Neediness, co-distress, and helping behavior in rats

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Abstract

Helping behavior is a crucial part of human prosocial dynamics. Studying the factors that increase helping behavior, as well as inhibit it, may help us understand why some people are more likely to help others or gain more access to help from others. Rats are highly social and exhibit prosocial behavior, including helping behavior, and therefore are an ideal species to use in research on propensity to help others. The present study will analyze how a free rat responds behaviorally to a trapped rat in need and how behavioral markers of distress, exploration, and hypervigilance may impact the likelihood of helping.

Introduction

Helping behavior

Helping behavior is an extremely vital component of human prosocial behavior. Therefore, it is important to understand what motivates people to help others, as well as what factors may inhibit helping behavior. It may be the case that people help others that they are familiar with, or others that have helped them in the past in order to display reciprocity. Displayed distress or signals of danger coming from someone in need may also have an impact on their likelihood of being helped. Rats are an ideal species to study in order to understand these dynamics, as they have been shown to be very social and empathic (Barnett, 1958; Barnett & Spencer, 1951, Bartal et al., 2011).

Prosocial behavior in rats

Many studies have established that rats display consistent prosocial behavior, specifically in a context in which one rat takes an opportunity to help another out of a restrainer (Bartal et al., 2011). This helping behavior increased in a follow up study in which the free rat and the rat trapped in the restrainer were familiar with each other. In this case, helping behavior (opening the restrainer) increased in frequency (Bartal et al., 2014). This increase was present regardless of the strain of the familiar rat, indicating that familiarity may overpower any bias that the rat has for other rats of its own strain.

This may be due partially to the evidence that exists for empathy and empathically motivated behavior in rats. One study examined rats' behavior in response to the affective state of another rat and found that avoidance behavior was displayed more often for adult rats displaying distress (Rogers-Carter et al., 2018). However, empathy may also motivate helping behavior due to the rats acting to reduce their own distress in response to the distress of a conspecific. Quantifying co-distress behaviors and the affective responses of the free rat to the trapped rats displays of distress may provide evidence for this line of reasoning.

In the present study, the restrainer model is used, and a free rat is given access to a lever that will open the restrainers in which two individual rats are trapped. A cold ice pack is placed on the floor of one restrainer in order to increase the distress of the trapped rat, creating a “higher need” condition for one of the rats. One rat is a familiar rat of a different strain, and the other is the same strain as the free rat, but is unfamiliar. Behavioral analysis will examine how distress behaviors from either trapped rat impact the behavior of the free rat. Potential behavioral responses from the free rat may include distress, avoidance, and exploratory behavior.

Distress Contagion Hypothesis

Affective empathy in humans is commonly defined as the sharing of affective emotion between people. There is evidence that rodents display a similar phenomenon, which has been referred to in recent literature as “emotional contagion” (Yu et al., 2024). This paper proposes that rodents may use olfactory cues, such as pheromones, in order to communicate threat, fear, and distress. Other sensory routes of emotional communication are also examined, including visual, auditory, and observable behavior. In our research, we focused on observable behavior as well as one physiological measure in the form of defecation in order to quantify distress signals. According to the rodent emotional contagion theory explored by Yu and colleagues, the rats in our study may be spreading their distress through emotional contagion to each other through observable distress behavior.

Keyser's and colleagues also explore the idea of emotional contagion in rodents as a function of affective empathy, citing that similar brain regions involved in human empathy are involved in emotional contagion in rodents. Vicarious freezing, a known phenomenon in which rats freeze in fear at the same time, is demonstrated significantly by both familiar and unfamiliar rats of different strains, implying that the effects of distress contagion are not solely limited to familiar rats (Keysers et al., 2023). This is confirmed in a meta-analysis including 80 years of work on rodent emotional contagion (Hernandez-Lallement et al., 2022). In our study, we explored the effects of both familiarity, need, and observable distress behavior in order to determine the impact that each has on rodent prosocial behavior.

Methods

"Experimental Design

All testing occurred with cagemate pairs. Each session consisted of ten consecutive trials. In a given session, one rat from the pair was assigned the role of "trapped rat," and the other rat was assigned the role of "free rat." Each session began with a two-minute habituation phase. Both rats were placed in the arena and allowed two minutes to freely explore the environment. The restrainer door was ajar to allow the rats to enter and explore the entirety of the testing environment. Following the habituation phase, the rats were placed in their separate cages while the experimenter prepared the arena for testing.

At the start of each trial, the trapped rat was placed in the restrainer, and the free rat was then placed in the arena, opposite the restrainer. The free rat could forcefully press the lever on the restrainer to open the door and release her partner. In each trial, the free rat was given two was removed at the two-minute mark, and the free rat was removed a few seconds later. If the free rat opened the restrainer, the two rats were allowed to interact for the remainder of the two minutes. If the free rat opened the restrainer with fewer than thirty seconds left in the trial, the two rats were given thirty seconds from the time the restrainer was opened. In trials where the restrainer was opened, at the end of the trial, the previously trapped rat was removed before the free rat. Regardless of whether the restrainer was opened, once removed from the arena, the free and trapped rats were placed in separate cages while the experimenter prepared for the next trial.

There were two conditions. In the *room-temp* condition, a room temperature ice pack (27.94 cm x 15 cm) was placed in a plastic bag and put on the floor of the restrainer. In the *cold* condition, a frozen ice pack was placed in a plastic bag on the floor of the restrainer. For both conditions, the ice pack was placed in a sealable plastic bag that was wiped with a dry paper towel between trials. New bags were used for each trapped rat. Multiple measures were recorded live by the experimenter including whether the restrainer was opened, the latency to open the restrainer, the temperature of the ice pack, and whether the restrained rat urinated or defecated. Between trials, the experimenter removed any feces and/or urine, changed the ice pack to the ice pack for the other condition, and recorded the temperature of the ice pack that was placed in the restrainer. At the end of a testing session, before another dyad was tested, the entire arena and restrainer was cleaned with 70% ethanol.

Each free rat (N = 18, 14 Sprague-Dawley and 4 Long-Evans rats) underwent four sessions. For the first three sessions, each rat was tested every six days. Due to scheduling issues, each rat had a two week break before they were tested in the final session. Each session was composed of ten trials where the conditions were alternate in successive trials (T1: *room-temp condition, T2: cold condition, T3: room-temp condition, etc). In sessions one and three, the odd numbered trials were the room-temp condition and the even numbered trials were the cold condition. In session* *two and four, the odd numbered trials were the cold condition, and the even numbered trials were the room-temp condition. Overall, each free rat went through twenty trials of each condition for a total of forty trials per free rat. All trials were recorded with two cameras: an overhead view captured the whole arena (forward facing camera of an iPhone 12) and a side view looked directly into the restrainer (Akaso EK7000)." As previously detailed in Winokur 2023.*

Source of data:

Emily Winokur has already run the previously described door-opening trials, and video footage of each trial has been provided for the video coding and analysis required for this project. Door opening behavior and defecation has also already been quantified and analyzed for each rat. Comprehensive behavioral coding and analysis will add important context to these preliminary results and help us to understand the rats' behavioral consistencies across different social contexts (Winokur et al., *in preparation,* Winokur 2024)**.** In a dyadic experimental contexts with rats all of the same strain, the affective state of each rat and co-distress behaviors affected the propensity of the free rat to help the trapped rat, however, it is unclear whether these results translate to a triadic experimental context where the rats are not all of the same genetic strain.

These completed trials have already been videotaped by Emily Winokur, and frame-by-frame video analysis will be manually conducted using ELAN (Winokur et al., *in preparation*). Door opening behavior has already been quantified and analyzed. Further video analysis using manual behavioral labeling with ELAN (version 6.3) will provide a better understanding and contextualization of the rats' behavior, helping us to determine whether the behavioral changes exhibited in response to stress in the other studies cited are consistent with the findings in this study regarding helping and stress.

ELAN Frame-by-Frame Coding

Frame-by-frame coding of social and distress behaviors that the rats display throughout the trials was used in order to detail and quantify the repertoire of behaviors exhibited during the experimental trials.

Behaviors to Record (Trapped Rats- Sprague Dawley and Long Evans):

Behavior	Definition	Categorization
Cephalic grooming	Face washing, circular movements of paws over the ears, face, and head. Includes head-directed unilateral and bilateral strokes.	Distress
Non-face licking, grooming, or scratching	Using the mouth or paws to lick or scratch the ventrolateral torso and/or anogenital region. Also includes scratching ears, neck and/or body with back paws.	Distress
Paw licking/nibbling	Licking or chewing on front/back paws.	Distress
Door contact	Any part of the body (except	Distress/escape

	tail) in physical contact with the restrainer door. Includes the rat touching the door with her nose, pushing on the door with paws, or leaning against the door.	
Immobility	Absence of movement except for that related to respiration for at least one second.	Distress
Defecation	Fecal boli excretion.	Distress

Behaviors to Record (Free Rat):

Behavior	Definition	Categorization
Cephalic grooming	Face washing, circular movements of paws over the ears, face, and head. Includes head-directed unilateral and bilateral strokes.	Distress

Non-face licking, grooming, or scratching	Using the mouth or paws to lick or scratch the ventrolateral torso and/or anogenital region. Also includes scratching ears, neck and/or body with back paws.	Distress
Restrainer entry	At least two paws, front or back, *inside* the restrainer.	Exploration
Interaction with the restrainer	Includes sniffing (within 1⁄2 inch of) the restrainer or the lever, touching the *outside* of the restrainer with any part of the body, and climbing on top of the restrainer.	Exploration
Interaction with the lever	Sniffing or whisking at the lever within ~1 inch. Also includes physical contact with the lever and climbing over/under the lever.	Exploration
Climbing on the side walls	Jumping up and remaining on the top of the walls of the	Exploration

	arena.	
Immobility	Absence of movement except for that related to respiration for at least one second.	Distress
Unsupported rearing	Standing on hind paws without placing forepaws on any surface.	Hypervigilance/safety monitoring
Social contact	Any physical contact between the two rats, one/both rat(s) sniffing the other rat. Includes: wrestling, following, climbing over/under, huddling, allogrooming.	Social
Defecation	Fecal boli excretion.	Distress

Analysis

Following completion of the manual behavior labeling using ELAN, we performed analysis in R in order to determine if there was significant correlation between time spent displaying distress between the free rat and either trapped rat.

Future analysis will focus on temporal correlations between the recorded behaviors of the free and trapped rats in order to determine if one rat's distress affects the timing of the other rats' displayed distress.

Frame-by-frame video analysis was used to examine the trapped rats' expression of distress, regardless of the experimenter-imposed condition. Multiple behaviors indicative of the trapped rat's distress were examined. This included the amount of time that the rat spent grooming, paw licking, immobile, and in contact with the restrainer door. Each behavior was normalized to reflect the total percent of time, while trapped, that the rat was engaged in each behavior. Because each distress behavior had differing distributions and ranges, min-max scaling was used to transform each behavior to the same scale of 0 to 1. This normalization technique enables equal weighing of each behavior. Finally, to calculate a total distress score for each trial, the scaled values for each behavior were added together. As a separate physiological distress metric, we recorded whether the trapped rat defecated, regardless of the number of times. A generalized linear mixed effects model was used to assess whether the trapped rats' distress score or defecation predicted whether the free rat would open the restrainer.

Results

Free Rats' Helping Responses to the Trapped Rats' Distress

"We examined how the trapped rats' distress scores predicted the free rats' probability of helping. As the distress score of the trapped rat increased, the probability that the free rat opened the restrainer decreased significantly (LRT: $X2 = 67.29$, $p < .001$, Table 5A), and the distress score of the trapped rat was higher in trials where she was not helped (LRT: $X2 = 72.66$, $p < .001$, Fig 5A, Table 5B). This suggests that when the trapped rat displayed higher levels of need, as indicated by her distress score, she was *less* likely to recruit help. Except for one trapped rat, all the other trapped rats had a higher distress score in trials where she was *not* helped (Fig 5A, above diagonal line). Additionally, when the trapped rat defecated, she was significantly *less* likely to be helped (LRT: X2 = 34.53, $p < .001$, Fig 5B, Table 5C, Supplemental Table 4). Only one free rat was more likely to help on trials where her partner defecated (Fig 5B, below diagonal line), but for that pair, there were very few trials where defecation occurred (Fig 5B, dot size). Given that the distress score was higher on trials where the trapped rat defecated (LRT: X2 = 9.56, $p = .0020$), additional analysis was conducted to ensure that the defecation was not the sole driver of relationship between the distress score and opening. With only trials where the trapped rat did *not* defecate, as the trapped rat's distress score increased, the probability of the free rat opening the restrainer decreased significantly (LRT: X2 = 32.36, $p < .001$). See Supplemental Table 5 for breakdown of how individual distress behaviors predicted helping behavior. "

Frame-by-frame behavioral analysis of shared distress

Twenty-four instances of behaviorally coded video footage were analyzed, and it was determined that, when distress was displayed by either trapped rat, there was a significantly high likelihood that distress would be shared by all three rats. This supports the hypothesis of distress contagion in rats, as not only did the free rat take on the distress of the trapped rat, but all three rats displayed similar distress levels regardless of level of need (cold condition or trapped condition).

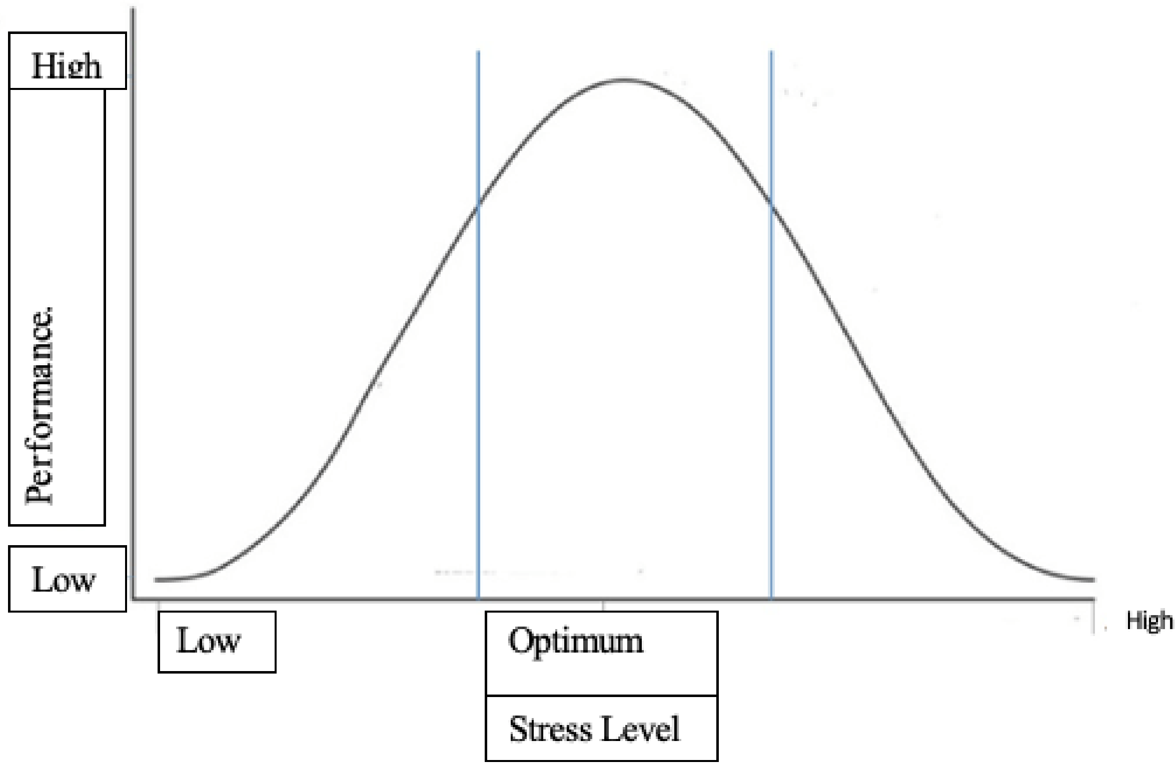
Interpretation

Empathy and Co-Distress

One potential explanation for the detrimental effect of expressed distress from the trapped rat on the free rat's propensity to help is distress contagion. One study interprets this pattern as one similar to affective empathy in humans, and proposes that rodents express fear vicariously as a way to mirror and communicate distress (Keysers et al., 2023). In the context of this experiment, distress contagion may cause the free rat to take on the distress experienced by the trapped rat. This would explain the significant shared distress scores among the free rat and both trapped rats.

Yerke's-Dodson Law

The Yerke's-Dodson Law proposes that there is an optimal level of stress at which performance peaks, and lower or higher levels of stress result in lower levels of performance. In this context, performance is demonstrated by door-opening by the free rat (helping behavior), and stress is demonstrated by the distress behaviors being quantified by frame-by-frame video analysis. According to the distress contagion theory proposed above along with the preliminary finding that the free rat and trapped rat share distress, the free rat taking on the trapped rat's distress may result in a lower probability of helping due to the rat's stress levels surpassing the optimal level for performance. Ideally, the free rat would experience a level of stress arousing enough to prompt action in the form of helping by pressing the lever. However, if the trapped rat displays higher levels of stress (through grooming, freezing, defecating), the free rat appears to take on this distress and helping behavior performance is therefore impaired due to high stress levels.



Shared distress as a detriment to helping behavior

The theory of distress contagion, as supported by our preliminary results above, may have a detrimental impact on the free rat's likelihood of helping either trapped rat. If the free rat takes on the trapped rats' distress, she may land further to the right of the Yerke's Dodson curve, demonstrating a level of stress that is inhibitory to performance and helping behavior. Therefore, we theorize that the shared distress demonstrated between the free rat and both trapped rats may be detrimental to the free rat's ability to help either trapped rat due to the inhibitory effects of high stress.

Discussion

Our results may shed light on helping behavior in humans. In our society, it is typical for those most in need of help to receive it the least. We often interpret the lack of helping behavior as a lack of empathic responding. Whereas this is sometimes the case, the converse can also be true; extreme empathic responding or shared distress might also result in a lack of helping behavior. Thus, a key element of helping behavior may be the ability to self-regulate following the experience of empathic distress (Weisz & Cikara 2021).

Future Work

Further analysis of the remaining video footage will involve coding the same behaviors as listed above in order to achieve greater statistical power. All frame-by-frame labeled videos will then be analyzed using the same methods described above, in addition to time-bin analysis of distress behaviors in order to determine whether a temporal correlation between the free and trapped rats' distress behaviors exists. We will also look at whether the temporal co-occurrence of distress behaviors has an effect on the free rat's likelihood of helping behavior.

We will perform a time-dependent cross-correlation analysis ("ccf" function in R) to explore the relationship between the behavioral expressions of the trapped rat and the free rat. To conduct this analysis, we will divide the time when the trapped rat was restrained into 0.1-second intervals and coded the occurrence of the behavior of interest (distress, rearing, or escape) as binary data: 0 for absence and 1 for occurrence. For each trial, separate streams of time-series data will be obtained for the trapped rat and the free rat, and a cross-correlation analysis will be conducted to investigate if the trapped rat's distress behavior predicted the occurrence of the behavior of interest in the free rat in a time-dependent manner. To assess the significance of the observed correlation, we will generate 1000 bootstrapped samples for each trial and calculate the maximum autocorrelation coefficient. This will allow us to create a null distribution and determine if the observed maximum autocorrelation coefficient between the two rats' behaviors is significantly greater than what would be expected by chance. Using a generalized linear mixed effect model assuming a binomial distribution, we will then investigate whether having a significant cross correlation predicted helping behavior and whether the behavior of the free rat alone predicted her likelihood of helping.

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