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Sara Khan April 10, 2023

Examining Projections to the Anterior Cingulate Cortex in Prairie Voles

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Abstract

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Social cognition is a set of processes necessary for individuals to interact with others. Among one of many socio-cognitive processes is empathy, which is impaired in individuals with psychiatric disorders such as Autism Spectrum Disorder (ASD). Consolation is an empathy-like prosocial behavior, defined as comforting another in distress, and is exhibited by humans towards each other. The prairie vole, a monogamous and highly affiliative species, is notable for expressing consolation behavior toward their mates. These animals exhibit consolation through allogrooming when their partner is in distress. Prior research has established that consolation induces neural activity in the vole anterior cingulate cortex (ACC). To gain a better understanding of the brain regions that project to the ACC in voles, we used a tracing strategy paired with a semi-automated cell counting software, Wholebrain, to map inputs to the ACC. Prairie voles received unilateral stereotaxic infusion of AAVrg-CAG-GFP to the ACC. Following two weeks of viral incubation, animals were perfused, and their brains sectioned at 40 μM on a cryostat. These sections were stained and imaged on a fluorescent microscope. WholeBrain software was then used to identify and quantify cells projecting to the ACC. Cortical structures found projecting to the ACC include the piriform area, claustrum, olfactory area, primary somatosensory cortex, insula, infralimbic area, and orbitofrontal cortex. A control chemogenetic study was also performed to determine whether the chemogenetic exogenous ligand Clozapine-N-Oxide (CNO) affects vole social behavior during a consolation test. Our study found that there was no significant difference between CNO and saline in behavioral outcomes. Future studies using DREADDs will involve manipulations to the neural pathways projecting to the ACC to determine how these circuits are implicated in consolation in prairie voles.

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Introduction

Social cognition is a selection of processes involved in helping an individual perceive, interpret, and act in their social environment (Frith, 2008).This concept falls under the broad field of cognition, which examines the ways in which humans and animals obtain knowledge and process information to make sense of the world. Social cognition is altered in several psychiatric disorders, including Autism Spectrum Disorders (ASD) (Sharma, Gonda, & Tarazi, 2018). Two key characteristics of individuals with ASD include difficulty communicating with others and a tendency to exhibit restrictive or repetitive behaviors. In addition to these common traits, a deficit in empathetic and prosocial behaviors is also seen in individuals with ASD (Wang et al., 2022).

There is no known cure for ASD, but various treatments and interventions are available that can help individuals with ASD manage their symptoms and improve their quality of life. Some of the most common treatments for ASD include behavioral interventions such as Applied Behavior Analysis (ABA), speech therapy, and occupational therapy. ABA therapy is an effective solution to reducing unwanted repetitive behaviors in children with ASD and improving social communication (Reichow, Barton, Boyd, & Hume, 2012). Additionally, the FDA has approved several medications for the treatment and regulation of some atypical ASD behaviors, such as risperidone for irritability and aripiprazole for repetitive behaviors (Levy et al., 2010). Among the common differences between ASD and neurotypical individuals is a deficit in empathic processing.

Empathy is defined as a process that enables an individual to detect the experiences, needs, and desires of others (Riess, 2017). There are three notable types of empathy: emotional contagion, sympathetic concern, and empathic perspective-taking (F. de Waal, 2008). Emotional

contagion is the most basic form of empathy and refers to social transmission of an emotional response or display of emotion across members of a group. An example of this behavior is a flock of birds taking off after one member of the group is startled. Sympathetic concern, or cognitive empathy, is the ability to understand the perspective of another person (F. de Waal & Aureli, 1996). An example of this behavior in nonhuman primates is when cries of an infant rhesus monkey elicit embraces from other infants in the group to reduce the distressed party's negative arousal. Finally, empathic perspective-taking involves not only understanding someone's emotions but also adopting their point of view, even if one has not exactly experienced that emotional experience themselves in the past. Each type of empathy is necessary for proper social function and collaboration.

Understanding the emotions of others and how to appropriately react to their behaviors enables an individual to act in a prosocial manner. Prosocial behavior is any behavior that can benefit another, including cooperation, comfort, and sharing (Ding et al., 2018). Prosocial behavior is considered positive and beneficial for society as it promotes social harmony and mutual aid. It can be motivated by a variety of factors, such as empathy, personal values, and social norms. Research in humans has found that engaging in this type of behavior can have lasting benefits on emotional well-being (Miles, Andiappan, Upenieks, & Orfanidis, 2022). In one study, participants were asked to perform prosocial behaviors three times a week for three weeks. Participant well-being was assessed prior to the experiment, and they were placed into one of three experimental conditions randomly: neutral acts, self-focused acts, and prosocial acts. After only two weeks of prosocial intervention, participants felt reduced anxiety-like behaviors, suggesting that prosocial behavior benefits well-being. Prosocial behavior is an important aspect

of human social cognition, though the neuronal mechanisms underlying prosocial behavior are largely unknown.

Studies have identified several brain regions involved in empathy and prosocial behavior in rodents, humans, and non-human primates. In rodents, the anterior cingulate cortex (ACC), insula, and medial prefrontal cortex (mPFC) are implicated in social transmission of fear and emotional contagion (Jeon et al., 2010). In humans, brain imaging studies have shown that the ACC, insula, and mPFC are activated when individuals observe or imagine others in distress or engage in prosocial behavior (Eisenberg & Eggum, 2009). Moreover, recent research has also highlighted the role of the temporoparietal junction (TPJ) and superior temporal sulcus (STS) in social cognition, including perspective-taking, mentalizing (the ability to understand the mental state of oneself and others), and empathy (Zaki & Ochsner, 2012). Similarly, in non-human primates, the ACC, insula, and STS have been implicated in empathy-like behaviors (Decety & Meyer, 2008). These findings suggest that empathy and prosocial behavior involve a distributed network of brain regions that are conserved across species, highlighting the importance of social cognition and emotional regulation in social behavior.

Though the aforementioned regions have been associated with social cognition, the circuitry and neuronal mechanisms underlying this type of behavior remain largely unknown due to limitations in research methods used in humans. Studies involving human empathy use functional magnetic resonance imagery (fMRI). fMRI measures the blood-oxygen-level-dependent (BOLD) signal, which is temporally delayed and correlative in nature (Glover, 2011). Additionally, it only examines blood flow in regions rather than direct anatomical connectivity, which can only be assessed in humans *postmortem*. This correlational BOLD signal only shows brain areas with activity when a certain task is performed, which does not confirm that those

brain regions are implicated in the social cognitive neuronal circuit and are essential for coordinating a behavioral output (Turner, 2016). Experimental manipulations of specific brain regions or circuits to understand their anatomical connectivity or function are limited by ethical considerations in humans. Investigations using animal models allow for the neural mechanisms underlying behavior to be studied utilizing a variety of techniques not available in humans.

Prairie voles are a highly affiliative rodent species that exhibits prosocial behaviors towards its partner in long-term, monogamous pair bonds (McGraw & Young, 2009). In addition to these bonds, rodents also exhibit mate guarding, biparental care, and preferential mating, making this species a novel translational model for examining affiliative behavior, bonding, and other social processes. One notable prosocial behavior that prairie voles exhibit is consolation, which is defined as comforting physical touch towards a distressed party (Burkett et al., 2016). Consolation is an empathy-like behavior that can be directly assessed in an animal model and is therefore a means by which we can examine the neural mechanisms underlying empathy-like prosocial processes.

In a recent study, pair-bonded prairie voles underwent a consolation test, where voles within each pair-bonded dyad were assigned as either an observer or demonstrator (Burkett et al., 2016). Observers were the target animals within each dyad, and their behaviors toward the demonstrator were assessed. Animals were given 30 minutes in the testing room before being separated for 30 minutes. During the separation, the animals were either separated into different cages or separated while the demonstrator animal received a stressor of mild footshocks. Demonstrators were then returned to the homecage, and behaviors exhibited by the observer toward the demonstrator were assessed. Prairie voles show a significant increase in allogrooming, defined as licking and grooming directed by observer animals towards

demonstrator animals, when the demonstrator animal received footshocks during separation as compared to demonstrators who were just separated and received no stressor. Consolation serves as an empathy-like prosocial behavior that can be examined in an animal model to elucidate the mechanisms driving prosocial and empathy-like behaviors. Mice have recently also been shown to exhibit consolation of a cagemate or sibling in distress; however, prairie voles exhibit lasting, complex affiliative behaviors towards their long-term mates that are not observed in other rodent species, making them an interesting model to study consolation and other socio-cognitive behaviors (Wu & Hong, 2022).

One brain region of interest in the examination of consolation is the anterior cingulate cortex (ACC). In the Burkett study (2016), after animals underwent behavioral testing, researchers found an increase in cFos expression in the ACC of animals whose partner had been shocked. cFos is an immediate early response gene that serves as an indirect marker for neuronal activity (Velazquez, Caputto, & Boussin, 2015). Presence of this increase in Fos production suggests that the ACC is active during rodent consolation behavior. Furthermore, in mice, optogenetic inactivation of the ACC disrupts social transfer of pain and analgesia, which are both types of emotion contagion and can be studied as empathy-like behaviors (Smith, Asada, & Malenka, 2021).

Additionally, the ACC is important for empathy in humans. A recent study performed a cue signaling experiment in which participants were asked to accurately predict the probability of receiving a reward and whether themself or another participant outside would receive the reward (Lockwood, Apps, Roiser, & Viding, 2015). The study found that the ACC was more active when the participant was making judgments about the other participant rather than themself, showing that the ACC is involved in empathetic behaviors in humans. Common

functions of the ACC shown in humans include emotional regulation, motivational drive, and social cognition (López-Gutiérrez et al., 2021). The ACC has robust connectivity across the primate brain: the cognitive control network (CCN), the salience network (SN), and the socio-emotional network (SEN). The CCN includes the dorsal ACC and the adjacent pre-supplementary motor area (pre-SMA) and is involved in cognitive control and task switching (Dosenbach et al., 2006). The SN includes the dorsal ACC and the anterior insula and is implicated in detecting and orienting attention to salient stimuli, both internal and external (Seeley et al., 2007). Finally, the SEN includes the subgenual ACC and the adjacent ventromedial prefrontal cortex (vmPFC) and is associated with affective processing, social cognition, and reward processing (Knutson, Katovich, & Suri, 2014). Together, these ACC networks allow humans to interpret relevant stimuli, process information, and effectively engage in social behavior. They facilitate responses to both internally-driven and externally-driven demands and play an important role in integrating sensory information.

Given the relevance of the ACC in social cognition and consolation in prairie voles, this thesis will examine connectivity of this area to other brain regions that may be implicated in social cognition. We administered a retrogradely-traveling tracer to the ACC of prairie vole brains to anatomically map projections to this region. Through analyzing brain sections from the anterior portion of prairie vole brains, we will determine which regions project to the ACC. This information will allow us to expand our understanding of social behavior in prairie voles by helping us develop targets for future chemogenetic experiments. We believe several cortical and subcortical regions involved in emotional and sensory processing will project to the ACC, and that some of those regions are implicated in social behaviors relevant to our lab's research. Once we have established which regions heavily project to the ACC and may be relevant for

consolation behavior, we will perform chemogenetic manipulations of ACC-containing circuits to determine their role in consolation. Chemogenetics use Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), which are G protein-coupled receptors, either excitatory (Gq) or inhibitory (Gi) that can be selectively activated by a non-endogenous ligand (CNO), allowing for researchers to control activity of specific neural populations and circuits (Roth, 2016). DREADDs cannot be activated by endogenous ligands; however, the exogenous ligand Clozapine-N-oxide (CNO) binds to DREADDs and is used in chemogenetic experiments. Prior to performing these experiments, we must validate our techniques, since chemogenetic tools in prairie voles have only recently been adopted and have not yet been used by our group to manipulate consolation. We performed a control experiment wherein we examined whether CNO alone, as compared to saline, had any effect on prairie vole behavior during the consolation test. We expected CNO to have no effect on consolation behavior, due to the absence of DREADDs in our experimental animal's brains. The results from the tracing study and the chemogenetic control study will set the basis for future chemogenetic manipulations of ACC-containing circuits during consolation, to expand our understanding of the neural mechanisms underlying empathy-like behavior in prairie voles.

Methods

Experiment 1

This experiment aims to identify brain regions projecting to the ACC and quantify cells within those regions.

Subjects

We selected 3 male and 3 female adults (8-10 weeks old) prairie voles (*Microtus ochrogaster*) from our laboratory colony at Emory originally derived from field-caught voles in Champaign, Illinois. The voles were weaned at 21 days and group-housed in same sex duos or trios at 22°C under a 14:10 h light/dark cycle in ventilated 26 × 18 × 19 cm Plexiglass cages with Bedo'cobbs Laboratory Animal Bedding (The Andersons; Maumee, Ohio). Animals were given access to *ad libitum* water and food (Lab Rabbit Diet HF #5326, LabDiet). All experiments were done following Institutional Animal Care and Use Committee protocol at Emory University.

Surgery

Animals were anesthetized under isoflurane and received an intracranial injection to the ACC of a retrogradely-traveling adeno-associated virus with a CAG promoter and tagged with a green fluorescent protein (AAVrg-CAG-GFP; 37825, AddGene, Lot #d180640). Animals received a total volume of 500 nL and the hemisphere of viral injection was counterbalanced across sexes (**Fig. 1**). The following coordinates were used: AP = 1.4, ML +/- 0.5, DV = -2.1 (mm, relative to bregma). A 10 **µ**L Hamilton syringe was lowered into the ACC and left for 5 minutes prior to injection. The injection then occurred across 5 minutes at a rate of 100 nL/min. Lastly, the injector was left in place for five minutes to allow for diffusion in the tissue.

Animals were then returned to the colony and given 2 weeks to recover from surgery before being transcardially perfused using a 4% paraformaldehyde solution. Brains were collected and stored afterward.

Immunohistochemistry

Brains were sectioned at 40 µm sections on a cryostat. Every other section was used for a subsequent immunohistochemistry protocol. Sections were stained with a rabbit anti-GFP primary antibody, with 4′,6-diamidino-2-phenylindole (DAPI), and with a goat anti-rabbit

secondary antibody conjugated with Alexa Fluorophore 488. Sections were then mounted and imaged on a fluorescent microscope (BZ-X710, Keyence, Japan) using green and blue filters to examine GFP and DAPI expression, respectively.

Sections were washed in 1X PBS three times at room temperature. They were incubated in 0.05% Triton X-100 in PBS (PBST) for 30 min and then 5% normal horse serum (16050122, Thermo Fisher Scientific) in PBST (NPBST) for 30 min. Sections were incubated with rabbit anti-GFP antibody (598, MBL, Japan, Lot #082) diluted 1/1000 in NPBST overnight at 4 °C. Then, the sections were washed in 1X PBS three times at room temperature and incubated with goat anti-rabbit IgG secondary antibody conjugated with Alexa Fluor™ 488 (A-31627A11008, Invitrogen, Lot #2431375 Thermo Fisher Scientific) diluted 1/1000 in NPBST with DAPI (62248, Thermo Scientific, Lot #3410231) diluted 1/400 for 1 h at room temperature. Sections were washed in 1X PBS three times at room temperature and mounted on the glass and dried for 20 minutes. Fluromount-G® anti-fade (0100-35, Southern Biotech, Lot #B0922-N722) was used for coverslipping slides.

Imaging and Image Analysis

Images were taken using a fluorescent microscope (BZ-X710, Keyence, Japan). ImageJ was used to process images and then a semi-automated cell counting software (WholeBrian, Fürth et al., 2018) was used to quantify neurons projecting to the ACC within discrete brain regions. Coronal sections were registered and fit to a coronal mouse brain atlas and cells expressing GFP quantified. This allows for segmentation of cell counts into discrete brain regions across the anterior-posterior axis. Registration of sections and quantification of cells is ongoing; however, upon completion, we will be able to quantify the proportion of cells projecting to the ACC within each region compared to all projections to the ACC. This analysis

will provide information on relevant ACC-containing circuits that our lab can later manipulate in behavioral experiments.

Experiment 2

This experiment aims to determine whether the ligand for future chemogenetic experiments, CNO, has a significant effect on prairie vole social behavior.

Subjects

Male prairie voles (3-7 months old) were used as the experimental "observer" animals in this experiment. All males were paired with a female prairie vole (3-7 months old) for 72 hours prior to the start of behavioral experiments. All housing conditions were consistent with those described in Experiment 1. Following behavioral experiments, animals were returned to the colony and reused in experiments where applicable.

Drug Preparation

Animals were administered either sterile saline or 0.3 mg/kg of Clozapine-N-oxide (CNO; 6329, Tocris, Batch #4A1274367) dissolved in 2% dimethyl sulfoxide (DMSO; BP231-100, Fisher Bioreagents, Lot #277912) and diluted in sterile saline.

Consolation test

After animals were confirmed to be non-related, opposite sex prairie voles within our cohort were paired with one another and allowed three days to cohabitate and form a pair bond with one another (**Fig. 2**). Following this period, animals underwent a three-day testing procedure. On each of the three days, animals remained in their homecage and were placed in the testing room for a 90-minute acclimation period. All nesting material and food hoppers were removed during testing. Animals were separated for 30 minutes following the acclimation period

and reunited in their homecage for 10 minutes at the end of the protocol. They were habituated to this research protocol for two days, and on the second day, the observer animal received a mock intraperitoneal (i.p.) injection during the separation period. On the third test day, the observer animal received an i.p. injection of either CNO or saline during the separation period before the reunion. The demonstrator animal was taken to a separate testing room during this period and administered five footshocks (0.8 mA, 0.5 sec) over the course of 30 minutes. This third reunion period was recorded using video equipment to allow for later behavioral scoring.

Scoring Behavior.

Behaviors exhibited by the observer animals toward the demonstrator animal during the 10-min reunion period were scored using Behavioral Observation Research Interactive Software (BORIS; Friard and Gamba, 2016). An ethogram of behaviors examined can be found in Table 1.

Data analysis.

Durations of behaviors were calculated and analyzed using a 2-way mixed factorial ANOVA with drug condition (saline or CNO) serving as the between-subjects factor and behaviors serving as the within-subjects factor. T-tests comparing behavioral durations between saline and CNO were also performed. All data analysis was performed using GraphPad Prism version 9.4.1 for Mac (GraphPad Software, San Diego, California USA, www.graphpad.com).

Results

Experiment 1

From this experiment, we obtained sections from six prairie vole brains. The project is currently ongoing, so we have only analyzed the anterior portion of two brains included in the

study. Both of these animals were injected with AAVrg-CAG-GFP virus in the ACC in the left hemisphere.

Due to the ongoing nature of this research, we can't draw full conclusions on the regions projecting to the anterior cingulate cortex and the lateralization of these connections. The range of AP coordinates for brain sections analyzed was +0.545 mm to +5.245 mm from bregma, the point at which sagittal and coronal sutures intersect in the prairie vole brain.

From those two brains, we examined several cortical and subcortical regions of the prairie vole brain using GraphPad Prism. We identified and quantified several relevant regions projecting to the ACC (**Fig. 3**). While most regions showed greater expression in the ipsilateral (left) hemisphere, the insula had more contralateral (right hemisphere) expression. Additionally, the infralimbic area and orbitofrontal cortex had high cell counts, both exceeding 1000.

Experiment 2

Within the behavioral paradigm, animals received either Clozapine-N-oxide (CNO) or saline injections in a between-subjects study model. 10 animals in the experiment received saline injections, whereas 9 animals received CNO injections. Durations of each behavior across the reunion period of the testing day were quantified for each animal (**Fig. 4**).

A mixed-model ANOVA was conducted with drug condition as the between-subjects factor and behavioral durations as the within-subjects factor. There was no significant main effect of drug condition ($F(1,85)= 0.5044$, $p = 0.4795$) or interaction ($F(4,85)= 0.1038$, $p = 0.9809$); however, there was a main effect of behavioral duration ($F(4,85)= 12.30$, $p < 0.0001$). Follow-up Bonferroni's multiple comparisons tests were conducted between durations for the saline and CNO conditions for each behavior, and there were no significant differences found (adjusted p value for all > 0.9999). Bonferroni's multiple comparisons test was also used as a

follow-up to compare durations of each behavior to the other behaviors. Huddling was found to be significantly different in its duration compared to all other behaviors (adjusted p value < 0.004 for all). Pair bonded prairie voles tend to spend a majority of their non-active time huddling with each other, and our findings further confirm this. These findings all suggest that CNO does not affect behavior in this paradigm in the absence of a chemogenetic receptor.

Discussion

Experiment 1

Though this research project is ongoing, several cortical regions were identified as important in relation to the anterior cingulate cortex and social cognition broadly: the claustrum, primary somatosensory cortex, primary motor cortex, infralimbic area, the olfactory area, and the piriform cortex.

The claustrum is an important region for social cognition in both humans and non-human primates. Damage to the claustrum can lead to impairments in social processing (Ayyildiz et al., 2022). Functional imaging also showed that the claustrum is activated when integrating sensory information, which is important for emotional experience and interpretation of others' emotions (Bennett & Baird, 2006). The claustrum has also been linked to higher-order social functions, such as empathy, theory of mind, and social decision-making (Atilgan et al., 2022). It is thought to be the seat of consciousness and a hub for the integrating sensory and limbic information (Crick & Koch, 2005). Optogenetic silencing of claustral neurons also impaired memory retrieval, which is important for building social relationships (Kitanishi & Matsuo, 2017). Additionally, evidence has also shown that lesions or inactivation of the claustrum can lead to increased anxiety-like behaviors in rodents (Niu et al., 2022). In our study, we evaluated consolation behaviors in the prairie vole species and the brain regions that are responsible for

these affiliative actions. In humans, consolation behaviors such as hugging reduce cortisol levels in both saliva and blood (Sumioka, Nakae, Kanai, & Ishiguro, 2013). The claustrum's role in mediating this behavior and the physiological benefits associated with consolation suggest that further research on this brain structure may have important implications for the development of new treatments for anxiety disorders and stress regulation.

In addition to the claustrum, other brain regions are implicated in social behavior. The somatosensory cortex is responsible for processing tactile and proprioceptive information related to social touch, while the motor cortex is involved in generating and controlling social motor behavior. The olfactory area and piriform cortex are critical for processing social olfactory information, such as pheromones and other social cues. The infralimbic cortex is a region within the ventromedial prefrontal cortex responsible for the inhibition of subcortical structures in suppression of emotional responses like fear. These regions are all connected to the anterior cingulate cortex, a key region involved in processing social and emotional information. Completion of this project and identifying brain regions projecting to the ACC will provide us with the first whole-brain connectivity map of the prairie vole ACC and will inform future ACC-containing circuit manipulations to better understand how the ACC is involved in social behaviors.

Experiment 2

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are a powerful tool used in behavioral neuroscience research to manipulate neuronal activity in a

targeted and reversible manner. In research, DREADDs have been used to study the role of specific brain regions and cellular populations in behavior. For example, DREADDs have been used to selectively activate or inhibit neurons in the medial prefrontal cortex of mice, a brain region involved in social decision-making, to investigate the effect on inhibitory control, habit formation, long-term memory, and spatial memory (Gunaydin et al., 2010). DREADDs have also been used in rats for example to investigate the role of the anterior insula cortex (aIC), a brain region critical for processing social and emotional information, in rescuing heroin-induced deficits in prosocial behavior (Tomek, Stegmann, Leyrer-Jackson, Piña, & Olive, 2020). Excitatory DREADDs localized to the aIC were found to rescue deficits in prosocial behavior brought on by heroin self-administration. Through use of DREADDs, researchers can obtain valuable information about the circuitry and neuronal mechanisms underlying social behavior throughout several regions of the brain.

In our study, we performed a CNO-Saline control experiment to confirm whether CNO affects behavior in the absence of DREADDs. Our findings indicate that CNO alone did not have a significant effect on affiliative behaviors studied in the research paradigm. CNO conditions did not alter the duration of allogrooming, sniffing, social rest, self-grooming, and huddling exhibited by the observer animal toward its partner. Given that CNO is the exogenous ligand that will be used in subsequent DREADDs experiments, our research indicates that CNO alone does not affect behavior in the consolation paradigm. In future studies, we can reduce the number of animals used by not having to include a control saline condition within the experimental paradigm. Comparisons can then be made between animals given a control vs. DREADD virus, with all animals receiving CNO during behavioral testing. Since CNO and saline do not produce significant differences in behavior, this finding will allow future DREADDs experiments to be

completed with greater efficiency. DREADDs are a novel technique in prairie voles, and our experiment was important to establish that CNO alone has no significant effect on behavior within the consolation paradigm to set a precedent for future DREADDs experiments in prairie voles. Moving forward, our lab plans to utilize the ligand CNO with either inhibitory or excitatory DREADDs in the ACC to better understand the role of this region underlying vole social behavior.

Conclusions

The anterior cingulate cortex (ACC) is a highly interconnected brain region that plays a critical role in social cognition. Based on functional neuroimaging studies in humans and anatomical connectivity studies in non-human primates, the ACC receives input from a wide range of brain regions involved in social processing, including the amygdala, prefrontal cortex, insula, and temporal lobe, and sends output to other regions involved in social cognition, such as the striatum and hypothalamus (Etkin, Egner, & Kalisch, 2011). This extensive connectivity allows the ACC to integrate sensory and socially salient information from multiple sources and modulate social behavior accordingly. For example, the ACC is involved in the regulation of emotional responses to social stimuli, such as facial expressions, and in the modulation of social decision-making, such as trust in humans and cooperation in Rhesus monkeys (Behrens, Hunt, Woolrich, & Rushworth, 2008; Haroush & Williams, 2015). Altered ACC connectivity has been linked to deficits in social cognition and emotion regulation in a variety of psychiatric disorders, including autism spectrum disorder and depression ((Kana, Libero, & Moore, 2011; Sheline, Price, Yan, & Mintun, 2010). In the Kana study, researchers used Positron Emission Tomography (PET) to produce images of the brains of human participants with autism. They found underconnectivity in the ACC, medial prefrontal cortex, and TPJ, among other regions. In

the Sheline study, researchers explored three different brain networks in humans, the cognitive control network, default mode network, and affective network (AN), and the impact of depression on their functionality. These regions are all connected to the dorsomedial prefrontal cortex (DMPFC), termed the dorsal nexus. Researchers found that the ACC had extremely high connectivity to the DMPFC, which shows potential targets for treatment of depressed individuals.

Through this ongoing research, we will be able to use information about various ACC inputs and their relative contributions to social behavior, along with chemogenetic manipulations to help us understand the role of brain regions projecting to the ACC in facilitating rodent social behavior. Findings outlined in this paper related to brain regions implicated in social cognition will inform subsequent studies on prairie vole social behavior. Due to the highly affiliative nature of monogamous prairie voles, they have emerged as an excellent model for translational social behavior, allowing for this work to inform investigations into human social processing. Work in human socio-cognitive function is essential for understanding how humans, a highly social species, process social stimuli and make decisions in social situations. Understanding social processing in humans also informs research into those who have atypical social processing such as individuals with ASD. Through investigation into human empathetic behaviors and affiliative decision-making, researchers can identify atypical social processing and appropriately create solutions for management or treatment of these deficits. Additionally, modulation or evaluation of these circuits in animals can help us better understand human social behavior and the mechanisms underlying decision-making processes.

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TABLES:

Table 1. Ethogram of relevant social behaviors

Social rest	Observer animal is groomed by the demonstrator animal
Allogrooming	Observer animal grooms the demonstrator animal
Sniffing	Demonstrator animal's body or anogenital region is sniffed by the observer animal
Huddling	Observer animal and demonstrator animal sit side-by-side
Self Groom	Observer animal grooms themselves

FIGURES:

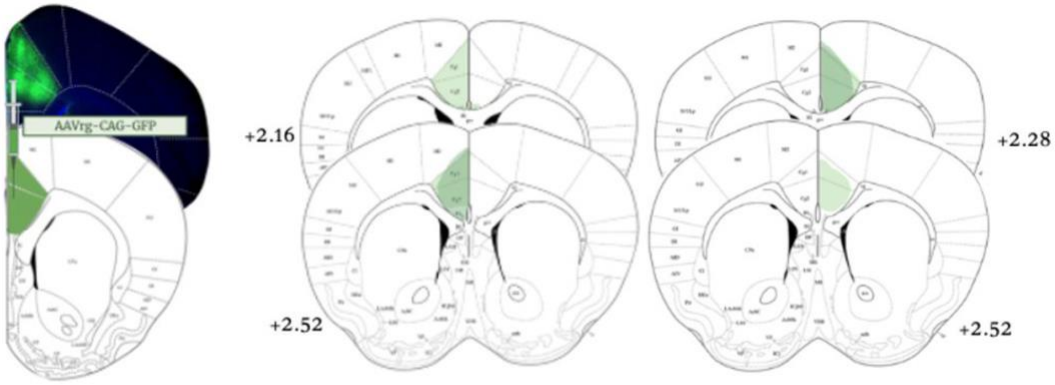

Fig 1. – Animals received injection of AAVrg-CAG-GFP to the ACC. Animals received an isoflurane anesthetic before an intracranial injection of a retrogradely-traveling adeno-associated virus with a CAG promoter and tagged with green fluorescent protein (AAVrg-CAG-GFP). The following coordinates were used for injection: AP = 1.4, ML +/- 0.5, DV = -2.1 (mm, relative to bregma). The injections occurred across 5 minutes at a rate of 100 nL/min. Animals were perfused two weeks after surgeries and their brains sectioned at 40 μM.

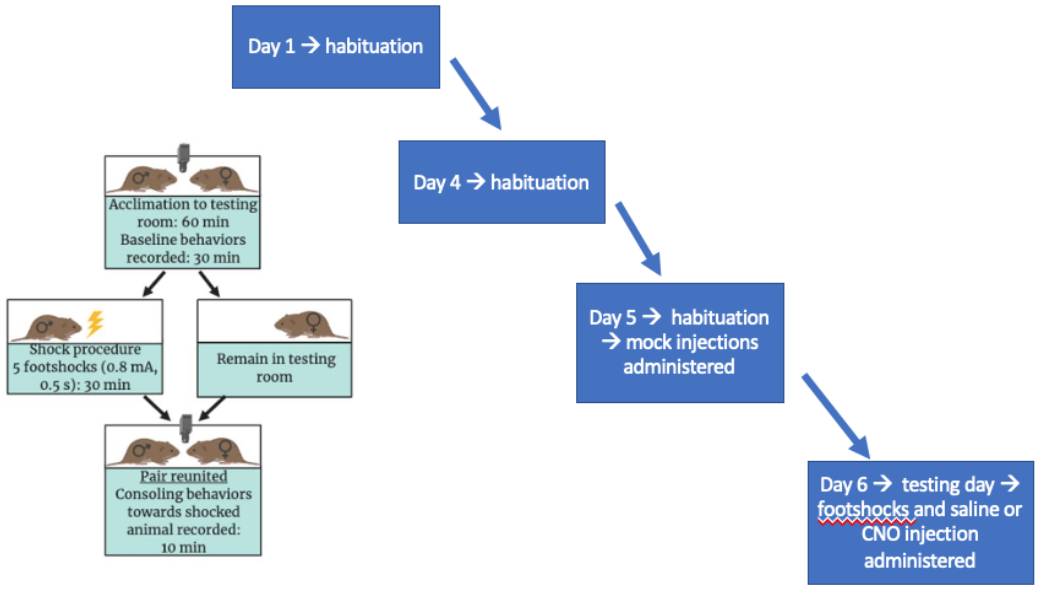

Fig. 2 – Animals underwent a consolation behavioral paradigm across six days. In this study, animals were given three days to pair bond and mate before beginning the three-day behavioral paradigm. The animals were paired on Friday of the previous week, and, on Monday of the subsequent week, animals pair-bonded and became monogamous partners. The fourth day of this procedure is the first day of the behavioral study. On this habituation day, animals were given 90 minutes to acclimate to the testing room, separated for 30 minutes, and reunited for 10 minutes. On the second habituation day, animals went through the same process except the observer animal received a mock saline injection, and the animal pair was recorded for 30 minutes during the acclimation period. On Day 6, the testing day occurred. Animals were recorded for 30 minutes during the acclimation period. When animals were separated, the observer animal received either a saline or CNO injection. During the reunion period, the animals were recorded for 10 minutes.

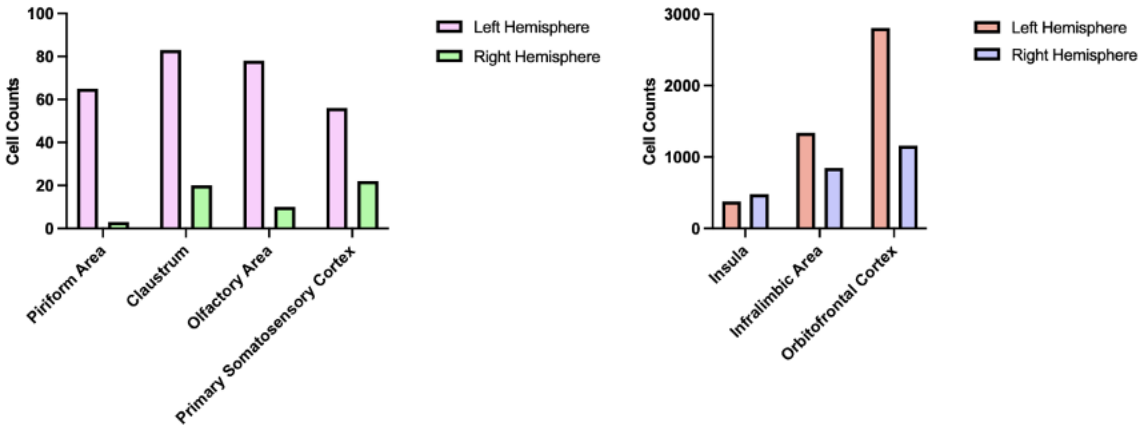

Fig. 3 –Brain regions projecting to the ACC. Cell counts from brain regions in the anterior portion of the brain (0.545 mm to 5.245 mm, relative to bregma) projecting to the ACC were quantified using WholeBrain analysis. (**A**) The piriform area, claustrum, olfactory area, and primary somatosensory cortex all project to the ACC, and greater cell counts for these brain regions were found in the left hemisphere which was ipsilateral to the injection site. (**B**) The insula, infralimbic area, and orbitofrontal cortex also showed projections to the ACC. The insula had greater cell counts in the right hemisphere which was contralateral to the injection site.

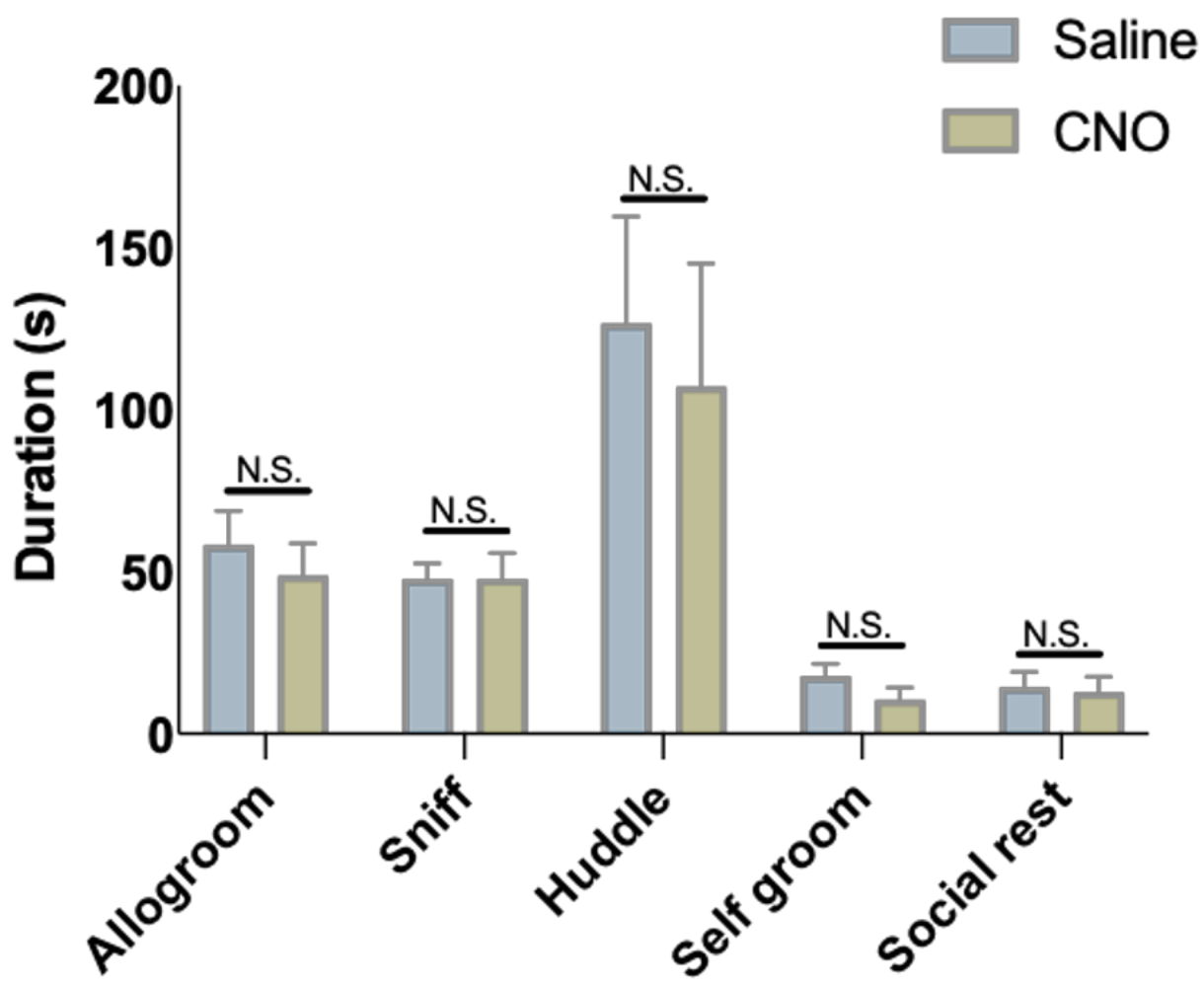

Fig. 4 – Durations of behaviors exhibited during reunion period. During the testing day of the consolation paradigm, animals were administered either saline or exogenous ligand CNO injections. There were observer and demonstrator animals included in the study, with the demonstrator undergoing footshocks following separation from their partner. Observer animals consoled their partners following the reunion period. Behaviors exhibited by the observer to the demonstrator during the reunion period were quantified. The following behaviors were examined: allogrooming, sniffing, huddling, self-grooming, and social rest. No main effect was found within injection conditions in the study, but there was a main effect within durations of

affiliative behaviors examined. There was no interaction found between behaviors and injection conditions. A significantly greater duration of huddling was found compared to other behaviors.