Title of Document: DOPAMINE SIGNALING AND OXYTOCIN ADMINISTRATION IN A RAT MODEL OF EMPATHY

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ABSTRACT

The rat model is commonly used to study prosocial and empathetic behavior. However, the neural underpinnings of such behavior are unknown. We investigated the potential roles of two neurotransmitters, dopamine (DA) and oxytocin (OT), in prosocial behavior of rats. Our first experiment used a Pavlovian association task with two rats to investigate how DA release was modulated by social context. This experiment used fast-scan cyclic voltammetry (FSCV) to measure subsecond DA release in the nucleus accumbens (NAc). Consistent with previous work, cues that predicted reward were associated with increased DA release, and cues that predicted shock inhibited DA release non-discriminately across trial types. However, during shock trials, DA release was modulated by social context in two ways. First, reductions in DA release during shock trials were weaker in the presence of the conspecific, suggesting a consoling effect which was supported by behavioral indicators. Second, DA release during shock trials increased when shock was administered to the conspecific, suggesting that recording rats used the reactions of the conspecific to verify personal safety. We concluded that DA release is modulated by social context in that rats use social cues to optimize predictions about their own well-being. In our second experiment, we investigated the influence of oxytocin on prosocial behavior. Oxytocin

was administered intranasally prior to a distress task in which a lever press resulted in reward delivery and one of three additional outcomes: no shock (‘reward-only’), shock to engaged rat (‘shock-self’), or shock to the conspecific (‘shock-other’). Results demonstrated that oxytocin did not significantly increase prosocial behaviors.

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By

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General Introduction

In consonance with prevalent social culture, empathy as a character trait in interpersonal interactions is undoubtedly valuable. Empathy promotes cooperation in social dilemmas, strengthens our leadership skills, maintains satisfying interpersonal relationships, mitigates negative behaviors such as hostility and delinquency, and predicts social competencies throughout development (Rumble, Van Lange, & Parks, 2010; Humphrey, 2013; Davis & Oathout, 1987; de Wied et al., 2010; Allemand, Steiger, & Fend, 2015). While the trait of empathy plays an important role in the daily lives of socialization and relationships for all humans, it is of special importance for those diagnosed with psychopathologies characterized by deficits in empathy, such as autism spectrum disorder, schizophrenia, and antisocial personality disorder (Baron-Cohen & Wheelwright, 2004; Blair, 1995; Seara-Cardoso et al., 2012; Horan et al., 2015; Derntl et al., 2012). Investigating the neurobiological basis of empathy would be especially beneficial towards creating more effective treatments for these disorders.

Empathy is generally categorized into cognitive empathy (conscious awareness of the affective state of others), affective empathy (the ability to relate to and share another person's emotional state), and primitive motor types of empathy (developmental milestones characterized by the ability to automatically process and mimic emotional facial expressions through observing the behavior of others) (Davis, 1994; Cohen & Strayer, 1996; Feshbach, 1997; Hoffman, 2001; de Wied et al. 2010). These three categories of empathy are integrated in the cognitive-affective model of empathic behavior. This model defines empathy as a set of three adaptive skills, all of which must be present for fully functional empathy: understanding and processing affective cues, placing oneself in the cognitive state of others, and displaying appropriate emotional responsiveness to social cues (de Wied et al., 2010). A lack of one or more of these skills can be a marker of psychopathology. Past literature has also demonstrated that empathy is involved in prosocial behavior (Eisenberg & Miller, 1987). While empathy has been defined in a variety of

ways within the literature, for the purposes of this thesis we define empathy as the awareness of the affective states of others, including the ability to conceptualize the perspective of another based on knowledge of the situation or context (de Wied et al., 2010).

Human imaging work has provided information regarding core brain structures involved in empathy, which we will elaborate on below. However, detailed work in animal models at the single-neuron, circuit, network, and neurotransmitter levels is limited. Knowledge of the neural basis of this process is critical for understanding the fundamental mechanisms that are necessary and sufficient for these behaviors to occur. The understanding of the neurobiological basis of empathy has the potential to guide the creation of novel pharmacological interventions, which may alter an individual's ability to recognize distress and modify their behavior accordingly.

Linking Empathy and Dopamine

Dopamine (DA) is a neurotransmitter known to be heavily involved in processes regulated by the cortico-striatal-limbic system. DA neurons are located in the ventral tegmental area (VTA) and substantia nigra pars compacta (SNpc) (Danjo et al., 2014). Midbrain DA neurons in VTA project vastly to many brain areas including the nucleus accumbens (NAc), ventral striatum, prefrontal cortex, anterior cingulate cortex (ACC), amygdala, and the limbic system (Danjo et al., 2014). Aside from their involvement in the dopaminergic pathways, all of these areas of the brain are known to be heavily involved in empathic response (Marsh et al., 2011; Apps & Ramnani, 2014; Danjo et al., 2014).

Many disorders which are characterized by deficits in empathy are also linked to abnormal dopamine (DA) activity (Marsh et al., 2011). For example, researchers hypothesize that many symptoms of autism spectrum disorder (ASD) may be related to hyperdopaminergic processes that arise during development (Previc, 2007). This claim is supported by findings of abnormal dopamine transporter bindings at the molecular and genetic level in ASD (Nakamura et al., 2010; Hamilton et al., 2013), and similar disruptions in DA release have been found in animal

models of autism (Mittleman & Blaha, 2015). In contrast, researchers hypothesize that individuals with schizophrenia have hyperdopaminergic processes in mesolimbic regions and hypodopaminergic processes in prefrontal regions (Brisch et al., 2014). These dopaminergic deficits are concentrated in cell populations that project into the mesolimbic and mesocortical pathways, and may be caused by an increased number of D2 receptors (Rice, 2016; Seeman, 2013). Another disorder that is likely characterized by dopamine dysfunction is antisocial personality disorder (APD). Past studies have found a relationship between HVA (dopamine metabolite) and impulsive behavior, as well as polymorphisms affecting dopamine receptors, in individuals with APD (Soderstrom et al., 2001; Reese et al., 2010).

Abnormal DA activity has also been linked to psychopathic traits in the typical population. An fMRI study found that individuals with psychopathic traits had hyper-reactivity of brain areas involved with DA release (i.e. NAc) during rewarding events (Buckholtz et al., 2010). Another study conducted by Pedroni et al (2014) discovered that L-DOPA administration, which temporarily increased levels of dopamine in human subjects, resulted in increased selfish behavior in the absence of punishment threat. These findings imply that atypical dopaminergic activity is likely involved with empathetic processing in individuals with and without psychopathologies.

Nucleus Accumbens (NAc)

Studies have demonstrated that the Nucleus Accumbens (NAc) is involved in learning, goal-directed behavior, and motivation (Day et al., 2007; Kringelbach et al., 2010; Jackson et al., 2001; Burton, Nakamura, & Roesch, 2015). For example, the NAc is responsible for motivating behaviors in response to rewarding and aversive stimuli, and seems to be a common “junction point” for signals of value and motivation (Bissonette & Roesch, 2015). This motivational function has been demonstrated in a variety of instrumental tasks, and experimental inactivation of DA neurons that project to the NAc results in reduced aversive behavior (Danjo et al., 2014).

The NAc, which has strong reciprocal connections with midbrain DA neurons located in the VTA, is theorized to generate value predictions which are used by DA neurons to compute prediction errors (Wolske et al., 1993; Bissonette & Roesch, 2015). For these DA neurons, increased activity in response to reward is referred to as a positive prediction error because the outcome is "better than expected," while decreased activity in response to a punishment is referred to as a negative prediction error because the outcome is "worse than expected." Notably, positive prediction error encoding is also present in aversive situations that involve the absence of fear. For example, DA release is elevated when rats expect shock but it does not occur (i.e., an event that is better than expected; positive prediction error). These prediction error (PE) signals do not change as motivation increases with training, but they shift to the time of outcome prediction cues within training paradigms (Bissonette & Roesch, 2015).

While we do not specifically know what role the NAc and DA play in decision-making in social contexts, it is likely that psychological constructs related to learning and prediction error encoding are impacted by social reward and distress (Bissonette & Roesch, 2015). Therefore, we are motivated to characterize how DA release in the NAc is impacted in a variety of social situations. Several studies have already suggested that neural processing in the NAc is important for socially mediated behaviors. For example, when oxytocin (OT) receptors within the NAc core are blocked, OT can no longer act as a social reinforcement signal, and thus the reinforcing properties of social interaction are diminished (Dölen et al., 2013). Another study conducted by Kashteylan et al (2014) demonstrated that reward delivery to a conspecific modulates DA release in the NAc. Both of these studies highlight the importance of the NAc in processing social reward, emphasizing the need for further investigation.

Anterior Cingulate Cortex (ACC)

The anterior cingulate cortex (ACC) is also implicated in empathy and modulation of dopamine (DA) release, as it is linked to the VTA via the mesolimbic pathway. The ACC is part

of the cerebral cortex, and can be anatomically divided into two relevant sections: the gyral ACC (ACCg), which are the "ridges" of tissue of the ACC, and the sulcal ACC (ACCs), which are the "depressions" of tissue of the ACC (Apps & Ramnani, 2014). The ACC has a prominent role in cost-benefit analyses and the processing of rewards, and also plays a large role in social decision-making (Chang & Sanfey 2013). A study conducted by Apps and Ramnani (2014) found that rewards given to the 'self' and rewards given to a confederate were processed differently by the ACC. The ACCs was found to process the amount of effort, or "cost" needed to undertake an action for both the individual and the confederate. However, it was determined that the ACC is responsible for the brain's ability to process the magnitude of a reward given to a confederate, whereas the nucleus accumbens (NAc), a part of the ventral striatum that has reciprocal projections with VTA, is responsible for the ability to process the reward given to the individual (Apps & Ramnani, 2014). The implication of this result is that the ACC and the NAc play a pivotal role in the expression of emotional empathy while maintaining a "self-other distinction" by means of dopaminergic pathways (Uzefovsky et al., 2014).

Evidence has revealed that the ACC is involved with one's ability to empathize when observing pain in others. A study utilizing fMRI, conducted by Mathur et al (2010), exposed subjects to pictures of others in pain. Whole-brain regression analysis revealed a positive and significant correlation between degree of self-reported empathy for pain scenes and neural response to pain targets within the ACC (Mathur et al., 2010). These results reveal that the ACC plays a role in one's perception of pain in another, and suggest that the ACC is involved in modulating one's affective experience of empathy when perceiving suffering in another. Additionally, a study which tested the ability of youths with psychopathic traits to perceive and empathize with pain revealed that in the rostral ACC, youths with psychopathic traits showed reduced responsiveness to increasing pain in self and pain in others (Marsh et al., 2013). This reinforces the likelihood of the ACC's involvement in affective experience of empathy by

showing that there was reduced activity in the ACC (relative to control subjects) in subjects with traits characterized by a lack of empathy. It is still unclear exactly how ACC modifies behavior in the context of social decisions, but the DA-NAc system likely works with the ACC due to their connectivity and roles in empathetic responses.

Oxytocin (OT)

Oxytocin (OT) is a peptide hormone that is recognized to play a major role in the modulation of behavior, especially in response to social context. Due to the prominent activation of the OT system during pregnancy, lactation, reproductive functions, and maternal behavior, OT was considered to be a "maternal hormone" that contributed to bonding between a mother and infant (Slattery & Neumann 2010). However, more recent studies have shown that the role of OT is more diverse than previously established. OT has been shown to affect stress-induced behaviors: upregulation of the OT system in the endogenous area of the brain, which encompasses circuits that anticipate future environmental events, results in strong anxiolytic effects in both males and female mice and rats (Slattery & Neumann 2010). This upregulation can also occur in males through sexual activity (Slattery & Neumann 2010), further demonstrating that the role of OT in mammals is not limited to maternal interactions.

Studies involving the oxytocin receptor (OTR) have shown that there is a significant link between OT and the regulation of pair-bonding consolation behaviors. Monogamous, pair-bonded prairie voles are known to offer consolation behavior to reduce stress in a partner through actions such as grooming. Further study has demonstrated that prairie voles also match the fear response, anxious behavior, and increased corticosterone of a stressed partner (Burkett et al., 2016). In prairie voles, high densities of OTR are found within the ACC, adjacent prelimbic cortex (PLC), and nucleus accumbens shell (NAcS). Prairie voles that contact with stressed cagemates also increase activity within the ACC, and the injection of an OT antagonist into the cerebral ventricle caused both the hormone response and consolation behavior of the unstressed voles to dissipate.

The consolation behaviors demonstrated by prairie voles are reflective of similar behaviors that are present in humans, and within humans, the ACC has been linked to the expression of empathy while the NAc has been associated with both social and non-social reward analysis, among other functions (Burkett et al., 2016). The evidence clearly suggests that OT plays a central role in the regulation of social behavior in rodents, and has implications regarding how OT could affect social behavior within humans.

Discoveries on the effects of OT on animal behavior generated interest within the scientific community in the effects of OT on humans. Studies showed that higher levels of OT within plasma correlated to more robust social bonds between family members and romantic partners (Gordon et al., 2008). Genetic variation in the gene that regulates OTR, OXTR, can affect support-seeking behavior, prosocial decision-making, social cognition within ADHD patients, and prosocial behavior in patients suffering from ASD (Walum et al., 2012). Further studies showed that the intranasal application of OT caused significant changes in a large variety of behaviors within humans. OT treatment affected social behaviors including trust (Kosfeld et al., 2005), generosity (Zak et al., 2007), facial recognition (Rimmele et al., 2009), emotional perception, and tendency to communicate in pair bonds (Domes et al., 2007). Another effect of OT treatment was the emergence of parochial altruism, which results in behaviors that involve self-sacrifice to promote "in-group" trust, welfare, and cooperation, but also promotes defensive, but not actively aggressive behavior toward competing "out-groups" (Dreu et al., 2010). Although the effects of OT on myriad human social behaviors are well-documented, there is still a gap in the research regarding the full scope of how OT can alter complex social decision-making and the mechanism by which it affects these changes. Previous research has demonstrated that some changes in social behavior are analogous between rodents and humans, thus an animal model may be used to investigate the influence of OT on complex decision-making in a social context.

Rat Model for Empathy

In order to investigate the neurobiological basis of empathy, a viable animal model is necessary for *in vivo* experimentation. The rat was chosen for several reasons, the first of which is pre-existing research demonstrating that rats are capable of exhibiting empathy (Rice & Gainer, 1962). Ben-Ami Bartal et al. conducted a major study demonstrating empathy in rats, in which two cagemates were each given a "free rat" or "trapped rat" role (Ben-Ami Bartal et al., 2011). In the experimental condition, named "trapped condition," the free rat was placed in a cage that contained a restraining tube in the center. The tube contained the trapped rat, and its door could be opened upon application of pressure by the free rat. The aim was to determine whether free rats would learn to open the restrainer door for the trapped rats as a result of prosocial empathy-driven behavior (Ben-Ami Bartal et al., 2011). Controls included a free rat with an empty restrainer ("empty condition"), a free rat with a toy rat in the restrainer ("object condition"), and a free rat with an empty restrainer and a live rat across a perforated divide that allows the rats to see, hear and smell each other ("2+ empty condition"). As anticipated, free rats in the experimental condition were more likely to become door openers than free rats in the control conditions, and their behavior demonstrated evidence that door opening was a learned, goal-driven, purposeful behavior.

To determine that door opening was truly an empathetic behavior and not a behavior driven by desire for social contact, a second experiment was created. The trapped condition remained the same, while the second experimental condition involved a free rat placed in a cage with a trapped rat, but the restrainer door opened into a different cage, so that upon opening the rats could not be in the same cage ("separated condition"). The separated condition had experimental condition ("separated cagemate") and a control condition ("separated empty"), where "empty" refers to the terminology explained earlier. In the separated cagemate condition, rats continued opening the door at the same latency as they did in the trapped cagemate condition,

but they stopped opening the door at the empty condition. This indicates that social interaction is not a necessary result of the prosocial door opening behavior; thus, it can be inferred that this is an empathy-driven behavior. While some believe that these results are inconclusive, this study has been highly regarded in the neuroscience community as a primary example of rats exhibiting empathy (Silberberg et al., 2014).

In addition to the Ben-Ami Bartal study, a wide base of research exists to support the presence of empathy in a rat model. When tested in pairs, rats' reactions to identical pain stimuli have been shown to be stronger in the presence of a visible conspecific, and when a rat receives a different dose of pain stimulus than a familiar neighbor, the pain responses of the rats influence each other bidirectionally (Langford et al., 2006). Also, researchers have shown that rats' reactions to shock cues are modulated not just by their own experience but also by observation of a conspecific; that is, that observing rats will freeze when a conspecific is shocked and are more likely to do so when they have experienced the shock themselves (Atsak et al., 2011). Additionally, when a rat that has experienced a shock before observes a "demonstrator" rat receive a shock, the observer rat will also react with a fear response that is specific to the type of stressor stimulus applied and changes in intensity with the intensity of the stimulus (Sanders et al., 2013). The propensity of rats for socially modulated reactions establishes them as a viable model for empathy and empathetic behavior.

Another benefit to using rats for this study is their demonstrated abilities for social learning and communicative transmission of shared experiences (Knapska et al., 2006; Bruchey et al., 2010; Knapska et al., 2010). Many experiments have shown that vicarious freezing behavior is a specific and consistent way for a rat to communicate fear to another rat, and it can trigger a fear response in another rat that has been pre-exposed to the fear trigger (Church, 1959; Kim et al., 2010; Atsak et al., 2011). This pre-exposure is necessary for the rat to display a fear response, as rats unexposed to the fear-response trigger have been shown to demonstrate little or no

empathetic fear behaviors in response to a rat experiencing the trigger (Guzmán et al., 2009; Sanders et al., 2013). These social communicative instincts will lend to easily observable and qualifiable observation of empathetic behavior in rats during experimentation.

Thirdly, all sources examined in this literature review demonstrate that rats are a widely accepted and utilized model for dopaminergic activity in the human brain. Similarities have been shown between dopamine activity in humans, monkeys and rats (Fiorillo et al., 2003; D'Ardenne et al., 2008; Kobayashi and Schultz, 2008; Zaghloul et al., 2009).

Broadly speaking, for *in vivo* experimentation, rats are the most viable option. Research has determined consistently that rats are empathetic and demonstrate empathy-related behaviors (Rice and Gainer, 1962; Ben-Ami Bartal et al., 2011), they have a propensity for social learning (Knapska et al., 2006; Bruchey et al., 2010; Knapska et al., 2010; Church, 1959; Kim et al., 2010; Atsak et al., 2011), they show verified, quantifiable indicators of affective states (Burgdorf et al., 2008; Burgdorf et al., 2011, Browning et al., 2011), and they have been established as a reliable model for dopaminergic activity in the human brain (Fiorillo et al., 2003; D'Ardenne et al., 2008; Kobayashi and Schultz, 2008; Zaghloul et al., 2009).

Summary and Overview

The current thesis investigated the potential roles of dopamine (DA) and oxytocin (OT) in empathy and prosocial behavior of rats. While empathy has been defined in a variety of ways within the literature, for the purposes of this thesis we define empathy as the awareness of the affective states of others, including the ability to conceptualize the perspective of another based on knowledge of the situation or context (de Wied et al., 2010). Furthermore, we define prosocial behavior as any action intended to benefit and/or prevent harm to another (Eisenberg & Miller, 1987). Furthermore, we operationalize affective states through measurements of beam breaks and other behavioral indicators, including freezing and approach behavior.

Three primary questions guided our research: (1) How is subsecond dopamine release

influenced by the presence and reward/shock of a conspecific? (2) Can this relationship be used to infer a partial neurobiological basis of empathy? (3) How does oxytocin modulate prosocial behavior in our animal model? These research questions were motivated by past literature which has demonstrated that various psychopathologies, such as antisocial personality disorder, schizophrenia, and autism spectrum disorder, are characterized by abnormal empathetic behavior and abnormal dopamine activity (Marsh et al., 2011).

Chapter 1 of this thesis addresses the first and second research questions, utilizing a Pavlovian association task to investigate how DA release was modulated by social context. It was hypothesized that within this animal model, dopamine release would be modulated by delivery for reward and punishment to a conspecific located nearby. More specifically, we hypothesized that if rats had 'empathetic signaling' of DA release, then cues that predicted reward and punishment to the conspecific would modulate DA release in the same manner as cues that predict reward and punishment to the 'self.'

Chapter 2 of this thesis addresses the third research question, investigating the influence of oxytocin on prosocial behaviors. In this experiment, oxytocin (OT) was administered intranasally prior to a distress task in which a lever press resulted in reward delivery and one of three additional outcomes: no shock ('reward-only'), shock to engaged rat ('shock-self'), or shock to a conspecific ('shock-other'). It was hypothesized that rats treated with OT would increase prosocial behavior compared to control rats treated with saline.

Chapter 1: Subsecond dopamine signaling of reward and punishment is modulated by social context

Introduction

As described above, it is well known that the activity of dopamine (DA) neurons and DA release into the nucleus accumbens (NAc) reflect reward prediction errors. That is, DA release in the NAc increases and decreases to events that are better (i.e. reward) or worse (i.e. shock) than expected, respectively (Schultz, 1997). This includes delivery of outcomes, as well as cues that predict those outcomes. For example, DA release is high to cues that predict reward and to delivery of reward itself, especially when that reward is unexpected (Bissonette & Roesch, 2015). Furthermore, it has been shown that in paradigms where the rat expects punishment, DA release increases when the expected punishment does not occur, reflecting that safety from said punishment is rewarding. In contrast, when cues that predict unavoidable shock are presented, or when predicted rewards are unexpectedly omitted, DA release is inhibited (i.e., negative prediction error). It is unknown how these signals are modulated when outcomes are delivered to a conspecific, or when outcomes are delivered to the 'self' in presence of a conspecific.

Past literature has investigated the role of DA signaling in a social context. A study conducted by Kashteylan et al. (2014) investigated the modulation of DA release from the ventral striatum. Results demonstrated that observation of a reward delivered to a conspecific resulted in an increase in DA release when rats found the situation appetitive, however when rats found delivery to the conspecific aversive, a reduction in DA release was observed. Further research is needed to investigate how DA release correlates with different outcome deliveries (i.e. shock and neutral outcomes).

To address this question, we designed a Pavlovian association task paradigm in which three different auditory cues predicted either reward, shock, or neutral (no outcome). In this task, two rats were placed side-by-side in a single behavioral box separated by a perforated wire mesh.

One of these rats – the recording rat – was equipped with a fast-scan cyclic voltammetry electrode in NAc to record subsecond DA release. Five seconds after the onset of the auditory 'outcome cue', a 'directional' light illuminated indicating which rat would receive the outcome. The conspecific rat was removed for half of the recording sessions to test for the effects of social context. Food cup entries, freezing, orienting, and approach were also used as behavioral measures to determine the recording rats' affective state and outcome predictions.

Consistent with previous work, DA release increased to cues that predicted reward and during reward delivery. Cues that predicted shock inhibited DA release non-discriminately across trial types; prior to shock onset, DA release was low when shock was delivered to either rat. However, during shock trials, DA release was modulated by social context in two ways. First, reductions in DA release to cues that predicted shock were weaker in the presence of the conspecific. During these trials, rats approached the conspecific more often, suggesting that they were seeking social interaction. Second, DA release on shock trials increased quickly when shock was administered to the conspecific, suggesting that recording rats were using the emotional reactions of the conspecific to verify personal safety.

We conclude that DA release is modulated by social context in that rats use social cues to optimize predictions about their own well-being. Social context also appeared to weaken signals associated with predicted shock. Finally, our results suggest that, at least in this context, that DA does not signal the valence of positive and negative events from the perspective of the conspecific (e.g. empathy).

Methods

Subjects. 16 male Sprague-Dawley rats (8 pairs) were obtained from Charles River Labs at 300-350g (90-120 days old). Animals were individually-housed in a temperature- and humidity-controlled environment and kept on a 12-h light/dark cycle (0700-1900 in light); all tests were run during the light phase. Animals had access to water ad libitum and body weight

was maintained at 85% of baseline weight by food restriction (15g standard rat chow provided daily, in addition to approximately 1g sucrose pellets during experimental trials). Of 15 animals undergoing electrode surgery, 8 animals provided reliable cyclic voltammograms; 40 recording sessions from these 8 animals were used in our final sample. All procedures were performed in concordance with the University of Maryland, College Park Institutional Animal Care and Use Committee (IACUC) protocols.

Chronic microelectrode fabrication. Electrodes were constructed according to the methods of Clark et al. (2010). A single carbon fiber with a diameter of 5 μm (Goodfellow Corporation) was inserted into a 15-mm cut segment of fused silica (Polymicro Technologies) while submerged in isopropyl alcohol. One end of the silica tubing was sealed with a two-part epoxy (T-QS12 Epoxy, Super Glue) and left to dry overnight, leaving untouched carbon fiber extending past the seal. The protruding carbon fiber was cut to a length of 150 μm. A silver connector (Newark) was secured to the carbon fiber at the opposing end of the silica tubing using silver epoxy (MG Chemicals) and was allowed to dry. A final coat of two-part epoxy was then applied to the pin connection to provide insulation and structural support for the electrode and was allowed to dry overnight.

Intra-cranial surgical procedures. All animals were anesthetized using isoflurane in O_2 (5% induction, 1% maintenance) and implanted with a chronic voltammetry microelectrode aimed at the NAc core (+1.3 AP, +1.4 ML, -6.9 DV), an ipsilateral bipolar stimulating electrode (Plastics One) in the medial forebrain bundle (-2.8 AP, +1.7 ML, -8.8 DV), and a contralateral Ag/AgCl reference electrode (Sigma-Aldrich). The reference electrode and anchoring screws were stabilized using a thin layer of dental cement (Dentsply), leaving the holes for the stimulating and recording electrodes unobstructed. The stimulating and recording electrodes were attached to a constant current isolator (A-M Systems) and voltammetric amplifier, respectively, and lowered to the most dorsal point of the target region (-6.6 DV for the working electrode and -

8.5 DV for the stimulating electrode). At this depth, a triangular voltammetric input waveform (-0.4 to +1.3 V vs. Ag/AgCl, 400 V/s; Heien et al., 2003) was applied to the recording electrode at 60 Hz for 30 minutes and then reduced to 10 Hz for the remainder of the surgery. Electrical stimulation (24 biphasic pulses, 60 Hz, 120 μA) was applied to the stimulating electrode in order to evoke dopamine release, which was monitored at increasing depths by the recording electrode. If neither an evoked change in DA nor a physical response (whisker movement or blinking) was observed, the stimulating electrode was lowered by 0.05mm until a response was achieved or to a maximum depth of 8.8mm. The working electrode was then lowered by 0.05mm until DA release was observed or to a maximum depth of 6.9mm. Once electrically-evoked DA release was detected in the NAc core, a thin layer of dental cement was used to secure the stimulating and recording electrodes in place. A Ginder implant (Ginder Scientific; constructed in house) was connected to the reference, stimulating, and recording electrodes and fully insulated using dental cement, leaving only the screw-top connector exposed, in order to reduce noise and prevent loss of connectivity during behavioral training.

Animals then received post-operative care: subcutaneous injection of 5 mL saline containing 0.04 mL carprofen (Rimadyl), topical application of lidocaine cream to the surgical area, and placement on a heating pad until full consciousness was regained. Animals were also given antibiotic treatment with Cephlexin orally twice daily post-surgery for two weeks to prevent infection of the surgical site. All subjects were allowed a month for full recovery and stabilization of the electrode before experimentation.

Fast-scan cyclic voltammetry. Fast-scan cyclic voltammetry (FSCV) is a method of measuring phasic changes in extracellular dopamine concentration, meaning changes that occur for only brief periods. This technology uses microelectrodes, which are small electrical conductors that make contact with the dopamine-releasing environment in the brain. Other methods to measure concentrations of extracellular dopamine include microdialysis and constant-

potential amperometry. Microdialysis has a high specificity and sensitivity between the chemicals being measured. However, it has slow temporal resolution and can only present changes in dopamine concentrations on a minute to hour basis (Robinson et al., 2003). Constant-potential amperometry offers a high temporal resolution, but it lacks sufficient ability to distinguish between chemicals being measured (Robinson et al., 2003). FSCV is advantageous as it is able to measure changes in extracellular concentration on a subsecond time scale, allowing researchers to associate changes in dopamine with very specific time points in the paradigm and thus specific points in the behavior being expressed.

For recordings, animals were connected to a head-mounted voltammetric amplifier (current-to voltage converter) and a commutator (Crist Instruments) mounted above the recording chamber. During each session, an electrical potential was applied to the recording electrode in the same manner as described above (see Intra-cranial surgical procedures). In order to detect changes in dopaminergic concentration over time, the current at its peak oxidation potential was plotted for successive voltammetric scans and background signal was subtracted. Two PC-based systems, fitted with PCI multifunction data acquisition cards and software written in LabVIEW (National Instruments), were used for waveform generation, data collection, and analysis. The signal was low-pass filtered at 2,000Hz. Event timestamps from Med Associates were recorded, in order to analyze behaviorally relevant changes in dopamine release.

Dopamine was identified by its stereotypical and specific cyclic voltammogram signature. Behaviorally-evoked DA signals met electrochemical criterion if the cyclic voltammogram was highly correlated to that of the DA templates produced during the training set. The training set is a template containing six each of background-subtracted cyclic voltammograms and corresponding calibrated concentrations for both dopamine and pH extracted from data pooled across animals acquired during electrical stimulations that are known to evoke DA release (stimulation at 1V: 30 Hz, 6 pulses; 30 Hz, 12 pulses; 30 Hz, 24 pulses; 60 Hz, 6

pulses; 60 Hz, 12 pulses; 60 Hz, 24 pulses). The data collected during a session were not analyzed if reward trials did not elicit DA release that satisfied these chemical verification criteria. Voltammetric data were analyzed using software written in LabView and MATLAB (Mathworks). A principal component regression (Tar Heel CV chemometrics software) was used to extract the DA component from the raw voltammetric data (Zachek et al., 2010; Zaghloul et al., 2009). Eigenvalues (principal components) are calculated that describe relevant components of our training set, and perform multivariate regression analysis to determine a correlation coefficient to describe our recorded behavioral data versus the training set. The number of factors selected to keep in our PCA analysis accounts for >99% of the variance (at least 3, but usually 4-5 factors are kept). Factor selection is a very important step, as retaining more factors than are necessary would add noise to our data but retaining too few could mean discarding potentially meaningful information (Zalocusky & Deisseroth, 2013). Importantly, the exact same method was applied to each trial-type allowing for fair comparison between conditions.

This experiment also uses the residual to examine the quality of the fit. In general, the residual is the difference between the experimental observation and the predicted value derived from a model/template (our regression values) and is a measure of the unknown portion of the signal that is not accounted for by the principal components of the regression. This is important when considering the accuracy and the applicability of the model and is important for identifying possible interfering molecules or noise (such as drift). The sum of squares of the difference between the template and the experimental data is the residual value (Q) and the threshold Qa establishes whether the retained principal components provide a satisfactory description of the experimental data; the discarded principal components should provide a measure of noise (Zaghloul et al., 2009; Zalocusky & Deisseroth, 2013). This Qa measure in combination with our regression analysis to establish our concentration corrections is utilized.

Chemometrics is a widely-used analytical method that separates changes in current that

are caused by DA release from those caused by pH shift or other electrochemical 'noise' by comparing eigenvalues derived from stimulated DA release and changes in pH to those derived from behavioral release (Hermans et al., 2008). See Appendix A for more information about FSCV.

Histology. Following completion of the study, animals were terminally anesthetized with an overdose of isoflurane (5%) and transcardially-perfused with saline and 4% paraformaldehyde. Brain tissue was removed and post-fixed with paraformaldehyde. Brains were then placed in 30% sucrose solution for 72 hr. and sectioned coronally (50μm) using a microtome. Tissues slices were mounted onto slides and stained with thionin for histological reconstruction.

Behavioral Paradigm. There has been much research examining aspects of empathy in rats using a wide variety of behavioral paradigms. While these paradigms are different, they usually manipulate similar variables. Thus, several variables should be considered including both positive and negative affective stimuli such as rewards (food) and punishments (shock); levers that allow rats to voluntarily stimulate an event; types of visual or auditory cues; the setup of the cage, including the degree the rats can observe each other during trials; the training the rats undergo before the paradigm; the number of trials and sessions run of the paradigm (precedent dictates around 60 trials per session); and the characteristics of the conspecific which includes the breed, conspecificity (cagemate vs. non-cagemate status), and how long the rats have been housed together.

All of these variables are manipulated when considering a unique paradigm that examines empathetic behavior in rats. To determine that empathy is truly being tested, and that no confounding variables are at play, all possible explanations for the data collected must be considered and either proven or disproven in order to establish conclusive results. For example, in order to prove that the spike in dopamine release when a rat sees a conspecific rewarded is due to empathy and not simply the observation of the reward being delivered, an additional protocol

must be run that shows an absence of additional dopamine release when a rat observes an empty reward chamber. Because there are many possible confounding variables involved, several control conditions must accompany each paradigm to rule out alternative explanations.

The experiment described in this chapter involved a pair of food-deprived conspecific rats located in a cage across a perforated divide (Fig. 1). The sides of the cage were fitted with food receptacles programmed to release a food reward upon a given cue, and the floors were fitted with equipment for shocking the rats. Each side of the cage had a small light (the 'directional light'), and a large 'houselight' over the center of the cage. Also located in the center of the cage was a tone emitter.

The paradigm began with the activation of the 'houselight cue,' which signaled the beginning of the trial to the rats. Five seconds later, one of three auditory cues (the 'outcome cue') was emitted from the tone emitter. Tone I indicated the upcoming administration of a positive reward of food, which was highly salient for the food-deprived rats. Tone II indicated an upcoming negative outcome of a shock. Tone III indicated a neutral outcome, with no reward or punishment. The tones lasted for the full 5-second duration of the epoch. Next, one of the two directional lights was activated, indicating to the rats which side of the cage would receive the positive, negative or neutral outcome (the 'directional cue'). Finally, the reward or punishment was administered to the rat indicated by the directional cue (the 'outcome'). The three tones were counterbalanced across all rats, and varied between a clicker, a single pitch sustained tone, and white noise.

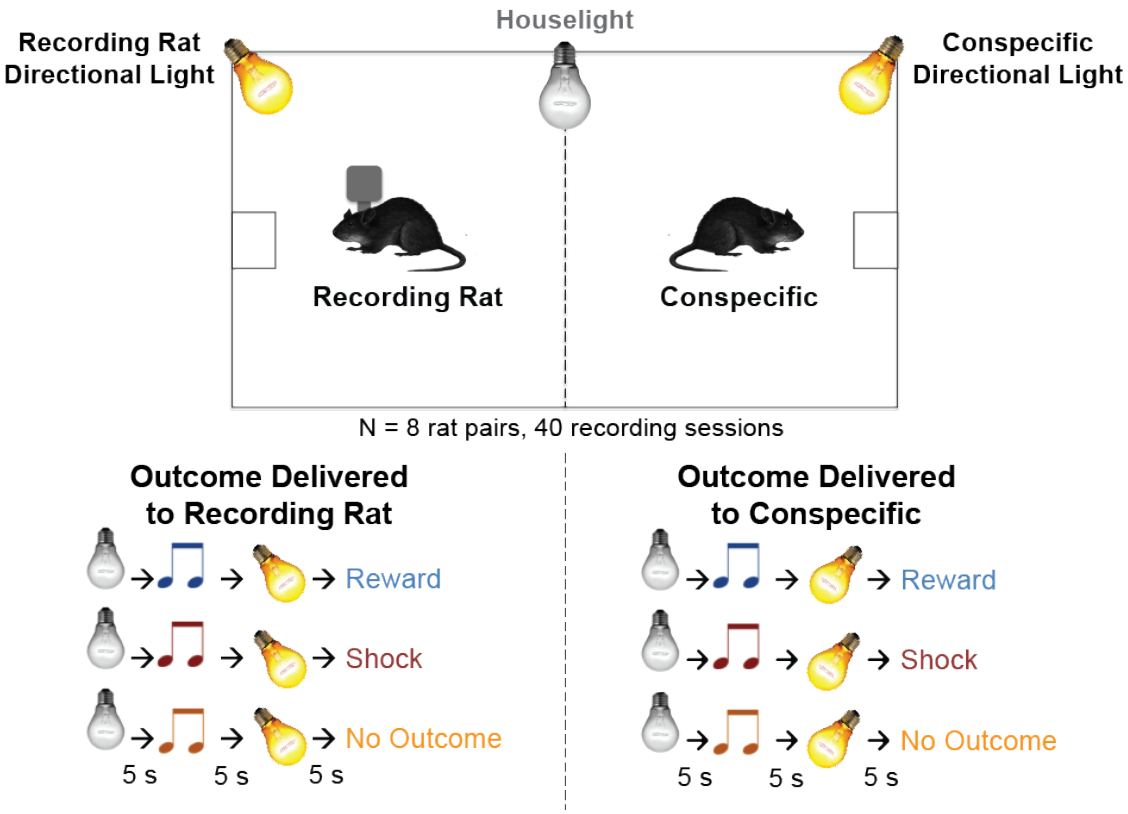

Figure 1. Once the house light (grey) was activated, an auditory cue sounded signifying one of three outcomes: reward, shock, or neutral (no outcome). Next, one of the directional light cues (yellow) on either the left half or the right half of the cage was turned on, signaling which rat would receive the outcome. Each event was separated by 5 s. Food receptacles used to offer rewards were also individually placed on each side of the box. FSCV technology was used to record dopamine from the 'recording rat'. The outcome was delivered to either the recording rat or the conspecific.

Beam Breaks. An infrared beam was placed at the entrance to the food cup on the recording rat's side of the cage. This beam was disrupted upon physical entry of the rat's nose into the food cup, and beam breaks served as a quantitative measure of this behavior. Beam status (intact or broken) was polled every 20 ms, and each time at which the beam was broken was recorded. For analysis, these data were aggregated as proportions across 1-second bins (i.e. divided by the number of possible breaks per second to yield a percentage).

Video Scoring. Cameras were positioned facing the recording rat who was placed in an experimental cage with a perforated mesh divider. As previously mentioned, the recording rat was placed in sessions with and without a conspecific on the other side of the mesh divider as described in the behavioral paradigm methodology. Briefly, the paradigm consisted of four

epochs lasting five seconds in length: house light; cue tone; directional light; and outcome. During each epoch, the behavior of the recording rat was assessed according to three criteria: (1) freezing behavior, defined as sudden cessation of movement by the rat; (2) nose orientation, defined as the direction in which the snout is pointed; (3) approach to mesh divider, defined as movement of the rat in the direction of the mesh divider. Additionally, we assessed (4) the presence of conspecific, defined as whether the conspecific could be seen by the camera standing at the mesh divider (indicating attentiveness). To reduce bias, each video was assessed by two pairs of independent observers; one pair scored the former three criteria while the second pair scored the latter.

Nose orientation was dummy scored with integer values ranging from 0 to 2 (Fig. 2) while approach was scored on a scale of 0 to 1 (Fig. 3). For nose orientation, the experimental box was divided into three dummy scored regions. For some of the scoring criteria, there were further specifications that were defined before video scoring began (Table 1).

Freezing was a challenging behavior for scorers to judge. When a rat was still before an outcome cue, it was challenging to discern if the rat froze as a result of the cue. The scorers were very careful to make sure they actually observed freezing behavior.

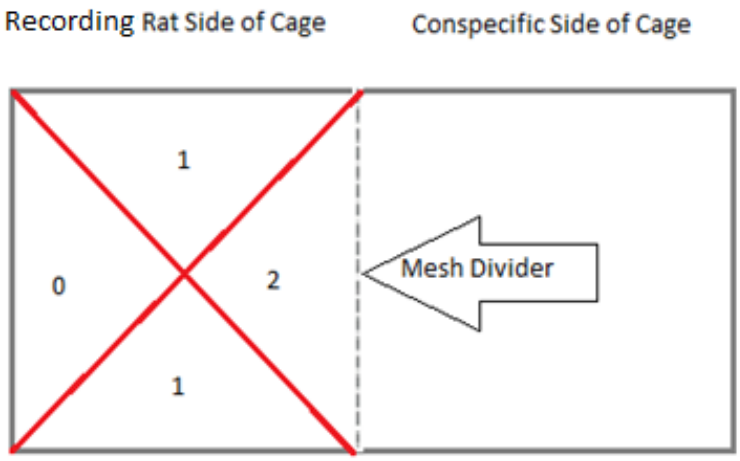

Figure 2. The nose orientation of the recording rat is divided into three distinct dummy coding regions: 0; 1; 2. A dummy coding of 0 indicates that the recording rat is facing the back of the cage and away from the mesh divider. A dummy coding of 1 indicates that the recording rat is facing one of the two sides of the cage. A dummy coding of 2 indicates that the recording rat is facing towards the mesh divider.

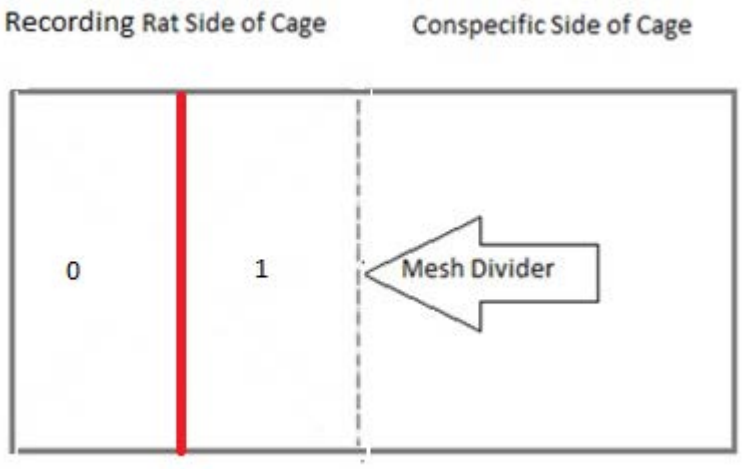

Figure 3. The nose orientation of the recording rat is divided into two distinct dummy coding regions: 0; 1. A dummy coding of 0 indicates that the recording rat's head points away from the mesh divider. A dummy coding of 1 indicates that the recording rat's head points toward the mesh divider.

	Dummy Code Value		
Criteria	**0**	**1**	**2**
Nose Orientation	Snout is pointed towards the back of the cage, away from the mesh divider	Snout is pointed one of the two sides of the cage	Snout is pointed towards the mesh divider
Approach	The recording rat moves away from the mesh divider, or his head points away from the mesh divider	The recording rat moves towards the mesh divider, or his head points toward the mesh divider	N/A
Freezing	The recording rat does not suddenly stop moving	The recording rat suddenly stops movement	N/A
Conspecific	The conspecific cannot be seen through the mesh divider	The conspecific can be seen through the mesh divider	N/A

Table 1. Dummy Coding Value Assignment. This table provides the rubric that was followed by the video scorers.

Data Analysis. Analysis was centered on various epochs, each 5 seconds in duration and delimited by a task event: first, the house light came on to illuminate the chamber ('houselight epoch'); then, a cue tone was played to indicate the type of outcome being delivered – reward, shock, or the no-outcome 'neutral' ('outcome cue epoch'); next, a directional light turned on for one side of the cage to indicate the recipient of the outcome – either the recording rat or the conspecific ('directional cue epoch'); and finally, the outcome indicated by the cue tone was delivered to the side of the chamber indicated by the directional light ('outcome epoch'). Behavioral videos from recording sessions were scored for measures that would indicate attention

and seeking behavior (freezing, rearing, orienting to the lever) during each epoch for all trial types. For behavioral analysis, each of these measures was assigned a score on either a binary (0 or 1) or a ternary (0, 1, or 2) basis for each epoch. These behavioral analyses were scored blindly. The values for each measure were normalized into percentages of the behavior. In addition, an infrared beam placed at the entry to the recording rat's food cup recorded the times at which the recording rat's nose entered the food cup, polling every 20 ms. This series of times was then converted to a percentage of positive polls for interpretation as a behavioral measure.

As described above, all voltammetric data were analyzed using software written in LabView and then further analyzed in MATLAB (Mathworks). The dopamine component of our signal was first isolated from the raw voltammetric signal using principal component regression and calibration to a CV/concentration matrix.

For data analyses, we directly compared all trial-types over the four predefined epochs. For each epoch, we also computed indices that reflected DA release differences between trials types for each session ('together' or 'alone' for reward, neutral and shock trials; etc.) and then plotted the distributions of indices to determine how many sessions showed differences between trial-types over all recording sessions. The indices were 'together minus alone' for reward, neutral, and shock trial-types, and 'reward minus neutral' and 'shock minus neutral' independently for 'alone' and 'together' trials. Finally, we conducted independent sample t-tests, which measured the difference between 'alone' and 'together' trials for every 100 ms bin across the trial, as well as sign rank tests comparing randomly paired. Results represent data from 80 sessions of randomized block trials across 8 distinct pairs of rats. 'Together' trials are defined as trials in which both the recording rat and the conspecific were present. 'Alone' trials are defined as trials in which only the recording rat was present.

Results

Beam breaks into the food cup reflect expected outcomes. Here, we describe beam break results in general comparisons between trial-types (reward vs. shock vs. neutral) during relevant epochs to demonstrate that rats understood the meaning of the auditory outcome cues and directional light cues. In the following sections, we then describe how social context (i.e., rats were alone or together) impacted food cup entries across the 3 trial types during 'self' and 'other' trials. For the purposes of the results and discussion, 'self' trials are defined as trials in which the outcome (either reward, shock, or neutral no outcome) was delivered to the recording rat; 'other' trials are defined as trials in which the outcome was delivered to the conspecific. 'Together' trials are defined as trials in which both the recording rat and conspecific were present; 'alone' trials are defined as trials in which only the recording rat was present. On 'alone' trials, the outcome was still delivered to the conspecific's side of the cage, even though the conspecific was absent. In the follow figures, blue, orange and red represent 'reward', 'neutral' and 'shock' trials, respectively, and the thickness of the line indicates whether the rat was alone (thin lines) or in the presence of the conspecific (thick lines).

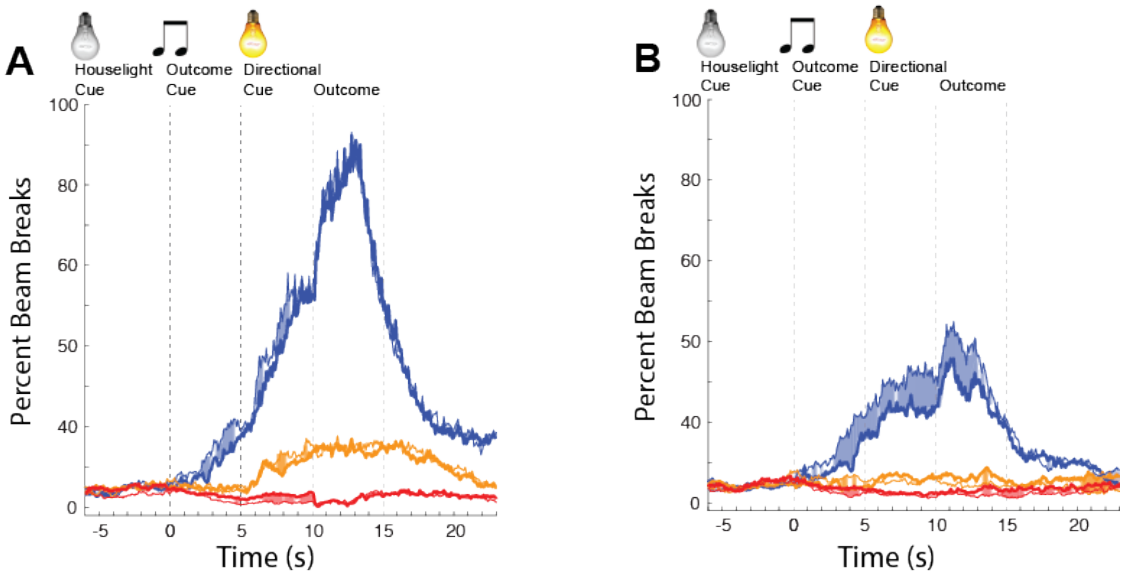

Figure 4. An infrared beam was placed at the entrance to the food cup on the recording rat's side of the cage. This beam was polled every 20 ms, and breakage of this beam is attributable to the recording rat's nose entering the food cup (no beam breaks were recorded during reward delivery to an empty cage). This plot shows the percentage of polls during which the beam was broken, using the same thick/thin lines and color scheme as the DA plots. The filled-in color between alone and together trials represent sliding windows (3-datum bins) where 'together' trials (thick lines) differed significantly from alone trials (thin lines) for that trial type, at a .05 significance level as determined by a 2-tailed paired-sample t-test. **4A:** reflects the beam breaks on trials with outcome delivered to the recording rat (i.e., 'self' trials); **4B:** reflects the beam breaks on trials with outcome delivered to the conspecific (i.e., 'other' trials).

Figure 4 shows the average beam breaks into the food cup across all recording sessions for the three trial-types. First, we examine the 'outcome cue epoch.' During the outcome cue epoch, rats learned what outcome to expect at the end of the trial, but they did not yet know which rat will receive it ('self' or 'other'). We found that during this epoch, beam breaks increased in response to cues that predicted reward relative to neutral and shock cues (Fig. 4A and 4B; *blue lines versus other colored lines in the outcome cue epoch;* all *p*-values < .001). As expected, there were no significant differences between reward-self trials (i.e. in which the reward was delivered to the recording rat) and reward-other trials (i.e. in which the reward was delivered to the conspecific) during the outcome cue epoch (p = .872 alone, .327 together). However, during the 'directional cue epoch,' in which rats learned who (either the recording rat or conspecific) would receive the outcome, there was a significant increase in beam breaks in the food cup for reward-self compared to reward-other trials ($p < 10^{-5}$). Note that both the outcome

cue epoch and the directional cue epochs occurred long before actual reward was delivered, reflecting that the recording rat anticipated the outcome and understood the meaning of the cues.

During shock trials, in which shock rather than reward was the outcome, the opposite pattern emerged; beam breaks into the food cup decreased during the outcome cue epoch to shock cues relative to neutral cues (Fig. 4A and 4B; *red lines versus orange lines in the outcome cue epoch*), and this difference became significantly stronger when the directional light cue conveyed to the recording rat that he would be shocked (i.e., shock-self; Fig. 4; *red lines versus orange lines in the directional cue epoch*; all *p*-values < .002).

These results demonstrate that rats understood cues and predicted future outcomes, with reward and shock trials eliciting more and less beam breaks into the food cup during 'self' trials, respectively. Notably, this pattern of beam breaks between trials types was also present during 'other' trials, but to a lesser degree.

Beam breaks into the food cup during predictive cues is modulated by social context. In the above section, we describe how cues that predicted reward and shock increased and decreased beam breaks into the food cup on reward and shock trials, respectively. Interestingly, the divergence in beam breaks on reward and shock trials (relative to neutral) was amplified when rats were alone (Fig. 4; *thin lines*) during epochs that preceded outcome delivery. There were significant differences between the 'alone' and 'together' trials (Fig. 4A; *thin blue line versus thick blue line*) during the outcome cue epoch on reward-self trials ($p = 0.047$), indicating that the recording rat entered the food cup more often when it was alone than when the conspecific was present. This effect did not obtain significance during the directional cue epoch ($p = 0.12$) or the outcome epoch ($p = .63$).

During shock-self trials (Fig. 4A; *thick red line versus thin red line)*, there were several significant tick marks from the sliding window t-test during the directional cue epoch (i.e. a significant difference was observed between the two groups in several .3-second time windows).

However, this was not significant over the course of the entire epoch ($p = 0.10$), and there was no difference in either the outcome cue epoch or outcome delivery epoch ($p = .53$ and .56, respectively). A similar pattern emerged between the neutral-together and neutral-alone trials: despite miscellaneous tick marks (Fig. 4A; *thick orange line versus thin orange line*), there were no significant differences in any analysis epoch (all p-values > .39).

During reward-other trials (Fig. 4B; *thick blue line versus thin blue line),* we see a similar pattern of divergence in beam breaks between 'together' and 'alone' trials. The percentage of beam breaks in trials when the reward was delivered to the conspecific's side of the cage were significantly higher when the recording rat was alone than when rats were together (Fig. 4B; *thick versus thin blue lines*) during the outcome cue ($p = .013$), directional cue ($p = .024$), and the outcome ($p = 0.031$) epochs. Furthermore, there were no significant differences between 'together' and 'alone' trials during the outcome cue, directional cue, or the outcome epochs for neutral trials (Fig. 4B; *thick versus thin orange lines;* all p-values > 0.22), or shock trials (Fig. 4B; *thick versus thin red lines;* all p-values > .30). Therefore, it appears that social context influenced beam breaks on reward trials, regardless of which rat received the outcome. Overall, we conclude that rats understood task contingencies and that behavior was modulated by social context, possibly reflecting less attention being paid to the valence of the cues in the presence of the other rat.

Video Scoring. Video scoring analysis was performed in order to better understand the rats' interests and behavior during the various trials and epochs. Four criteria (1) Freezing, (2) Approach, (3) Nose orientation, and (4) Conspecific Visibility, were assessed at each epoch of a given trial. We focus specifically on freezing and approach behavior in this section of the main text (see Appendix C for other behaviors). Here, we compared observations of these behaviors in 'self' vs 'other' trials for each trial type in alone and together contexts and across each epoch.

Rats froze to 'self' but not 'other' shock cues. Freezing, which was defined as absence

of movement except for respiration, was a difficult measure to score as it could not be concluded that the rat froze in reaction to a cue if it was already still before the cue. This difficulty is reflected in the low percentages given on the y axis of Figures 5A and 5B, as many of the rats were mostly static throughout all trials in each session. Before discussing the significance of the freezing results in relation to social context, it is important to confirm that the recording rat understood the meaning of the cues. P-values less than 0.001 computed from t-tests reveal that the average number of times the recording rat froze during alone-shock-self trials during the directional cue is significantly different than the number of times the rat froze during all 11 other scenarios (for the directional light epoch) except together-shock-self. Moreover, the data in Fig. 5 show that the rat froze a higher percentage of the time during self-shock trials (alone and together) than any other trial. These data imply that the recording rat understood the cue tone and directional light. During the self-shock trials, the rat knew from the tone that there would be a shock and knew from the directional light that they would be shocked. The rat's understanding is further supported by the fact that the recording rat freezes less during the directional light epoch during reward and neutral trials than it does during shock trials.

Social context increased freezing behavior during the outcome epoch of shock-self trials. As mentioned earlier, there was no significant effect of social context on freezing during the directional cue epoch of shock-self trials, implying that the freezing behavior of the rat is unaffected by the presence of the conspecific. However, the recording rat froze significantly more during the outcome epoch of these trials ($p = .0148$). In other words, the shock administered to the recording rat in the outcome epoch of shock-self trials resulted in more freezing in the presence of the conspecific than when the recording rat was alone.

The results from several metrics suggest that the recording rat does not express fear when the conspecific receives a shock. During the directional light epoch, the percent of freezing for alone-other-shock is not significantly different than the percent of freezing for together-other-

shock (p = 0.94), denoting that the recording rat does not exhibit fear towards the fate of the conspecific. Similar behavior occurs during the outcome epoch of shock-other trials: the freezing behavior of the recording rat during alone-shock-other is not significantly different than the freezing behavior during together-shock-other (p = 0.449). In one definition, these relations give proof to the idea that the recording rat does not display empathy or understanding towards the state of the conspecific during shock-other trials.

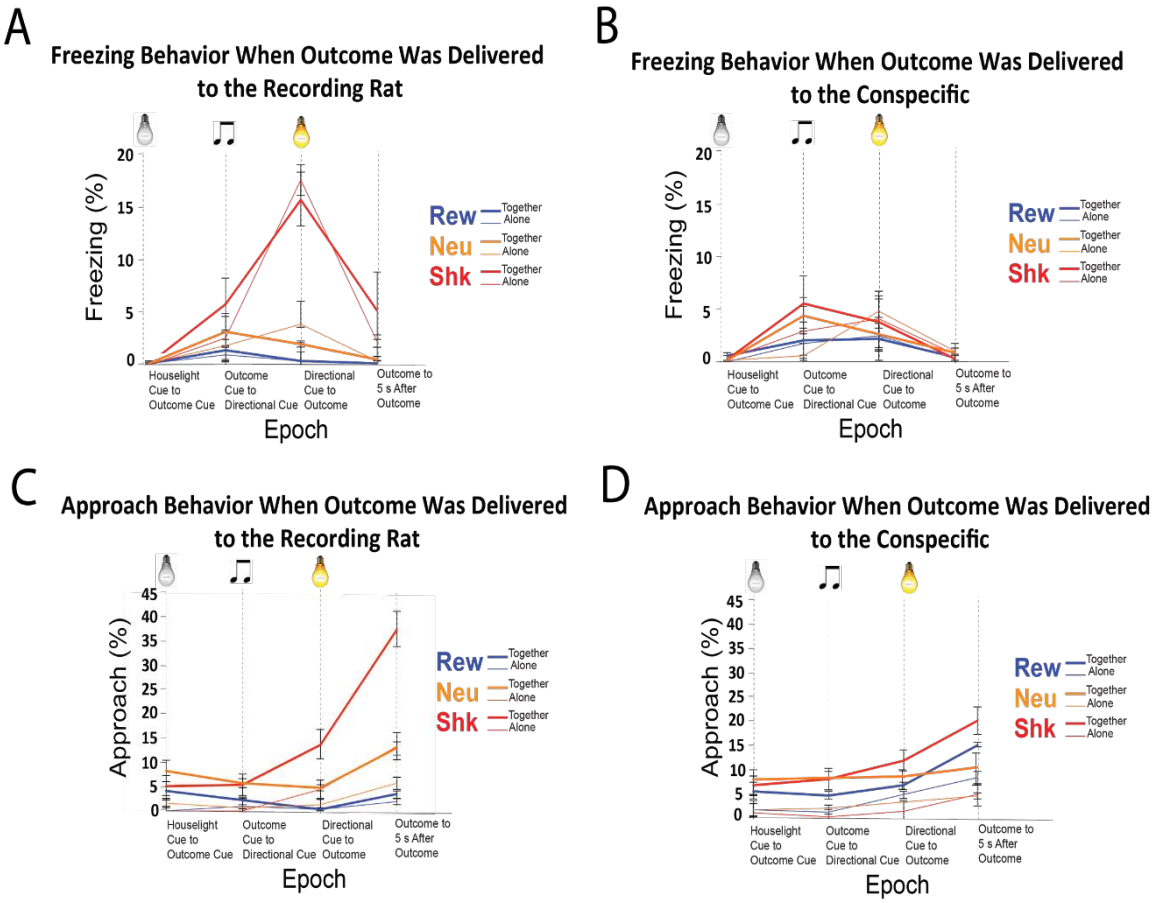

Figure 5. Plots A and B show the percentage of trials in which freezing behavior was observed during each specific epoch. Thick lines represent 'together' trials, while 'thin' lines represent 'alone' trials. **5A:** reflects freezing behavior on trials with outcome delivered to the recording rat ('self' trials) and **5B:** reflects freezing behavior on trials with outcome delivered to the conspecific ('other' trials). Plots C and D show the percentage of trials in which the recording rat approached the mesh divider. **5C:** reflects approach behavior on trials with outcome delivered to the recording rat and **5D:** reflects approach behavior on trials with outcome delivered to the conspecific. See Appendix C for result of other scored behaviors.

The recording rat approached the conspecific on shock trials. Approach was defined as the movement of the recoding rat in the direction of the mesh divider. The motivation for this

behavior varied by trial type and outcome recipient. Rats approached the mesh divide more in together trials than alone trials across trial types during outcome cue, directional light, and outcome epochs (Figure 5C-D; all *p*-values < .01). This suggested that the presence of the conspecific was significant in motivating approach behavior during shock trials and that approach was socially motivated.

When the rats are together, approach behavior appears to be less salient during reward trial types. When the rats were together, approach occurred less in reward trial types than neutral trial types during the outcome cue ('self' trials $p < 0.001$, 'other' trials $p = 0.03$), directional light ('self' trials $p < 0.001$), and outcome ('self' trials $p < 0.001$) epochs. This could be attributed to the recording rat going to the food cup during the reward trial types as eating is a more salient behavior, which explains why there were no significant differences in approach between reward and neutral trial types during the directional light ($p = 0.09$) and outcome ($p = 0.81$) epochs for other trials. Additionally, the significant differences in approach between reward and neutral trial types suggested that the rats were able to discern trial types by audio cues and direction of outcome with the directional light.

Approach was more salient during shock trial types when the shock was delivered to the recording rat than when it was delivered to the conspecific. When rats were together, the recording rat approached the mesh more for the shock trial types than reward (for 'self,' $p < 0.001$) or neutral (for 'self,' $p < 0.001$; for 'other,' $p = 0.02$) trial types during the outcome epoch. The differences in approach during the outcome epoch between shock and neutral types were 25.6% for 'self' trials and 12.0% for 'other' trials, and between shock and reward types were 36.6% for 'self' trials and 11.2% for 'other' trials. While there was no statistically significant difference when the rats were together between shock and reward trial types during the outcome epoch (for 'other,' $p = 0.06$), shock trial types did have more approach behavior than reward trial types. When the rats were together, approach occurred significantly more when the outcome was

delivered to the recording rat than when it was delivered to the conspecific ($p < 0.001$). Similar results were observed for the conspecific in scoring of conspecific presence (see Appendix C).

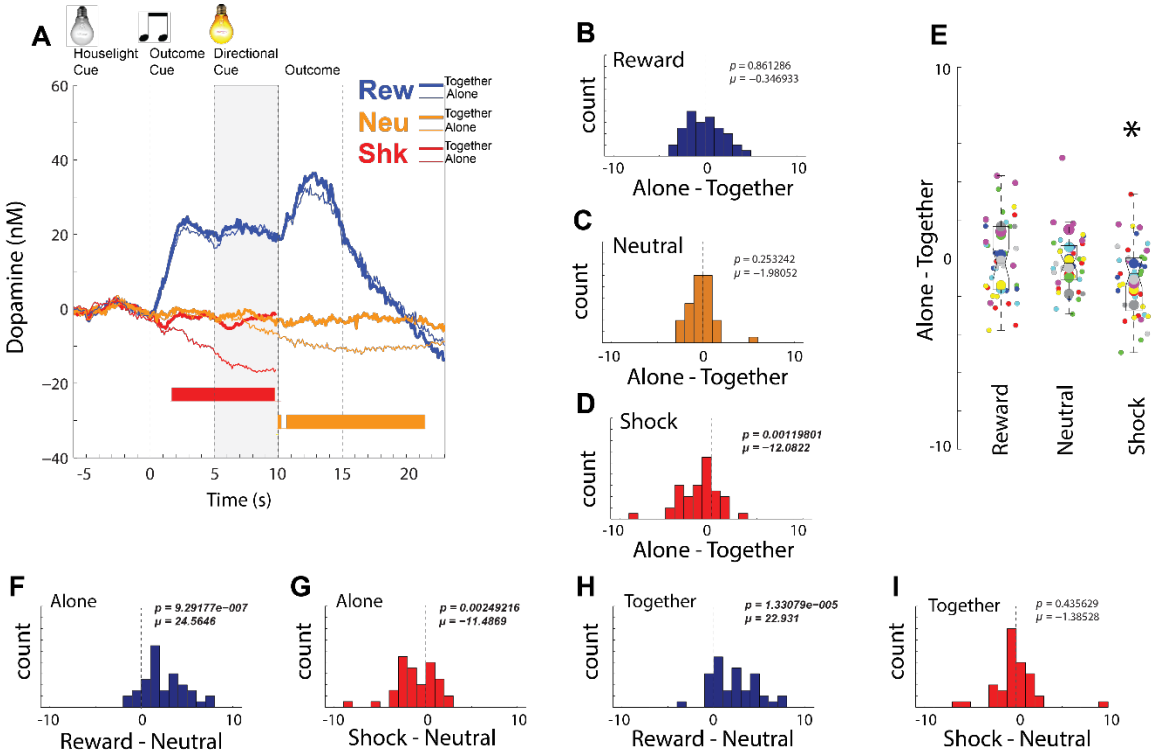

Figure 6. Dopamine release when the outcome was delivered to the recording rat. Epochs refer to the 5 second time periods after each relevant trial event (house light, outcome cue, directional cue, and outcome delivery). **6A:** DA release measured every 100 ms (n=40 sessions, 8 rats represented by each line). The colored ticks underneath represent sliding windows (3-datum bins) where 'together' trials (thick lines) differed significantly from alone trials (thin lines) at a .05 significance level as determined by a 2-tailed paired-sample t-test. DA release was excluded from analysis during the outcome epoch due to electrical noise from the shock interfering with the recording electrodes. **6B-6D, 6F-6I:** Histograms show distribution plots between groups: each data point is a difference between the mean DA release of each sample group in the directional cue epoch, and the test statistic assesses the degree to which the collection of points constitutes a significant difference between the groups. **6E:** The multicolored plot on the right shows these individual differences as points: each point represents a recording session, and each larger dot represents the collection of all recording sessions from each pair of behaving animals. Note that no single recording session or animal is responsible for the trends that we see between 'together' and alone trials across these groups. The asterisk indicates a Wilcoxon signed-rank test *p*-value <.05 (as shown in **6D**).

Dopaminergic signaling when outcome was delivered to the recording rat. The results from trials in which outcomes were delivered to the recording rat (i.e., 'self' trials) are shown in Fig. 6. Consistent with patterns established in previous literature, when the recording rat was alone, DA release increased to cues that predicted reward-self trials significantly more than neutral trials in the outcome cue, directional cue, and outcome epochs (Fig. 6A; *blue lines versus other colored lines;* all *p*-values < 10^{-8}) and DA release was inhibited in response to cues that

predict shock (Fig. 6A; *red lines versus other colored lines*; all *p*-values < 10^{-7}).

During reward-self trials (Fig. 6A; *blue lines*), we found no significant difference in DA signaling between 'alone' and 'together' trials. While there appeared to be some separation in the outcome epoch (Fig. 6A; *thick blue line versus thin blue line after outcome delivery)*, this effect was not significant ($p = 0.63$). During neutral-alone trials, we observed a steady decrease in DA release towards the end of the trial that was mitigated in neutral-together trials (Fig. 6A; *thin orange line versus thick orange from 10 s through 15 s)*. However, this effect was not significant: despite the sliding window t-test showing significance in 46 out of a possible 48 windows in the outcome epoch, the 'together' and 'alone' groups were not significantly different in the more general t-test across the outcome epoch ($p = 0.061$).

During shock-self trials (Fig. 6A; *red lines*), we found that the decline of DA release related to cues predicting shock was reduced in the presence of the conspecific *(*Fig. 6A; *thick versus thin red lines after outcome cue onset)* during the outcome cue ($p = 0.019$) and directional cue epochs ($p = 0.001$). To further quantify differences between 'alone' and 'together' trials we computed an index for each session that simply subtracted DA release on 'alone' trials from DA release on 'together' trials during the directional epoch. The signed-rank plot in Figure 6D shows that the paired differences in session averages of DA release, was significantly shifted in the positive direction during shock trials only, demonstrating that in the majority of sessions DA release was lower during shock-self-alone trials compared to shock-self-together trials (Wilcoxon; $p < 0.05$). Additionally, the box and whisker plot for alone/together in shock trials (Fig. 6E; *"shock"*) plots these paired difference values, demonstrating that the bulk of the sessions (small circles) and all of the averages across individual rats (large circles) fall between the first and third quartiles, and that the overall deviation from 0 (no difference) is due to a general effect across rats and sessions.

Dopaminergic signaling when outcome was delivered to the conspecific. The results from trials in which outcomes were delivered to the conspecific rat (i.e., 'other' trials) are shown in Fig. 7. After the outcome cue signaled a reward trial, there was an increase in DA release above neutral cues similar to what was observed during reward-self trials. Note, during this phase the rat was unaware of whether it was going to be a 'self' or an 'other' trial-type. However, after the directional cue was illuminated, and the recording rat became aware that reward was going to be delivered to the other side of the box, DA release declined to pre-cue levels for both 'alone' trials (Fig. 6A *thin blue line vs* Fig. 7A *thin blue line; p* = 3.12E-06 after the outcome cue) and 'together' trials (Fig. 6A *thick blue line vs* Fig. 7A *thick blue line; p* = 3.06E-06 after the outcome cue). As a result, DA release was significantly lower when reward was delivered to the conspecific compared to when reward was delivered to the recording rat ($p < 0.05$).

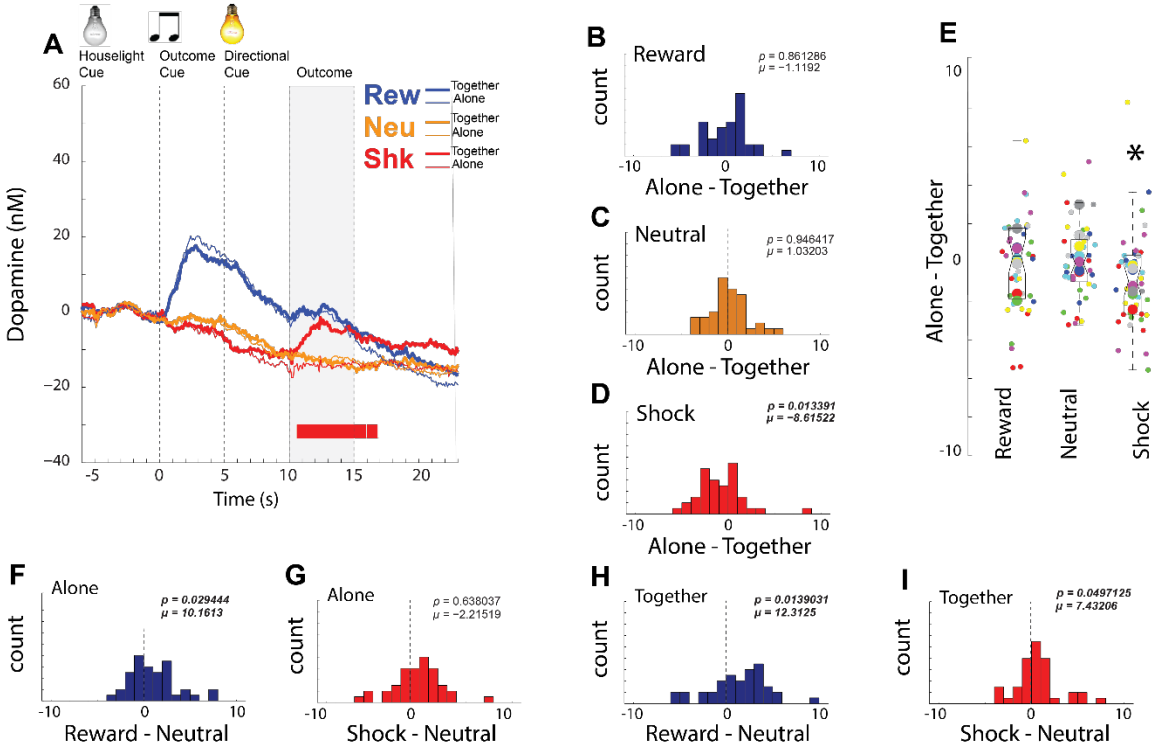

Figure 7. Dopamine release when the outcome was delivered to the conspecific (see Fig. 6 caption for details). The asterisk again indicates a Wilcoxon signed-rank test *p*-value <.05 (as shown in **7D**).

This was true for both alone (Fig. 7A; *thin blue line versus thin orange line; p* = 3.27E-06 after the outcome cue, *p* = 1.51E-04 after the directional cue, *p* = .012 after the outcome) and

'together' trials (Fig. 7A; *thick blue line versus thick orange line; p* = .001 after the outcome cue, *p* = .007 after the directional cue, *p* = .018 after the outcome) and there was no significant difference between them (*p* = .66 after the outcome cue, *p* = .31 after the directional cue, *p* = .59 after the outcome), which suggest that DA patterns during the trial were not specific to social context. Likewise, the reward-neutral signed-rank plot shows significant difference over the course of the outcome epoch across all recording sessions for 'alone' (Fig. 7F; *p* = 0.029) and 'together' trials (Fig. 7H; *p* = 0.014).

Further evidence that social context did not impact reward and neutral trials come from direct comparison of 'alone' versus 'together' trials individually for reward and neutral trial types. During reward-other and neutral-other trials, there were no significant differences in DA release between 'together' and 'alone' trials across all trial epochs (Fig. 7A; *thick blue line versus thin blue line; p* = .255 after the houselight cue, *p* = .845 after the outcome cue, *p* = .951 after the directional cue, and *p* = .780 after the outcome; Fig. 7A; *thick orange line versus thin orange line; p* = .720 after the houselight cue, *p* = .720 after the outcome cue, *p* = .781 after the directional cue, and *p* = .742 after the outcome).

Although reward-other and neutral-other trials did not differ between alone and neutral trials, remarkably, DA release on shock-other trials was higher when rats were together. When shock was delivered to the conspecific's empty side of the cage in 'alone' trials, DA release did not significantly diverge from neutral-alone trials for all epochs (Fig. 7A; *thin red line versus thin orange line; p* = .335 after the houselight cue, *p* = .327 after the outcome cue, *p* = .396 after the directional cue, *p* = .605 after the outcome). However, when the conspecific was present, DA release was higher after shock was delivered to the conspecific compared to neutral trials in which no outcome was delivered to the conspecific (Fig. 7A; *thick red line versus thick orange line; p* = .054 after the outcome). DA release was also significantly higher after shock was delivered to the conspecific compared to when shock was delivered to the conspecific's empty

side of the cage (Fig. 7A; *thick red line versus thin red line; p* = .036 after the outcome), which suggests that DA release during shock trials was influenced by the conspecific's presence. Fig. 7I demonstrates significant difference over the course of the outcome epoch across all recording sessions ($p = 0.013$); no rat or recording session was driving this significant result, as reflected by the box and whisker plot (Fig. 7E).

Discussion

These results suggest that DA signals that may be construed as 'empathetic' were not present within the context of this experiment. That is, DA release during self-trials differed from DA release during other-trials, in that DA release in response to cues that predicted positive and negative cues and outcomes directed to the 'self' differed from DA release in response to cues and outcomes directed to the conspecific. However, social context, as defined by the presence of a conspecific, did clearly modulate DA release in the nucleus accumbens in ways that might reflect consolation and self-preservation.

In order to interpret our data, we first needed to confirm that the rats understood the task fully, and that their responses were thus interpretable within the context of the paradigm. In Figure 4, we see that the distribution of average beam breaks for each trial type clearly indicates that the rats had a basic understanding of the trial cues. The beam break data reflect the average frequency with which the recording rats checked their food cup. A perfect understanding of the light and sound cues would lead to the rat checking their food cup during trials in which they would receive reward. Indeed, we see that the percentage of beam breaks was highest when the recording rat received the reward and the lowest during the shock outcome, both for the recording rat and the conspecific. This pattern suggests that the recording rats associated each sound cue with the correct respective outcome – they were more likely to check their food receptacle when the delivery of a reward was indicated than when a shock outcome was indicated. The overall pattern of DA release followed expected trends: increased DA release (relative to neutral) to cues

that predict reward, and inhibited DA release (relative to neutral) to cues that predicted shock, consistent with the notion that DA release reflects predicted value.

As described in the introduction, 'empathy' is defined as the awareness of the affective states of others. We hypothesized that 'empathetic signaling' would be reflected as a similar DA release when an outcome was delivered to the 'self' (recording rat) and to the 'other' (conspecific); this hypothesis was not supported. During reward trials, we observed that DA release increased significantly during the outcome cue when the reward was delivered both to the conspecific and to the recording rat; this was expected, as the rats had not yet discovered who would receive the reward. During the directional cue however, DA signaling diverged: while DA release increased for 'self' trials, it decreased and remained low for 'other' trials. This is in direct contrast to an empathetic signaling hypothesis, in which we would expect increases in DA release for both 'self' and 'other' trials. It could be argued that DA release during reward-other trials was higher than neutral-other trials. However, there was no significant difference in DA release between reward delivered to the conspecific ('together') and reward delivered to an empty cage ('alone'). This indicates that reward-related DA signals in NAc were not modulated by the presence of the conspecific. Based on these findings, social context does not seem to significantly affect DA release during reward trials.

DA release during shock trials revealed different effects. For trials in which the outcome and delivery cues signaled delivery of shock to the recording rat, for example, the inhibition of DA release that is normally associated with the prediction of shock to the 'self' is entirely mitigated by the presence of a conspecific. This pattern is visualized as a dissipation of inhibition – a small decrease followed by a large increase in DA release immediately after the sharp drop associated with the shock cue. This dissipation is seen only when the conspecific is present (thick red line) and not when the conspecific is absent (thin red line), suggesting that DA responses during shock-self trials are dependent on social context. More specifically, each aspect of this

pattern – the small dip and the immediate rise back to baseline (Fig. 6A thick red) – tell us about the impact of social context in different ways. The initial dip indicates that reward processing occurs as expected; naturally, DA release should decrease when a shock – an unpleasant, painful experience – is predicted. However, the bounce back in the presence of a conspecific indicates that the presence of another rat modulates DA reward prediction. We propose that 'consolation' at least partially explains this result. As described in the introduction, animals such as prairie voles are known to demonstrate conciliatory behaviors to purposely reduce stress in partners (Burkett et al., 2016). In that context, consolation was defined as an increase in contact by an uninvolved bystander toward a distressed individual, which produces a calming effect. In our experiment, the partition separating the cages prevents physical contact between rats, but approach behavior during certain trials can be interpreted as an attempt to engage in a conciliatory interaction. When the conspecific received the shock outcome, recording rats did not approach the conspecific. However, when the recording rat received the outcome, the recording rats did approach the conspecific, suggesting that they were seeking social interactions prior to and during shock delivery. This could be interpreted as 'consolation-seeking' behavior, which might be reflected by the return to baseline of DA release in the recording rat. Thus, the definition of consolation from Burkett et al. does not fit the data of our experiment, as uninvolved bystanders (i.e. recording rats) did not approach distressed individuals (i.e. conspecific rats). Instead, we propose that the recording rats' approach behavior during their own shock could be defined as consolation-seeking behavior. The presence of this behavior further suggests that social context modulates DA reward prediction.

Alternatively, we found that shock delivered to the conspecific corresponded with an increase in DA release during the outcome delivery, compared to no change when the shock was delivered to an empty chamber. This is likely due to confirmation of the recording rat's safety, mediated by either (1) the recording rat's ability to physically see and hear the effects of shock

delivered to the conspecific, making the rat more certain that it would not be shocked, or (2) the sense of relief corresponding with the conspecific's presence. The latter seems unlikely, as the presence of the conspecific could be expected to have changed DA release earlier in the trial. Therefore, it appears that this increase in DA release when a conspecific rather than an empty cage is being shocked is modulated by a confirmation of the recording rat's safety.

These results are counter to our original hypothesis. A positive empathetic signaling hypothesis of DA release in rats would suggest that when the conspecific was shocked, the recording rat would exhibit DA inhibition similar to its own shock. Instead, we see an increase in DA release during the outcome cue during shock-other trials. This is likely due to the self-preservation instinct being more salient than empathy in this situation. While some empathetic signaling is seen in shock-self trials, it is mitigated by what appears to be a sense of self-preservation or "relief" that the recording rat itself is not shocked**.** An alternative explanation for this phenomenon could be the idea of negative empathetic ("vindictive") signaling, the idea that the recording rat recognizes the affective state of the conspecific but that this recognition is reflected in DA signaling that is opposite of what it would be had the recording rat received the treatment. However, this negative empathetic hypothesis of dopamine signaling would also call for inhibition of DA release when reward was delivered to the conspecific as compared to an empty cage, which we did not observe.

We found that DA release during neutral trials was also affected by social context, but only during 'self' trials in which the neutral outcome was delivered to the recording rat. After the outcome epoch, DA release remained at baseline when rats were together and significantly dipped when they were alone during neutral-self trials. Although 'neutral' was not a negative outcome, it is possible that the recording rat was appeased by the presence of the conspecific. This could be due to social engagement, defined as the positively valanced interactions or preference of animals to socialize with conspecifics. Although the rats were not necessarily socializing across the

chamber division, the simple preference for the company of another animal may be more relevant than any implications of empathy (Silberberg et al., 2014). One limitation of this social engagement explanation is that we do not see a similar finding during 'other' neutral trials; there was no significant difference between 'together' and 'alone' conditions when neutral outcome was delivered to the conspecific. If social engagement was influencing DA release in the presence of the conspecific, we would expect to see a similar decrease in DA release on 'alone' neutral-other trials; instead we see a similar decline in DA release on both 'together' and 'alone' neutral other trials. In fact, DA release drops in all three trial types after the directional cue. This is most likely because said cue indicates that the events of the trial are no longer salient, as they are not delivered to the recording rat.

Behavioral beam break data provide an alternative explanation. These data demonstrated that the recording rat was more likely to check the food cup during 'alone' neutral-self trials. Because these were neutral trials, there was no reward in the food cup, therefore the decrease in DA release could reflect the rats' "disappointment" (i.e. neutral outcome was worse than expected).

In conclusion, we did not find any evidence of empathetic signaling as previously defined, however we did find self-preservation signaling patterns in shock-other trials, consolation in shock-self trials, and preference for presence of the conspecific in neutral-self trials, which indicates that social context does in some ways modulate DA release in the rat nucleus accumbens.

Limitations and Future Directions. While social context effects discovered in this experiment provide a promising stepping stone to further research, this experiment had some limitations. First, the electrical noise caused by shock delivery did not allow accurate interpretation of DA data during the outcome epoch of shock-self trials (see Fig. 6A, abrupt stop in thick red line at 10 s). Therefore, we relied on past literature to inform our expectations about

DA signaling during delivery of shock. Additionally, DA data in an animal model are not wholly illustrative of emotional and cognitive processing, an understanding of which is necessary for elucidating true empathy. Rather, they provide insight into signaling effects or correlates of our definition of empathy. We attempted to understand more about the internal affective states of the rats by collecting ultrasonic vocalizations (USVs). However, these data were unusable due to artifact noise in the ultrasonic range from some of the cue tones; we also only collected USV data from one microphone, and therefore were unable to determine which rat was emitting which vocalizations. Yet another limitation of this experiment was the passive nature of the paradigm, which restricted inferences about the potential influences of social context on behavior. We attempted to address this problem of passivity in our second experiment by introducing a force-choice lever task into the paradigm.

Chapter 2: Impact of oxytocin during reward-guided decisions that impact conspecific distress

Introduction

In recent years, oxytocin (OT) has been established as an important peptide hormone with an array of behavioral and neurochemical effects in both animal and human models. For example, OT has been shown to affect the perception of trust in human behavioral studies by altering neural circuitry. Examination of brain activity revealed that humans who received intranasal OT tended to display decreased activity within the amygdala and midbrain regions, which mediate fear processing, as well as in the dorsal striatum, which mediates behavioral adaptation to feedback information (Baumgartner et al., 2008). Within the OT treatment group, there were no changes in trusting behavior after human subjects learned that their trust had been breached multiple times. In contrast, the control group experienced a marked decrease in trusting behavior (Baumgartner, 2008). This study, and others like it (as described in the general introduction), suggest that oxytocin plays a critical role in decision-making within social contexts.

The relationship between OT and empathy has also been examined with animal models, particularly in the study of relationships between pair-bonded prairie voles. Research demonstrated that paired prairie voles offered consolation behavior in response to stress, and often showed correlated changes in stress hormones when only one vole in the pair was shocked (Burkett, 2016). When oxytocin receptors were blocked in prairie voles, this correlation was absent, and the voles failed to offer consolation to their bonded partner during shock (Burkett, 2016). This suggests that interference in OT pathways within the brain has significant effects on both behavior and neurochemical processes, as shown by the occurrence of more qualitatively "selfish" behavior with greater frequency when OT receptors are blocked.

Within another animal model, the rat, past literature shows that OT influences the behavior of rats in a social context. One study conducted by Calcagnoli et al. (2015)

demonstrated that the application of OT to male rats had both anti-aggressive and pro-affiliative effects, which was especially prominent when OT was introduced to rats intranasally. Specifically, introduction of intranasal OT tended to reduce aggressive behavior towards a male cagemate, increase social exploration toward an unfamiliar male conspecific, and strengthen bonding between a male rat and its female partner. Thus, intranasal application of OT has been shown to produce a significant alteration of behavior in male rats within a social context, but the precise mechanism and neural circuits involved in this process remain unclear.

Previous research indicates that the presence of OT enhances social bonding within a community in both humans and animals, but is unclear whether OT can impact other prosocial behaviors that result in personal gain at the cost of harm to others. To address this issue, we trained rats on a novel instrumental task where lever pressing for reward sometimes produced harm to a nearby conspecific. We predicted that intranasal administration of OT would reduce lever pressing when it also resulted in shock to a conspecific. However, as we will describe below, OT administration had little effect on behavior, and it did not make rats more prosocial.

Methods

Subjects. Twenty male Sprague-Dawley were used in this experiment. The first round of the OT experimentation was run with eight rats, with four in the experimental group and four in the control group. The second round of the experimentation was run with twelve total rats, with five rats in the experimental group and six rats in the control group; one rat in the experimental group was excluded due to experimental error. Rats were obtained from Charles River Labs, and were individually housed on a 12-hr light–dark cycle and tested during the light phase. Experiments were conducted during the day. Water was available *ad libitum* and body weight was maintained at no less than 85% of pre-experimental levels by food restriction (14-15g of laboratory chow daily in addition to approximately 2.5g of sucrose pellets (Test Diet) consumed during daily experimental sessions). All experiments were approved by the University of

Maryland, College Park under university and NIH guidelines.

Oxytocin Administration. OT solution was prepared by mixing a stock solution of 10 mg/ml OT from 15 mg OT powder and 1.5 ml of saline solution. The stock solution was then further diluted so that the solution given to the subjects was a 1:9 ratio of OT stock solution to saline to make a 1 ml aliquot of 1 mg/ml solution. Rats in the experimental group were given a dose of OT solution applied intranasally thirty minutes before the start of each experimental session, as in Calcagnoli et al. (2015). This allowed the OT to be fully taken into rat's system and reduced the effects of stress from the procedure on the results of the experiment. The OT solution was applied by two experimenters. One experimenter held the conscious rat in the supine position upon a table, with the body held with one hand and the head held in the other, with the head in a negative pitch (Fig. 8A). The other experimenter used a 100 μl pipette to apply the OT solution equally on the squamus epithelium of the rhinarium (Fig. 8B). An equal amount of OT solution was applied to each side of the rhinarium, while ensuring that OT solution did not fall into the nostrils and no direct contact was made between the pipette tip and the rhinarium (Calcagnoli et al., 2015). The control group was given a 0.01M solution of saline using the same intranasal method. Before using this administration procedure, rats were gradually handled for several days in increasing periods of time to further minimize the stress of intranasal application during the experimental period.

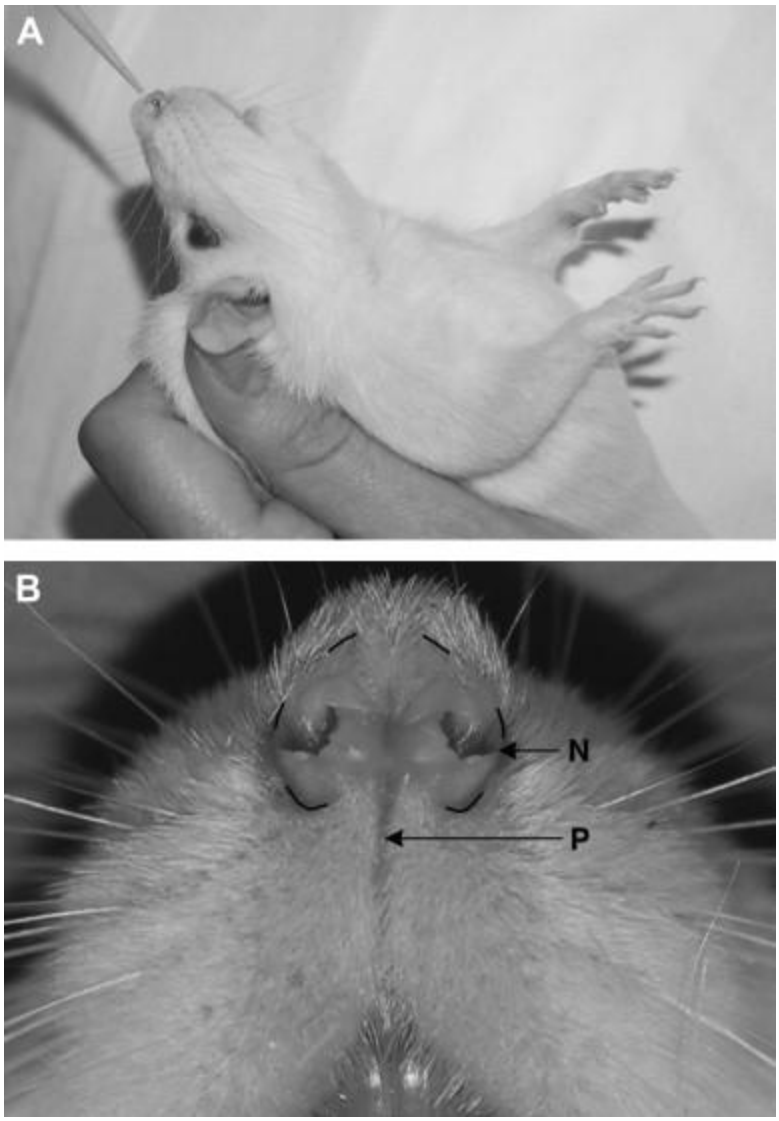

Figure 8. (Calcagnoli et al., 2015) 30 minutes prior to experimentation, oxytocin (OT) or saline was administered intranasally to rats. **8A.** Rats were held in the supine position with the head in a negative pitch during administration. **8B.** 1 mg/ml OT solution or 0.01M saline solution was applied equally on the squamus epithelium of the rhinarium (N). An equal amount of solution was applied to each side of the rhinarium, while ensuring that it did not fall into the nostrils and no direct contact was made with the rhinarium.

Behavioral Task. Animal subjects underwent behavioral training in a modified shuttle box chamber (16 in x 6.25in x 8.375 in; WDH; Med Associates). A modified guillotine door with wire mesh covering the opening divided the chamber in two equal compartments. Animals were trained to lever press for palatable sucrose pellet rewards; on each day, rats were placed on one side of the chamber and allowed to instrumentally respond until about 100 pellets were earned or 45 min had elapsed. During conditioning, a cue light turned on and a lever remained extended

until a response at the lever occurred. No auditory stimuli were presented. A lever press resulted in offset of the cue light, reward delivery into a food cup, and lever retraction. Conditioning sessions took place for 9 days after the rat acquired an instrumental response. This was done so that the rat could understand the meaning of each cue type (excluding shock), learn how to use the lever in the active task, and become familiar with their conspecific. For each subject, a conspecific rat was placed on the opposite side of the chamber on the last two days of conditioning in order to familiarize the rats with each other; animals could see and smell each other through the wire mesh. Each rat was paired with the same conspecific throughout the experiment. Conspecifics were of the same age and sex, were ordered at the same time, and housed next to each other in separate cages.

On day 10, the distress task was introduced (Fig. 9) in which a lever press resulted in lever retraction, reward delivery, and one of three additional outcomes: no shock ('reward-only'), foot shock to OT or saline treated rat ('shock-self'), or foot shock to conspecific ('shock-other'). As long as the OT or saline treated rat pressed the lever during the trial, it was given a reward for all trial types. The paradigm began with the activation of the 'houselight cues;' instead of having one houselight in the center of the cage, simultaneous activation of one light on each side of the cage signaled the start of the trial. Next, one of three discrete auditory tones (tone, white noise, clicker) corresponding with three discrete outcomes (reward-only, shock-self, or shock-other) began 5 seconds prior to lever extension and terminated upon a lever press. A lever press for all trials resulted in either an immediate reward delivery or an aversive foot shock (3 seconds, 0.56 mA) 2 seconds after the lever press, depending on the trial type. Auditory cues were counterbalanced across animals. During trials in which the animal did not press, the lever retracted and the auditory cue terminated 5 seconds after lever extension; reward or shock did not occur. Trials were randomly delivered in a session and the ratio of 'reward-only', 'shock-self', and 'shock-other' trials was 1:1:6 (shock trials occurring 12.5 percent of the time each, and

neutral trials occurring 75 percent of the time). Daily recording session lengths were about 1 hour. Lever pressing was calculated by average number of levers pressed per each trial type across all animals in the group for each day of the experiment.

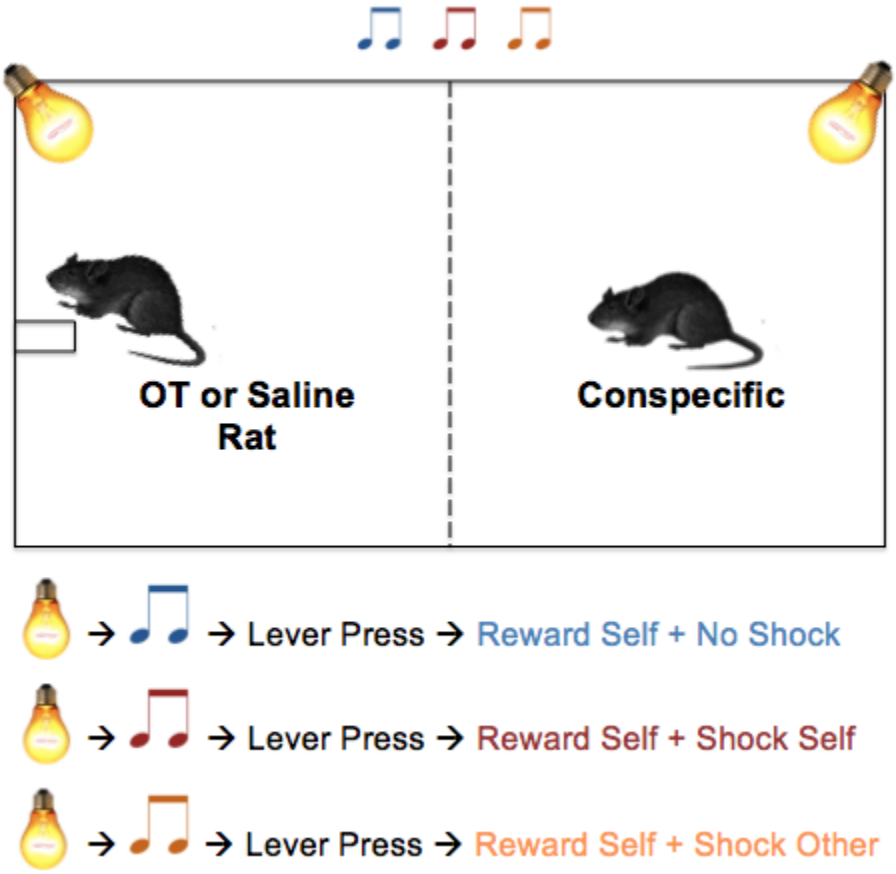

Figure 9. First, the house lights (yellow) were activated to signal the start of the trial. Next, one of three discrete auditory tones (tone, white noise, clicker) corresponding with three discrete outcomes (reward-only, shock-self, shock-other) was delivered. 5 seconds later, a lever was extended on the side of the OT/saline rat. A lever press for all trials resulted in an immediate reward delivery to the OT/saline rat (reward-only); on some trials, this reward was accompanied by an aversive shock to 'self' or to 'other'. During trials in which the animal did not press, the lever retracted and the auditory cue terminated 5 seconds after lever extension; reward or shock did not occur.

Reaction Time. In addition to measuring percent lever pressing during the experimental sessions, we measured reaction time. Reaction time is defined by the latency at which the rats pressed the lever after it was extended. Examination of reaction times can provide further insight into the psychological processes involved in decision-making and behavior (Blokland, 1998). Faster reaction times are often associated with automatic or habitual responding, and can be used as a measure the motivational level of animal, e.g. rats respond faster if they predict a large

reward compared to a small reward (Avila & Lin, 2014). Slower reaction times during decision making often reflect that the decision is difficult or there is not enough information to make a decision or that the animal is taking longer to contemplate their options when directing behavior in a goal-directed fashion (as opposed to automatic or habitual responding). Reaction times were averaged across each trial type across all animals in the group for each day of the experiment.

Experimental Periods. Conditioning without tones occurred for 9 days. During the main experiment, there were four experimental periods that lasted 4 days each. In experimental period 1, the experimental (oxytocin) and control (saline) groups were given no treatment and run together with conspecifics. In period 2, the two groups (saline and oxytocin) were given treatment and run with conspecifics. In period 3, the two groups were given treatment but run without conspecifics present. Lastly, period 4 was identical to period 1.

Data Analysis. For data analyses, we measured averaged the average lever pressing and average reaction times per trial type across all animals in the group for all days in an experimental period. We performed a multifactor ANOVA with groups, time, and trial-type as factors. We also performed post-hoc t-tests with the same factors.

Results

The average lever pressing across experimental phases for each trial type and each group is shown in Figure 10. In this figure, reward-only, shock-self, and shock-other trial types are represented by blue, red, and orange, respectively, whereas groups are denoted by solid (oxytocin) and dashed (saline) lines. Below, we break data down by experimental period; however, initially we performed a multifactor ANOVA with groups, time and trial-type as factors for both average lever pressing and average reaction times. In the average lever pressing ANOVA, there were significant main effects of trial-type ($F(464166) = 1114.02$, $p < 0.05$) and time ($F(2352) = 5.8$, $p < 0.05$) with no main effect of group ($F(36) = 0.09$, $p = 0.77$). Although the interaction between trial-type and time was significant ($F(1813.2) = 4.47$, $p < 0.05$),

interactions between group by time($F(360) = 0.89$, $p = 0.57$), group by trial-type ($F(405) = 0.89$, $p = 0.57$) and group by time by trial-type ($F(148) = 0.37$, $p = 0.99$) were not significant.

In the average reaction times ANOVA, there was a significant main effect of trial-type ($F(114) = 145$, $p < 0.05$) with no main effect of group ($F(0.06) = 0.08$, $p = 0.78$) or time ($F(1.2) = 1.6$, $p = 0.08$). Although the interaction between trial-type and time was significant ($F(1.637) = 2.08$, $p < 0.05$), interactions between group by time ($F(0.5) = 0.67$, $p = 0.80$), group by trial-type ($F(0.4) = 0.54$, $p = 0.59$), and group by time by trial-type ($F(0.6) = 0.73$, $p = 0.84$) were not significant. Below, we perform post-hoc t-tests for each experimental period.

Experimental Period 1 (Days 1-4). In Experimental Period 1, the rats in both groups received no treatment and were together with their conspecific during the experimental sessions. As expected, rats pressed significantly more for reward-only trials compared to shock-self trials (Fig. 10) (t-tests; all p-values < 0.05). Recall, that for both of these trial types, the rat received reward, but only on shock trials did they also receive a shock upon the lever press. Rats tended to avoid pressing the lever when the tone indicated that the lever pressing rat would be shocked in addition to receiving reward (i.e., shock-self trials). Thus, the rats distinguished between tones and decided that the expected reward was not worth personally enduring the shock.

Next, we asked if shock directed toward the conspecific deterred the experimental rat from seeking reward. During Experimental Period 1, the rats pressed the lever significantly less during shock-other trials (Fig. 10; *orange lines*) compared to reward-only trials (Fig. 10; *blue lines*). Although lever pressing was significantly reduced when there were aversive consequences to the conspecific, it was not to the same degree as was observed on shock-self trials. We conclude that rats did reduce reward seeking on trials during which the conspecific would be shocked, however rats were more sensitive to manipulations that impacted their own well-being.

As previously mentioned, both groups of rats did not receive treatment during this experimental period. Therefore, significant differences in lever pressing nor reaction times were

neither expected nor observed (t-tests; all *p*-values > 0.05).

Experimental Period 2 (Days 5-8). In Experimental Period 2, the rats in the oxytocin group received intranasal oxytocin administration (Fig. 10 and Fig. 11; *solid lines*), and the rats in the saline group received intranasal saline administration 30 minutes prior to the start of the experiment (Fig. 10 and Fig. 11; *dashed lines*). The rats in both groups were together with their conspecific during the experimental sessions. For the oxytocin group, the average lever pressing for reward-only trials increased from 77% to 93%; the average lever pressing for shock-other trials increased from 51% to 64% (Fig. 10). For shock-self trials in the oxytocin group, the average lever pressing decreased from 16% to 7%. For the saline group, the average lever pressing for reward-only trials increased from 78% to 90%; the average lever pressing for shock-other trials increased from 53% to 68%. For self-shock trials in the saline group, average lever pressing decreased from 21% to 10% (Fig. 10).

Thus, average lever pressing for both groups decreased during self-shock trials (Fig 10.; *red lines*; p) and increased during reward-only trials (Fig. 10; *blue lines*). Note, however, that lever pressing was significantly less on shock-other trials compared to reward-only trials (t-test; *p*-values < 0.05), demonstrating that rats still took into account the conspecific's distress during this phase. Counter to our hypothesis that oxytocin would make rats more prosocial, intranasal oxytocin administration had no significant impact on lever pressing during any of the trial types (t-tests; $p = 0.19$ for reward-only trials, $p = 0.06$ for self-shock trials, $p = 0.53$ for shock-other trials).

Experimental Period 3 (Days 9-12). In Experimental Period 3, the rats in the oxytocin and saline group received intranasal oxytocin and saline administration, respectively, 30 minutes prior to the experimental session, as in Experimental Period 2. However, in this period, both groups were alone without their conspecific during the experimental sessions. The trends established from Experimental Period 2 continued between the oxytocin and saline groups in

average lever pressing. Average lever pressing for both groups decreased further during shock-self trials and increased further during reward-only and shock-other trials.

Although we saw no significant differences between oxytocin and saline groups in the prior experimental period, differences were present and significant during period 3. The average lever pressing for shock-other trials for the oxytocin group was significantly different than the saline group (t-test; *p*-value < 0.05). The average lever pressing for reward-only trials for the oxytocin group, however, approached significance compared to the saline group (t-test; *p*-value = 0.057).

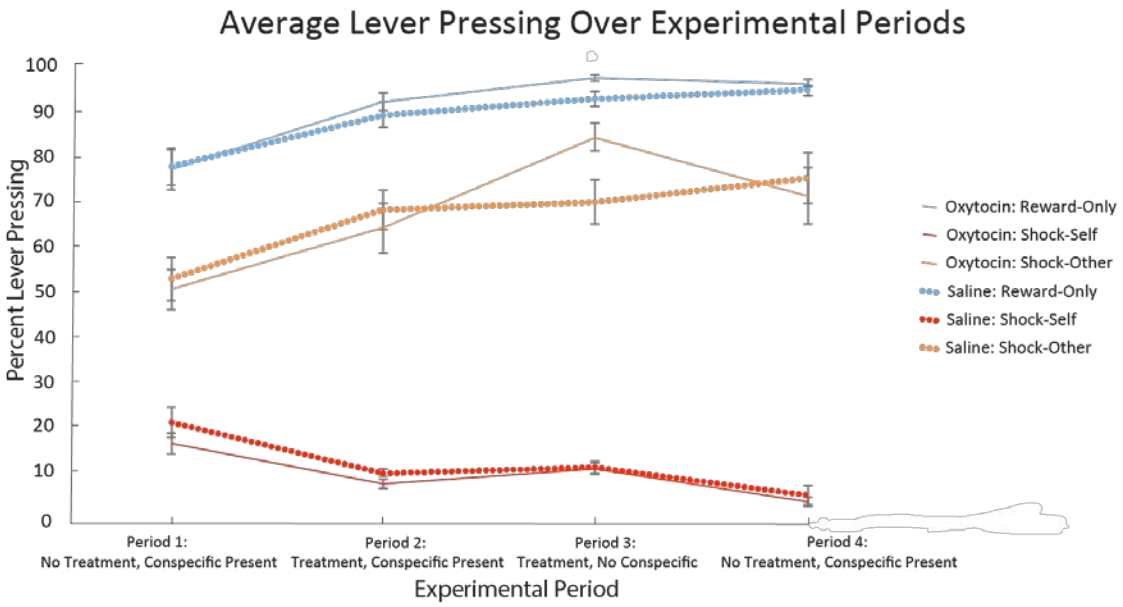

Figure 10. Percentage of times lever pressed during each trial type and treatment averaged among all rats. Experimental periods refer to a period of four days when rats received the same treatment during their testing session (one session per day per rat), except for the last experimental period, in which there are three days. The percentage of lever pressing for each experimental period is an average of the average of each rat's lever-pressing behavior in a specific trial type and treatment during each day in the experimental period. During days 1-4 (period 1), rats were given no treatment and were tested together. During days 5-8 (period 2), rats were given treatment and were tested together. During days 9-12 (period 3), rats were given treatment but were tested without the presence of a conspecific in the adjacent cage. During days 13-15 (period 4), rats were not given treatment and were tested with the presence of the conspecific again. The average lever pressing each experimental period for each trial type is shown with standard error bars to show significant differences in lever pressing behavior. Solid lines represent oxytocin treatment group and dashed lines represent saline treatment group (n = 9 for oxytocin treatment group, n = 10 for saline treatment group). Blue lines represent reward-only trials, red lines represent shock-self trials, and orange lines represent shock-other trials. Daily trajectory of percentage of lever pressing are plotted in Fig E1 (Appendix E).

Experimental Period 4 (Days 13-15). In Experimental Period 4, the conditions were replicated from Experimental Period 1; the rats in both groups received no further treatment, but they were together with their conspecific during experimental sessions. The objective of this experimental period was to test the strength of any significant differences between the two groups, in the absence of treatment but in presence of the conspecific. The least response in average lever pressing was for shock-self, and the greatest response was for reward-only. Significant differences between the two groups observed in shock-other trials during the previous experimental period were absent in this experimental period (t-tests; $p = 0.41$ for reward-only trials, $p = 0.59$ for self-shock trials, $p = 0.35$ for shock-other trials).

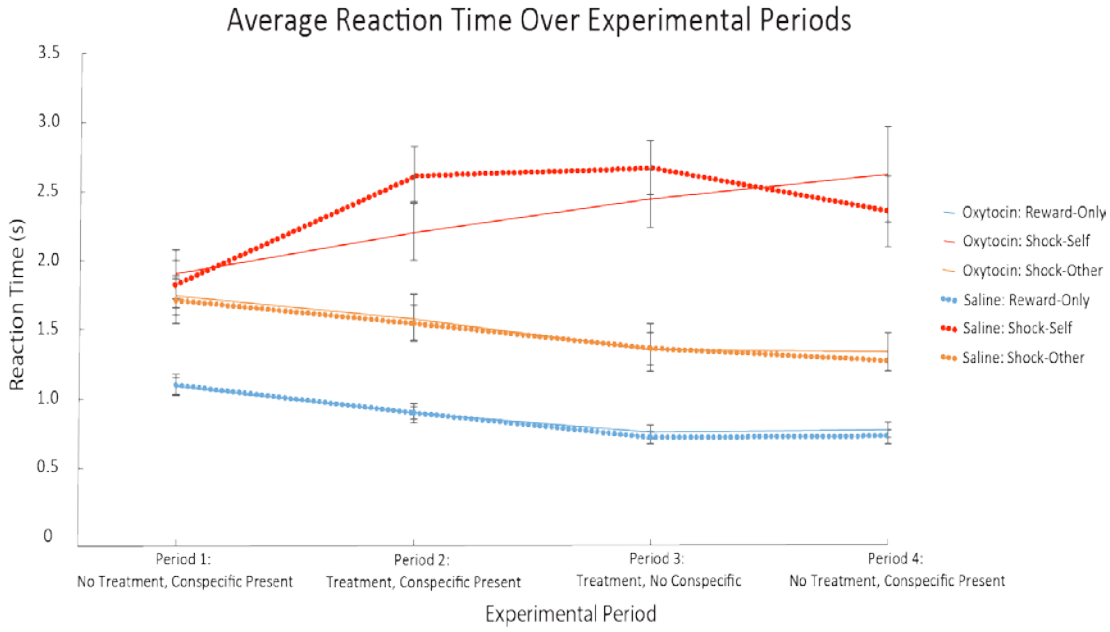

Figure 11. This figure shows the reaction time during each trial type averaged among all rats in a given experimental period. See Fig. 10 caption for explanation of legend. Daily trajectory of reaction times is plotted in Fig E2 (Appendix E).

Oxytocin treatment does not influence reaction time. Figure 11 illustrates the average reaction time across the four experimental phases. Oxytocin had no impact on reaction time (Fig. 11; *solid versus dashed lines*) across trial-type or phase. However, reaction times did vary over the course of the experiment. Initially, during Experimental Period 1, rats were significantly faster on reward-only trials compared to shock-self and shock-other trials, which did not significantly

differ from each other. Over time, rats became faster on reward-only and shock-other trials, but became slower on shock-self trials. Consistent with the lever pressing results presented in Fig. 10, rats were aware of the trial types in that they were fastest and slowest to press the lever for reward-only and shock-self trials, respectively, with reaction times for shock-other falling in between.

Discussion

The objective of this experiment was to observe the effects of oxytocin on the prosocial behavior of Sprague-Dawley rats. Prosocial behavior was operationalized through shock-other lever pressing behavior (reaction time and percent lever press) for both groups (saline and OT). In Experimental Period 1, the two groups did not receive any treatments prior to their sessions with their conspecifics, which established the baseline lever-pressing behavior of the rats in the absence of treatment. During this period, there were no significant differences in lever pressing behavior (reaction time or percent lever press) between the saline and OT groups, demonstrating homogeneity across groups before treatment. For both groups pre-treatment, weak 'prosocial behavior' was demonstrated in Experimental Period 1, as the lever was pressed on only half of the shock-other trials. While this was significantly lower than the lever pressing for reward-only trials during the same experimental period, the rats were still shocking their conspecific for a reward in about 50% of the shock-other trials, suggesting that prosocial behavior is not the predominant behavior in rats. This finding of weak prosocial behavior early in our experiment is consistent with prior studies; if we had stopped our experiment after Experimental Period 1, we would likely be able to make conclusions of prosociality in rats which are consistent with prior literature. However, results from subsequent Experimental Periods 2 through 4 demonstrated that this prosocial behavior diminishes over time, as reflected by the gradual increase in average lever pressing during shock-other trials, and the gradual decrease in average reaction times for shock-other trials. The decrease in average reaction time for shock-other trials over the course of the

experiment, regardless of experimental group or social context within the experimental period, suggests that some level of habituation to lever pressing in the experiment, or dissipation of cue distinction between reward-only and shock-other trials, may have occurred.

Results also demonstrated that this diminution of prosocial behavior over time was not remediated by OT administration. The oxytocin group largely followed the same average lever pressing and average reaction time trends as the saline group. Expected decreases in average lever pressing during shock-other trials after intranasal oxytocin administration did not appear in Experimental Period 2, suggesting that the treatment had negligible effects on prosocial behavior.

The only significant differences between the OT and saline groups were found in average lever pressing in shock-other trials during Experimental Period 3. In this experimental period, the rats were given treatment but were tested without the presence of the conspecific. During reward-only trials, rats in the OT group pressed the lever more often than rats in the saline group. Therefore, OT may have increased self-serving reward behavior, particularly when conspecifics were not present. Contrary to our predictions, during shock-other trials, rats in the OT group pressed the lever more often than rats in the saline group. Because the conspecific was not present during this experiment period, there was no visible or auditory feedback resulting from the shock-other lever press. Therefore, OT may have further increased self-serving reward behavior, as the consequences (i.e. seeing and hearing another rat in pain) of harming a conspecific were no longer present.

Similar results from other experiments suggest that administered oxytocin does increase and reinforce self-serving behavior, as well as positive reinforcing effects (Chang et al., 2012; László et al., 2016). Chang et al. found that oxytocin-induced rhesus monkeys would increase social donation behavior by giving a reward to other rhesus monkeys more frequently than compared to an empty cage. However, the researchers also found that when the monkeys had the choice of rewarding self vs. other, oxytocin-induced rhesus monkeys would slightly increase self-

rewarding behavior. On a larger scale, this would suggest that oxytocin administration may influence the processing of social information, but does not play a significant role in increasing behavioral expression of prosocial behavior.

Limitations and Future Directions. This experiment had some limitations. First, we did not methodologically confirm that intranasal administration of oxytocin reached the brain with any post-experimental procedures, such as histology. We chose intranasal administration based on prior literature with methodology showing demonstrated behavioral effects through intranasal oxytocin administration (Lukas & Neumann, 2012; Calcagnoli et al., 2015). This method is also commonly used pharmaceutically with humans, and we wanted to conserve some translational aspects of the treatment. However, there was some behavioral effect present in the oxytocin group, which could arguably serve as verification that oxytocin had crossed the blood brain barrier. In future experiments, we would like to replicate the paradigm with more direct administrative methods, such as cannulation or microinjection to oxytocinergic neural systems (Pedersen & Prange, 1979; László et al., 2016).

Another limitation of this study is that video has not been scored yet, so we do not know if other behaviors (freezing, approach) corresponded with lever pressing behavior. These data could help support claims that oxytocin improved social information processing; one potential supportive result would be increased approach or orientation towards the conspecific during shock trials in the oxytocin group compared to the saline group. To further study the effects of oxytocin on prosocial behavior, it would be beneficial to repeat the experiment with lever-pressing rats and conspecifics paired as cagemates. While the rats were housed in adjacent cages in our experiment, we predict that there would be a stronger effect of oxytocin administration on prosocial behavior with a cagemate.

General Discussion

Empathy, defined within the context of this thesis, is the ability to conceptualize the internal state and perspective of another based upon contextual knowledge (de Wied et al., 2010). Previous research has determined that empathy plays a crucial role in complex social interactions involving leadership, hostility, and social competency between typical humans, as well as in humans with psychopathologies such as autism spectrum disorder, schizophrenia, and antisocial personality disorder (Baron-Cohen & Wheelwright, 2004; Blair, 1995; Seara-Cardoso et al., 2012; Horan et al., 2015; Derntl et al., 2012).

In order to study the nature of empathy in a manner that does not place undue stress or harm on humans, researchers developed an alternative model to study how subjects are influenced by and acted in response to empathy. The animal model, particularly the rat, has been used frequently to study empathy and empathetic behavior within the literature. Our research attempted to take this model one step further and investigate the underlying neural mechanisms of empathy within the rat, in hopes that our findings would (1) provide evidence of signals modulated during empathy and (2) provide translational evidence to support our understanding of typical and atypical empathy in humans.

Previously, empathy was thought to be limited to only humans and some primate species, and claims that other animal species displayed the trait were met with skepticism from the greater scientific community. This convention was eventually challenged by a series of experiments conducted by Ben-Ami Bartal et al. (2011) which demonstrated the tendency of rats to display rescue behavior when exposed to another rat in distress, even when given the choice between the trapped rat and a food reward. This article was published in Science, demonstrating for the first time that rats are prosocial. However, this study has been met with heavy resistance from the scientific community. For example, one study replicated the result and then subsequently demonstrated that rats would not learn to free other trapped rats if they were denied social

interaction once the rat was freed. They concluded that rats were not prosocial but were simply were seeking social interaction, which rats find rewarding (Silberberg et al., 2014).

Others have taken an evolutionary approach to discredit the finding that rats are prosocial. One of the more prominent criticisms came from another study in which ants were shown to display "prosocial" rescue behavior towards other members of their colonies when trapped. Vasconcelos et al. (2012) argued that the rescue behavior present in both species could be a simple acknowledgement of the reproductive benefits of survival, and thus was not indicative of a higher form of empathetic processing in which there was genuine motivation to benefit the conspecific.

Our research adds to this literature of skepticism around rats' prosociality in two ways. First, we show that DA release in NAc – which we know reflects values of cues and outcomes – does not increase and decrease to appetitive and aversive events, respectively, when they are directed at the conspecific. Second, we show in an instrumental task, where rats have to give up reward to prevent shock from occurring to another rat, that rats with extended training only show prosocial behavior on less than 30 percent of trials, whereas they spare themselves from shock on nearly every trial. In this experiment, oxytocin, which is known to have anti-aggressive and pro-affiliative effects in rats, did not impact behavior.

Our results suggest that rats are not as prosocial or 'empathetic' as has been argued. In the Pavlovian task described in Chapter 1, rats did not freeze for cues that predicted harm to the conspecific, but significantly froze to cues that predicted personal shock. Further, rats approached the conspecific more often on shock-self trials compare to shock-other trials, suggesting that rats were more interested in their own well-being than that of the conspecific. These 'selfish' behaviors were reflected in the DA signal as well. Although it is difficult to determine what the rats were 'thinking' or 'feeling' during our task, it is well-known that DA release in NAc increases and decreases to events that are better and worse than expected, respectively. Indeed, in

our task, reward delivery and cues that predicted reward delivery increased DA release when they were directed to the recording rat. Similarly, cues that predicted shock inhibited DA release when it was the recording rat that was to endure the shock. Cues and outcomes directed to the conspecific did not produce the same pattern of results, suggesting that the rat – if DA signals genuinely reflect value – did not find reward and shock directed to the conspecific to be appetitive and aversive, respectively. Specifically, reward and cues that predicted reward to the conspecific did not elevate DA release. Furthermore, shocks directed to the conspecific actually increased DA, instead of inhibiting it, suggesting that rats were using social and emotional cues from conspecific to determine who would receive the shock. Support for this conclusion comes from the finding that the recording rat and conspecific did approach each other on shock trials.

The results of this first experiment imply that phasic changes in extracellular DA may not be the neural system to assign value to expected outcomes from the point of view of the conspecific. The DA data revealed no convincing evidence for the presence of empathetic signaling in the recoding rat in response to positive or negative outcomes experienced by a conspecific. The closest positive social behavior and correlated DA signal that we observed may reflect a correlate of consolation behavior that has been recently described in prairie voles (Burkett et al., 2016). DA signaling revealed that the presence of a conspecific caused the DA inhibition associated with the activation of the shock cues to dissipate (Fig. 6, thick red line). This suggests that the recording rat found the shock cue to be less aversive when in the presence of the other rat. This pattern in signaling could indicate the existence of consolation behavior between rats, defined as the comfort received by an individual after a loss or disappointment. Video scoring revealed that there was a behavioral component to this signal: rats tended to approach the partition separating the two sides of the cage during shock ‘self’ trials in which both rats were present. Even this behavior was seemingly one-sided, as video scoring showed that the recording rat moved toward the partition during the shock ‘self’ trials, and did not do the same for shock

'other' trials; rats seek consolation in stressful situations, but do not offer consolation when they are not directly affected.

These results suggest that DA release does not represent predictions and prediction errors from the point of view of the conspecific, but it is also a possibility that the rat model and behavioral task used in our first experiment are inappropriate to uncover these neural correlates and associated behaviors. Despite the work of Bartal et al. and others, rats may not be capable of the same internal and external empathy that have been observed in humans and some primate species. Further evidence for this comes from our second experiment, in which we showed that with extended training rats were not as prosocial as suggested in previous reports, and this prosociality was not improved with pharmaceutical intervention.

Although our results do not provide a translational link to how humans make prosocial and 'empathetic' choices, they do provide key insights into how DA signals are modulated in social contexts. For the first time, we show that DA release is modulated by social context in at least two ways. First, reductions in DA release during cues that predict shock were weaker in the presence of the conspecific, suggesting a consoling effect which was supported by finding that rats approached the other conspecific prior to and during shock delivery. Second, DA release during shock trials increased when shock was administered to the conspecific, suggesting that the DA signal is modulated by the affective state of the conspecific to verify personal safety. Overall, we concluded that DA release is modulated by social context in that rats use social cues to optimize predictions about their own well-being.

Future Directions

Given the time constraints of the Gemstone Honors Program, we were not able to investigate all of our different research questions. Therefore, there are additional data collection methods and analyses that we suggest for future researchers. First, we would be interested in investigating individual differences within a sample of rats. For example, we would like to

examine the relationship between DA release and behaviors within each rat to determine the heterogeneity of empathy as a trait within the rat species. It is possible that some rats have greater empathetic signaling and corresponding behaviors than other rats. We would also be interested in running investigating how DA release changes over time, as past research has demonstrated that DA signaling may differ between early and late trials (Kashtelyan et al., 2014). Furthermore, we would suggest the investigation of sequence effects within each recording session to determine if the previous trial type influences the DA signaling and behavior in the subsequent trial.

In addition, we would like to rerun a similar lever press paradigm and see how oxytocin interacts with dopamine (DA) release using FSCV technology. Oxytocin has been shown to modulate many monoamines and likely has an influence on DA, yet their cellular-level interaction is currently unknown (Insel & Young, 2001). It is likely that oxytocin interacts with DA to modulate rewarding social situations (Decety, 2011).

We recognize that a large limitation of our study is that the rats did not get to interact outside of the experimental paradigm testing sessions. Past literature has demonstrated that social bonding, and more specifically physical touch, is related to empathy (Watt, 2005; Montague et al., 2013; Jones & Yarbrough, 1985). Therefore, it would be interesting to rerun both experiments with cagemates. Alternatively, we could rerun both experiments with siblings and mother-offspring pairs to see if there is an influence of familial relationships on empathy, as past research implies that empathy and prosociality may differ within these relationships (Pepler, Corter, & Abramovitch, 1982; Bryant & Crockenberg, 1980). Furthermore, several studies have demonstrated a difference between empathy and response to oxytocin in males and females, so we suggest rerunning this experiment with female rats and conducting group difference analyses (Hoffman, 1977; Rilling et al., 2014; Dumais et al., 2016). It is our hope that the investigation of these questions, methods, and analyses will further our understanding of the neurobiological basis of empathy and prosocial behavior across species.

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APPENDIX A: Glossary

All citations are from the Medline Plus dictionary, unless otherwise noted.

Medline Plus, (2012). Bethesda, MD: National Institutes of Health.

Antisocial personality disorder: A mental health condition in which a person has a long-term pattern of manipulating, exploiting, or violating the rights of others.

Autism spectrum disorder (ASD): A heterogeneous, neurodevelopmental condition that is characterized by deficits in social reciprocity and communication, repetitive, restrictive behaviors.

Analog background subtraction: A method to eliminate background noise, where the charging current is recorded and played back at the summing point to eliminate the output.

Cannula: A thin tube inserted into a vein or body cavity to administer medicine, drain off fluid, or insert a surgical instrument (Barthas et al., 2015).

Commutator: A commutator is the moving part of a rotary electrical switch in certain types of electric motors or electrical generators that periodically reverse the current direction between the rotor and the external circuit.

Conspecific: A neighboring rat, relative to the experimental rat in a given paradigm (Kashtelyan, 2014).

Cyclic voltammogram: A pattern of current vs. applied potential specific to a chemical compound.

Dopamine: A neurotransmitter associated with reward processes.

Empathy: Conscious awareness of the affective states of others, including the ability to conceptualize the perspective of another person based on knowledge of the situation or context.

FSCV: Fast-Scan Cyclic Voltammetry; technology which allows for rapid acquisition of a voltammogram within several milliseconds and ensures high temporal resolution; used to measure neurotransmitter release.

Microelectrode: A small electrode which is inserted in a living biological cell or tissue in order to study its electrical characteristics.

Oxytocin: A neurotransmitter associated with sociality.

Paradigm: A designed situation which is used to model behavioral empathy.

Prosocial Behavior: Any action intended to benefit and/or prevent harm to another (Eisenberg & Miller, 1987).

Psychopathy: A mental disorder which is characterized by amoral, antisocial behaviors, lack of ability to establish meaningful personal relationships, extreme egocentricity, and failure to learn from experience. Now referred to as Antisocial Personality Disorder (APD).

Reference electrode: An electrode which has accurately maintained and pre-determined potential, which is used as a reference for measurement by other electrodes.

Schizophrenia: A long-term mental disorder involving a breakdown in the relation between thought, emotion, and behavior, leading to faulty perception, inappropriate actions and feelings, withdrawal from reality and personal relationships into fantasy and delusion, and a sense of mental fragmentation.

Temporal (time) resolution: The precision of a chemical measurement with respect to time.

Trial Types

Alone Trials: Trials in which only the recording rat was present.

Together Trials: Trials in which both the recording rat and the conspecific were present.

Self Trials: Trials in which the outcome (reward, neutral, or shock) was delivered to the recording rat.

Other Trials: Trials in which the outcome (reward, neutral, or shock) was delivered to the conspecific (during together trials) or an empty box (during alone trials).

APPENDIX B: FSCV Method

Fast-scan cyclic voltammetry (FSCV) is a method of measuring phasic changes in extracellular dopamine concentration, meaning changes that occur for only brief periods. This technology uses microelectrodes, small electrical conductors that make contact with the dopamine-releasing environment in the brain. FSCV is advantageous as it is able to measure changes in concentration on a subsecond time scale, allowing researchers to associate changes in dopamine with very specific time points in the paradigm and thus specific points in the behavior being expressed. In our experiment, the recording electrodes were placed in the NAc due to its integral role in the brain's dopamine pathways. The exact placement of the electrodes was 1.3 mm anterior to the bregma, 1.8 mm laterally, and between 6.6 mm and 8.5 mm ventral to the skull.

During the use of FSCV, the potential of the electrode was increased and decreased linearly, causing dopamine molecules adjacent to the electrode to be oxidized into dopamine-quinone and eventually reduced back to dopamine, creating a current (Robinson et al., 2003). Our use of FSCV was very similar to the one described in the Robinson et al. (2003) publication, but with some variations. A triangle waveform was applied intermittently to the electrodes using an Ag/AgCl reference electrode. This means that the voltage began at -0.4 volts (V) and increased at a linear rate until it reached 1.3 V. At the moment when it reached 1.3 V, the change in voltage was halted, and then decreased at a linear rate back to -0.4 V, thus yielding the triangle waveform. The voltage change occurred at a scan rate of 400 V/s, and therefore each individual scan lasted 8.5 milliseconds (ms). These scans occurred at intervals of 100 ms, yielding 10 samples per second. These specificities were adjusted to improve sensitivity, selectivity, and time resolution of measurements (Robinson et al., 2003).

A shortcoming of FSCV is that the background current greatly exceeds the faradaic current (the current created by oxidizing dopamine) from redox reactions of solution species

(Hermans et al., 2008). The background current is caused by charging of a layer of ions that is absorbed onto the surface of the electrode as well as redox reactions with functional groups near the surface of the electrode (Robinson et al., 2003). Due to these other factors causing current, the oxidation of dopamine may only cause a small increase in current, making it hard to distinguish changes caused by dopamine. Fortunately, we corrected for this limitation by making use of background subtraction. Since the carbon fiber microelectrodes recorded relatively stable background levels over several seconds, the background was digitally subtracted to reveal changes in current induced only by the oxidation and reduction of dopamine (Robinson et al., 2003). Furthermore, effects on the current from interfering pH signals were also subtracted to measure pure dopamine changes (Robinson et al., 2003).

Analyzing the corresponding cyclic voltammogram, a measure of current vs. applied potential, allowed us to verify that the change in current was due to the oxidation and reduction of dopamine. Dopamine is oxidized most rapidly occurring around +0.6V (Robinson et al., 2003). This point corresponds to the greatest increase in current, and observing the peak's location and shape, can confirm that the change in current is due to dopamine. Since norepinephrine and dopamine have nearly identical cyclic voltammograms (Robinson et al., 2003), the electrodes were placed in known areas of high dopamine concentration and low epinephrine content. The changes in dopamine concentration were identified by plotting the current levels that occurred at the peak levels of oxidation vs. time. The points in this plot were the peak current levels of numerous scans (10 scans/s) that occurred continuously for several seconds. The plot of current was then converted to dopamine concentrations via calibration. For example, (X nmol/L) / Y nA.

The electrodes were connected to a head mounted voltammetric amplifier, an instrument that converts current to voltage, and a commutator (an instrument that indicates the extent to which a binary operation is not commutative) that was mounted above the recording chamber. In order to generate waveforms and collect and analyze data, we used two PC-based systems fitted

with two multifunction data acquisition cards and software written in LabVIEW. Software written in LabVIEW generated cyclic voltammograms as well as extracted the voltammograms that indicate changes in dopamine concentration. LabVIEW converted the peak currents to dopamine concentrations. As described earlier, by plotting these peak currents, changes in dopamine that occurred over several sections were visualized. This visualization of spikes in dopamine and approximate direct values of change were compared in analysis.

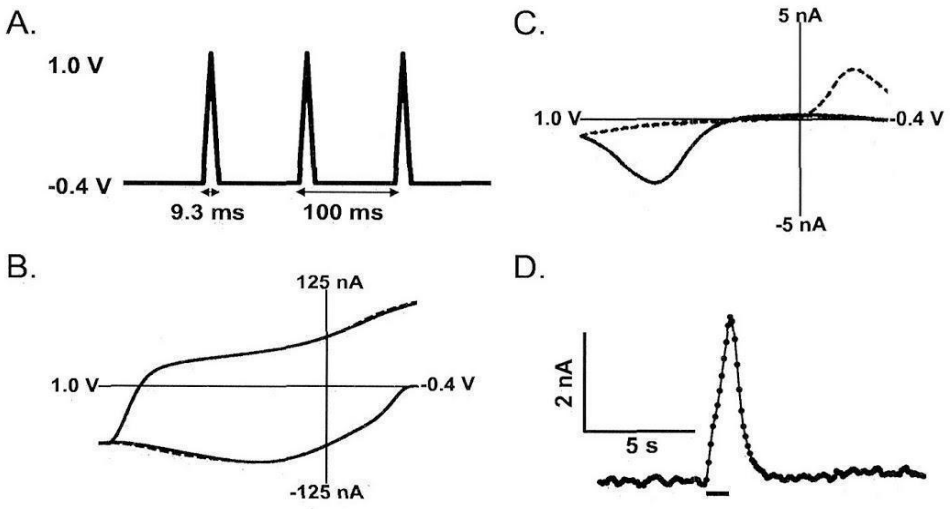

Figure B1. FSCV (Robinson et al., 2003). *Panel A* indicates a graph of the voltage change over time. In this example, the voltage is scanned from -0.4V to +1.0V in 9.3s, and these scans occurred every 100ms. However, we ramped the voltage to 1.3V, and the scans lasted for 8.5ms. In *Panel B*, the background current is graphed vs. applied potential. The solid line is background current and the slightly visible dashed line is a 3% change in the current; when the solid line is subtracted from the dashed, the current yielded represents the current contributed by dopamine. *Panel C* represents the new graph, which is called a cyclic voltammogram. The solid line represents oxidation (-0.4V to +1.0V scan), while the dashed line represents reduction (+1.0V to -0.4V scan). The cyclic voltammogram is the chemical identifier. *Panel D* represents dopamine current plotted at its peak oxidation voltage vs. time. Each point represents current from one voltammogram. The current can then be converted to dopamine concentration using in vitro calibration.

APPENDIX C: Supplementary Chapter 1 Video Scoring Figures

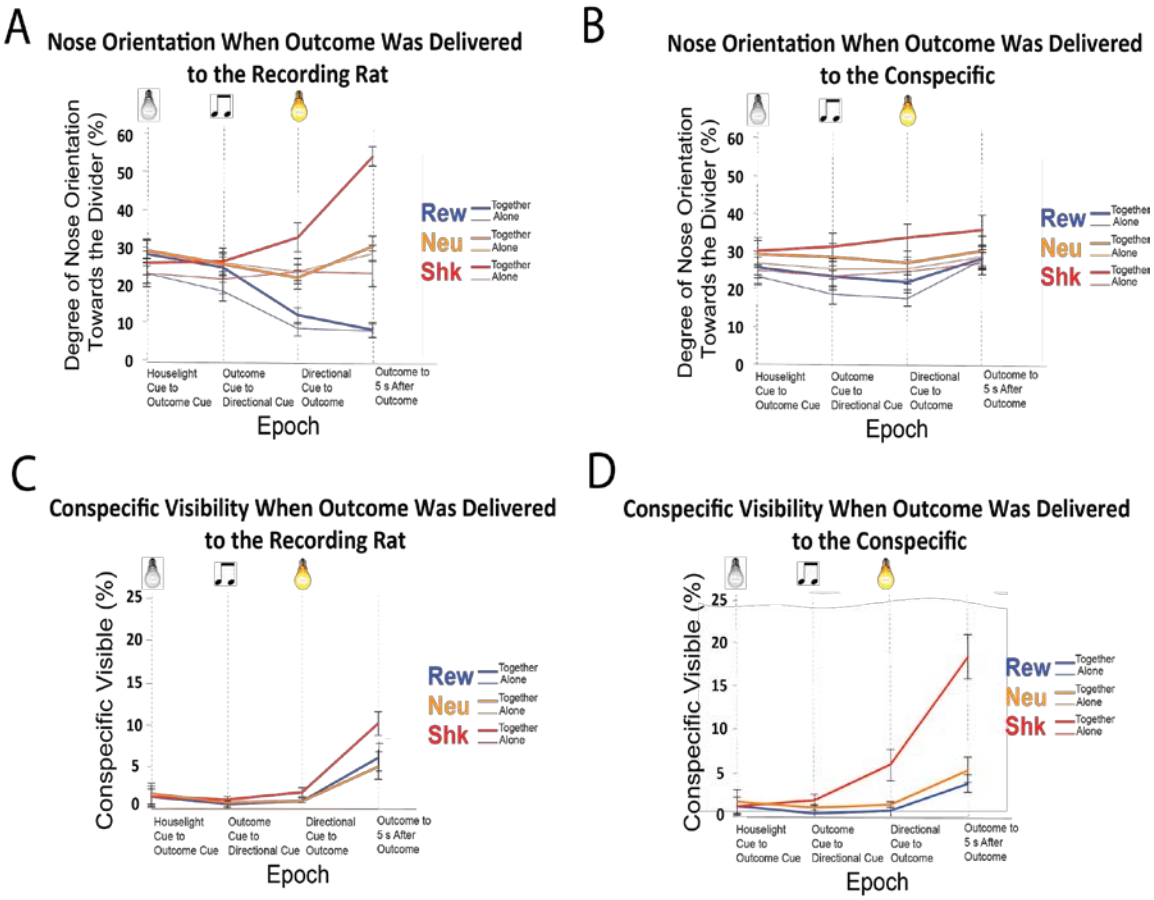

Figure C1. Plots A and B show the percentage of trials in which the recording rat's nose was oriented towards the divider during each specific epoch. Thick lines represent 'together' trials, while 'thin' lines represent 'alone' trials. **C1.A:** reflects degree of nose orientation on trials with outcome delivered to the recording rat ('self' trials) and **C1.B:** reflects degree of nose orientation on trials with outcome delivered to the conspecific ('other' trials). Plots C and D on the other hand show the percentage of trials where the conspecific was visible through the mesh window. Only the 'together' trials are plotted for this behavior, as the conspecific was absent on 'alone' trials. **C1.C.:** reflects conspecific visibility on trials with outcome delivered to the recording rat and **C1.D:** reflects conspecific visibility on trials with outcome delivered to the conspecific.

Conspecific presence varies by trial type and outcome recipient. Conspecific presence was defined as the visibility of the conspecific through the mesh divider. The conspecific seemed to be more concerned with outcomes that impact themselves than the recording rat. Figures C1.C and C1.D show that the conspecific approached the mesh divider the most during the outcome epoch, in particular during shock trial types. Differences between shock trial types and other trial types varied between outcome recipients with more statistically significant differences observed when the outcome was administered to the conspecific. When the outcome was administered to

the recording rat, differences between shock and reward trial types were significant ($p = 0.04$), while differences between shock and neutral trial types were not significant ($p = 0.06$). When the outcome was administered to the conspecific, conspecific presence was significantly greater during shock trial types than any other trial types (all p values < 0.001). During the directional light epoch, the conspecific was at the mesh divider more during shock trial types than reward and neutral trial types (all p values $= 0.01$). This suggests that the conspecific was able to discern trial types by audio cues and direction of the outcome by the directional light. Additionally, this is very similar to how to the recording rat approaches the mesh divider when it received a shock. The presence of the conspecific at the mesh divider may have been motivated by a desire for social consolation when a shock was administered to it.

Nose orientation. Nose orientation was used to assess attention. As mentioned earlier, nose orientation was dummy coded with ternary values (0, 1, 2). Figure C1.A and C1.B show that the recording rat faced the mesh divider significantly more in shock together trails than shock alone trials when the outcome was delivered to the conspecific in the outcome epoch (p value < 0.01). This effect can also be observed when the outcome was delivered to the recording rat. This signifies that the recording rat shows interest during these trials, but as stated in the main text, the rats did not necessarily approach the mesh divider.

APPENDIX D: Supplementary Chapter 1 Ultrasonic Vocalization Figures

Method

The ultrasonic vocalizations emitted by rats have been studied extensively as an indicator of affective states. While positive affective states can be measured in humans through a variety of methods including self-reporting of feelings, researchers working with laboratory animals must rely on approach behavior (tendencies of the animals to approach each other) and facial-vocal displays to detect positive affect (Burgdorf et al., 2011). 50 kHz USVs have been established in a variety of studies as an indicator of positive affective states in rats, especially the frequency-modulated variety (Burgdorf et al., 2008; Burgdorf et al., 2011). These vocalizations have been directly related to behaviors associated with positive affect, including mating and reward (Burgdorf et al., 2008). Additionally, alternative hypotheses regarding the cause of 50 kHz USVs have been systematically rebutted: the frequency-modulated variety is not a byproduct of locomotion or thoracic compressions; they are not merely a non-affective response to social interactions; only the flat variety (not frequency-modulated) is involved in aggressive behavior and only before the onset of aggression; they are expressed in anticipation of reward but are diminished if reward is not received, proving that they do not merely express a "wanting" state; and finally, the hypothesis that ultrasonic calls reflect a non-affective state of high arousal was rebutted by studies relating the rates of 50 kHz vocalizations to reward tasks and inversely relating them to highly arousing aversive stimuli such as bright light and shocking (Burgdorf et al., 2011). Because 50 kHz frequency-modulated USVs have been established to be related to positive affect in rats, they can be valuable discrete indicators of positive affective states during empathy related-behavioral tasks.

As mentioned above, another variety of USVs, those with a frequency of 22 kHz, have been associated with negative affective states (Burgdorf et al., 2011). Various studies have shown that these vocalizations are similar to human anxiety and/or depressive states. They occur during

social defeat and avoidance behavior, and are positively correlated with drug-conditioned avoidance (Burgdorf et al., 2008; Burgdorf et al., 2011). The rates of these vocalizations are usually inversely related to the rates of occurrence of 50 kHz USVs, which makes sense as they represent opposite affective states (Burgdorf et al., 2008; Burgdorf et al., 2011). 22 kHz USVs are associated with negative emotional states but are not as well-documented as 50 kHz vocalizations.

In particular, a study which monitored USVs emitted during self-administration of cocaine and sucrose concluded that less than 1% of the frequencies emitted during both the sucrose and cocaine self-administration were at 22 kHz (Browning et al., 2011). Additionally, during extinction, or when rats expect a reward but do not receive one, a 22 kHz frequency was found to be present (Browning et al., 2011). A frequency of 22 kHz was displayed to increase as a response to cues that predicted shock in the future. Therefore, using USVs to differentiate between rats' affective states is a noninvasive method of accurately quantifying emotions in rodents.

Ultrasonic vocalizations (USVs) emitted by rats are an additional quantitative measure of the affective and motivational states of the animals. We recorded vocalizations during the experimental paradigm using a Med Associates USV detector, which scanned ultrasonic frequencies every 30 ms and differentiates between specific bandwidths defined by the user (22 kHz and 50 kHz). MED-PC was used in conjunction to allow for temporal alignment to task events such as cue, reward delivery, and shock. For data collection, a decibel level threshold was used to define ultrasonic vocalizations. The results of this processing were a categorical pair of quantitative measures that could be interpreted to describe the affective states of rats during protocols: USVs per second, for both the 50 kHz and 22 kHz varieties. However, these USV data collected from the experiment in chapter 1 were inconclusive due an inability to control for cue sound artifacts or to identify the source of USVs (i.e. recording rat or conspecific). In future

experiments, we would utilize 2 microphones (one on each side of the cage) to determine which rat was emitting the USV.

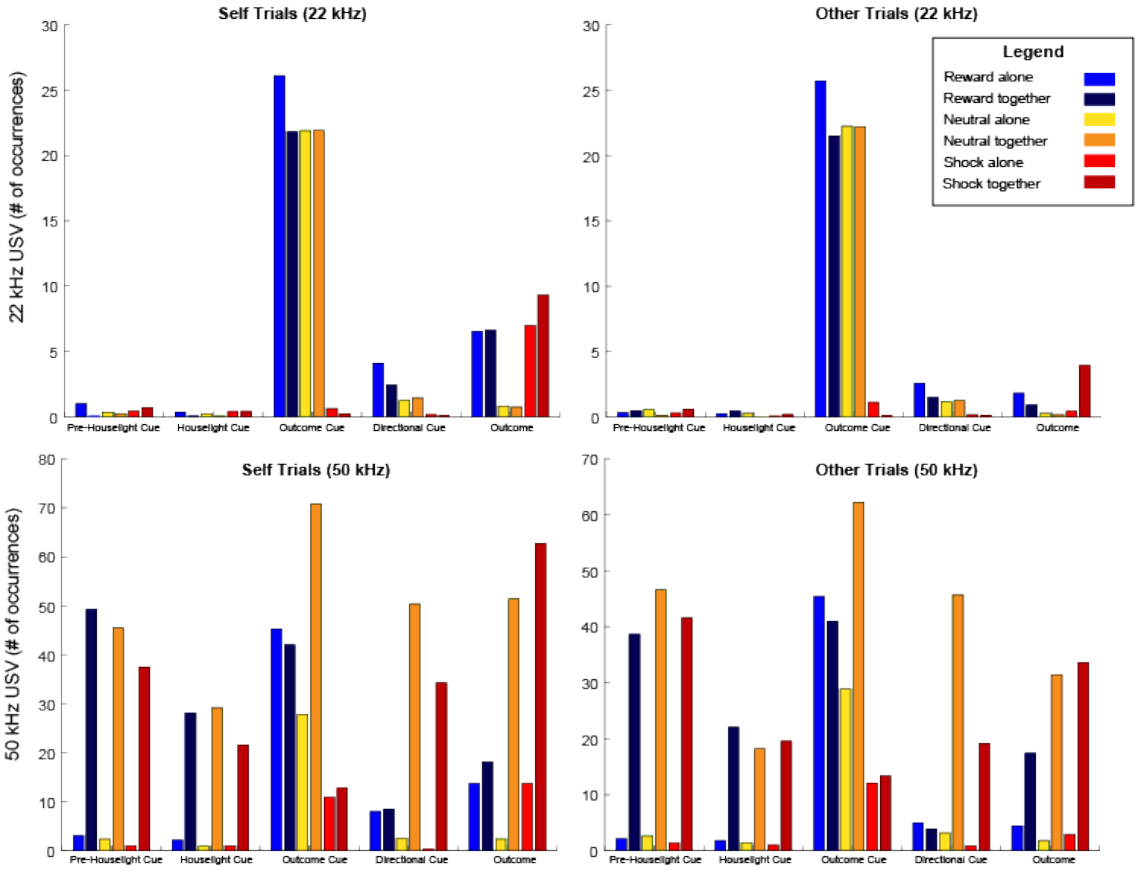

Figure D1. 22kHz and 50kHz USVs during experimentation. Results were inconclusive due to the fact that we could not 1) separate vocalizations from electrical noise, and 2) could not determine which rat was emitting which vocalizations. Thus, results were not analyzed for significance.

APPENDIX E: Supplementary Chapter 2 Figures

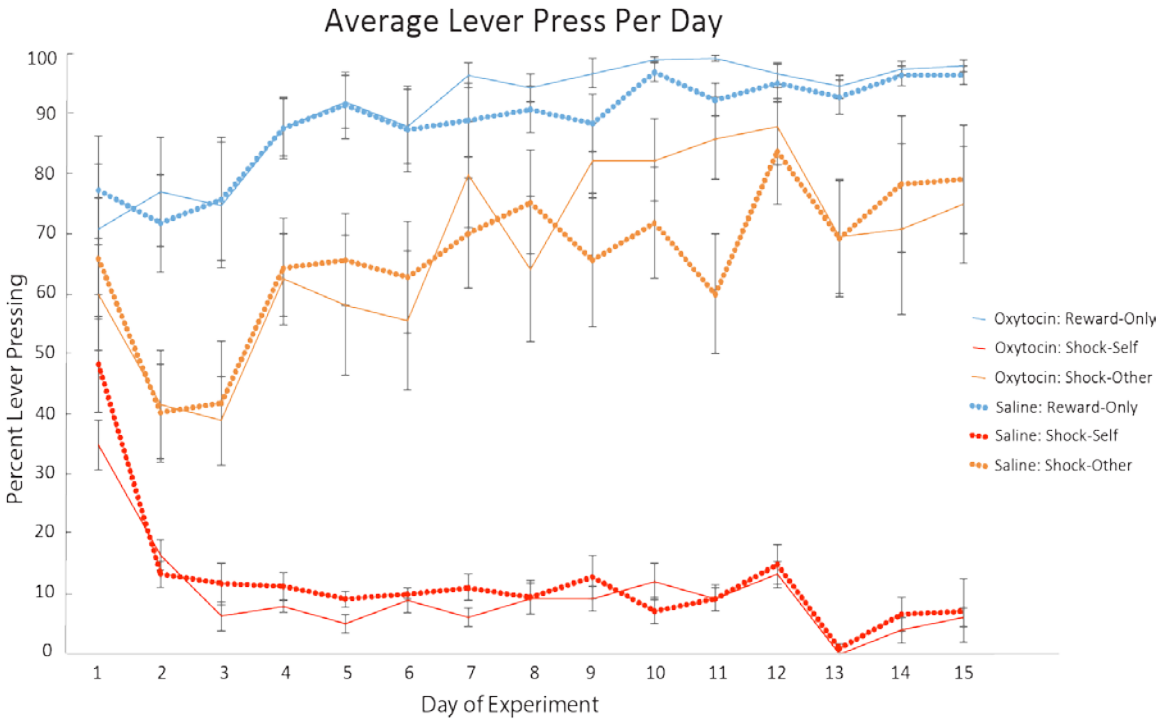

Figure E1. This figure shows the percentage of lever pressing during each day of the experiment for each trial type and treatment averaged among all rats. The percentage of lever pressing for each day is an average of the lever-pressing behavior for all rats in a specific trial type and treatment for that day. During days 1-4, rats were given no treatment and were tested together. During days 5-8, rats were given treatment and were tested together. During days 9-12, rats were given treatment but were tested without the presence of a conspecific in the adjacent cage. During days 13-15, rats were not given treatment and were tested with the presence of the conspecific again. The average lever pressing each experimental period for each trial type is shown with standard error bars to show significant differences in lever pressing behavior. Each line represents a trial type paired with a treatment, with solid lines indicating oxytocin treatment and dashed lines indicating saline treatment. Each trial type is represented by a color shown in the legend on the right.

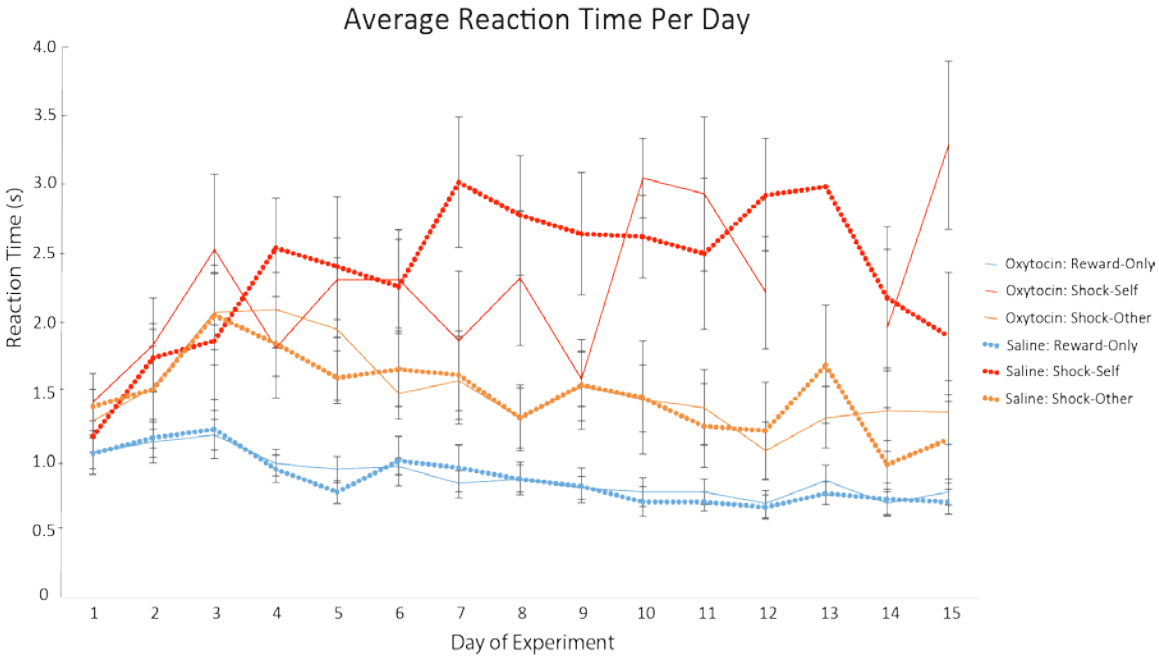

Figure E2. This figure shows the reaction time during each trial type averaged among all rats in a given day of the experiment. The reaction time for each day is an average of the lever-pressing behavior for all rats in a specific trial type and treatment for that day. During days 1-4, rats were given no treatment and were tested together. During days 5-8, rats were given treatment and were tested together. During days 9-12, rats were given treatment but were tested without the presence of a conspecific in the adjacent cage. During days 13-15, rats were not given treatment and were tested with the presence of the conspecific again. The reaction time for each experimental period for each trial type is shown with standard error bars to show significant differences between trial types. Each line is paired with a treatment, with solid lines representing oxytocin and dashed lines representing saline. Each trial type is represented by a color shown in the legend on the right. Reaction time on the 13th day of experiment was 0 because the rats did not press the lever on this day (see Fig. E1).

Reaction times had a non-significant increase over the 15-day period in both oxytocin and saline treatments during shock-self trials with no significant difference between the treatments (t-values > 0.05). In the day-by-day breakdown of average reaction times shown in Fig. I2, the difference in error bars suggest that there were significant differences in shock-other trial types for both treatments during Day 7 and Day 9. However, subsequent t-tests show that there are no significant differences in reaction times for shock-other trial types in both treatments for both Day 7 and Day 9 (t-values > 0.05). Average reaction time data for shock-self trials in the oxytocin treatment is not present for Day 13, as shown in Fig I2 Day 13, as previously described, is the first day of the last experimental period where the rats do not receive any treatment and were tested in the presence of the conspecific. Lever-pressing data for all rats in the oxytocin

treatment on Day 13 showed that there were zero percentage of levers pressed for shock-self trials. Thus, there is no average reaction time to report. On Day 14 and Day 15, average reaction times are reported and continue to increase.