TITLE. Of Fear and Fellowship, the Neural Dynamics of Social Emotion

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SUMMARY
Social contexts allow for the influence of emotions between agents. Emotional experiences of an individual can both be modulated by the presence of another as well as modulate the experience of another. We found that the presence of a conspecific in stressful, novel contexts reduced the occurrence of fear behaviors in rats. The mean amplitude of prominent local field potential (LFP) oscillations within the Basolateral Amygdala (BLA), Anterior Cingulate Cortex (ACC), and Insular Cortex (INS) during freezing behaviors were also reduced during social versus non-social conditions. These decreases in amplitude occurred despite increases in oscillatory coherence between the BLA and INS as well as between the BLA and ACC in the same ranges. Conversely, during a novel head swaying behavior that resembles freezing, there were no regional differences in amplitude despite a difference in coherence. These findings suggest that during social conditions, there may be a lower demand for cooperative processing across the BLA, ACC, and INS to process and respond to potentially fearful stimuli, despite more activity overall. Additionally, despite occurring in similar situations to freezing, the novel head-swaying behavior constitutes an electrophysiologically distinct state. As a whole, these findings indicate distinct behavioral outcomes and neurophysiological correlates of fear and vigilance when comparing cases of social and non-social contexts.

INTRODUCTION
In social situations, emotions of an individual can have an impact on the state of the group as a whole. The presence of social support can reduce the degree of negative emotions[2], while positive emotions can influence others to exhibit similar positive sentiments[9]. Thus emotions in one individual can both be influenced by the presence of others as well as impact the emotional experience of another. With a growing importance in neuroscience to understand social contexts, and an evolving modern space with larger than before social networks, understanding how individuals influence others becomes increasingly important.

When conditions of fear, stress, or uncertainty arise, animals often use the existence of others as a way to respond to and mediate these emotions. Rodents, especially rats, have been shown to exhibit a freezing behavior, which can be socially transmitted across a population[3]. Additionally, pairs of rats have been observed exhibiting a head-swaying behavior in which the core of the body remains still while the head moves horizontally back and forth. For the sake of this paper, these behaviors together will be referred to as “Vigilance Behaviors.” While these behaviors have been shown to be socially transmitted, the reasoning remains unclear. One possibility is that

these behaviors are leveraging social buffering[13]. Alternatively, this may be seen as a social contagion behavior, as the fear response or stress transfers from an initial rat to another[11]. Understanding the causes of these behaviors can inform future goals in the interpretation of neural underpinnings of compassion or empathy. To expand on these behaviors, I also aim to understand the underlying processes that give rise to them and how they may differ depending on the relevant information.

In an effort to understand this, I investigated three regions of interest: the Anterior Cingulate Cortex (ACC), the Basolateral Amygdala (BLA), and the Insular Cortex (INS). These regions are of relevance as they work together to process external sensory information with interoception to evaluate the environment as well as other entities in order to inform behavior. The ACC has been shown to contain "emotional mirror neurons"[4], and inhibition of this region disrupts observational fear learning[8]. The BLA holds importance in the processing of emotions like fear and anxiety[1], and also is relevant to the processing of social recognition[17]. Lastly, the INS is related to signs of individual subjective emotional awareness[5], and inactivation of the region inhibits social affective preference[16]. These regions provide key information in understanding how the representation of the emotional states of both subjects might impact the behavioral outcomes. As such, I investigated these regions to examine how differences in behavior might be based on affective state (of self and others), and how relationships between subjects might impact the social transmission and engagement with these behaviors.

RESULTS

To observe emotion both within social and non-social contexts, we used a paradigm designed to elicit reactions to potentially threatening novel stimuli in which rats are allowed to roam an open field (Supplementary Figure 1), occasionally being presented with large moving stimuli (Supplementary Figure 2) for brief periods of time. High-density extracellular recordings were collected from each region (BLA, INS, ACC) for 5 rats. A single trial consisted of a 20-minute recording in which stimuli were presented 10 different times to elicit behavioral responses, however behaviors were analyzed regardless of whether they occurred after the presentation of a stimulus or not. Rats experienced two *solo* and two *cagemate* recording sessions (counterbalanced for order of appearance) in which they experienced the stimuli alone or in the presence of a cagemate, respectively.

Vigilance Behaviors Decrease in Social Settings

We first examined whether there would be behavioral differences between the two conditions. We found significant differences in the number and duration of head-swaying events as well as the duration of freezing events across solo and cagemate conditions (Figure 1). Non-vigilance behaviors on the other hand didn't show this significant change between conditions (Figure 2). Grooming was used as a point of comparison due to it being similarly linked to potentially stressful situations[10]. Social relationships were then evaluated during the cagemate conditions

based on the occurrence of social behaviors such as grooming each other, wrestling, following, and sniffing each other. These were then used as regression parameters to examine whether the relationship between occurrences of these behaviors were affected by social behaviors exhibited between rats, none of which had significant effects.

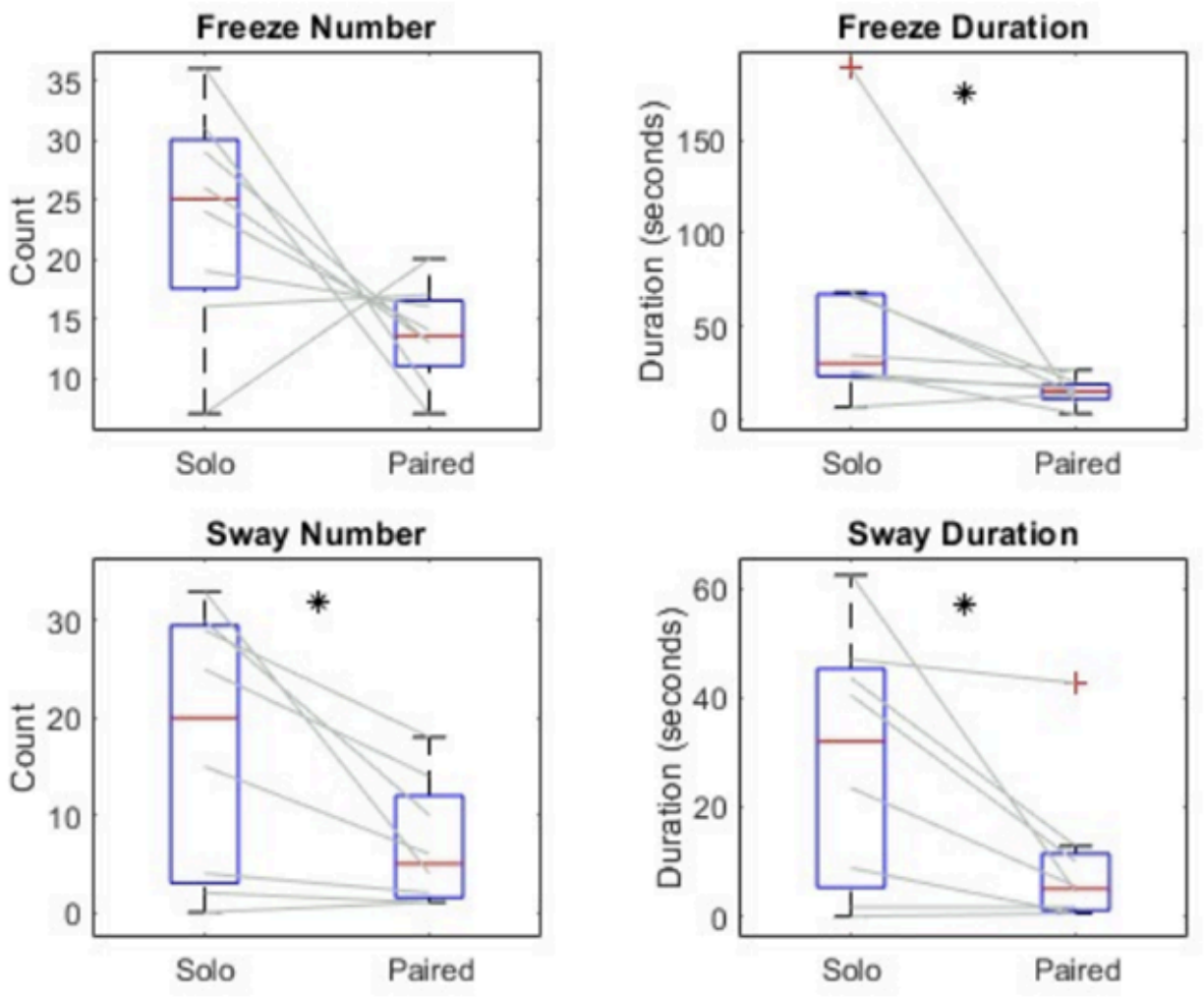

Figure 1: The existence of a cagemate in the open field arena condition results in decreased duration of freezing and head-swaying behaviors along with reduced numbers of head-swaying. Left column: the count of unique instances of each behavior. Right column: The total duration of all instances of each behavior. Significance determined by a Wilcoxon signed rank test with threshold $\alpha=0.05$ with $n=8$ rats used for behavior comparisons. Significance in the freeze duration still holds even with the outlier removed.

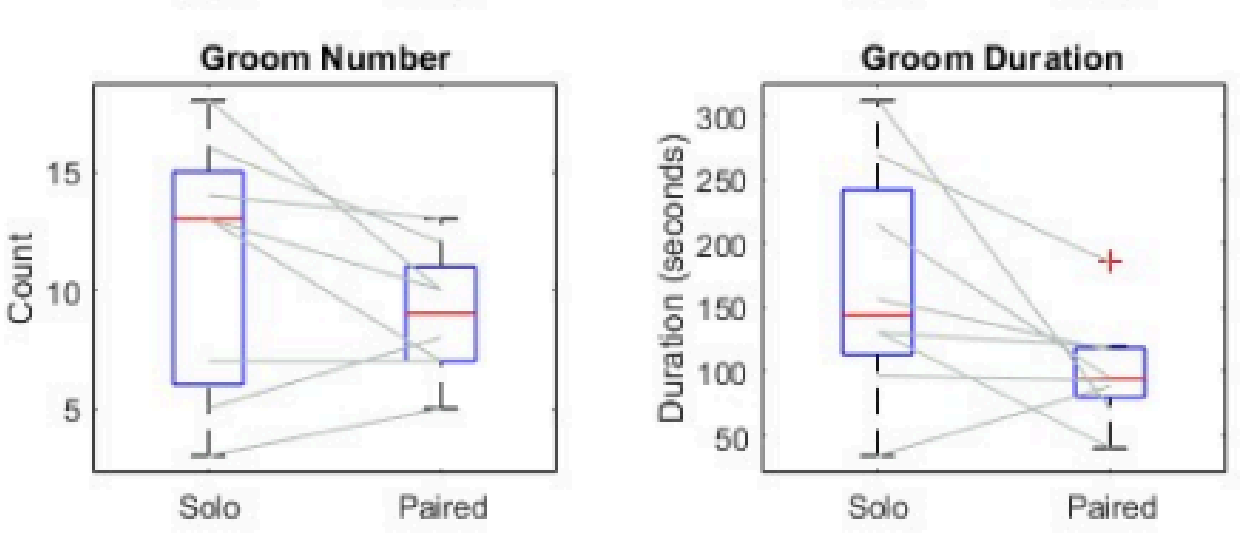

Figure 2: The existence of a partner in the open field did not have any significant effect on the occurrence of non-vigilance behaviors. Left column: the count of unique instances of each behavior. Right column: The total duration of all instances of each behavior. Significance determined by a Wilcoxon signed rank test with threshold $\alpha=0.05$ with $n=8$ rats used for behavior comparisons.

Regional Neural Signatures of Vigilance Behaviors

We then looked to understand how the underlying neural activity changed, to examine if there might be neurophysiological contributions to the behavioral outcomes we observed. Frequency bands of interest were identified by observing prominent rhythms in spectrograms, power spectral densities, and magnitude squared coherence between regions, which were then isolated to observe the average amplitude of these regions during the times surrounding behaviors of interest. Given no significant differences in the occurrence of grooming behaviorally, we opted to only look at the neural signatures of vigilance behaviors as a means to evaluate social effects. Mean amplitude was selected to be averaged over 2 second periods of interest: the 1.5 seconds before a behavior occurred to the 0.5 seconds following the onset of behavior. These results are based on a bootstrap analysis to create an equal number of occurrences across different conditions despite any significant differences which were present in the original numbers. For this, each cagemate session was matched with either the solo session that preceded or followed it, at which point the session which had higher numbers of the behavior of interest were randomly subsampled to match the session with lower behavioral numbers. Subsamples were then generated 100 times, averaged and a 95% confidence interval was constructed to test for significant differences between conditions.

In one particular range of interest (22-28 Hz), we noted a significant difference in the average amplitude between solo and cagemate conditions across all three regions (Figure 3). Notably, this only occurred surrounding freezing behaviors and not head-swaying behaviors. When compared

to another frequency range selected for comparable findings across spectrograms, power spectral densities, and magnitude squared coherence, this difference was not found (Figure 4). With distinct dynamics existing within a given region, we further looked to investigate the relationships between regions, so we elected to further investigate the coherency.

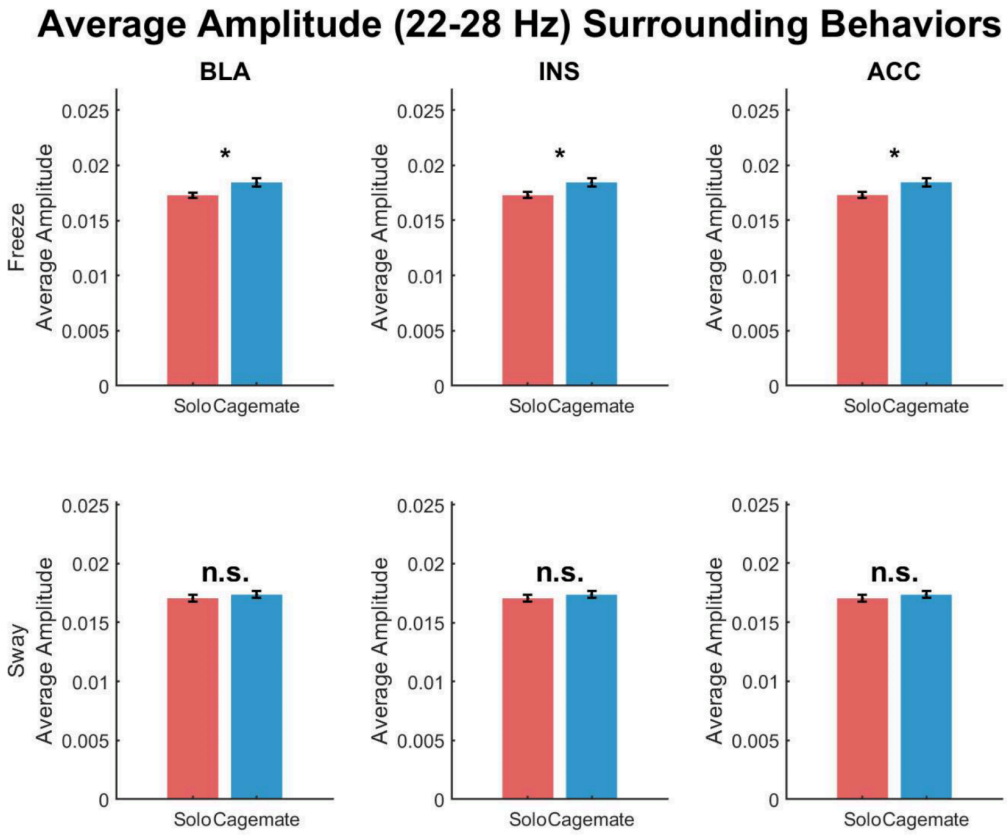

Figure 3: Average amplitude across all behavior region combinations. Statistical comparisons were done using bootstrapped samples. The top 3 subplots correspond to the freezing behaviors while the bottom 3 subplots correspond to the head-swaying behaviors. Each column corresponds to a different region, from left to right being Basolateral Amygdala (BLA), Insular Cortex (INS), and Anterior Cingulate Cortex (ACC) with n=5 rats used for neural comparisons.

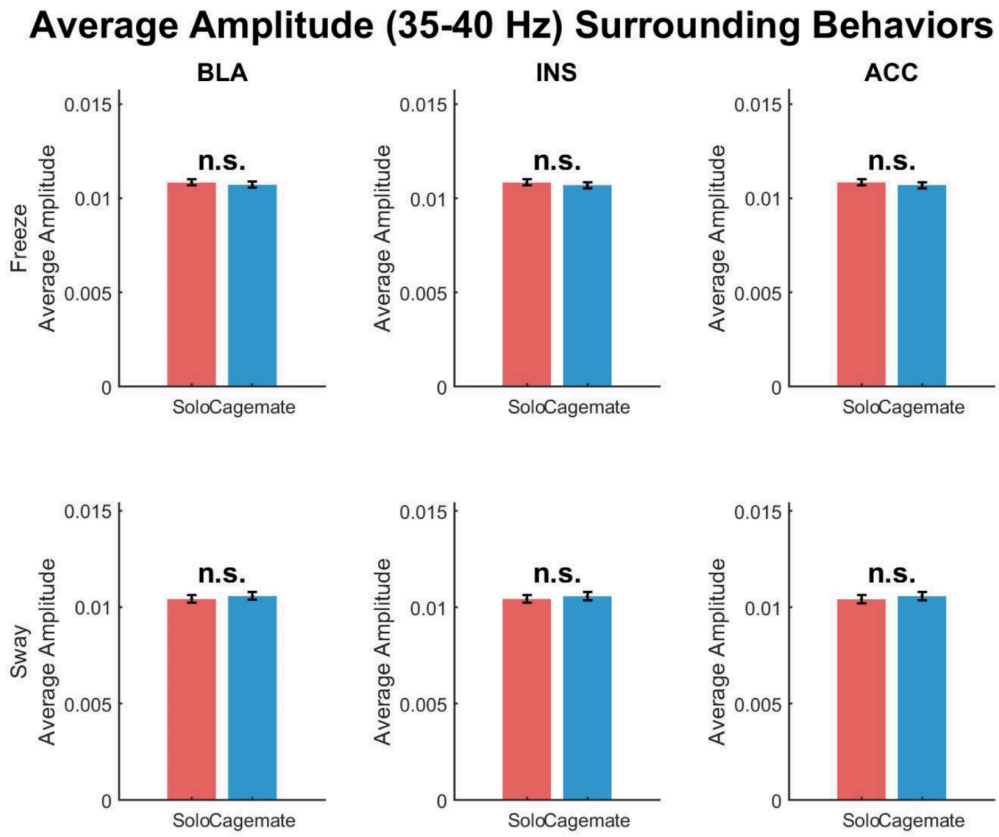

Figure 4: Average amplitude across all behavior region combinations. Statistical comparisons were done using bootstrapped samples. The top 3 subplots correspond to the freezing behaviors while the bottom 3 subplots correspond to the head-swaying behaviors. Each column corresponds to a different region, from left to right being Basolateral Amygdala (BLA), Insular Cortex (INS), and Anterior Cingulate Cortex (ACC) with n=5 rats used for neural comparisons.

Neural Signatures Across Regions

When observing relationships across regions, we found notable increases across both the BLA and INS coherence as well as between the ACC and BLA in the 22-28 Hz frequency range (Figure 5). This occurred during both the freezing and head-swaying behaviors while in the mean amplitude there was an inverse effect only during freezing behaviors. despite the mean amplitude not showing significant differences for head-swaying. Similar effects can also be seen in the 35-40 Hz frequency range, with no significance for any behavior and region interaction in the average amplitude.

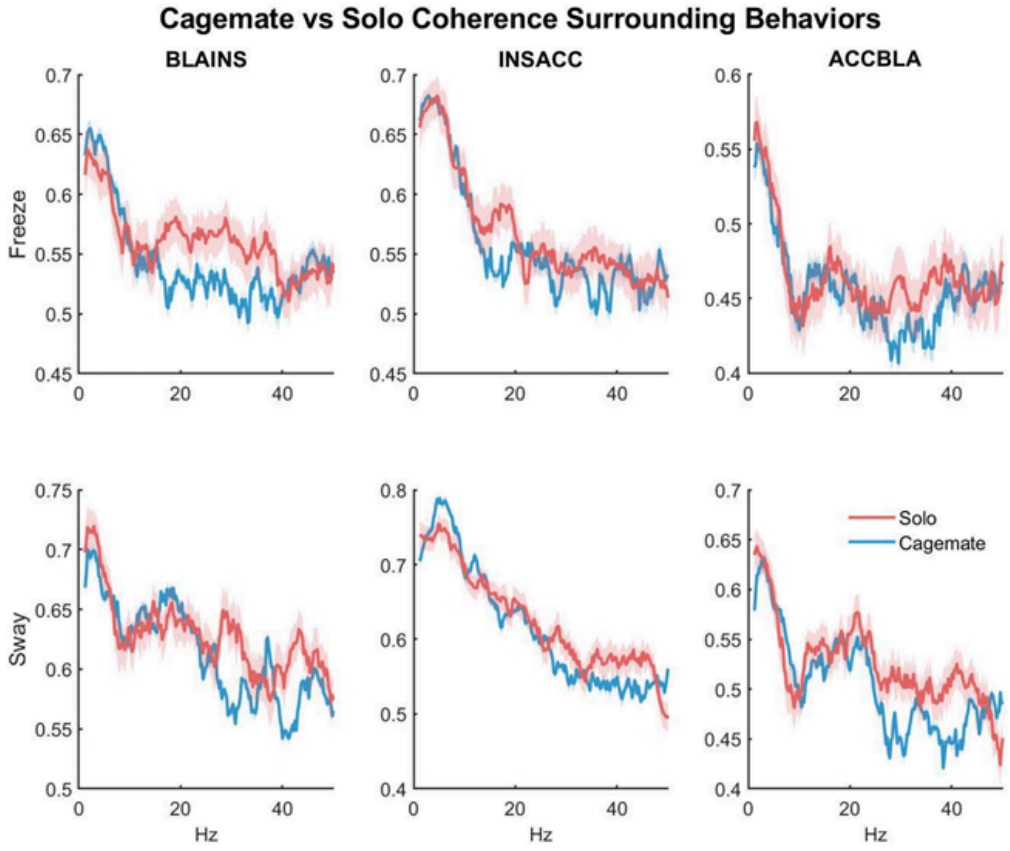

Figure 5: Coherence between regions surrounding behaviors. The top 3 subplots correspond to the freezing behaviors while the bottom 3 subplots correspond to the head-swaying behaviors. Each column corresponds to a different region pairing, from left to right being Basolateral Amygdala with Insular Cortex (BLAINS), Insular Cortex with Anterior Cingulate Cortex (INSACC), and Anterior Cingulate Cortex with Basolateral Amygdala (ACCBLA). Plots contain lines for both Solo and Cagemate conditions. 95% confidence intervals based on bootstrapped samples are shown surrounding each line.

DISCUSSION

Social situations provide both the possibility of and prior evidence that emotions can be influenced by and influence others. In this study we found that different types of behaviors may be differentially impacted by the presence or absence of a known conspecific. Vigilance behaviors exhibited decreases in either the duration or both number and duration across trials when in the presence of a conspecific. Grooming, another type of stress related behavior, didn't exhibit change. This may be due to the roles of these behaviors in specifically social contexts, as freezing has prior evidence showing its effects in social buffering[12], while the novel head-swaying behavior exhibits similar physical properties to freezing. This finding indicates

that despite grooming also having relevance as a stress-related behavior[10], the functions of these types of behaviors and how they can change across contexts may differ.

To understand potential mechanisms resulting in these behavioral differences, we sought to look at neural signatures and how they differed between situations. For freezing behaviors, we observed a decrease in the mean amplitude when looking at the non-social contexts as compared to the social contexts, which was opposite of the difference observed in the coherence between the same regions. This may be an effect that arises due to a tighter coordination between the regions such as what happens when lidocaine-induced sodium channel blockage amplifies coherence across disparate regions of cortex[15]. This finding could indicate that in non-social settings, these regions have stripped away unnecessary activity to increase functioning of a fear-related circuit.

The decrease in mean amplitude is an interesting result, however, as within this frequency range these regions are implicated in a number of significant ways. There's evidence that a stronger emergence of beta (13-30 Hz) in the BLA is associated with anxiety[7], associated deviations from expectation and significant stimuli in the INS[6], or continued exposure to stressors in the ACC[14]. Finding higher mean amplitude within this frequency range during social situations suggests that there may be more total information processed surrounding the stimuli and situation, potentially involving information surrounding the state of the present conspecific, but that the decrease in coherence may result in less down-stream effects or less temporally consolidated regional activity.

These neural findings also indicated a significant difference between the two vigilance behaviors despite what appeared to be potentially similar behavioral outcomes. Significant differences in mean amplitude were observed during freezing between the across social and nonsocial conditions, but not during head-swaying. Looking at oscillatory coherence profiles across these two behaviors, we do find some differences in multi-regional activity as well (Supplementary Figure 3) which could be further explored to investigate the differences between these two similar behaviors.

This work presents an early understanding of how behavioral outcomes between non-social and social situations might have underlying neural differences. Future work from this group aims to further investigate the dynamics of social behaviors through analyzing differences in neural correlates based on the role of the actor in socially transmitted behaviors, whether the origin or recipient of one, or based on the capability of the recipient of socially transmitted behaviors to observe the stimulus prompting them. Further research also aims to address how the degree of social bond might influence social behaviors, and investigate more thoroughly the interactions and relationships between these regions during social contexts.

METHODS

Behavioral Paradigm

All protocols involving animals were performed in accordance with National Institutes of Health (NIH) guidelines and with the prior approval of the Institutional Animal Care and Use Committee (IACUC) at the University of California, San Diego.

Animals

All animals used were female Sprague-Dawley rats from Charles River Laboratories. Rats were raised in pairs until surgically implanted at which point they were separated. There were a total of 4 pairs or 8 rats. Rats were enriched 2 times weekly in their pairs both before and after surgical implantation. Rats were between 7 and 14 months of age at the time of recording. Animals that were raised in pairs together are considered "cagemates" for this study.

Apparatus

The open field arena consists of a 4-foot diameter painted wood base surrounded by 3-foot clear plexiglass walls. The arena was filled with a variety of plastic enrichment toys including but not limited to dog toys, balls, springs, and bottles. Enrichment toys were changed between recording days. Animals were exposed to the arena to habituate them for a 20 minute session with no stimuli first. The animals were then exposed to one set of trials before chronic implantation for behavioral data, as well as one set of trials after chronic implantation for behavioral and neural data.

Trials

Each recording consisted of a 20-minute session. During each session, 10 fearful stimuli were presented, with randomly generated spacing between each stimulus for 60 to 150 seconds. Fearful stimuli presented included Party City Hanging Halloween Decorations, experimenters with masks on, and cardboard predator imitations. Stimuli were presented for 15 seconds in motion at a distance of roughly 2 feet from the arena. Locations in which stimuli were presented were also randomized, occurring in one of 4 positions spaced around the perimeter of the arena. A white noise machine was present and on for the duration of the trial to mitigate the effect of any noise made by the experimenters when presenting stimuli. The order of trials was counterbalanced between pairs.

Experimental Conditions

Two experimental conditions were used throughout the recordings, "Solo" in which a single rat was present, and "Cagemate" in which a rat was placed in the arena with the cagemate as mentioned above. Each of these experimental conditions consisted of 2 days of recording totaling 4 days of recording for each rat.

Behavioral Coding

All video recordings were independently scored by two trained raters. Scored recordings were then compared between raters using Cohen's Kappa, with a minimum score of 0.6 required to be included in the dataset. Scorings were then randomly selected between the two raters who met criteria for a given recording. Behaviors of <0.5 seconds in duration were dropped from neural analyses to ensure a minimum duration for comparison.

Electrophysiological Recordings

High-density extracellular tetrode recordings were performed with independently movable tetrodes implanted in the Anterior Insula, Basolateral Amygdala, and Anterior Cingulate Cortex. Recordings were conducted at 40 kHz using Plexon's Omniplex Neural Data Acquisition system. Signals were bandpass filtered between 1-400 Hz to isolate local field potentials (LFPs). A total of 5 rats were implanted into these regions.

Analyses

Mean Amplitude. A third-order Butterworth filter was used to bandpass-filter LFPs for each of the ranges of interest for mean amplitude. A Hilbert Transform was used to compute instantaneous phase estimates within a given frequency range. These were then averaged over a window of 2 seconds from 1.5 seconds prior to the beginning of the behavior to 0.5 seconds after the behavior started. Mean Amplitudes were then bootstrapped with 100 unique bootstrap samples by pairing solo and cagemate trials following each other and selecting the minimum value of each behavior between the days to create equal numbers between groups for comparisons to avoid differences arising from biased estimation.

Coherence. Coherence was calculated using the Chronux package in matlab with taper time-bandwidth=5, numbers of tapers=9, padding=1, error bars between 2 and 0.05, and filtered between 1 and 150 Hz. Coherence was similarly bootstrapped to remove bias of the estimator.

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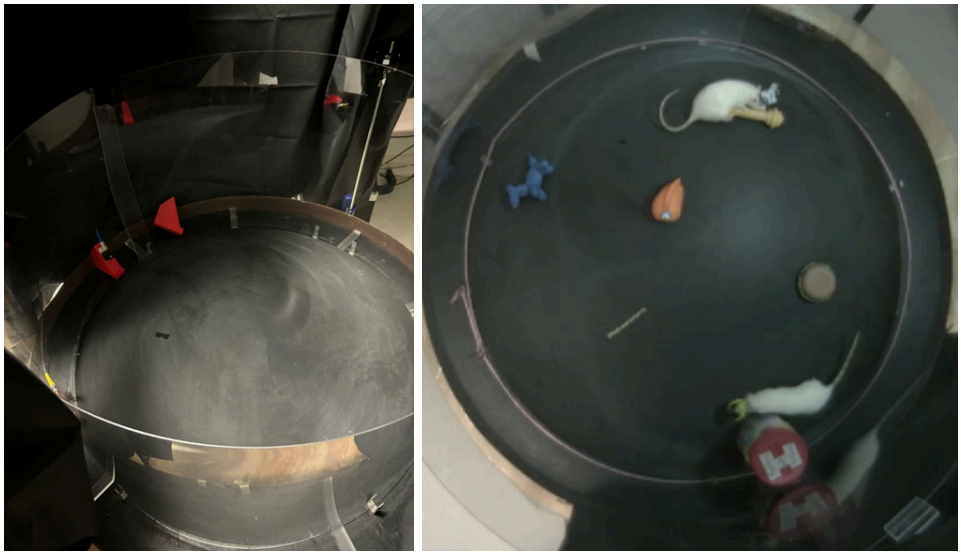
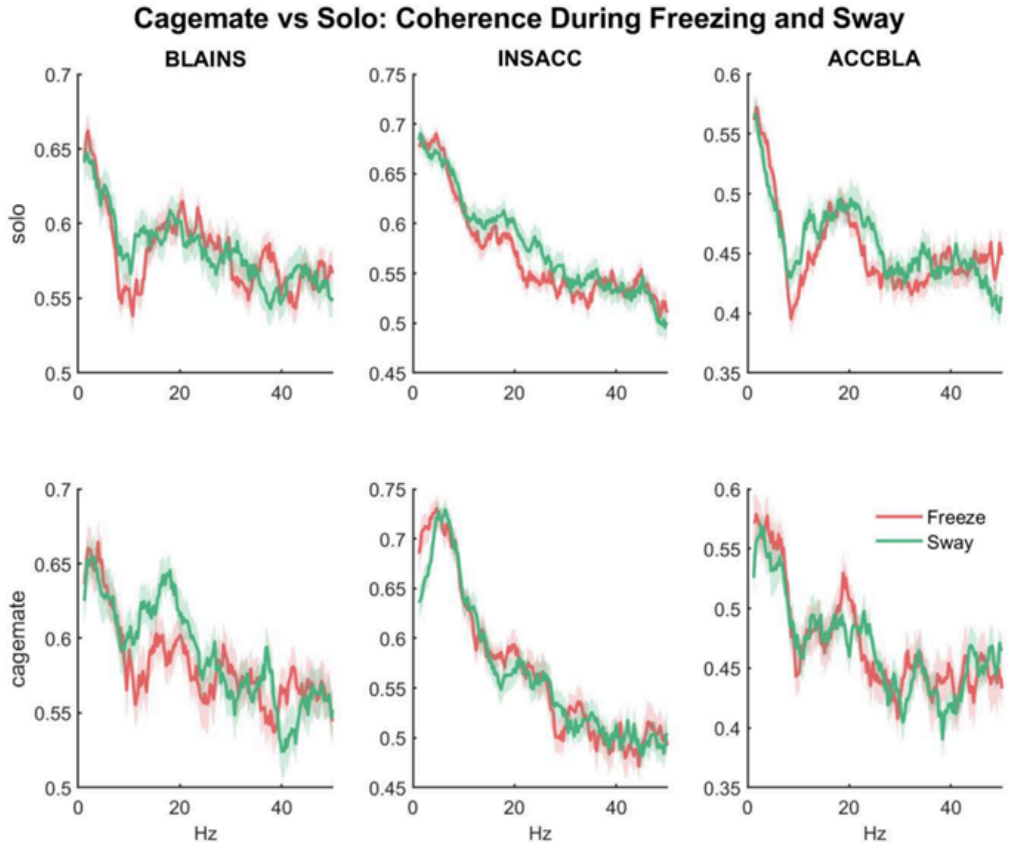
SUPPLEMENTARY MATERIALS



Supplementary Figure 1: The arena in which the experiment was conducted. The completely empty arena from a partial side view (Left). The arena from a top down view as seen during one recording with toys present (Right).

Supplementary Figure 2: Example stimuli presented during trials. Stimuli were divided into 3 categories, "masks" (left), in which an experimenter wearing a mask would be present and moving during the presentation time and "scarecrows" (middle) or "cardboard predators" (right), for which an experimenter would hold the object in question at the desired location and move it around.



Supplementary Figure 3: Coherence comparison between different behaviors. The top 3 subplots correspond to the solo condition while the bottom 3 subplots correspond to the cagemate condition. Each column corresponds to a different region pairing, from left to right being Basolateral Amygdala with Insular Cortex (BLAINS), Insular Cortex with Anterior Cingulate Cortex (INSACC), and Anterior Cingulate Cortex with Basolateral Amygdala (ACCBLA). Plots contain lines for both freezing and head-swaying behaviors. 95% confidence intervals based on bootstrapped samples are shown surrounding each line.