) and muscimol-treated (gray bar) rats had higher SI time during T1 session, during which they interacted with a novel conspecific, than T2 session, during which they interacted with a familiar conspecific (*2-way RM ANOVA main effect of session $F_{1,5}$= 28.39, P=0.0031). n=3 vehicle, 4 muscimol. **(c)** Muscimol-treated rats spent comparable amount of time with a novel conspecific in T1 session as a novel conspecific in T2 session but less time with a familiar conspecific in T2 session (*Fisher's LSD, p=0.0365). n=5 novel partner, 2 familiar partner.



(c)

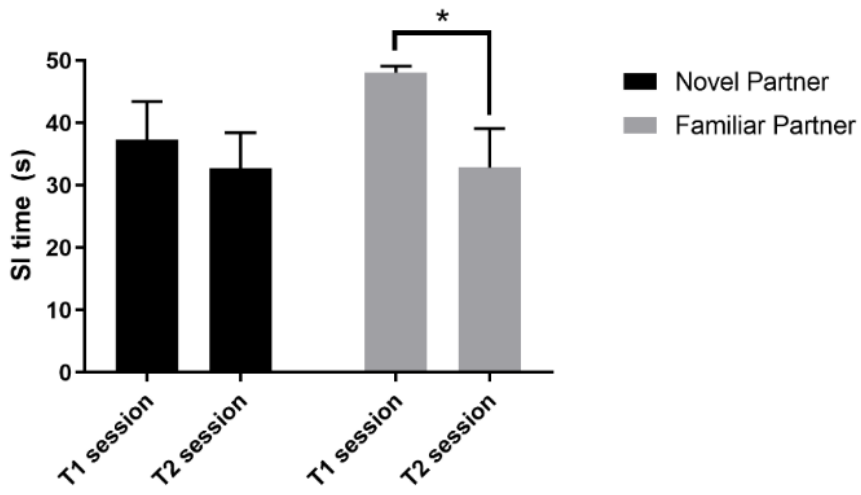
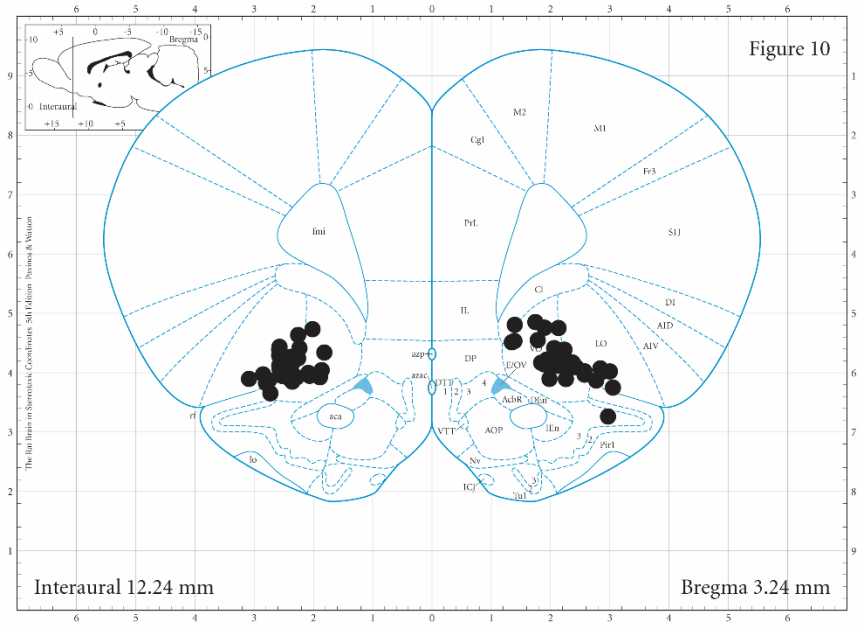

*
50
40
30
20
10
0
SI time (s)
T1 session
T2 session
T1 session
T2 session
Novel Partner
Familiar Partner

Figure 3.7. OFC injection sites for all Chapter Three experiments

Approximate location of all injections for Chapter Three experiments. Injections ranged from bregma +2.76 mm to +4.68 mm but are shown here representatively at +3.24 mm to bregma. Criteria for inclusion was at least unilateral injection within or bordering ventral or lateral OFC. Locations based on (Paxinos & Watson, 2004).



Discussion

Rats treated with intracranial injection of saline into OFC, when presented with a familiar and novel conspecific simultaneously, spent greater time in the zone with the novel conspecific compared to the familiar conspecific, which is considered typical rodent behavior (Engelmann et al., 1995). Many species, including rodents, demonstrate an innate drive to seek novelty, which is theorized to reflect an organism's need to gather environmental information and is in constant balance with neophobia, or fear of novel stimuli (for review, see (Pisula, 2009)). Rats treated with intracranial injection of the $GABA_A$ agonist, muscimol, into OFC, in order to temporarily inhibit OFC signaling, spent equivalent time in the zones with novel and familiar conspecifics. Interpretation of these data are challenging given the innate complexity of the SR task. The most direct interpretation is that inhibition of OFC impaired discrimination of a novel versus familiar conspecific, however this test alone does not provide information as to why discrimination has been impaired.

Discrimination of conspecifics may be impaired at a cognitive level. OFC inhibition may impair social memory, such that muscimol-treated rats can no longer form a memory of a familiar conspecific or recall the memory of a familiar conspecific. Alternatively, OFC inhibition may impair the ability to discern novel versus familiar conspecifics for reasons such as dysregulated olfactory recognition or impairment of some other skill necessary for the interpretation of identifying social cues. OFC inhibition may impair valuation of a novel conspecific over a familiar conspecific, or the drive to seek novelty. OFC inhibition may impair decision-making capacity or result in impulsive-like behavior, causing a chaotic pattern of investigation that resulted in apparent

indiscrimination of conspecifics. Lastly, OFC inhibition may result in anhedonia, or the lack of drive to investigate either conspecific to any meaningful degree. Alternatively, discrimination of conspecifics may be affected by changes in emotion-like state, particularly if rats were experiencing increased anxiety-like behavior due to OFC inhibition. Lastly, discrimination of conspecifics may be impaired at a more action-oriented level. Even if OFC inhibition did not affect cognitive processing of the discrimination of two conspecifics, these rats may have impaired ability to act upon this knowledge.

The multitude of possibilities in interpreting these data drove us to use other behavioral measurements to narrow down the role of OFC in social processing. First, in looking at the three-zone SR test, we observe that both vehicle- and muscimol-treated rats spent equivalent amount of time in the middle zone of the arena. This suggests that OFC inhibition is likely not leading to a state of social anhedonia, since both groups of rats were engaged in social interaction for equivalent amounts of time during the test.

Next, we observed in a NOR test that OFC inhibited rats were able to discriminate between two inanimate objects. This finding was significant in that it suggested that despite OFC inhibition, rats were able to form and recall the memory of a familiar object. In addition, OFC inhibition did not impair discernment of a novel versus familiar object and suggested that sensory systems were intact such that the rats could interact with environmental stimuli in expected ways. Furthermore, OFC inhibition did not impair decision-making ability since both vehicle and muscimol treated rats were able to spend greater time with one object over another. In addition, OFC inhibition did not impair novelty-seeking or valuation of a novel over familiar object, as both vehicle and muscimol treated rats spent greater time investigating the novel object compared to familiar object.

The NOR test does not contain a social aspect and therefore these data cannot speak to the OFC's role in social contexts. However, the negative findings in the NOR test suggest that OFC may have a unique role in social contexts. Given the extensive literature on OFC's role in valuation, reward learning, and value-based decision making in rats (for review see (Izquierdo, 2017)), it was possible that OFC inhibition was uniquely impairing the positive valuation of social experiences or the ability to make social decisions. To test this hypothesis, the SP test was used.

SP testing revealed that OFC-inhibited rats spent greater time investigating a conspecific than an empty corner of the arena. Most broadly, this suggests that OFC inhibition did not impair discrimination of social and non-social contexts. Furthermore, these data suggest OFC inhibition did not impair valuation of investigating a conspecific over a not investigating a conspecific. And, in conjunction with NOR test findings, the SP test reiterated that OFC inhibition did not impair decision making, as rats clearly made a decision to investigate one portion of the arena over another, and OFC inhibition did not seem to impair sensory processing of environmental stimuli, including social stimuli.

Impulsive behavior from OFC inhibition was ruled unlikely because in most behavioral tests examined, both vehicle- and muscimol-treated rats traveled comparable distances. The only exception is muscimol-treated rats traveled significantly less distance than vehicle-treated rats in the three-zone SR test. This is a caveat to this study and is not readily explainable by the present measures. Because no other test demonstrated difference in distance traveled between vehicle- and muscimol-treated rats, and OFC alteration by mbTBI did not result in motor deficits, it is unlikely this finding is a result of motor deficits in muscimol-treated rats. One hypothesis is that if rats have impaired ability to act upon

the discrimination of two simultaneously present conspecifics, this may result in less distance traveled overall.

Lastly, inhibition of OFC was demonstrated to not affect baseline anxiety-like behavior or anxiogenic response to the BLC. This suggested that OFC inhibition was unlikely to be leading to an altered anxiety-like status that could affect performance on a SR test (or any of the behavioral tests mentioned thus far).

Taking all of these findings into consideration, we limited our interpretations of impaired SR after OFC inhibition to one of the following: OFC inhibition impaired social memory specifically (rather than all forms of memory), OFC inhibition impaired the cognitive discrimination of two simultaneously present social cues, or OFC inhibition impaired the ability to act upon the discrimination of two conspecifics.

In a similar study by Rudebeck and colleagues, rats with complete OFC lesion were found to have intact SR behavior, however SR was measured very differently than in the presently discussed two- and three-zone SR tests. Rudebeck's SR test was performed via consecutive presentation of a single conspecific separated by an interval of time. Intact SR was present if the experimental rat spent significantly less time investigating the conspecific during the second presentation compared to the first presentation (Rudebeck et al., 2007).

In replicating the findings of this study with our present use of muscimol injection into OFC, we eliminated another possible interpretation of the data. The one-zone SR test, executed comparably to the SR test in Rudebeck's paper, revealed that OFC inhibition was not impairing social memory itself. OFC-inhibited rats still demonstrated less SI time

during the second presentation of the conspecific compared to the first presentation, comparable to vehicle-treated rats, suggesting that OFC-inhibited rats can still form and recall a social memory. This finding was not the result of interaction fatigue in the second presentation compared to the first presentation, as muscimol-treated rats demonstrated comparable time spent in the second presentation as the first presentation when a novel conspecific was given for each presentation.

In summary, the present findings accumulate to suggest OFC is important for either: discrimination of two simultaneously present social cues in a way unrelated (or unmeasured by current means) to impulsivity, gregariousness, social decision-making, social memory, anhedonia, or changes in anxiety-like behavior or, OFC is important for the ability to act upon the knowledge of the difference between these cues, which would require goal-based decision making.

Previous literature shows that rodents with OFC lesion display increased aggression and changes in dyadic social interactions, specifically the ability to adapt defense techniques in response to varying conspecifics during play fighting (Pellis et al., 2006; Rudebeck et al., 2007), but there is scant literature on the direct role of rodent OFC in social valuation or social decision-making. Human and non-human primate literature conflict on whether OFC plays a role in social valuation (Moretti, Dragone, & de Pellegrino, 2008; Noonan, Sallet, Rudebeck, Buckley, & Rushworth, 2010).

There is considerable evidence that OFC serves as a site for choosing outcomes based on real-time valuation, therefore guiding action (for review, see (Rudebeck & Murray, 2014)). In rodent OFC, populations of neurons selectively encode either reward

value or outcome, suggesting that either interpretation of the current findings is plausible (Furuyashiki, Holland, & Gallagher, 2008).

The current study reaffirms roles for OFC in interpreting social cues and serving as an important site for directing behavioral outcome, while presenting new ideas on the role of OFC in social valuation and social decision-making, particularly in the rodent. As a potential regulator of social safety learning, the present findings support OFC as a processor of complex social scenarios and their conjunction with cognitive efforts. Either failure to discriminate two social cues or the failure to act upon the discrimination of two social cues would serve as possible explanations for how OFC inhibition destabilizes social safety learning as observed in Chapter Two. Lastly, the present findings highlight the importance of interpreting SR data in conjunction with compatible behavioral tests to best interpret behavioral findings.

Chapter Four. Discussion and Conclusions

Summary of findings

Briefly, rats exposed to mbTBI demonstrated elevated levels of the neurotrauma marker acrolein in OFC, as well as resting state fMRI signaling abnormalities in lateral PFC (including OFC). Therefore, it is likely that mbTBI-exposed rats have alterations in OFC signaling, and behavioral measures of mbTBI rats may serve as measures of OFC dysfunction. This was confirmed by measurement of elevated mGluR1/5 expression in OFC of mbTBI rats. mbTBI rats demonstrated impaired social safety learning, as measured by the SoFiA paradigm, as well as increased time spent with a familiar over novel conspecific in a measure of SR behavior. When exposure-naïve rats were administered mGluR1/5 agonist into OFC, social safety learning measured via SoFiA was attenuated, similarly to after mbTBI exposure, and distinction of a novel and familiar conspecific was impaired in a measure of SR behavior.

Inactivation of OFC via intracranial injection of the $GABA_A$ agonist, muscimol, revealed a selective role for OFC in measures of SR behavior. Specifically, distinction of consecutively presented novel and familiar conspecifics in a one-zone measure of SR behavior was intact, while distinction of simultaneously present novel and familiar conspecifics in two- and three-zone SR tests was impaired. OFC inactivation did not impact NOR or SP behaviors. Measures of distance traveled across all tests were comparable between muscimol and vehicle treated rats. OFC is broadly implicated in cognitive, emotion-like, and social processes, and the cumulative data presented here suggest OFC is particularly important for social processing, as detailed in the following sections.

The influence of OFC alteration on cognitive, emotion-like, and social processing

Social safety learning was dysregulated in rats with OFC alteration via mbTBI but not sham injury. Social safety learning, as measured by the SoFiA paradigm, is a complex, innate behavior requiring the interaction of cognitive, emotion-like, and social processes. Specifically, SoFiA requires rats to integrate perception and interpretation of a socially familiar cue, then learn to associate this cue with a reduction in an emotion-like behavior, namely anxiety, then generate anxiolytic behavior accordingly.

Congruently, OFC is a region broadly implicated in cognitive, emotion-like, and social processes. Dysregulation of SoFiA alone did not reveal whether OFC was influencing the cognitive, emotion-like, or social components of SoFiA behavior. Instead, to dig more deeply at the specific role of OFC in social safety learning, a variety of behavioral tests were performed to explore cognitive, emotion-like, and social behaviors following OFC alteration.

Cognition

There is a robust collection of literature on OFC's role in cognition, which is relatively consistent across species (Neubert, Mars, Sallet, & Rushworth, 2015; Wallis, 2011). Most concisely, OFC is implicated in goal-directed behavior. This implication encompasses all stages of goal-directed behavior including identification (Gottfried & Zelano, 2011; Howard, Gottfried, Tobler, & Kahnt, 2015), cognitive judgement (Golebiowska & Rygula, 2017) and valuation (Hosokawa, Kato, Inoue, & Mikami, 2007; Padoa-Schioppa & Assad, 2006) of situations or options, re-evaluation as contextual information changes (Gardner, Conroy, Shaham, Styer, & Schoenbaum, 2017; Gottfried,

O'Doherty, & Dolan, 2003; Howard & Kahnt, 2017; Nogueira et al., 2017) (which is consistent with findings from paradigms of reversal learning (Chudasama & Robbins, 2003; Panayi & Killcross, 2018), reinforcer devaluation (Valentin, Dickinson, & O'Doherty, 2007), association learning (Luk & Wallis, 2013), credit assignment (Noonan, Chau, Rushworth, & Fellows, 2017), and extinction learning (Gottfried & Dolan, 2004; Zelinski et al., 2010)), decision-making (Fellows, 2011; Fellows & Farah, 2007), and the execution of these decisions to generate behavioral outcomes (Feierstein, Quirk, Uchida, Sosulski, & Mainen, 2006; Furuyashiki et al., 2008). Differences in which anatomical components of OFC contribute to each of these processes has begun to be parcellated (for review, see (Rudebeck & Murray, 2011)).

Humans with bilateral OFC lesion demonstrate reduced reversal learning, suggesting impaired ability to update changing values of choices (Hornak et al., 2004; E T Rolls, J Hornak, D Wade, & J McGrath, 1994), as well as inconsistent decision-making (Fellows & Farah, 2007), increases in risk-taking during decision-making (Clark et al., 2008), and failure to recognize long-term consequences of decisions (Anderson, Bechara, Damasio, Tranel, & Damasio, 1999). Interestingly, OFC lesion is not associated with deficits in general intelligence (Bechara, Tranel, Damasio, & Damasio, 1996; Eslinger & Damasio, 1985).

The present investigation revealed neither inactivation of OFC by muscimol or OFC alteration via mbTBI impacted rats' performance on a classic cognitive test, the NOR test. The NOR test measures the ability of a rat to discriminate between two inanimate objects, one familiar and one novel. In this test, the rat must not only discriminate between these objects but internally decide which object to investigate and for how long, and then

act out this decision. Because OFC-affected rats performed comparably to OFC-intact rats, it is likely that OFC is not influencing these particular cognitive processes. In addition, OFC-inactivated rats performed comparably to OFC-intact rats in a SP test, suggesting again that the ability to make a decision, even in social contexts, is intact.

Emotion-like processing

The role of OFC in emotion is likely related to its cognitive functions (Rudebeck, Bannerman, & Rushworth, 2008), and OFC may serve as an integration site for emotional/affective valuation and cognitive processes (Rolls & Grabenhorst, 2008). One theory, called the Somatic Marker Hypothesis, suggests that emotions are one of the main influencers in decision making (for review, see (Bechara, 2004; Bechara, Damasio, & Damasio, 2000). It is thought that disruption of emotional behavior and emotion regulation by vmPFC (including OFC) lesion may contribute to irrational economic decision-making (Koenigs & Tranel, 2007).

Still, humans with OFC lesions demonstrate deficits directly related to emotional functioning, including lower scores on measures of empathy and emotion recognition (Bramham, Morris, Hornak, Bullock, & Polkey, 2009). OFC lesion in humans also leads to poor self-insight, as observed in a study in which violation of typical social behaviors did not readily produce embarrassment, which the authors suggest may reflect a role for OFC in the integration of emotion processing, self-monitoring, and interpersonal behavior (Beer et al., 2006). Importantly, lesions in vmPFC (including OFC) result in emotional dysfunction that correlates with deficiencies in real-world competencies, such as planning

and initiation (Anderson, Barrash, Bechara, & Tranel, 2006), suggesting emotional dysregulation from OFC aberrancy has important implications for daily functioning.

Studies in animals present more mixed results on the effect of OFC lesion on emotion-like behavior. Rodent studies have found OFC lesion increases overgeneralization of fear (Zelinski et al., 2010) and OFC inactivation by muscimol increases anxiety-like behavior while decreasing depressive-like behavior (Kuniishi et al., 2016); however, a non-human primate study suggests OFC lesions lead to a decrease in fearful behaviors and anxiolysis (Kalin, Shelton, & Davidson, 2007). There is also some evidence that OFC is not critical for interpreting affective representations of a conditioned reinforcer (Burke, Franz, Miller, & Schoenbaum, 2008).

The present investigation does not implicate OFC in emotion-like processing in the behavioral measures used. This is because both sham- and mbTBI-exposed rats had comparable baseline anxiety-like behavior as measured in the OF and SI tests, as well as comparable response to the anxiogenic BLC. The same result was found in rats with inhibited OFC in SI tests under dim red lighting and anxiogenic BLC. In addition, sham- and mbTBI-exposed rats performed comparably in the TST, a measure of a depressive-like phenotype.

Social processing

Social processing can broadly be defined as mechanisms by which an organism receives, interprets, and responds to social stimuli. Social behaviors are the outcome of complex internal processes that may require the integration of cognitive, emotion-like, and/or social processes (P. Chen & Hong, 2018). Multiple studies using OFC lesion or

injury have demonstrated an important role for OFC in social behaviors and interactions. In humans, OFC damage leads to increased social inappropriateness, antisocial behavior, poor self-awareness in social situations, and impaired interpretation of social cues (Beer et al., 2006; Bramham et al., 2009; Cicerone & Tanenbaum, 1997; Radochonski, Perenc, & Radochonska, 2015). Human vmPFC (inducing OFC) damage can result in what is described as "acquired sociopathy", which is a reflection of the dramatic shift in social function that can follow a frontal lesion, leaving an individual with originally normal social functioning in a state of severely dysregulated social and emotional behavior. This particular constellation of behavior includes inappropriate affect and social behavior, low frustration tolerance, irritability, and low emotional expressiveness (Barrash, Tranel, & Anderson, 2000). This sudden dysregulation can have profound effects on a person's life; for example, one notable case of bilateral OFC surgical lesion resulted in an originally successful community leader quickly divorcing a long-term partner and rushing into a second, short-lived marriage, investing in numerous poor business ventures, and failing to remain employed (Barrash et al., 2000; Eslinger & Damasio, 1985). Surprisingly, this same person demonstrated normal ability to respond to social situations, understand the consequences of various social responses, understand how to achieve certain social objectives, and predict the outcome of a given social scenario (Saver & Damasio, 1991), suggesting the role of OFC in social processing is nuanced and likely influenced by concurrent deficits in cognitive and emotional processes.

In non-human primates, OFC lesions result in altered social investigation habits and increased aggressive-like behavior towards certain conspecifics (Babineau et al., 2011; C. J. Machado & J. Bachevalier, 2006) but less aggressive-like behavior towards a human

intruder (Izquierdo, Suda, & Murray, 2005). In rodents, OFC lesion results in increased aggressive-like behaviors (B. Kolb, 1974; Bryan Kolb & Nonneman, 1974; Rudebeck et al., 2007).

Surprisingly, there is little evidence OFC is important for social decision making or social processing, and the anterior cingulate cortex (ACC), another prefrontal region, is often considered a more prominent locus for directing complex social decisions and behaviors (Rudebeck et al., 2007; Rushworth, Behrens, Rudebeck, & Walton, 2007). However, the present investigation identifies a selective role for OFC in social processing.

First, while none of the behavioral tests used in this study were direct measures of general social behavior, very few overt aggressive-like behaviors were observed across all behavioral testing. In addition, there is some evidence that rodents with lesioned OFC demonstrate lower social investigation time (Bryan Kolb & Nonneman, 1974); however, this was not observed in our measure of SP, and rats with OFC overactivation had similar baseline SI times to control rats.

In addition, social processing of individual social cues appears intact, as SP and one-zone SR behavior were both unaffected by OFC inactivation. In measures of SR, mbTBI, but not sham injury, caused an increase in time spent with the familiar conspecific over the novel conspecific, which is the opposite of what is typically found in rodent species (Engelmann et al., 1995). Rats with inactivated OFC demonstrated impaired SR behavior in two- and three-zone SR tests but not a one-zone SR test. In addition, OFC inactivation did not impair SP behavior. These findings converge to suggest that OFC is important either for the discrimination of two simultaneously present social stimuli or the ability to act out a decision based on the discrimination of two simultaneously present

social stimuli. Either conclusion implies that inactivation of OFC destabilizes social processing in select scenarios.

Findings are not confounded by alternative explanations of behavioral change

Because of the variety of functions of the OFC, it is important to consider alternative explanations for the present findings. As a mediator of all five sensory modalities (reviewed in (Rolls, 2004a, 2004b)), OFC may facilitate the sensation of social stimuli. Rats predominantly use olfaction to investigate conspecifics and olfaction is well known to be mediated in part by OFC in rodents (Ramus & Eichenbaum, 2000; Schoenbaum & Eichenbaum, 1995a, 1995b). However, the current findings do not point to OFC-mediated disruption of sensation. Because OFC inhibition did not impair NOR, SP, or one-zone SR test behavior, this suggests that rats with inhibited OFC can still observe and interact with their environment in expected ways. Because both inanimate objects and conspecifics could be distinguished, it is very unlikely that OFC alteration was impairing social processing simply by impeding olfaction or other pertinent sensory modalities.

In addition, because of its rich role in both sensory processing and valuation, OFC is predicted to play an important role in hedonic experience (Kringelbach, 2005), and OFC abnormality is correlated with anhedonic behaviors (Gorwood, 2008; Luby et al., 2018). Intact SP behavior following OFC inactivation suggests an absence of social anhedonia. Furthermore, rats receiving vehicle injection or OFC inactivation spent comparable amounts of time in the middle zone of the three-zone SR test. The middle zone, which is separated from both conspecifics in the three-zone chamber, represents a non-social region.

OFC inactivation did not lead to a greater (or lesser) amount of time spent in this non-social middle region relative to vehicle treated rats, suggesting the impairment of three-zone SR behavior is not a result of OFC inactivated rats simply having low social investigation time, which might otherwise reflect a social anhedonia. In addition, OFC alteration did not impact distance traveled in all but one behavioral test, again suggesting OFC inactivation did not simply result in a refusal to investigate the given tests' presented stimuli. Although none of the behavioral measures used in the present study were direct measures of hedonic behavior, there is little evidence to suggest this is being affected by OFC alteration.

OFC is also implicated in impulsivity or impulsive-like behaviors. Both humans and rodents show elevations in impulsivity or impulsive-like behavior following OFC lesion (Berlin, Rolls, & Kischka, 2004; Mar, Walker, Theobald, Eagle, & Robbins, 2011). None of the current measurements used were direct tests of impulsivity. However, there is evidence to suggest impulsive-like behavior was not increased following OFC manipulation. Comparing mbTBI and sham rats, both groups traveled comparable distances during testing. In addition, comparing vehicle- and muscimol-treated rats, both groups traveled comparable distances during testing.

A significant portion of current literature studying OFC focuses on its role in valuation and value-based decision making. These behaviors are not often examined in social contexts; however, the present findings may encourage this line of study. OFC's regulation of valuation and value-based decision making is observed across species (for review see (Wallis, 2011). However, the present data do not suggest an impairment of valuation of social behavior, as SP was intact. This is consistent with prior literature in non-human primates (Noonan et al., 2010) but not prior literature in humans (Moretti et

al., 2008). As previously stated, decision-making likewise does not seem impaired, based on findings from NOR and SP tests. Value-based decision making requires the integration of value assessment of two or more options, followed by the execution of a decision based on the higher valuation of one option over another. Because OFC alteration is not affecting valuation or decision-making independently, it is unlikely OFC alteration is affecting value-based decision making based on the presented data. However, this was not directly tested for and cannot be completely ruled out as a possible result of OFC alteration.

OFC has been implicated in a variety of learning-related behaviors, particularly Pavlovian-style learning and reversal learning. SoFiA is a measure of safety learning in a social context, and therefore may rely on associative type learning which may be in part regulated by OFC.

The influence of OFC alteration on cognitive, emotion-like, and social processing: the commonality of processing social familiarity

In aggregate, the data presented here suggest that OFC is influencing social processing more than cognitive or emotion-like processing. Specifically, OFC alteration is not impacting measures of anxiety or depression-like behaviors, decision making capacity or novelty seeking in either social or non-social contexts, social memory, or gregariousness. OFC alteration is also likely not affecting sensation, hedonic behavior, impulsive-like behavior, or general social behaviors. Instead, OFC alteration is impacting social safety learning and SR behavior selectively.

Therefore, how the OFC is influencing social processing is nuanced and puzzling. Social safety learning and SR share the common concept of the importance of

distinguishing social familiarity. In social safety learning, a familiar social cue serves to signal safety, while in SR, social familiarity results in the recognition and devaluation of a familiar compared to a novel conspecific. In both examples, an inability to either recognize, interpret, or respond to (defined earlier as social processing) social familiarity would result in disruption of these complex behaviors.

Processing of social familiarity

As described in Chapter One, social support is critical for mental and physical wellbeing. OFC size is linearly related to the size of one's social network, and this is mediated by a measure of social cognition (Powell, Lewis, Dunbar, Garcia-Finana, & Roberts, 2010). Therefore, it is likely that OFC is contributing toward social cognition in a way that promotes the accrual of social relationships. One aspect of this social cognition may be the social processing of familiarity.

There is scant literature on the relationship between OFC and social familiarity. In addition, the neural mechanisms of social familiarity are not well understood. Presumably, one would first need to interpret a social cue, integrate this with a social memory to determine familiarity, then assign value to the familiarity, which in turn guides a decision based on the familiarity, resulting in a behavioral outcome, which can then be used to reassess behavior and further characterize the social relationship.

The present investigation does not suggest that OFC is important for interpreting social cues, forming or retrieving social memories, valuing familiarity, or making general decisions. However, deficits in social safety learning and two- and three-zone SR following OFC alteration may suggest OFC is important for using familiarity to guide

social behavior. In social scenarios, familiarity is a contextual descriptor that can act as a cue to guide social learning (seen in SoFiA) or social decision making (seen in SR). Similarly to how a light can cue a food reward, which ultimately allows the light to become associated with a positive reward outcome, familiarity may cue the potential or presence of social support, ultimately allowing familiarity to become associated with the positive outcomes created by social support.

Although OFC is not well studied in terms of social learning and social decision-making, OFC does have an established role in associative learning and goal-directed behavior. The ability to learn associations between cues, rewards, and outcomes is reliant on OFC (reviewed in (Mainen & Kepecs, 2009; Ostlund & Balleine, 2007; Young & Shapiro, 2011). Furthermore, OFC is necessary for appreciating future outcomes (Bechara, Damasio, Damasio, & Anderson, 1994; Bechara et al., 1996). Therefore, OFC lesion may impair the ability to learn to associate familiarity with positive outcome.

On the other hand, OFC lesion in rats impairs the ability to alter response following devaluation of an outcome (Gallagher, McMahan, & Schoenbaum, 1999; Pickens, Saddoris, Gallagher, & Holland, 2005). In these studies, rats were conditioned to receive food based on a light cue. Then, the food was devalued by being paired with a toxin. While sham-operated rats demonstrated reduced response to the light cue, OFC lesioned rats demonstrated no reduction in response to the light cue (Gallagher et al., 1999; Pickens et al., 2005). A similar result is found in non-human primates (Fiuzat, Rhodes, & Murray, 2017). In the present studies, an experimental rat investigates a novel conspecific more than a familiar conspecific presumably because the value of the novel conspecific is evaluated to be higher than that of the familiar conspecific. However, if OFC lesion

impairs devaluation of previous reward stimuli, OFC inactivated rats in the present study may be demonstrating a failure to devalue the familiar conspecific specifically when a decision must be made to spend time with either the novel or familiar conspecific. It is important to note that familiar conspecific devaluation is apparent when measured via the one-zone SR test, reiterating that the need to make an acute decision between two simultaneously present choices may be important in the selectivity of OFC involvement.

Furthermore, OFC is thought to contribute to behavioral flexibility, including reversal learning and extinction learning (for review see (Hamilton & Brigman, 2015)). Interestingly, rats with OFC lesion fail to extinguish conditioned fear responses and display overgeneralization of fear response (Zelinski et al., 2010). Safety learning is often measured in rodents using extinction learning paradigms, and SoFiA is similar to extinction learning in that anxiolysis is learned over time by repeated presence of a cue associated with nonoccurrence of adversity. Therefore, it is plausible that OFC alteration dysregulates SoFiA because of its regulation of extinction learning behaviors and role in behavioral flexibility in general. Particularly, in the case of SoFiA, the extinction cue is a familiar social partner; if processing of social familiarity is disrupted, then extinction learning would be disrupted as a whole.

Dysregulation of processing of familiar social cues could in part explain clinical phenomena following OFC damage, either by TBI or other source of lesion. By disrupting the processing of familiar social cues, a person's entire social network may be destabilized. As discussed in Chapter One, failure to perceive and/or receive social support, particularly from socially familiar sources, can have devastating effects on one's mental and physical wellbeing. In the context of social safety learning, if a person cannot use a socially familiar

cue, such as a friend, to signal safety, then a chronic anxiety or fear state may persist despite the absence of a threat. This in turn could develop into an anxiety disorder. Furthermore, dysregulation of social familiarity could contribute to some of the social inappropriateness (such as being overly familiar with a stranger) exemplified by people with OFC damage.

Putative circuits and substrates of OFC-mediated social processing

OFC shares bilateral connectivity with the amygdala (Hoover & Vertes, 2011; Murphy & Deutch, 2018) and IL (Hoover & Vertes, 2007, 2011), the two regions thus far implicated in SoFiA circuitry (Lungwitz et al., 2014; Truitt et al., 2007). Amygdala and OFC connectivity is critical for guiding goal-directed behavior (Baxter, Parker, Lindner, Izquierdo, & Murray, 2000), and amygdala contributes to neuronal encoding of value in OFC (Rudebeck, Mitz, Chacko, & Murray, 2013). In addition, OFC regulates the medial PFC (mPFC), particularly IL, to amygdala signaling pathway as an inhibitory modulator, and this may be GABA-dependent. In addition, potentiation of OFC pathways dysregulated OFC control over mPFC-amygdala circuitry (Chang & Grace, 2018).

Together, these findings suggest that OFC, IL, and amygdala are tightly co-regulated and their coordination is important for behavioral outcomes. We hypothesize that OFC may serve as a downstream site for integration of emotion-like information from amygdala and cognitive information from IL to produce the behavioral outcome of SoFiA. In addition, OFC may provide input to IL regarding the valuation of social familiarity in a real-time fashion, facilitating association learning (such as that required in safety learning) that may be directed by IL. OFC may also provide input to amygdala in the cognitive

processing of emotion-like behaviors, potentially facilitating anxiolysis as a form of top-down regulation.

Both OFC activation via mGluR1/5 selective agonist and OFC inhibition via $GABA_A$ agonist provided evidence for OFC-mediated regulation of social safety learning and processing of social familiarity. The molecular activity regulating such complex behaviors is largely unknown. The present findings suggest an important role for mGluRs in regulating complex behaviors, which is consistent with mGluR literature (for review, see (Mukherjee & Manahan-Vaughan, 2013)). Particularly, mGluR5 may be important for sociability, however this study demonstrates mGluR5 is not important for discrimination of novel versus familiar conspecifics (Mesic et al., 2015). Overall, it appears that balance of excitatory/inhibitory activity within OFC may be critical for social processing.

Future directions

There are several plausible and exciting future directions for this project. First, for Chapter Two, the next critical step would be to determine if normalizing the effects of elevated mGluR1/5 expression in OFC of mbTBI-exposed rats could rescue SoFiA deficit following blast exposure. Because mGluR1/5 is critical for regulating postsynaptic signaling, application of a mGluR1/5 antagonist may be just as detrimental to OFC signaling as a mGluR1/5 agonist. Rather, the use of a glutamatergic stabilizer such as memantine may be a better alternative for normalizing glutamatergic/GABAergic balance in OFC. Memantine is an uncompetitive NMDA receptor antagonist shown at therapeutic doses to promote synaptic plasticity and protect against excitotoxicity (Rogawski & Wenk, 2003). Importantly, memantine is already approved by the United States Food and Drug

Administration (FDA) as a therapy for Alzheimer's Disease and therefore could readily be applied to the TBI patient population if efficacy is found. Alternatively, a mGluR1/5 negative allosteric modulator could be used to try to regulate mGluR1/5 levels in blast-exposed OFC, which again may be a safer and more effective alternative than a mGluR1/5 antagonist.

It would also be interesting to further investigation of the altered SR behavior observed in mbTBI-exposed rats compared to sham-exposed rats. The increased time spent in the familiar zone compared to novel zone suggests a preference for familiarity that contradicts typical rodent behavior. However, in the same cohort of rats, attenuated SoFiA acquisition was observed. It would be interesting to detangle these two somewhat competing observations. Furthermore, altered SR preference was observed following mGluR1/5 agonism in OFC but not following OFC inhibition, suggesting OFC requires specific glutamatergic/GABAergic signaling balance. It would be interesting to see if memantine or a negative allosteric modulator of mGluR1/5 would normalize SR behavior.

For Chapter Three, the next most intriguing step would be to determine if OFC inhibition impairs SoFiA. This experiment has been attempted by our lab but results are inconclusive and require repeating. Although OFC overactivation is shown to destabilize SoFiA, as discussed previously overactivation demonstrates OFC is sufficient but does not determine if OFC is necessary for SoFiA. Inhibiting OFC, via muscimol, would directly measure if OFC is necessary for high order social processing like social safety learning, as measured by the SoFiA paradigm.

Furthermore, it would be excellent to detangle whether OFC is involved more in action selection or discrimination of two simultaneously present social cues. It would be

interesting and important to directly explore the effect of OFC inactivation on action selection and execution, particularly in social contexts which to my knowledge has not been studied before in rodents. This could be executed using real-time imaging of neuronal signaling to establish a pattern of OFC involvement in complex social interactions between rodents that require decision or action; this technique would provide the high temporal resolution required to distinguish these rapid and fluid behaviors.

In addition, there are limitations to all of the behavioral studies presented here that warrant expanded procedures. All muscimol injections were administered prior to original interaction with either familiar object or familiar conspecific, so only the ability to form a memory has been examined, not the ability to consolidate a memory or recall a memory after a long period of time (long-term memory). These phenomena could be explored by injecting muscimol following the initial familiarization trial, or by increasing the inter-trial interval between familiarization trial and novel probe trial.

Ultimately, it would be ideal to measure the role of OFC in human social encounters, which may be facilitated by neuroimaging during the execution of social tasks, particularly those that require decision-making or identification and discrimination of familiarity cues. While this work is in its infancy, the OFC is an intriguing region of interest worthy of continued investigation across species to ultimately pinpoint its roles in behavior.

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Curriculum Vitae

Katharine DiAnn Andrews

Education

- Bachelor of Science (BS), *Cum laude with dual honors and undergraduate research thesis*
    - Texas A&M University, College Station, TX
    - Double Major: Genetics; Biochemistry
    - Completed May 2013
- Doctor of Philosophy (PhD) in Medical Neuroscience
    - Indiana University-Purdue University Indianapolis
    - Completed May 2019
- Doctor of Medicine (MD)
    - Indiana University-Purdue University Indianapolis
    - 2013-present

Academic Positions

- Medical Scientist Training Program; 2013-present
    - Indiana University-Purdue University Indianapolis
    - Mentors: Raghu Mirmira, MD, PhD and Maureen Harrington, PhD
- Graduate Student; 2015-2019
    - Indiana University-Purdue University Indianapolis
    - Mentors: William Truitt, PhD and Thomas McAllister, MD
- Summer Undergraduate Research Fellow

    - University of Texas Southwestern Medical Center
    - Mentor: Juan Pascual, MD, PhD
- Undergraduate Research Assistant
    - Texas A&M Health Science Center
    - Mentors: Helene Andrews-Polymenis, DVM, PhD and Lydia Bogomolnaya, PhD

Honors, Awards, and Fellowships

- 2019: Elite 50, Indiana University Purdue University at Indianapolis (IUPUI)
- 2017-2019: Predoctoral Training Award (TL1), Indiana Clinical and Translational Sciences Institute
- 2018: Larry Kays Fellowship, Paul and Carole Stark Neurosciences Research Institute, Indiana University School of Medicine
- 2013: University Honors Graduate and Foundation Honors Graduate, Texas A&M University
- 2012: Honorable Mention, American Society for Microbiology Undergraduate Fellowship

Teaching Experience

- 2018: Private Instructor for Non-Didactic Small Group Sessions of Neuroscience and Behavior (MED-X-660) for remediating first year medical student
- 2018: Small Group Instructor, Neuroscience and Behavior (MED-X-660), IU School of Medicine curriculum for first year medical students

- 2016: Small Group Instructor and Session Co-Leader, Neuroscience and Clinical Neurology (ANAT-D-505), IU School of Medicine legacy curriculum for second year medical students

Mentorship Experience

- Mentor of 1st place recipient of Indiana Medical Student Program for Research and Scholarship (IMPRS) competition and Buckner Family Scholarship, M. Bruce
- Student research mentor for 3 rotating PhD students, 2 medical students, 2 undergraduate students, and 4 high school students
- Career mentor for 1 undergraduate student

Professional Affiliations

- 2018-present: Society for Neuroscience
- 2018-present: Association for Clinical and Translational Science
- 2016-present: American Psychiatric Association
- 2016-present: American Physician Scientists Association

Oral Presentations

- "Traumatic brain injury: Mild to wild?" Presented on Sept 16, 2015 as part of Translational Neuropsychiatric Topics Seminar Series, IU School of Medicine
- "Psychosocial learning and the orbitofrontal cortex: Implications for traumatic brain injury" Presented on Dec 14, 2017 as part of the Stark Neuroscience Research Institute Seminar, IU School of Medicine

Abstracts and Posters

- **Andrews, K.D.**, Lungwitz, E.A., Dietrich, A.D., Race, N.S., Majumdar, S., Burke, A.R., Bruce, M.L., Acri, D.J., Shi, R., McAllister T.W., Truitt, W.A. (2018) "Role of orbitofrontal cortex in social recognition and social-enhanced safety learning in rats" Presented by KA at Society for Neuroscience 2018, San Diego, CA
- Bruce, M.L., **Andrews, K.D.**, Lungwitz, E.A., Truitt, W. A. (2018) "Characterizing the role of orbitofrontal cortex in social memory" Presented by MB at Indiana Medical Student Program for Research and Scholarship (IMPRS) poster and oral presentation competition; 1st place; Indiana University School of Medicine, Indianapolis, IN
- **Andrews, K.D.**, Lungwitz, E.A., Dietrich, A.D., Race, N.S., Majumdar, S., McAllister, T.W., Shi, R., Truitt, W.A. (2018) "Role of orbitofrontal cortex in social-enhanced safety learning and TBI-induced psychosocial deficits in rats" Presented by KA at Greater Indiana Society for Neuroscience; Purdue University, West Lafayette, IN; Presented by KA at Translational Science 2018 (American Clinical and Translational Science), Washington, DC; Presented by KA at The National MD/PhD Conference, Keystone, CO
- Hudson, C., **Andrews, K.D.**, Lungwitz, E.A., Truitt, W.A. (2017) "Novel rodent behavior paradigms for measuring the effect of social support on anxiety" Presented by CH at Indiana University School of Medicine annual SEED program for high school students; Indianapolis, IN

- Majumdar, S., Lungwitz, E.A., Du, R., **Andrews, K.D.**, Dietrich, A., Truitt, W.A. (2016) "Elucidating the neural circuit of social familiarity-induced anxiolysis" Presented by SM at Society for Neuroscience; San Diego, CA
- Gimeno, A., **Andrews, K.D.**, Du, R., Truitt, W. (2016) "mTBI in Rats: An animal model of concussion" Presented by AG at Indiana University School of Medicine annual SEED program for high school students; Indianapolis, IN
- **Andrews, K.D.**, Ma, Q., Tondo, M., Pascual, J.M. (2012) "Elevated pyruvate dehydrogenase activity in GLUT-1 deficient mouse brain" Presented by KA at UT Southwestern Medical Center annual Summer Undergraduate Research Fellowship poster session; Dallas, TX

Manuscript Publications

- Majumdar, S.*, Lungwitz, E.A.*, **Andrews, K.D.***, Chambers, J.E., Truitt, W.A. (2018) "Animal models to investigate social support induced anxiety reductions" In S. Sangha & D. Foti (Eds.), Neurobiology of abnormal emotion and motivated behaviors. Cambridge, MA: Academic Press. ISBN: 9780128136935 *co-first author
- Rangarajul, S., Levey, D., Nho, K., Jain, N., **Andrews, K.D.**, Le-Niculescu, H., Salomon, D., Saykin, A., Petrascheck, M., Niculescu, A. (2016) "Mood, stress, and longevity: Convergence on ANK3" *Mol Psychiatry* Immediate Communication 1-13.
- Bogomolnaya, L.M., Aldrich, L., Ragoza, Y., Talamantes, M., **Andrews, K.D.**, McClelland, M., Andrews- Polymenis, H.L. (2014) "Identification of novel factors

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