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The evolution of empathy: through the lens of a rodent model.

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THE EVOLUTION OF EMPATHY:
THROUGH THE LENS OF A RODENT MODEL

By

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M.S., Winthrop University, 2017

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A Dissertation Approved on

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DEDICATION

To my parents,

Marilyn Dumont-Driscoll and Dan Driscoll,

for your endless support of my love for animals,

and cultivating a family built on empathy for others.

To my husband, Beau,

for your incredible patience,

for your love and support through

the highs and lows of my doctoral program,

and for willing to adopt and home all the rats

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ABSTRACT

THE EVOLUTION OF EMPATHY: THROUGH THE LENS OF A RODENT MODEL

Caroline Marie Driscoll-Braden

14 April 2023

Empathy is the capacity to be affected by and share the emotions of others, to discern the circumstances prompting another's emotional state, and to identify with another by adopting their perspective. Research investigating the empathically motivated behavior of rats can help inform the evolutionary history of empathy and provide additional support of the continuity of empathy in animals and humans. In this dissertation, I examine the helping behavior of rats to explore the complexities of rodent empathy. In Chapter I, I review the multiple layers of empathy and describe both historical and contemporary research examining empathy in non-humans. I explain why rats provide an ideal study system to explore empathy in other animals and the insights provided from behavioral, physiological, and neural research on rats. Chapter II details a pilot experiment aimed at discerning whether personal distress or concern for others motivates helping behavior in rats by introducing a separated escape area that could be easily accessed by rats exposed to a cagemate in distress. Although rats in the pilot experiment exhibited significantly less successful helping behavior in comparison to previous studies, ways to improve the protocol were gleaned for future work. Chapter III describes research determining whether the presence of a cost tempers the expression of helping behavior by introducing a water barrier that had to be crossed to help a trapped cagemate. This experiment also

allowed me to test whether the presence of an escape area affected helping behavior. Chapter IV explores the communicative antecedents to helping behavior to determine the ultrasonic vocalizations that motivate prosocial action. Not only were distress vocalizations recorded and analyzed to discern the effects of these calls on subsequent behavior, but positive calls were also recorded and analyzed. Multiple explanations are offered to interpret the role of these calls. The work detailed is concluded in Chapter V in which I discuss the implications of my findings and their place in the current state of the field. I also discuss interesting avenues of exploration for future research.

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CHAPTER I

THE EMPATHIC CAPACITIES OF OTHER ANIMALS

I. General Introduction

Empathy, and the behaviors motivated by it, have been contemplated by evolutionary biologists and ethologists since Darwin, who once believed sympathetically motivated prosocial behavior may not be favored by natural selection (Darwin, 1874). In a world where survival of the fittest reigns, how could such an other-oriented ability evolve and persist?

Empathy is the ability to feel the emotions of others, where an observer vicariously experiences the affective state of another without facing the emotion-inducing stimulus firsthand (Preston & de Waal, 2002b). In many cases, empathy is believed to underlie helping behavior, facilitate cooperation, improve coordination, and mitigate against aggression (de Waal, 2008; Parr, Waller, & Fugate, 2005); thus its origins, motivators and inhibitors are important factors to uncover. Much of the work exploring the empathic capacities of other animals was pioneered by primatologist Frans de Waal, who after noticing that chimpanzees “make up” after fights by kissing, began his exploration into reconciliation and consolation in other animals at a time when others were focused on their aggressive tendencies (de Waal, 2021).

In social species, the environment has favored abilities and behaviors that facilitate the formation and maintenance of strong social bonds where the consequences of these bonds provide powerful benefits that outweigh associated costs (Tomasello, 2014). Empathy is believed to have evolved in association with parental care where mothers better equipped to understand the needs of their offspring, conveyed through vocalizations and body language, and to respond appropriately, conferred benefits in the form of enhanced reproductive success (de Waal, 2008; Decety, 2011; MacLean, 1985; Preston & de Waal, 2002b). Once the ability to adopt and understand the emotional states of others was established, it could be coopted and used to enhance other social relationships. Social animals are better able to overcome challenges when working with others, such as the cooperative hunting in Tai chimpanzees (Boesch, 1994) or defensive bunching in elephants (McComb, Moss, Durant, Baker, & Sayialel, 2001). While empathy is not necessary for helping behavior, such as in the case of rescue behavior in ants (Nowbahari, Scohier, Durand, & Hollis, 2009), or cooperation, like that seen between unrelated desert leafcutter ant queens prior to worker emergence (Bernasconi & Strassmann, 1999), empathy can enhance both helping behavior and cooperation in highly social and intelligent animals (Parr et al., 2005; Joshua M Plotnik, Lair, Suphachoksahakun, & de Waal, 2011). Furthermore, empathy acts as a bridge between individuals, strengthening social bonds, which serve to buffer against stress and adversity (Cohen & Wills, 1985; Morrison, Eckardt, Colchero, Vecellio, & Stoinski, 2021; Young, Majolo, Heistermann, Schülke, & Ostner, 2014).

Other animals, both wild and captive, provide opportunities to research how empathy evolved, the internal and external stimuli that promote empathy, and the behaviors empathy elicits. Empathy is often evaluated by measuring an individual's response to another in distress, rendering systematic investigations in large-brained mammals rare, as they are often limited by ethical constraints and restricted to observational studies. These observational accounts have provided support to suggest other animals engage in empathically motivated behavior and in doing so, provide a suite of associated life history traits to guide future research. The variety of reactions other animals display in response to the emotions of others demonstrates the empathic gradient found in the animal kingdom and provides a blueprint that can be employed to investigate the evolutionary history of empathy and the biological roots of its manifestation.

II. Levels of Empathy & Supporting Examples

de Waal (2008) utilizes the analogy of a Russian nesting doll to illustrate the multiple layers of empathy where more complex abilities are built upon the more primitive. The analogy establishes the evolutionary continuity of empathy in which earlier capacities aren't replaced but provide the functional scaffolding that support more advanced forms (de Waal, 2008; de Waal & Preston, 2017).

i. Affective State-Matching – Emotional Contagion

The automatic transfer of emotions between individuals, where an observer assumes the affective state of another, is at the core of the empathetic response and provides the foundation for more complex expressions of empathy (de Waal, 2008). Emotional contagion has been extensively documented in multiple animal species where an individual, often referred to as the 'observer,' upon witnessing the emotional state of another subsequently demonstrates marked changes in behavior and physiology that match those of the ‘demonstrator’ (Y. Han, Sichterman, Maria, Gazzola, & Keysers, 2020; Langford et al., 2006; Masserman, Wechkin, & Terris, 1964; Miller, 1967; J. M. Plotnik & de Waal, 2014; Romero, Konno, & Hasegawa, 2013; Silva, de Leaniz, & Luchiari, 2019).

Emotional contagion is a vital component of social life as it allows individuals to gain valuable information about their environment through the experiences of others (Pérez-Manrique & Gomila, 2022; Preston & de Waal, 2002b). This ability is highly adaptive, as it enables behavior to be quickly altered to respond appropriately to potential threats or environmental challenges before encountering them. While emotional contagion can elicit self-benefitting behavior, it also provides opportunities for emotional connectivity between individuals, which may serve as the backbone from which prosocial behavior can be initiated (Decety, 2011; Pérez-Manrique & Gomila, 2022).

Studies on emotional contagion in nonhumans date back to the late 1950s, and in one of the first studies investigating the capacity in rodents, Church (Church, 1959) trained rats to press a bar until the behavior was repeated reliably. However, once bar pressing was matched with the shocking of another rat, bar pressing significantly declined. Consistent with behavior suggestive of empathy, where previous experience with a stressor enhances emotional responses in witnesses, rats who experienced foot shocks prior to experimentation demonstrated even more severe declines in their bar-pressing.

A similar experiment was conducted with rhesus macaques where individuals were trained to pull a chain to receive food pellets (Masserman et al., 1964). Consistent with the response of Church’s (1959) rats, once pulling the chain was also coupled with delivering a shock to another individual, most participants consistently opted to remain hungry over pulling the chain for food. Miller (Miller, 1967) incorporated a physiological measure of distress and discovered that rhesus macaques experiencing distress and those witnessing distress had matched elevations in heart rate. This inclusion of a physiological component provided an important addition to the study of emotional contagion, as it provided an objective and quantifiable measure of an emotional response to the distress of another. These early studies demonstrated that other animals are behaviorally and physiologically affected by the emotional arousal of others and provided the foundation for investigations into the complexity of these responses.

As empathy studies expand to include exploration in non-mammals, evidence is mounting that suggests emotional contagion is ubiquitous within the chordate phylum as the capacity is also afforded to some bird and fish species. Much like the rats and macaques in the experiments designed by Church (1959) and Masserman et al. (1964), pigeons chose hunger over pecking a lever that dispensed food while delivering an electrical shock to another (Watanabe & Ono, 1986). Zebrafish demonstrate fear contagion where exposure to conspecifics engaged in antipredator behavior elicits similar behaviors (Fernandes da Silva, Garcia de Leaniz, & Luchiari, 2019) and increased cortisol levels (Oliveira et al., 2017) in observers. Consistent with the familiarity bias typical of empathy, the magnitude of the response to another's antipredator behavior was enhanced with familiarity (Fernandes da Silva et al., 2019).

The transfer of positive affect is less represented in the empathy literature; however, the presence of this vicarious stimulation of positive emotions demonstrates the robust nature of empathy and offers an additional route to explore empathy. Manual tickling of rats by human handlers is believed to induce positive affect, as its performance elicits recruitment of opioid receptors and induces the dopaminergic reward system (Burgdorf & Panksepp, 2001; Panksepp & Burgdorf, 1999, 2000), and recently the vicarious transfer of this positive affect to observers has been exhibited. Kaufmann et al. (L. Kaufmann, M. Brecht, & S. Ishiyama, 2022) discovered that rats witnessing live tickling, as opposed to recordings, of conspecifics exhibited “Freudensprünge” or “joy jumps” in response. Upon discovering that both experienced and witnessed tickling induced the emission of 50 kHz ultrasonic vocalizations, a potential homolog to human laughter (Panksepp & Burgdorf, 2003), and the activation of the somatosensory cortex, authors speculated this matched neural response may underpin contagious laughter.

Continued research on the most basic empathetic responses is important to determine how widespread the capacity for emotional state-matching is within the animal kingdom, define the neural substrates necessary for its expression and infer the selection pressures that facilitate its manifestation.

ii. Sympathetic Concern – Consolation

Sympathetic concern provides the next layer of empathy, where the capacity to vicariously experience the emotions of others is built upon and includes attempts to understand the source of another’s emotional reaction. Unlike emotional contagion, sympathetic concern is mediated by the addition of self-other differentiation, which dampens the emotional response as the observer recognizes the demonstrator as the source of distress (de Waal, 2008). While emotional contagion can produce self-oriented behavior that simultaneously benefits another, sympathetic concern is responsible for the induction of consolation behavior which appears to be aimed at alleviating another’s distress by offering comfort and reassurance (de Waal, 2008; Pérez-Manrique & Gomila, 2018).

The presence of consolation behavior is often used as a proxy for sympathetic concern and was first systematically detailed in other animals when de Waal and van Roosmalen (de Waal & van Roosmalen, 1979) were examining post-conflict behavior in a captive colony of chimpanzees. They discovered that after an agonistic interaction between individuals, third-party bystanders would offer comfort to the aggressed party, most notably by instigating an embrace. This behavior is potentially risky, as the third party could be targeted by the initial aggressor. To determine if the consolation behavior seen in chimpanzees actually reduced the distress of the recipient, Fraser et al. (Fraser, Stahl, & Aureli, 2008) measured rates of two self-directed behaviors induced by anxiety, self-scratching and self-grooming, following aggressive interactions. Both behaviors were elevated post-conflict in aggressed parties and remained high when consolation and reconciliation events were absent. However, when recipients of aggressions were consoled by a third-party bystander, rates of both self-directed behaviors reduced to rates of matched-control levels. These results provided some of the first evidence that the consolation behavior demonstrated by chimpanzees reduces the receiver's negative arousal. Furthermore, the strength of the chimpanzee's social bonds was predictive of consolation behavior, thus fitting traditional constructs of empathy where familiarity enhances empathy. Similar to chimpanzees, bonobos console victims of aggression and protect them from continued assaults, a potentially risky act (Clay & de Waal, 2013; E Palagi & Norscia, 2013; E. Palagi, Paoli, & Tarli, 2004)

Sympathetic concern is marked by an emphasis on the other, where the benefits received by an observer's actions are predominately bestowed to the distressed demonstrator; thus, the allomaternal care commonly exhibited in wild elephant herds, in which individuals respond with care to the needs of another individual's offspring, made elephants a compelling species to explore consolation behavior outside the hominids (Bates et al., 2008). Plotnik and de Waal (2014) recorded the behavioral responses of Asian elephants to the distress of others in a semi-natural environment to explore the presence of consolation behavior beyond primates. Within the first minute of an elephant expressing agitation or distress via posturing and vocalizing, bystanders responded by assuming similar behavior by chirping and emitting distress vocalizations, engaging in bunching behavior and affiliative touching of the distressed individual. These responses were seen significantly less often during control periods and suggest that bystanders were motivated to provide comfort to others experiencing distress. Since de Waal and van Roosmalen's (1979) discovery of chimpanzee consolation behavior, this other-oriented behavior has been documented in multiple other mammals, such as prairie voles (Burkett et al., 2016), dogs (Cools, Van Hout, & Nelissen, 2008), and dolphins (C. Yamamoto et al., 2015), and provides additional evidence to support the evolutionary continuity of empathy.

iii. Empathic Perspective-Taking – Targeted Helping Behavior

Empathic perspective-taking (EPT) is the outer layer in the Russian nesting doll of empathy and is thought to be present only in large-brained animals, as it involves higher order processing facilitated by encephalization (de Waal & Preston, 2017). EPT allows an individual to understand and assume the perspective of another while discerning the contexts eliciting the affective state of another (de Waal, 2008). EPT involves emotional regulation where the observer's emotional response is dampened compared to that of the demonstrator, likely supported by a heightened distinction between self and other (de Waal, 2008; Pérez-Manrique & Gomila, 2018). Targeted helping behavior involves appropriately appraising the circumstances and dispensing aid specific to the context; thus, targeted helping is often employed to investigate the expression of EPT.

Anecdotes of animals engaging in targeted helping behavior suggest that other animals are emotionally invested in the welfare of others to the extent that they are motivated to act strategically with intent to provide aid. Take for example the occurrence documented by Pruetz (Pruetz, 2011)following the return of an infant chimpanzee to its mother after being captured by poachers. For two days, a young unrelated male chimpanzee was recorded carrying the infant for the mother struggling to keep pace with the group. As social bond strength enhances the expression of empathy, the uncharacteristic level of connectivity and cohesion previously recorded in this chimpanzee troop potentially facilitated this overt display of targeted helping behavior (Pruetz & Bertolani, 2009).

Given the constraints limiting investigations into helping behavior in large-brained mammals, protocols designed to measure the proclivity of individuals to help others achieve goals, such as obtaining food and other items, have been incorporated to provide quantifiable results, as anecdotes fall short of the scientific rigor of statistical analyses (Melis et al., 2011; S. Yamamoto, Humle, & Tanaka, 2012). Unfortunately, the absence of overt emotional cues displayed prior to initiation of help fails to provide evidence that assistance was motivated by emotional understanding and facilitated by empathy. Observational studies provide the means to explore targeted helping behavior in other animals and these accounts become increasingly relevant when long-term studies demonstrate targeted helping is performed repeatedly under typical circumstances over many years. Bates et al. (2008) recorded and analyzed a collection of observations of African elephant interactions collected over 35 years where consolation, helping and protection of both kin and non-kin were commonplace. Building evidence to demonstrate a pattern of behavior can also be achieved by combining results from similarly structured research. For example, the work of Lilly (Lilly, 1963), Caldwell & Caldwell (Caldwell & Caldwell, 1966), Kuczaj et al. (Kuczaj et al., 2015), and Cheng et al. (Cheng et al., 2018) can be combined to demonstrate that dolphins perform epimeletic behavior, defined as providing aid and protection to others in need. Together these accounts reporting the attempts of individuals and groups forming life rafts to support injured conspecifics and lift them to the surface to facilitate their breathing indicates this behavior is not uncommon and suggests the capacity for empathic perspective-taking.

Information garnered from anecdotes and observational studies involving large-brained mammals inform the suite of characteristics and contexts that elicit behavior demonstrative of empathic capacities. Attempting to answer remaining questions surrounding the evolution of empathy and the proximate mechanisms supporting associated behaviors requires replication and technological interventions uniquely afforded to controlled studies of small mammals, particularly rodents.

III. Rodent Empathy Model

Much like great apes, dolphins and elephants, rats exploit socially complex landscapes, coordinate cooperative behaviors, invest parental energy in raising altricial young, and form bonds of varying degrees where bond strength is predictive of longevity and reproductive success (Davis, 1996; Foote & Crystal, 2007; Modlinska & Pisula, 2020; Proops, Troisi, Kleinhappel, & Romero, 2021; Schweinfurth, 2020; Vermaercke, Cop, Willems, D'Hooge, & Op de Beeck, 2014). Expanding the study of empathy to these smaller-brained mammals allows for experimental manipulations, such as the induction of distress, and invasive surveillance of the neural and hormonal correlates of empathically associated responses (Ben-Ami Bartal et al., 2021; Ben-Ami Bartal et al., 2016; Carrillo et al., 2019; L. V. Kaufmann, M. Brecht, & S. Ishiyama, 2022; Nazeri, Nezhadi, & Shabani, 2019; Wu, Cheng, Liang, Lee, & Yen, 2023; Yamagishi, Lee, & Sato, 2020)

Ben-Ami Bartal et al. (Ben-Ami Bartal, Decety, & Mason, 2011) introduced a Rodent Empathy Paradigm designed to test helping behavior in rats, incorporating a low-tech protocol that would allow for future manipulations to examine additional aspects of rodent empathy. The helping behavior test (HBT) introduces pairs of rats to an experimental arena where one individual is trapped in a rodent restrainer outfitted with a door that can only be opened from the outside and the other rat is free to roam the arena. The focus is on the reaction of the free rat to the distress of the trapped individual, mainly whether the free rat opens the door to release its conspecific. Rats demonstrated an overwhelming preference for opening the door, but only when the restrainer contained another rat, and not when it was empty or contained a toy rat. Furthermore, the study discovered that rats would continue to help even when the trapped rat was released into a separate area, impeding subsequent social contact. Since the study's publication, multiple iterations of the design have been implemented, revealing the complexity of rodent prosocial behavior. Rats display in-group preference when dispensing help that is a function of age and neural recruitment (Breton et al., 2022) and can be overridden through social experience (Ben-Ami Bartal, Rodgers, Bernardez Sarria, Decety, & Mason, 2014). Furthermore, the helping behavior of rats appears to follow the expectations of reciprocity (V. Dolivo, Rutte, & Taborsky, 2016; V Dolivo & Taborsky, 2015) and its performance is subject to the behavior of bystanders (Havlik et al., 2020). In a cooperative food-sharing experiment, rats were trained to pull a stick that provided food to a partner in an adjacent area separated by mesh. When a focal rat was given the opportunity to provide food to another, their decision to help was influenced by their previous experience with the other rat and the amount of work required to provide food to the other individual. Rats were less likely to pull the stick providing food to the other rat if the other rat had not provided food to the focal rat previously; this was especially true when doing so required more effort, such as when pulling the stick was increased by one Newton step (Schneeberger, Dietz, & Taborsky, 2012). Even when tested with a new unfamiliar partner, previous experience with a different rat affected one's willingness to provide food to another; if a focal rat had previously received food from a cooperating partner, they were more likely to provide food to a stranger than if they had previously experienced a defector (Rutte & Taborsky, 2007). Not only do rats remember those who previously helped them, they also remember the quality of help they received. In a similarly designed experiment, rats were exposed to cooperating partners who shared either pieces of banana (highly palatable) or carrot (less palatable). When roles were reversed, rats provided bananas and carrots according to which partner they were paired with (Dolivo & Taborsky, 2015). Similar to the rats whose subsequent food-sharing behavior was dependent on their last encounter, the decision of rats to help those trapped in a restrainer was dependent on the behavior of their partner. Havlik et al. (2020) tested the bystander effect in rats using the HBT where a focal rat was placed in an arena with a trapped individual and a second or third free rat. The other free rats were either competent or pharmacologically induced defectors. Testing with competent partners had a "superadditive" effect where helping behavior was significantly more frequent and was initiated even more quickly than control levels; however, when tested with defectors, helping behavior drastically declined. Together these studies demonstrate the effects previous experience and social contexts have on the helping behavior of rats.

Beyond elucidating the environmental and social contexts that mediate the dispense of aid, research on rodent empathy has provided insights into the endogenous factors governing empathic responses and the ability of exogenous treatments to inhibit these reactions. Emotional contagion is believed to provide the initial connection between individuals necessary for provocation of empathically associated behaviors; however, the shared affect between observer and demonstrator that promoted helping behavior previously performed can be eliminated through observer treatment with an anxiolytic (Ben-Ami Bartal et al., 2016). Yet, the same anxiolytic is not potent enough to diminish the distress of the trapped cagemate, suggesting observer rats experience a dampened emotional response compared to that of the demonstrator (Vieira Sugano, Shan, Molasky, & Mason, 2022). Rats display similar neuronal recruitment in response to the distress of others as humans, most notably oxytocinergic and both mirror and anti-mirror neurons in the anterior cingulate cortex (Kemp & Guastella, 2011; Wu et al., 2023; Yamagishi, Lee, et al., 2020; Yamagishi, Okada, Masuda, & Sato, 2020). The disruption of empathic functioning due to opioid abuse seen in humans can be induced in rats (Carlyle, Rowley, Stevens, Karl, & Morgan, 2020; Kurtines, Hogan, & Weiss, 1975; Tomek, Stegmann, & Olive, 2019). Using the HBT, baseline helping rates were established before rats were divided into two groups that could self-administer either sucrose or heroin. Those self-administering sucrose matched their pre-treatment helping behavior rates, while those self-administering heroin saw drastic declines in their proclivity to help release a trapped cagemate (Tomek et al., 2019).

Rodent empathy studies have provided novel insights into the environmental and biological influences that govern empathically motivated behaviors and serve to inform the evolutionary history of empathy and the proximate mechanisms mediating empathic activation. Future work will continue to provide answers to unresolved questions surrounding empathy.

IV. Directions Explored Through Dissertation Experiments:

i. Does personal distress or concern for others promote rat helping behavior?

Exposure to distress in another can elicit personal distress in the observer via emotional contagion (Eisenberg & Eggum, 2009; Eisenberg et al., 1989). Personal distress can motivate self-directed behavior or, under certain contexts, elicit other-oriented behavior where motivations underlying the behavior are independent from the consequences for the demonstrator, as the intention is to mollify the distress of the observer (Eisenberg et al., 1989). Those skeptical of the empathic capacities afforded to rats often invoke personal distress as the driving force behind the helping behavior documented in studies utilizing the HBT or similar designs (Blystad, 2021; Hiura, Tan, & Hackenberg, 2018; Jentsch & Ringach, 2014; Lavery & Foley, 1963; Preobrazhenskaya & Simonov, 1974). This alternative explanation is not new and was offered in response to Rice and Gainer (Rice & Gainer, 1962), arguing the arousal caused by the distress calls of a dangling individual motivated the helping behavior performed (Lavery and Foley, 1963). First, this argument lends itself to an inconsistency surrounding the definition and requirements of empathy. While de Waal (2008) considers emotional contagion, and thus personal distress, a primitive form of empathy, others have a stricter, and thus more restrictive, definition of empathy that requires an individual to recognize that their emotional state was influenced by another's and thus initiates an other-oriented reaction, therefore excluding behaviors elicited by personal distress (Schwartz, Silberberg, Casey, Kearns, & Slotnick, 2017). The aversion to another's distress denotes artifacts of more complex empathic capacities where concern for the welfare of others is paramount, which would also encompass an aversion to the distress of others. Therefore, the question that requires resolution is not one investigating if rats possess empathic capacities, but to what degree do their empathic qualities qualify as actual empathy.

Only two studies have attempted to determine whether simple personal distress or more complex forms of empathy are involved in helping behavior in nonhumans, by introducing an escape area to the HBT (Carvalheiro et al., 2019; S. Han, Chen, Zheng, Wang, & Yin, 2022). Both studies found that the presence of an escape option did not hinder helping behavior in rats; rats forewent an easier option to alleviate their own personal stress, suggesting that their helping behavior was motivated by their cagemate's distress and not, at least entirely, by their own distress. Graphic renderings from both studies depict escape areas adjacent to the experimental arena where the restrainer is located and neither study compared the rate of vocalizations indicating distress between the two areas; thus, whether the escape area truly provided reprieve from the distress signals of the trapped rat remains unclear. The experimental arena detailed in the following dissertation was almost 100 cm longer than those previously used and the escape area was connected to the opposite end of the arena via a three-foot tunnel, allowing for greater distance to impede visual perception and attenuate the ultrasonic vocalizations of the trapped individual, as confirmed by comparing rates of ultrasonic vocalizations between the two locales; thus the escape area provided a low-stress environment the free rat could exploit to ameliorate their own personal distress.

ii. The role of communication in facilitating helping behavior:

Animals utilize vocalizations and additional modes of communication to convey information to others. These signals can evoke emotional, as well as behavioral, responses from listeners. For example, alarm and distress calls elicit vicarious arousal in conspecifics and provide a means by which individuals can solicit help from others (Preston & de Waal, 2002a). "True" altruism in humans has been studied to determine the "pure" incentives associated with helping others, using constructs in which social interaction between those needing help and those able to provide help have been experimentally eliminated (Bolton & Ockenfels, 2000; Fehr & Schmidt, 1999). To determine the factors that heighten empathic responses, Andreoni and Rao (Andreoni &

Rao, 2011) tested pairs of individuals where one was the allocator of money and the other was the receiver, manipulating who in the pair could speak. They found when the receiver was allowed to talk, giving by the allocator significantly increased, suggesting the power of communication in promoting the performance of altruistic behavior. The ability to convey that a problem exists and how it can be resolved with the help of another increases the accuracy of the help dispensed, whereas the ability to affect the emotions of another enhances the likelihood that help will be awarded.

While the role of distress calls in young offspring to elicit parental care is obvious, the retention of distress calls into adulthood suggests their adaptive value (de Waal, 2008); yet, little research on the vocal communication preceding helping behavior exists (Kuczaj et al., 2015; Lilly, 1963; Russ, Racey, & Jones, 1998). Dolphins, elephants, bats, and rats all demonstrate direct increases in aid provided to others following exposure to distress signals (S. Cox et al., 2022; Kuczaj et al., 2015; Lilly, 1963; J. M. Plotnik & de Waal, 2014; Russ et al., 1998); however, other types of calls and the responses of observers may play an equally important role in facilitating helping behavior. Ultrasonic vocalizations (USVs) produced by rats are used as reliable indicators of affective state and the emission of USVs can have behavioral and physiological effects on producers and listeners. Both the production of USVs and exposure to the USVs of conspecifics invokes neural substrates associated with emotions; USVs indicative of distress elicit activation of the cholinergic system, while USVs indicative of a positive emotional state elicit activation of the dopaminergic reward system (Brudzynski, 2007; Sadananda, Wöhr, & Schwarting, 2008). Research on rodent models of empathy can include further analyses of signals associated with attempted or successful displays of helping that could provide insights into the communicative traits natural selection has favored to support empathetic behavior.

iii. Does the presence of cost temper the performance of helping behavior?

Helping behavior is not uncommon in the animal kingdom, in which non-human animals will forgo or delay benefits for themselves in their pursuit to help others, and this behavior can be repeatedly induced in controlled studies utilizing rats. For example, Bartal et al. (2011) found that rats chose to help a distressed individual before opting to retrieve chocolate chips, and then shared the coveted treats with their newly released cagemate. However, helping behavior is typically characterized as benefitting another while incurring either a temporary or long-term cost to oneself, Yet, this aspect of helping behavior has been seldom investigated in nonhumans (Fehr & Fischbacher, 2003).

Studies on human helping behavior have found that individuals are willing to help others at some personal cost, though as the magnitude of an associated cost increases, subsequent helping behavior decreases (Shotland & Stebbins, 1983; Wagner & Wheeler, 1969). Few studies on other animals have coupled salient costs with providing help to others (Masserman et al., 1964; Schneeberger et al., 2012), likely due to ethical constraints. While rats experimentally tested for displays of helping behavior must overcome their fear of open spaces (Sivaselvachandran, Acland, Abdallah, & Martin, 2018), rats readily explore spacious experimental arenas during habituation periods prior to experimentation; thus, presently the effect of a cost on the expression of helping behavior in rats remains unknown.

V. Concluding Remarks

Kin selection and reciprocal altruism provide technical explanations for the evolution and maintenance of helping behavior, in which individuals dispense costly aid to kin and non-kin and the benefits are garnered by increased inclusive fitness(Hamilton, 1964) or "promised" future repayment (Trivers, 1971). However, decisions to help are typically instantaneous, as animals are motivated by salient cues to help others without consciously determining the future benefits rendered to them in return, and result in aid delivered to both kin and non-kin and occasionally even to those unable to offer symmetrical compensation (Douglas-Hamilton, Bhalla, Wittemyer, & Vollrath, 2006; Preston & de Waal, 2002a).

As evidence for empathically motivated behavior in other animals continues to mount, further research will provide additional insights into how empathy has evolved and the contexts that promote its expression. Behavioral studies will improve our understanding of the environmental and social factors that elicit empathic responses while neuroendocrine studies will pinpoint brain regions and neurotransmitters that underlie the capacity for empathy and how psychotropic drug use potentially impedes the neural mechanisms supporting its expression. These studies not only illuminate the evolutionary history of empathy and inform how we treat and understand the social impairment of those with substance use disorders (Tomek et al., 2019) and those with neural disorders marked by a lack of empathy (Win-Shwe et al., 2018); they can also inform societal efforts to develop effective methods to reduce xenophobia and foster empathy for out group members (Bartal et al., 2016, 2021), and promote empathy in schools and the workplace to enhance learning outcomes, increase productivity and decrease burnout (Satapathy, Pahwa, & Pareek, 2020).

CHAPTER II

PILOT EXPERIMENT: DOES PERSONAL DISTRESS OR CONCERN FOR OTHERS MOTIVATE HELPING BEHAVIOR IN RATS

I. Introduction

Emotional connectivity between individuals is rooted in the automatic and vicarious transfer of emotions from one individual to another (de Waal, 2008). This emotional contagion is fundamental to the lives of social animals where relevant information can be acquired through shared affect, hence its ubiquity in the animal kingdom (for review see: (Pérez-Manrique & Gomila, 2022). Preston & de Waal (Preston & de Waal, 2002b) offer the perception-action mechanism (PAM) to explain the autonomic and somatic facilitation of affective state-matching in which the perception of another's affective state triggers an unconscious and automatic neural representation of the same state in the observer. The resulting matched emotional and behavioral expressions are enhanced by similarity, familiarity, and shared experiences between individuals (Preston & de Waal, 2002a, 2002b)

de Waal's (2008) Russian Nesting Doll analogy (**Figure 2.1**) portrays the multiple levels of empathy with emotional contagion providing the foundation for which the more complex empathic abilities, sympathetic concern, and empathic perspective-taking, can build upon as they are facilitated by increased intelligence and self-other discrimination.

By exploring the empathic abilities of other animals, research can shed light on the selection pressures that have favored empathy, the neuroendocrine mechanisms that facilitate its expression, and the life history traits that are often coupled with the capacity to understand the emotions of others.

For example, chimpanzees and bonobos console victims of aggression despite potential retaliation from aggressors (de Waal & van Roosmalen, 1979; Fraser et al., 2008; Clay & de Waal, 2013), elder female elephants assist new mothers following the birth of their first calf (Lee, 1987; Moss, 1988), and dolphins act as life rafts lifting injured conspecifics to the surface for breath (Lily, 1963; Kuczaj et al., 2015). These examples lend support of the prediction that intelligent animals who provide prolonged parental care to altricial young and live in highly complex social environments, particularly fission-fusion societies, will possess sophisticated empathic aptitude.

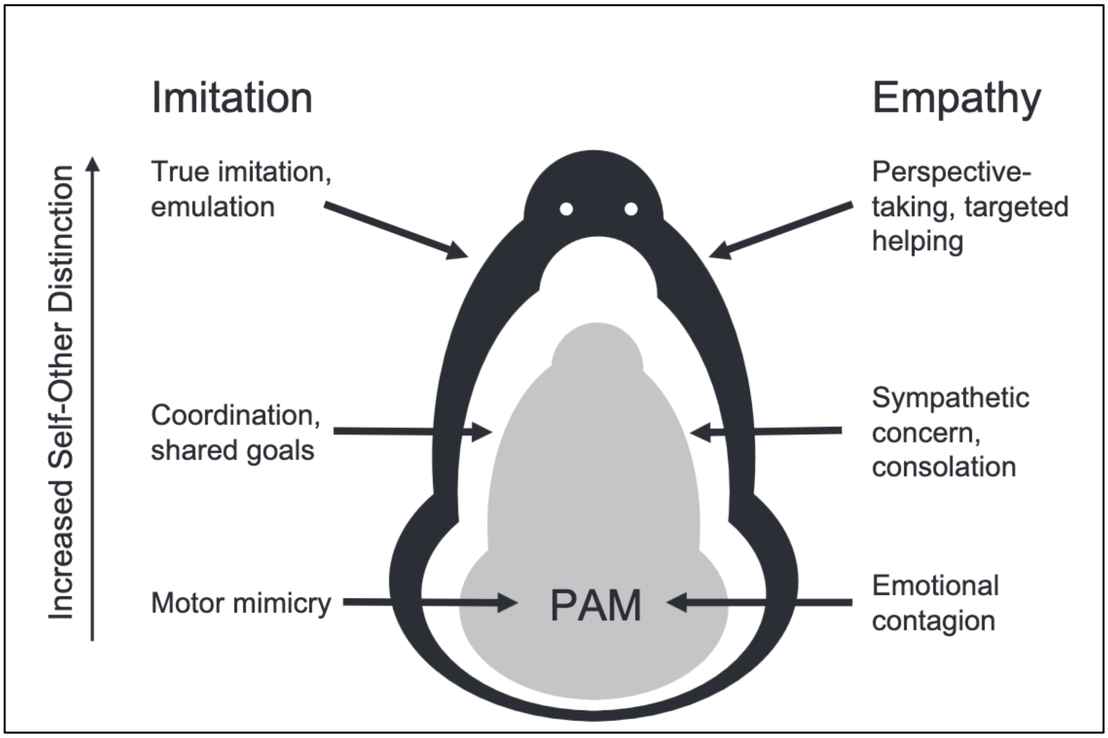

Figure 2.1 de Waal's Russian Doll of empathy and imitation depicts the building of more complex empathic abilities from more primitive ones in concert with increased distinction of self.

Akin to the aforementioned large-brained mammals, rats are adept problem-solvers (Davis, 1995) living in socially complex colonies, often numbering in the hundreds, with varying degrees of social bonds (Proops et al., 2021). Furthermore, rat dams must provide extensive care to their underdeveloped pups, demonstrating increased care toward pups in response to distress and separation (Smotherman, 1983). Rats fulfill the necessary empathic prerequisites and their ease of maintenance in laboratories and permissive ethical constraints make rats an ideal study subject to investigate the behavioral, endocrinological and neural responses of observers to the distress of another during an array of environmental and induced physiological conditions (Abbott, 2010). Early studies on the behavioral responses of rats to distressed conspecifics discovered that rats will act to cease the continuance of the distressing stimulus by decreasing lever pushing that delivered a shock to a conspecific or pressing a lever to lower a suspended individual (Church, 1959; Rice & Gainer, 1962; Greene, 1969). Follow up studies suggested rats were not acting altruistically but were motivated by the transference of fear, indicating that the empathic capacities of these "lower animals" were limited to emotional contagion (Lavery & Foley, 1963; Preobrazhenskaya & Simonov, 1974; Rice, 1964).

Recent studies have adopted more nuanced methods to investigate rodent helping behavior and have revealed that rats repeatedly act in a purposeful manner to reduce the distress of a conspecific (Bartal et al., 2011. 2014, 2016, 2021; Breton et al., 2022; Carvalheiro et al., 2019; Cox et al., 2020, 2022; Havlik et al., 2020; Sato et al., 2015; Yamagishi, Lee et al., 2020; Tomek et al., 2019). Despite incorporating more intricate experimental designs to provide evidence of empathically motivated behavior in rats, the authors of these contemporary studies are met with the same critiques as their predecessors; critics argue that the helping behavior demonstrated can be explained via more simplistic routes, often offering pursuit of social contact or amelioration of personal distress, rather than concern for another, as likely motivations (Blystad, 2021; Hiura et al., 2018; Schwartz et al., 2017; Silberberg et al., 2014). While multiple rodent empathy studies have addressed the former (Ben-Ami Bartal et al., 2011; Stewart S. Cox & Reichel, 2020; Sato, Tan, Tate, & Okada, 2015; Vieira Sugano et al., 2022), the latter has received little attention (Carvalheiro et al., 2019; Han et al., 2022). Carvalheiro et al. (2019) and Han et al. (2022) demonstrated that helping behavior did not significantly decline when rats were provided an option to escape to a separate darkened space. However, both protocols provided an escape area that was adjacent to the experimental arena housing the restrainer, potentially not providing sufficient reprieve from the distress cues of the trapped individual; thus, it remains to be determined if the helping behavior demonstrated by rats is motivated by empathetic capacities beyond personal distress.

Combining insights from Bartal et al. (2016) and Vieira Sugano et al. (2022) may provide further clarification regarding rat empathy. When investigating whether an emotional response is necessary to motivate helping behavior, Bartal et al. (2016) found that treatment with the benzodiazepine midazolam significantly hindered helping behavior in rats. Midazolam reduces anxiety by increasing the inhibitory effects of gamma-aminobutyric acid (GABA) and appears to have anxiolytic effects potent enough to block the transfer of the affective state from the trapped rat to the free rat. However, when Vieira Sugano et al. (2022) attempted to inhibit the stress response of the trapped rat to determine if sympathetic concern or pursuit of social contact motivates helping behavior in rats, they found midazolam did not successfully dampen the distress of the trapped rat, requiring the use of metyrapone, a drug that blocks the production of corticosterone, to overcome induced distress via confinement. Considering the distress elicited in an observer rat could be overcome by a less potent anxiolytic than that required by an individual experiencing the distress firsthand, it appears observer rats experience a dampened affective response in comparison, a quality suggestive of empathic abilities beyond personal distress.

Personal distress triggered by witnessing another in distress is expected to elicit helping behavior when one is unable to escape witnessing another in distress; thus, helping behavior in this circumstance is expressed for selfish means (reducing one's own distress). The presence of an opportunity to escape should prompt the observer to exploit this option, as it allows for the simplest means to reduce their own anxiety (Batson, 1987; Eisenberg et al., 1989). This pilot study aimed to examine if door-opening behavior exhibited by a free rat that results in the release of a trapped cagemate is motivated by pursuing alleviation of personal distress or the distress of their cagemate by providing a darkened escape alternative where the visual distress cues of their trapped cagemate are fully obstructed and the auditory and olfactory distress cues are attenuated.

If helping behavior is motivated by personal distress, rats given access to the escape area are expected to release their trapped cagemate at significantly lower rates than rats without access to the escape area. Conversely, if helping behavior is motivated with intent to reduce the distress of the trapped cagemate, rats will forgo the opportunity to quickly and easily reduce their own distress by retreating to the escape area and instead will proceed to the other end of the arena and open the restrainer door releasing their cagemate. Consistent with the findings of Carvalheiro et al. (2019), door-opening latencies are expected to increase when an escape option is present, as rats may choose to explore the escape area prior to helping.

II. Methods

i. Animals

Fifty-six adult Sprague-Dawley rats (30 females, 26 males), aged 63-70 days old upon arrival, were acquired from Charles River (Portage, MI). Rats were housed in same sexed pairs, kept in a 12-hour light/dark schedule and had ad libitum access to food and water. Animals were monitored by veterinary staff to ensure their health and safety and their use in this experiment was approved by the Institutional Care and Use Committee at the University of Louisville.

ii. Restrainer

The Plexiglas rodent restrainer (25cm x 8.75cm x 7.5cm, Harvard Apparatus, **Figure 2.2A**) was outfitted with a modified door (**Figure 2.2B)** that could only be opened from the outside. The restrainer door had two panels, one panel reached the bottom of the restrainer preventing the trapped rat from opening the door, and the second was slightly shorter which allowed for free rats to prod with their snouts to knock the door up and open. The shaft of the restrainer has several openings facilitating communication between trapped and free rats.

iii. Experimental Arena

Trials were conducted in a 150 x 50 x 50 cm clear Plexiglas arena that was lifted 5 cm off the ground by four steel legs (**Figure 2.3**). One wall of the arena had a circular opening 13 cm in diameter that was plugged and closed off in treatments in which the escape option was absent. For treatments where the escape option was accessible, one end of a 3-foot Plexiglas tunnel fit into the circular opening and led to into an escape area. The escape area was a 50 x 50 x 50 cm black Plexiglas box with a blackout curtain draped over the top.

iv. Handling

Upon arrival, rats were given 14 days to acclimate to their new home cage and establish familiarity with their cagemate before experimental trials began. On days 1-3, rats were handled for 5 minutes a day, and then 15 minutes a day on days 4-14 to reduce handler anxiety. During handling sessions, each rat was picked up from the cage and set in researcher's lap where it was gently stroked and lifted from lap and placed back down ten times. Following handling sessions, pairs of rats were released into the experimental arena for 60 minutes and were free to explore and gain familiarity with the area. The restrainer without the modified door was present during habituation.

v. Boldness Measure – Selection of Free Rat

Starting on day 15, pairs were assessed for boldness each day for the next three days. To measure boldness within a pair, the lid of their home cage was opened halfway and the latency until each rat lifted their upper body and placed both paws on the edge of cage was timed. Latencies were averaged across the three days and the individual with the shorter average latency was assigned the role of the free rat and the individual with the longer average latency was assigned the role of the trapped rat (Bartal et al., 2011).

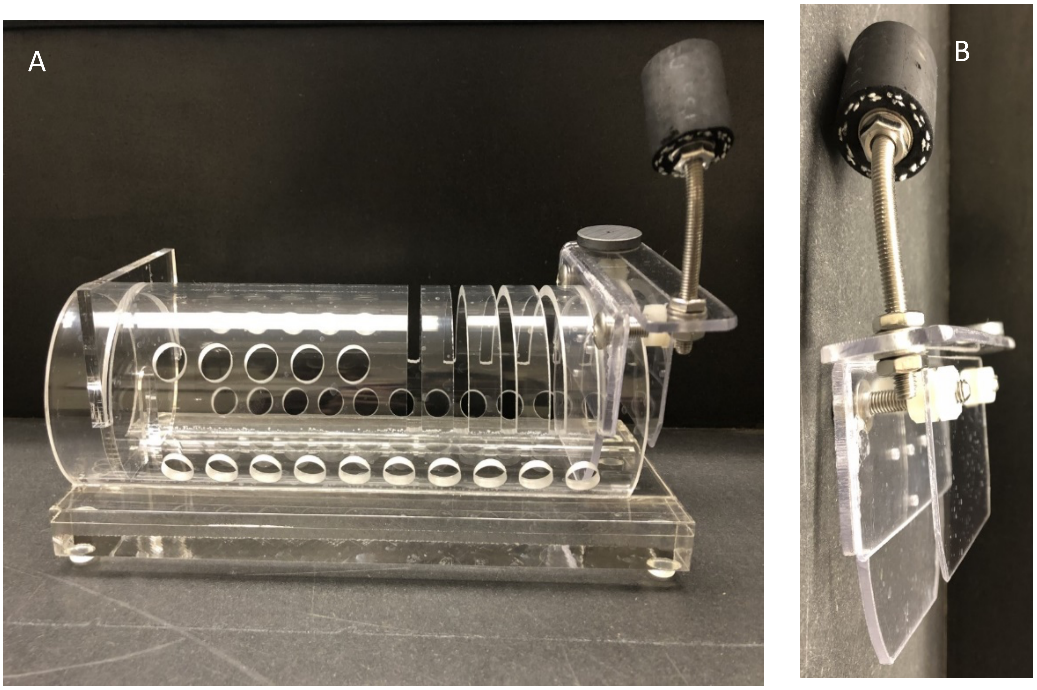

Figure 2.2 (A) Rodent restrainer. (B) Modified door.

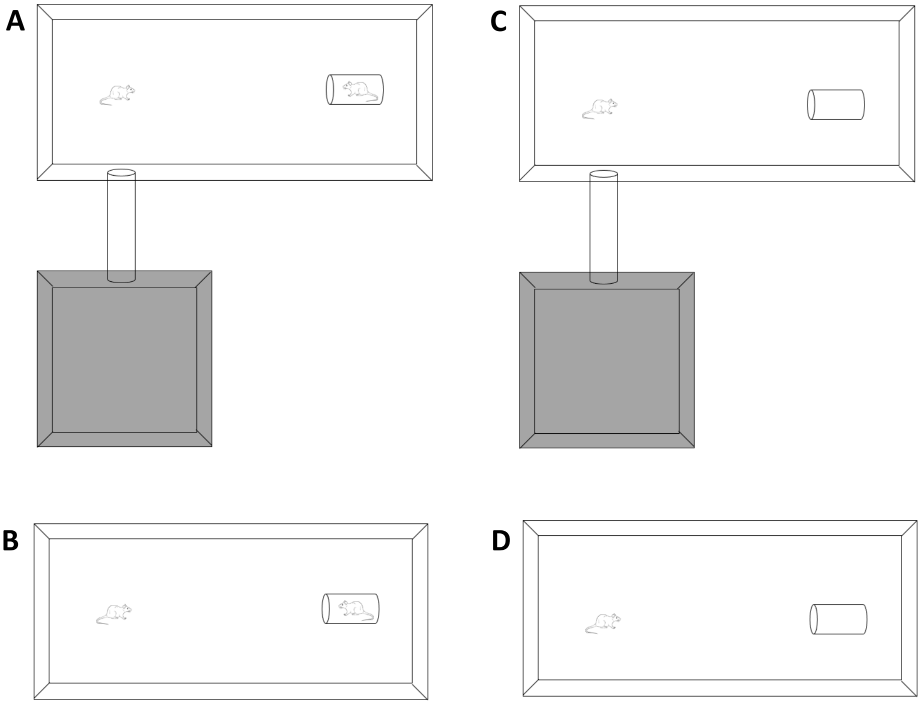

Figure 2.3 Experimental arena for treatments: (A) cagemate is trapped in the restrainer and free rat has access to escape area, (B) restrainer is empty and free rat has access to escape area, (C) cagemate is trapped in restrainer and free rat does not have access to escape area, (D) restrainer is empty and free rat does not have access to escape area.

vi. Experimental Trials

Free rats were tested in one of four treatments:

(1) Cagemate + no escape treatment: restrainer contained cagemate and the free rat did not have access to the escape area (n = 11).

(2) Cage mate + escape treatment: restrainer contained cagemate and the free rat had access to the escape area (n = 11).

(3) Empty + no escape treatment: restrainer was empty, and the free rat did not have access to the escape area (n = 6).

(4) Empty + escape treatment: restrainer was empty, and the free rat had access to the escape area (n = 6).

Before each trial began, the restrainer was placed at the end of the arena opposite the opening to the escape area. Each trial commenced when the free rat was placed in the arena. Trials lasted 40 minutes and each free rat underwent twelve trials. If the free rat did not open the door by the 25th minute, the door was opened halfway (manually), allowing the trapped rat to open the door fully, to reduce learned helplessness in the trapped rat (Martin EP Seligman, 1972; Martin E Seligman & Beagley, 1975); this was also done in empty restrainer conditions. The arena, restrainer, tunnel, and escape area were cleaned with Peroxiguard after every trial.

Two video cameras (Canon ZR200) recorded all trials. One camera was placed above the experimental arena, and another was set to night mode and placed above the escape area, under the blackout curtain. Trials were audio recorded using ultrasonic microphones (M500-384, Pettersson Elektronik, Uppsala, Sweden) and Audacity, open-source audio recording software. One microphone was mounted to a wall in the experimental arena next to the restrainer, and another was mounted to a wall in the escape area.

Rats emit 22 kHz ultrasonic vocalizations (USV) when in pain or distress, ranging from 18-32 kHz (Ben-Ami Bartal et al., 2011; Blanchard, Agullana, McGee, Weiss, & Blanchard, 1992; Blanchard, Blanchard, Agullana, & Weiss, 1991; Calvino, Besson, Boehrer, & Depaulis, 1996; Litvin, Blanchard, & Blanchard, 2007). To determine if 22 kHz USVs of the trapped cagemate were reduced in the escape area, 22 kHz USVs were recorded via the ultrasonic microphones, analyzed with the software DeepSqueak (Coffey, Marx, & Neumaier, 2019) and call rates (USV/minute) were compared between the escape area and the experimental arena. Bartal et al. (2011) discovered 22 kHz calls were produced at the highest rate during the first trials; thus, in the current experiment, 22 kHz USVs from the first trial of all the cagemate treatment pairs were recorded and statistically analyzed.

III. Results

Rats that opened the door at least two of the last three days of trials qualified as 'door-openers' (Bartal et al., 2011) and the number of door-openers were compared when (1) the restrainer contained a cagemate, (2) the restrainer contained a cagemate and an option to escape was present, (3) the restrainer was empty, (4) the restrainer was empty and an option to escape was present **(Figure 2.4).** In this study, only 3 of 11 rats tested with a trapped cagemate when an escape option was absent met the criterion for door-opener, while only 2 of 11 rats met the criterion for door-opener when tested with a cagemate and the option to escape was present. When free rats were tested with a trapped cagemate, there was no difference in the number of door-openers when an escape option was present or absent (Fisher's exact test, p = 1). None of the 12 free rats tested with an empty restrainer met the criterion for door-opener. There was no difference in the number of door-openers when the restrainer contained a cagemate or when the restrainer was empty (Fisher's exact test, p = 0.1366). Conversely, in Bartal et al.'s (2011) study, 23 of 30 rats qualified as door-openers when a cagemate was trapped in the restrainer, while 5 of 40 rats qualified as door-openers when tested with an empty restrainer or a restrainer containing a toy rat.

For the trials where the free rats opened the door to release their trapped cagemate, the latency to door-opening was measured (**Figure 2.5**). A significant difference in door-opening latency was not detected when compared between trials where an escape option was absent and trials where an escape option was present (t-test, t = 1.9574, df = 50, p = 0.0559).

22 kHz call rates were significantly lower in the escape area than in the experimental arena near the restrainer (t-test, t = 10.1989, df = 21, p < 0.0001), suggesting the 22 kHz USVs emitted by the trapped rat in the experimental arena are greatly reduced in the escape area (**Figure 2.9**).

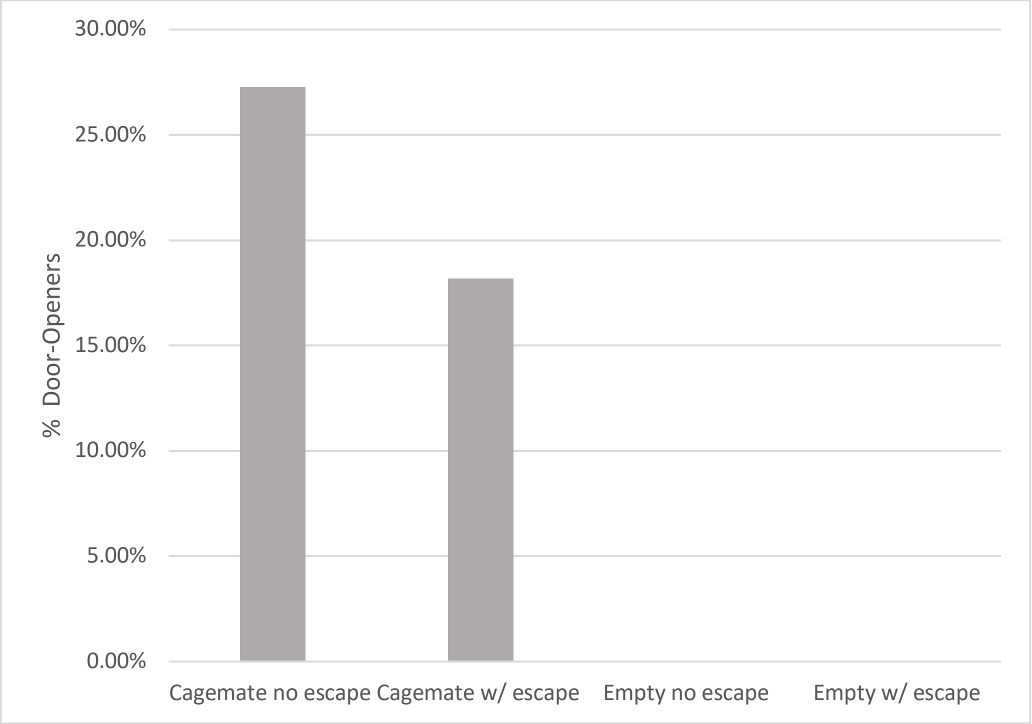

Figure 2.4 The percent of rats that were door-openers when (1) restrainer contained a cagemate and option to escape was absent, (2) restrainer contained a cagemate and option to escape was present, (3) restrainer was empty and option to escape was absent, and (4) restrainer was empty and option to escape was present.

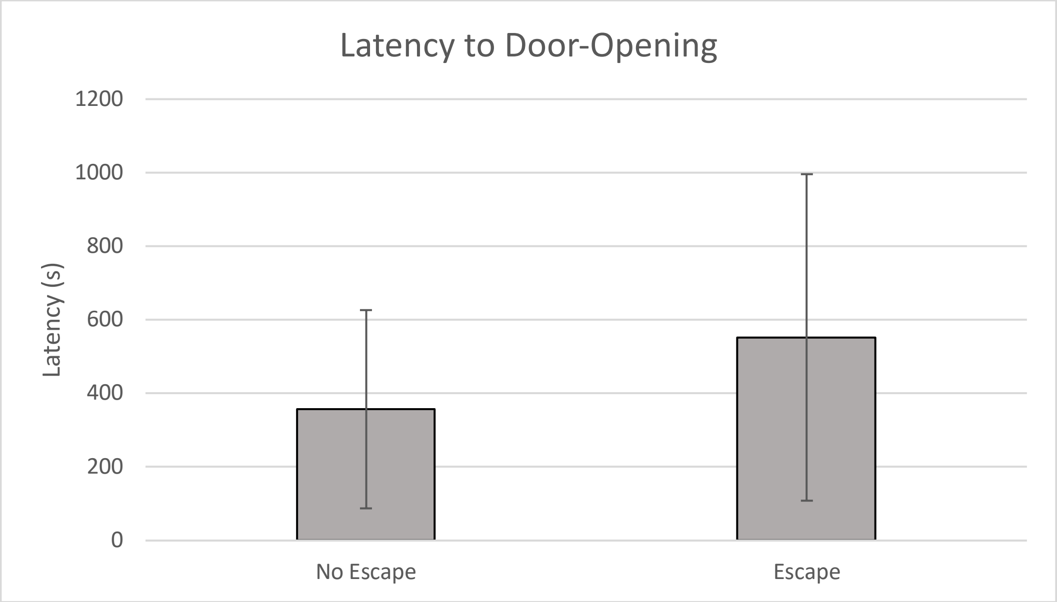

Figure 2.5 Latency to door-opening when the free rat opened the door to release a trapped cagemate when (1) an option to escape was absent, (2) an option to escape was present (x ± SE). No significant difference in latency was detected.

Figure 2.6. The percentage of rats that opened the door across the 12 trials when (1) restrainer contained a cagemate and option to escape was absent (black dotted line), (2) restrainer contained a cagemate and option to escape was present (dashed grey line), (3) restrainer was empty and option to escape was absent (solid black line), and (4) restrainer was empty and option to escape was present (solid grey line).

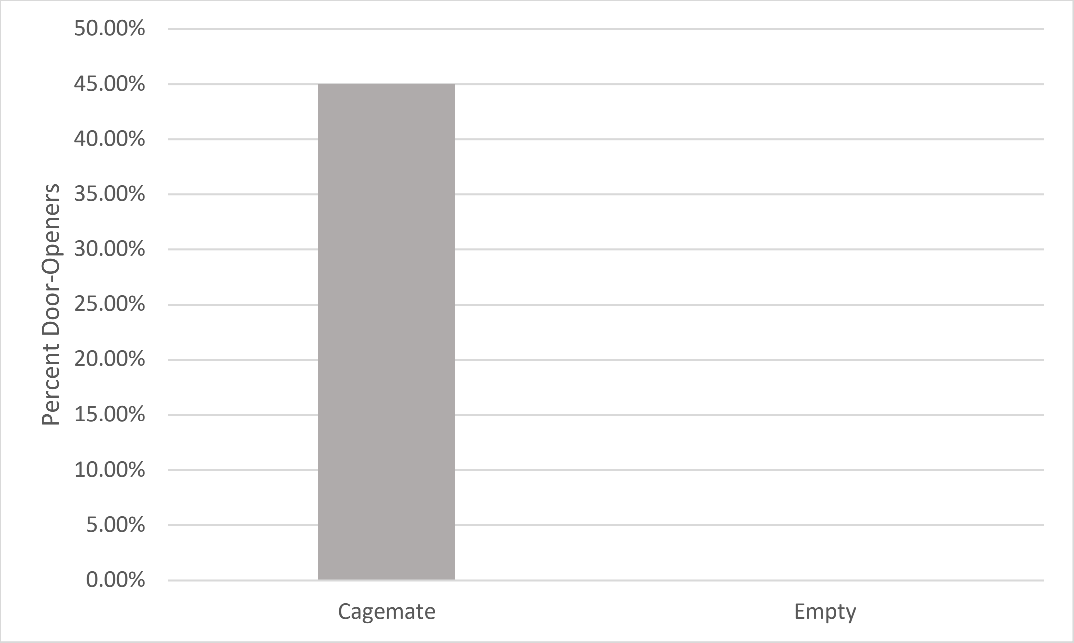

Figure 2.7 The percentage of rats that were door-openers when cagemate treatments were combined and empty treatments were combined.

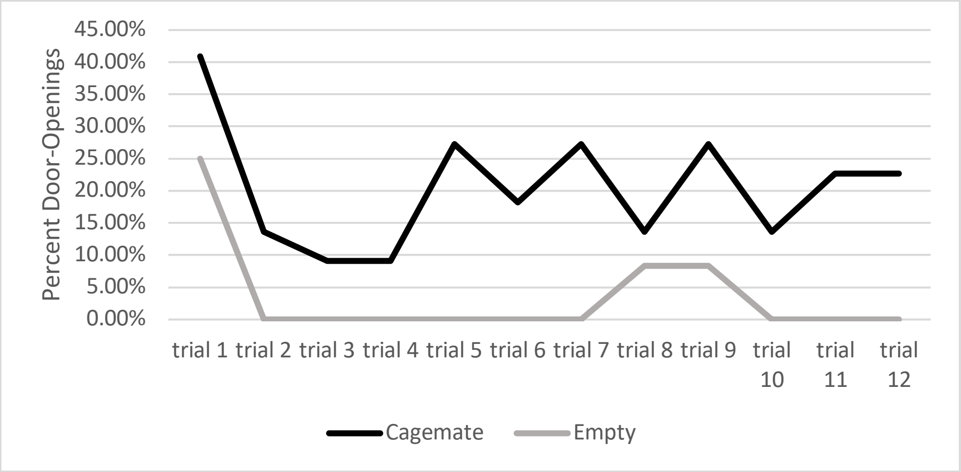

Figure 2.8 The percent of rats that opened the door across the 12 trials when cagemate treatments were combined and empty treatments were combined.

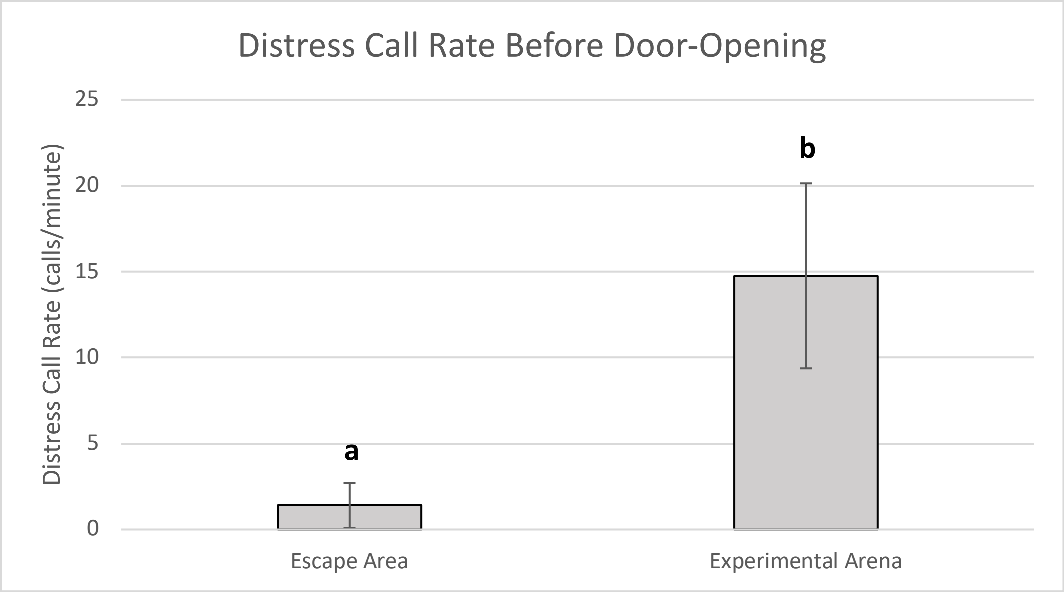

Figure 2.9 22 kHz call rate (USVs/minute) prior to door-opening during the first trial of all cagemate treatment groups (x ± SE). 22 kHz USVs were recorded and analyzed from ultrasonic microphones placed in the escape area and in the experimental arena mounted adjacent to restrainer. Bars labeled with different letters are statistically different from one another at the $p<0.05$ level.

IV. Protocol Updates for Future Experiments

i. Door-opening training:

Unlike the paradigm Helping Behavior Test (HBT) from Bartal et. al (2011), future experiments will include a door-opening training protocol as the pilot experiment rats demonstrated difficulty learning to open the modified door. Despite constructing the modified restrainer door from dimensions detailed in Bartal et al. (2011, supplemental methods), the door used in the current study was potentially harder to open than those used in other studies. Considering door-opening is the focal parameter measuring empathically motivated behavior in the HBT, confirming free rats possess the ability to help will ensure success of intended actions. Furthermore, by ensuring free rats are capable of door-opening, it will protect against accidental openings due to exploratory behaviors. Only rats that demonstrate the ability to fully open the door during the final four consecutive days of training will be used in the experimental trials of future studies.

ii. Total door-openings instead of door-openers criteria:

Free rats tested in the HBT designed by Bartal et al. (2011) had to open the door during at least two of the last three trials to be considered a 'door-opener.' The door-opener criterion was the predominant measure of helping behavior and was incorporated for its ease of use during statistical analysis; however, the number of days required to meet this criterion could be arbitrary, thus, for future experiments we will analyze total door-openings.

iii. Increase pre-trial handling and habituation:

Bartal et al. (2011, supplemental methods) handled rats for 5, 10, 15 and 15 minutes a day, respectively, for four days followed by 60 minutes of time to explore the experimental arena. This handing and habituation protocol was followed prior to experimental trials in the current study; however, potentially the rats were too anxious, thus, inhibiting helping behavior. To prevent potential inhibition of helping behavior due to suboptimal anxiety levels, future experiments will incorporate a more extensive handling and habituation protocol.

V. Concluding Remarks

The findings of the current study remain inconclusive as free rats exposed to distressed cagemates in the control treatment failed to open the restrainer door at a rate similar to those of previous studies (Bartal et al., 2011, 2014, 2016, 2021). Although the addition of an escape area in recent iterations of the helping behavior test revealed rats opted to release their trapped cagemate at rates comparable to those without the option to escape, albeit at higher latencies, the escape area provided in these studies was adjacent to the experimental area where the restrainer was located and thus may not have provided enough distance from the distress cues of the trapped cagemate (Carvalheiro et al., 2019; Han et al., 2022). The darkened escape area used in the current study was a greater distance from the restrainer designed to block all visual distress cues and demonstrated attenuation of 22 kHz USVs, thus providing enhanced reprieve from the distress of the trapped individual. Therefore, the arena and escape area in this study provided a more appropriate design to test for the influence personal distress has on the performance of helping behavior.

An important criterion distinguishing emotional contagion, and thus personal distress, from more complex empathic abilities is the capacity to discriminate the self from the other (de Waal, 2008). While behaviors motivated by personal distress may confer benefits for others, the intent behind the actions is self-serving (Batson et al., 1987). Conversely, the emphasis is directed to the other, not to self, when sympathetic concern and empathic perspective-taking are elicited and involves actions that predominately benefit another.

The motivations underlying the door-opening behavior demonstrated in previous studies have yet to be fully elucidated, and many remain skeptical that rats possess empathic abilities beyond personal distress (Lavery & Foley, 1963; Rice, 1964; Preobrazhenskaya & Simonov, 1974; Schwartz et al., 2017; Blystad, 2021). de Waal (de Waal, 2011) introduces the term ‘preconcern’ to the rodent empathy literature describing reactions of individuals to the distress of others where, instead of avoiding another’s distress and acting to alleviate one’s own aversive arousal, an individual is seemingly attracted to the source of distress. Preconcern is expressed without understanding the situation provoking distress in another, yet the observer seeks contact with the demonstrator and appears to offer comfort nonetheless, even if in part to improve their own affective state. Thus, preconcern acts as a precursor to sympathetic concern and may provide a missing link in the evolutionary history of empathy where more sophisticated layers have built upon earlier manifestations. The attraction to the distress cues of a trapped rat and the proclivity for releasing a conspecific confined to a restrainer suggests a capacity more nuanced than personal distress and may fit the criteria of preconcern. Future research will need to determine if rats are able to discern what is causing another’s distress and if they are capable of a primitive form of perspective-taking, potentially by incorporating a design similar to the one used by Menzel (Menzel, 1988). Menzel demonstrated that chimpanzees know when another has information they lack and will show greater attention to an individual who possess knowledge the rest of the group lacks (Menzel, 1988). Rats could be tested in groups in an open arena with multiple areas attached by Plexiglas tunnels. A single rat could be exposed to a positive or negative stimulus in one of those compartments prior to reuniting the entire group in the central arena. The openings to the attached tunnels could be blocked for a set amount of time to monitor the interactions of the naïve groupmates with the focal rats and then monitor behavior after access to connected areas was granted. If the naïve group members followed the focal rat to areas previously endowed with the positive stimulus, especially if the focal rat was of low status or younger than the others, it would suggest they recognized another possessed valuable information they lacked.

From there, an experiment could habituate a focal rat to an otherwise distressing stimulus, such as a toy predator, or couple a positive stimulus with an aversive consequence, such as eating a palatable food item and receiving a foot shock. Naïve individuals would then be exposed to the toy predator or the food item with the focal rat to measure the behavioral and physiological responses of the focal rat and the naïve rats. If the naïve individuals demonstrated changes in their fear responses to the different stimuli, it may suggest the capacity for empathic perspective-taking. Understanding the empathic capacity of rats can enhance knowledge regarding the evolution of empathy and would further inform the neural mechanisms involved in empathic perspective-taking, an ability diminished in people with schizophrenia and those on the autism spectrum (Jones, Happé, Gilbert, Burnett, & Viding, 2010; Langdon, Coltheart, & Ward, 2006).

CHAPTER III

THE EFFECTS OF COST ON HELPING BEHAVIOR IN RATS

I. Introduction

The study of empathy in non-humans, particularly in rodents, has seen a surge in experiments in the last decade where rats have demonstrated a reliable and repeatable ability to help others in distress (Ben-Ami Bartal et al., 2021; Ben-Ami Bartal et al., 2011; Ben-Ami Bartal et al., 2014; Ben-Ami Bartal et al., 2016; Carvalheiro et al., 2019; Havlik et al., 2020; Sato et al., 2015; Vieira Sugano et al., 2022; Yamagishi, Okada, et al., 2020). These studies have worked to uncover the proximate mechanisms facilitating helping behavior, the ultimate mechanisms maintaining its expression and the contexts that elicit and inhibit its performance. Enhancing our understanding of empathy through rodent studies may provide insights into its evolution and inform diagnoses and treatment of disorders marked by a lack of empathy (Stewart S Cox & Reichel, 2021).

The helping behavior test (HBT) has become the paradigm for examining helping behavior in rodents, and since Bartal et al (2011) first introduced the HBT, multiple experiments have used the design with various modifications (Bartal et al., 2014, 2016, 2021; Blystad et al., 2019; Breton et al., 2022; Carvalheiro et al., 2019; Cox & Reichel, 2020; Cox et al., 2022; Sato et al., 2015; Vieira Sugano et al., 2022). The HBT focuses on a pair of rats introduced to an experimental arena, in which one is trapped inside a rodent restrainer customized with a door that can only be opened from the outside, while the other is free to roam the arena with unobstructed access to the restrainer. The behavior of the free rat is monitored, particularly focused on whether they open the door to release their trapped counterpart. The HBT serves as an ideal model, as the consequences of the action, or inaction, of the free rat are explicit (i.e., they either open the door or they do not) and immediate (i.e., the trapped rat is released or remains trapped in the restrainer). When paired with appropriate controls, the frequency of door opening, as well as other components of the observing rat's behavior, can be measured and compared to better understand underlying motivations of helping behavior. The HBT can also be easily modified to assess helping behavior across various contexts and treatments.

Behaviorally, rats have exhibited flexibility in their response to distressed conspecifics, which is dependent on their previous experience with the stressor (Sato et al., 2015) and level of familiarity, both individually and by strain (Ben-Ami Bartal, 2014). However, rodent empathy studies have been critiqued by skeptics offering alternative explanations for the motivations underlying the prosocial behavior seen in rats, most commonly alleviation of personal distress and the pursuit of social contact (Silberberg et al., 2013; Schwartz et al., 2017; Hiura et al., 2018). While our current study addresses the former, the most compelling evidence found to dispute the latter comes from a recent study published by Vieira Sugano et al. (2022) which considered how free rats respond when tested with a trapped rat that was either anesthetized or treated with metyrapone, a drug used to reduce corticosterone production. Interestingly, when the trapped rat was unconscious, free rats opened the door to the restrainer and dragged the immobile rat out of and away from the restrainer. However, when the trapped rat was treated with metyrapone, and showed no signs of distress, door-opening significantly reduced. Together these results suggest that cognitive cues and affective cues are both singularly effective at motivating helping behavior in rats.

While the aforementioned studies have examined rodent helping behavior across various contexts, none, to my knowledge, have examined the effects of cost on helping behavior. Since helping behavior is typically accompanied by some type of cost in the natural world, whether it be risk of injury or energetic expenditure, it is thus relevant to understand the implications of cost on helping behavior (Eisenberg et al., 1989; Fehr & Fischbacher, 2003). If rats are capable of a primitive form of empathetic perspective-taking, observing another in distress should elicit targeted helping behavior intended to alleviate this individual's distress, even when the observer has the option to escape. However, this behavior may be altered if the cost associated with helping appears to be too high (Batson, O'Quin, Fultz, Vanderplas, & Isen, 1983; Shotland & Stebbins, 1983).

To test whether the presence of a cost hinders helping behavior in rats, I modified the traditional HBT by introducing a cold-water barrier that separated the free rat from the restrainer. Since rats dislike being soaked in water, this provided a relevant cost associated with helping (Sato et al., 2015). We expected rats would still be motivated to help their trapped cagemate even in the presence of a cost but anticipated a decline in the rate of door-opening when coupled with a cost.

To account for the possibility that motor mastery motivates door-opening, behavioral responses of rats presented with an empty restrainer were also compared when a cost was present and absent. Comparisons of these groups allowed us to make inferences about whether cost tempers the empathetic response to a distressed cagemate and determine if motor mastery, instead of targeted helping behavior, motivates risk associated door-opening behavior.

Considering rodents display affective state-matching (Burkett et al., 2016; Rogers-Carter, 2018; Han et al., 2020), it is possible that personal distress, not concern for another's welfare, could provoke helping behavior where rats are motivated to stop the aversive distress cues expressed by a trapped cagemate. Therefore, in all treatments, free rats had access to a dark escape area where distress cues from their trapped cagemate were greatly minimized and served as a reprieve from stress inducing stimuli. Escaping the distress cues of the trapped cagemate is a more efficient way to alleviate personal distress; thus, the addition of the escape area allowed us to explore the possibility of its usage to impede helping behavior. If helping behavior in rats is driven solely to ameliorate the free rat's own personal distress, we'd expect free rats to remain in the escape area and not leave to open the restrainer door.

II. Methods

i. Animals

Forty adult female Sprague-Dawley rats, aged 63-70 days old, were obtained from Charles River (Portage, MI). Only female rats were used in this study, as very few males could be trained to open the door before trails commenced, this may be due to the fact that males generally display lower activity levels and exhibit less exploratory behavior compared to females who spend more time in open spaces (Bonuti & Morato, 2022; Scholl, Afzal, Fox, Watt, & Forster, 2019; Van Hest, Van Haaren, &

Van de Poll, 1987). Rats were kept on a 12-hour light/dark schedule and had ad libitum access to food and water. Their use in the experiment detailed below was approved by the Institutional Animal Care and Use Committee at the University of Louisville.

ii. Experimental Arena

Trials were conducted in a 150cm x 50 cm clear Plexiglas arena that was lifted 5 cm off the ground by six steel legs. The bottom of the arena had two pieces of 50cm Plexiglas separated by a 50cm x 50cm x 5cm plastic tub (**Figure 3.1**). A cost was introduced in some treatments: because rats are averse being soaked in water (Sato et al., 2015), the cost was passing through a bin that was filled with cold water (16° C) to a height of 45mm. For conditions where the cost was absent, the bin was empty. The Plexiglas rodent restrainer (25cm x 8.75cm x 7.5cm, Harvard Apparatus) was outfitted with a modified door that could only be opened from the outside and was placed in the center of one end of the arena. The other end of the arena provided access to a 3-foot Plexiglas tunnel that led to into an escape area. The escape area was a 50cm x 50cm black Plexiglas box with a blackout curtain draped over the top. In the escape area, any distress cues given off by the trapped cage mate were greatly minimized.

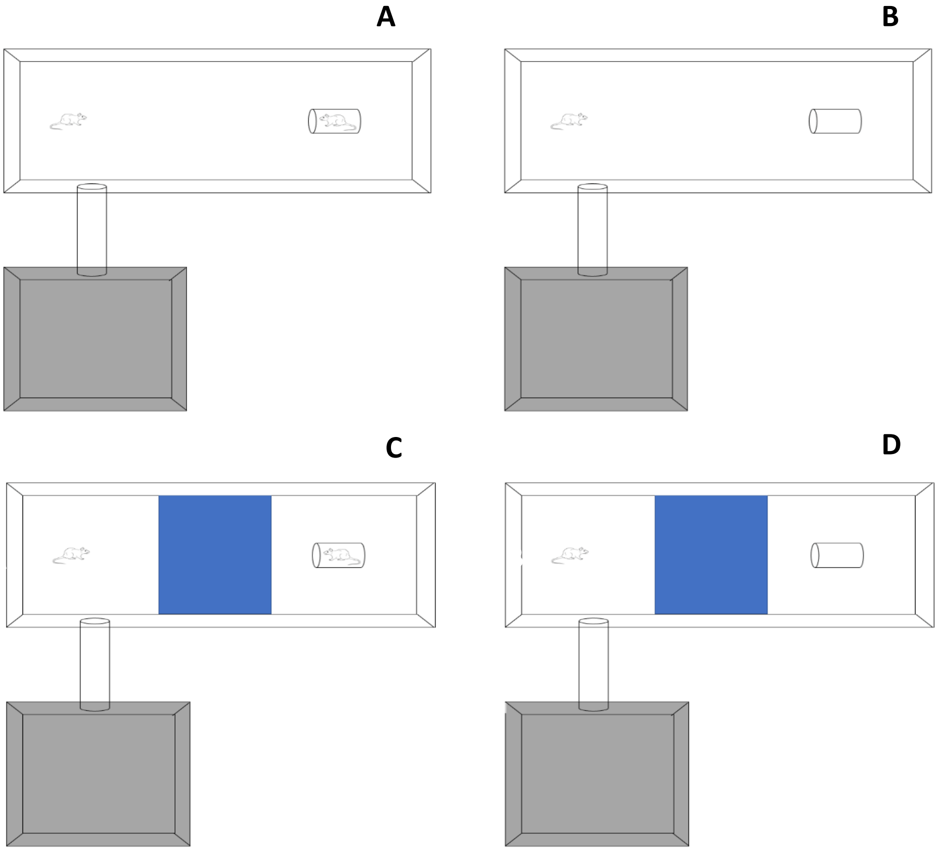

Figure 2.1 Experimental arena. Restrainer was placed at one end of the arena and tunnel leading to darkened escape area was at the opposite end, where the free rat started each trial. Free rats were subjected to one of the following treatments: (A) cagemate trapped in restrainer, (B) restrainer is empty, (C) cagemate trapped in restrainer and cold-water barrier separates free rat and restrainer, or (D) restrainer is empty and cold-water barrier separates free rat and restrainer.

iii. Handling & Boldness Measure

Upon arrival, rats were housed in pairs, and were given 14 days to acclimate to their new home cage and become familiar with their cage mate. As was done in the pilot study, starting on day 15, pairs were assessed for boldness each day for three days. To measure boldness within a pair, the researcher opened the cage lid halfway and timed the latency until each rat lifted their upper body and placed paws on edge of cage. Latencies were averaged across the three days and the individual with the shorter average latency was assigned the role as the free rat and the individual with the longer average latency was assigned the role as the trapped rat (Bartal et al., 2011). Handling time was increased from the pilot study so that after each boldness measure, rats were handled for 15 minutes a day to reduce handler anxiety. During handling sessions, each rat was picked up from cage and set in researcher's lap where it was gently stroked and lifted from lap and placed back down ten times. Following handling sessions, pairs of rats were released into the experimental arena for 30 minutes.

iv. Training

Starting at day 18, and the for the next two weeks, only the free rat was regularly handled and released into the experimental arena to reduce exploratory behavior typical after introduction to a new place and to begin door-opening training. During each 30-minute introduction, the free rat had the opportunity to open the restrainer door for half a chocolate chip. During the first five training days (18-22), the restrainer door was opened halfway to facilitate door opening. During the next nine days (day 23-31), the restrainer door was fully closed.

Unlike the paradigm Helping Behavior Test (HBT) from Bartal et. al (2011), we introduced the door-opening training because in our pilot experiment rats demonstrated difficulty learning to open the modified door, suggesting it was heavier and possibly harder to open than those used in other studies. Furthermore, by ensuring free rats were capable of door-opening, this reduced the occurrence of accidental door-openings due to exploratory behavior (Blystad et al., 2019) and ensured rats could open the door if they felt motivated to do so. Only rats that demonstrated they could fully open the door during the final four consecutive days of training were used in experimental trials.

v. Experimental Trials

Free rats were tested in one of four treatments (n = 10):

(1) Cage mate + no cost treatment: the free rat's cage mate was trapped in the restrainer and the free rat did not incur a cost to open the restrainer door.

(2) Cage mate + cost treatment: free rat's cage mate was trapped in the restrainer and the free rat had to cross the cold-water barrier (incur a cost) to open the restrainer door.

(3) Empty + no cost treatment: the restrainer was empty, and the free rat did not incur a cost to open the restrainer door.

(4) Empty + cost treatment: the restrainer was empty, and the free rat had to cross the cold-water barrier (incur a cost) to open the restrainer door.

At the beginning of each trial, the free rat was placed at the end of the arena opposite of the restrainer. Initially, free rats underwent twelve trials. However, about half of the free rats participating in treatments where the cost was present appeared to have habituated to the cold-water barrier by the eighth trial and thus to ensure crossing the barrier remained costly throughout all trials, the number of trials was truncated to six. Each trial lasted 40 minutes. If the free rat did not open the door by the 25th minute, the door was opened halfway (manually), allowing the trapped rat to open the door fully, to reduce learned helplessness in trapped rat (Seligman, 1972; Seligman and Beagley, 1975), but this was also done in empty restrainer conditions.

Trials were video recorded using a Canon z200 and activity levels were measured as cm/min using the animal tracking software AnyMaze. Trials were audio recorded on a M500-384 ultrasonic microphone from Pettersson Elektronik (Uppsala, Sweden) using Audacity, an opensource audio recording software. Ultrasonic calls were detected using DeepSqueak, a high frequency detection and analysis software.

vi. Statistical Analysis

To determine if the presence of a cost influences the expression of helping behavior, four groups of "free" rats (n = 10 rats/group) were subjected to six trials where they were placed at one end of an experimental arena, where a rodent restrainer with a modified door was stationed at the other end. Free rats were tested in one of the following four groups: (1) trapped cagemate + no cost, (2) trapped cagemate + cost, (3) empty restrainer + no cost, and (4) empty restrainer + cost. Comparisons of door-opening behavior, latency to door-opening, activity levels prior to door-opening, and percent time spent in escape area prior to door-opening were made among the four groups. To make these comparisons, we fit mixed effects models – a logistic model for the probability of door opening, a Cox frailty model to evaluate time to door opening, and linear models to evaluate activity levels and time spent in the escape area. Each model had a random intercept per rat to account for covariation among repeated trials per rat. The fixed effects were cagemate (yes/no), cost (yes/no), and an interaction term, which effectively defined the four groups. Comparisons were derived as linear contrasts from the estimated model coefficients. In an exploratory analysis, we fit a logistic mixed effects model for door opening with cagemate, trial number, and their interaction as fixed effects, to explore differences in success probability trends over the trials. All analyses were conducted in the open-source R software environment (R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.r-project.org/).

III. Results

i. Door-Opening

Door-opening frequencies varied among the four groups (p = 0.008, **Figure 3.2**). Both the presence of a cagemate and the presence of a cost were found to be significant contributors to probability of door-opening. Free rats opened the door significantly more when a cagemate was trapped in the restrainer than when the restrainer was empty (cagemate: 101/120, 84%; empty: 65/120, 54%, p = 0.006). Free rats opened the door significantly less when a cost was present than when a cost was absent (cost: 66/120, 55%; no cost: 100/120, 83%, p= 0.007). No interaction was found between cagemate and cost (p = 1.0), meaning the magnitude of the effects of cagemate and cost were independent.

Although free rats in the cagemate + no cost treatment (57/60, 95%) opened the restrainer door more often than rats in the cagemate + cost treatment (44/60, 73%), the difference was not significant (Wald $X^2 = 3.51$, p = 0.061). Thus, when a cagemate is trapped, door-opening is not significantly hindered by the presence of a cost. Free rats in the cagemate + cost treatment (44/60, 73%) opened the door more often than rats in the empty + cost treatment (22/60, 37%). Rats are significantly more likely to incur a cost to open a restrainer door when a cagemate is trapped than when the restrainer is empty (Wald $X^2 = 4.03$, $p = 0.045$), suggesting the cues of a distressed cagemate motivate engagement in costly behavior. Despite free rats in the empty + no cost treatment (43/60, 72%) opening the door more often than rats in the empty + cost treatment (22/60, 37%), there was insufficient evidence to a show a difference at the population level (Wald $X^2 = 3.49$, $p = 0.062$). Thus, when the restrainer is empty, door-opening is not significantly inhibited by the presence of a cost.

i. Progression of Trials

The proportion of rats that opened the door during each trial were compared between the cagemate and empty groups. A trend suggests the rate of opening the door to an empty restrainer declined as trials progressed relative to that of opening the door to release a trapped cagemate (**Figure 3.3**). When the restrainer was empty, door-opening declined as trials progressed, while door-openings remained relatively consistent when a cagemate was trapped in the restrainer (likelihood ratio $\chi^2 = 9.13, p = .033$). This suggests releasing a distressed cagemate provides sufficient reinforcement to motivate continued door-opening.

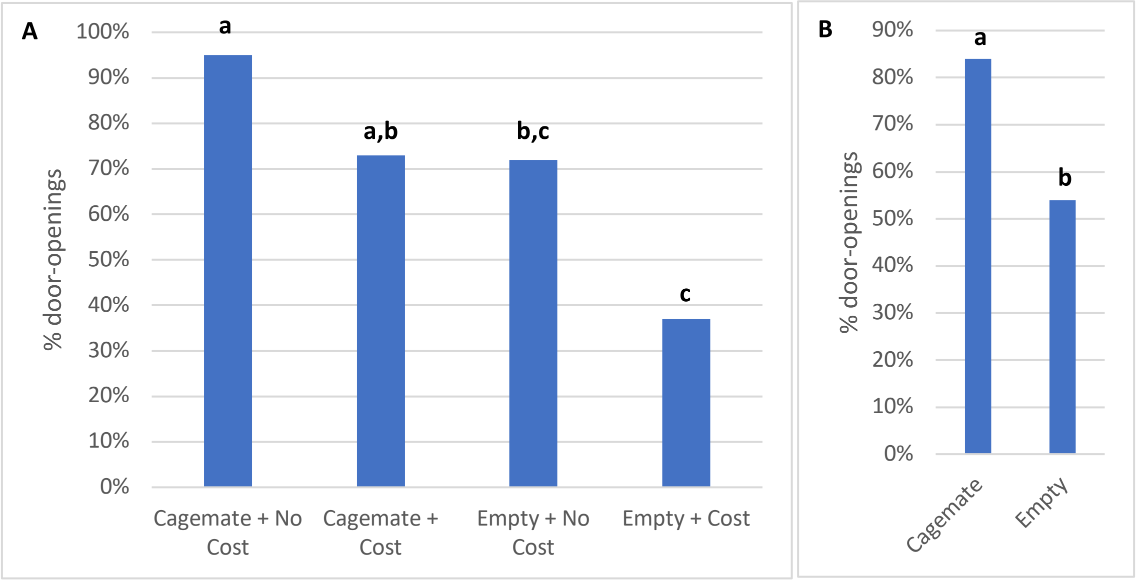

Figure 3.2 Performance of free rats. (A) Proportion of trials where the free rat opened the door when the restrainer contained a cagemate and no cost was present, when the restrainer contained a cagemate and a cost was present, when the restrainer was empty and no cost was present, and when the restrainer was empty and a cost was present. (B) Proportion of trials where the free rat opened the door when the restrainer contained a cagemate and when the restrainer was empty. Bars labeled with different letters are statistically different from one another at the p<0.05 level.

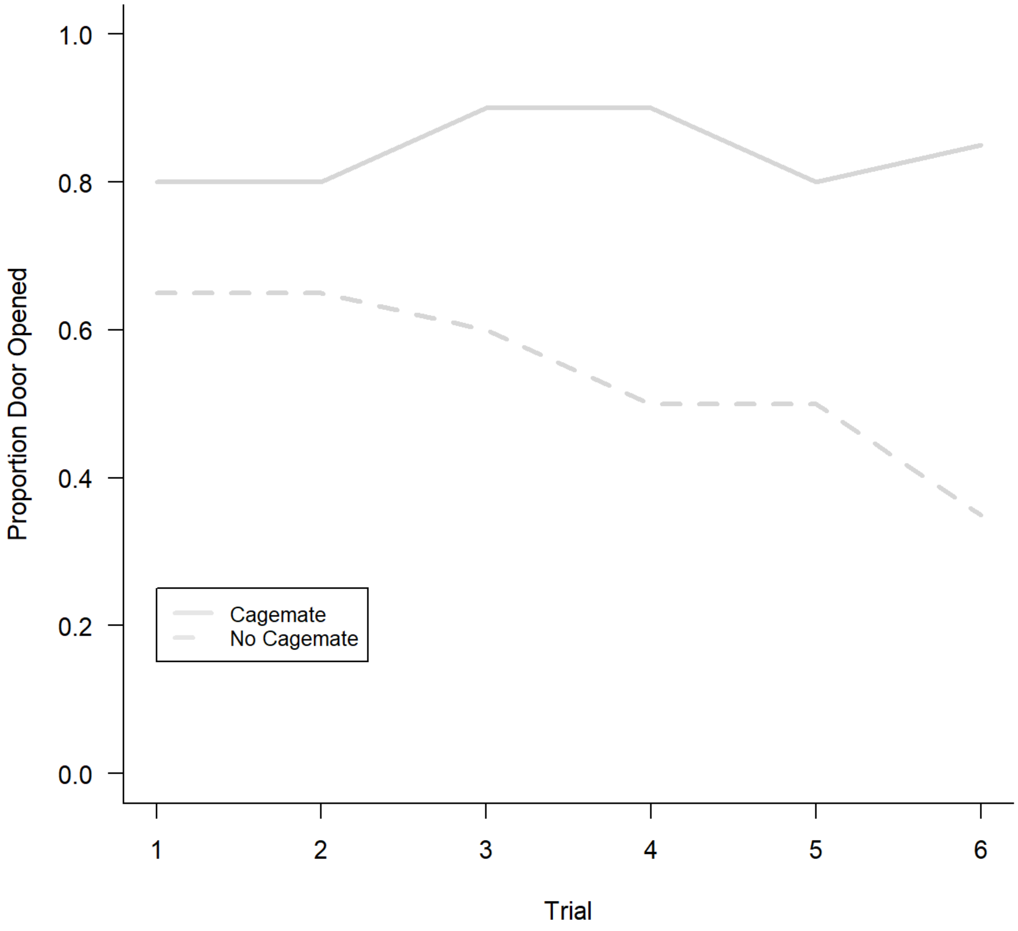

Figure 3.3 Proportion of rats that opened the door during each of the six trials when cagemate treatments are aggregated and empty treatments are aggregated. The solid line represents rats tested with trapped cagemates. Dashed line represents rats tested with an empty restrainer.

ii. Activity Levels

In studies utilizing the HBT, activity levels have been used as a behavioral proxy to measure increased levels of arousal, which is suggestive of anxiety. Activity levels were compared to understand how cost and the presence of a cagemate affect arousal levels (**Figure 3.4**). To determine if the presence of a trapped cagemate increases arousal, a targeted comparison was conducted between an aggregated cagemate treatment (i.e., cagemate/cost and cagemate/no cost) and an aggregated empty treatment (i.e. empty/cost and empty/no cost). Free rats demonstrated significantly higher activity levels when a cagemate was trapped in the restrainer than when the restrainer was empty (Wald $X^2 = 4.73$, $p = 0.030$).

To determine if the presence of a cost increased activity levels when a cagemate was trapped in the restrainer, a targeted comparison was conducted between the cagemate + no cost treatment and the cagemate + cost treatment. When a cagemate was trapped in the restrainer, there was no difference in activity levels whether a cost was present or absent (Wald $X^2 = 0.88$, $p = 0.349$). Thus, the addition of an associated cost does not heighten anxiety levels when required to help a cagemate.

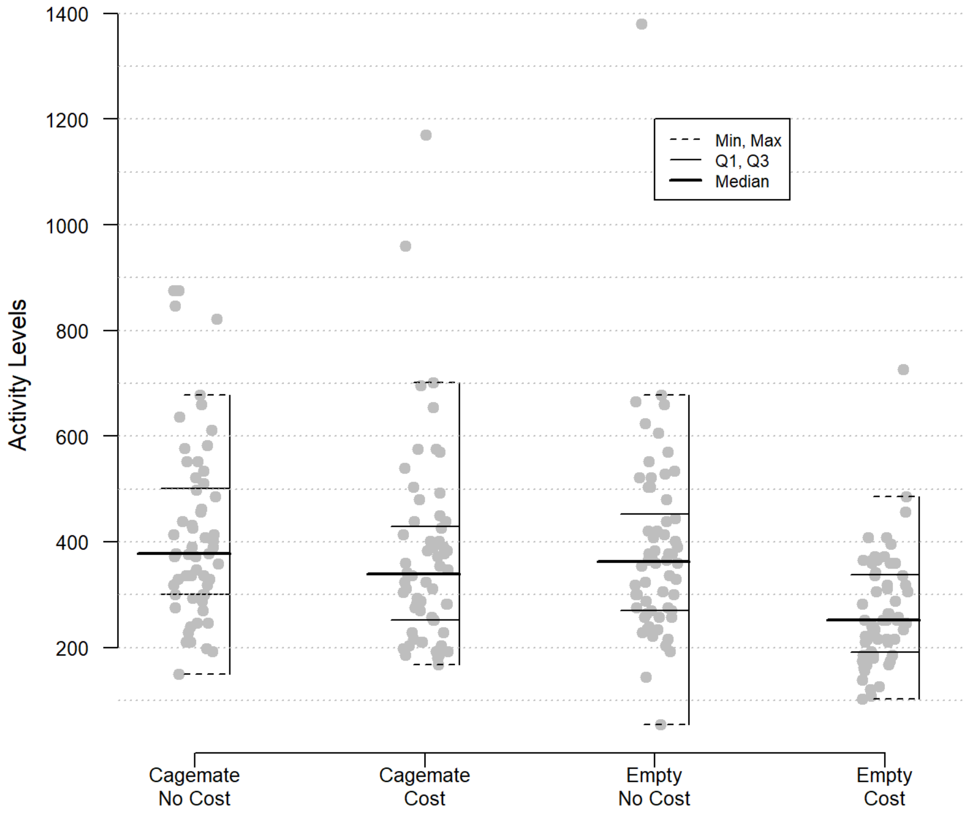

Figure 3.4: Strip chart with boxplot statistics of activity levels (cm/min) prior to door-opening when the restrainer contained a cagemate and no cost was present, when the restrainer contained a cagemate and a cost was present, when the restrainer was empty and no cost was present, and when the restrainer was empty and a cost was present. Note: In cases where the free rat did not open the restrainer door, researcher opened the door half-way at the 25-minute mark.

iii. Time Spent in Escape Area

The escape area provided free rats with an opportunity to alleviate the stress associated with subjection to a brightly lit open space and/or the distress cues of their cagemate. To determine if the presence of a trapped cagemate promotes self-directed behaviors, the proportion of time free rats spent in the escape area prior to door opening was compared between an aggregated cagemate treatment and an aggregated empty treatment (**Figure 3.5**). Free rats spent a significantly greater proportion of time in the escape area before door opening when the restrainer was empty than when the restrainer contained a trapped cagemate (Wald $X^2 = 30.1$, $p < 0.001$). Thus, free rats were less likely to move to a low stress environment when a trapped cagemate was present, suggesting helping behavior was at least partially motivated to reduce their cagemate's distress.

To determine if the presence of a cost influenced time spent in the escape area when a cagemate was trapped in the restrainer, a targeted analysis was conducted to compare the cagemate + no cost treatment and the cagemate + cost treatment. When a cagemate was trapped in the restrainer, there was no difference in percent time spent in escape room before door-opening whether a cost was present or absent (Wald $X^2 = 0.73$, $p = 0.39$). This suggests the presence of a cost does not elicit a higher level of stress that would motivate free rats to seek an opportunity to reduce their own personal distress.

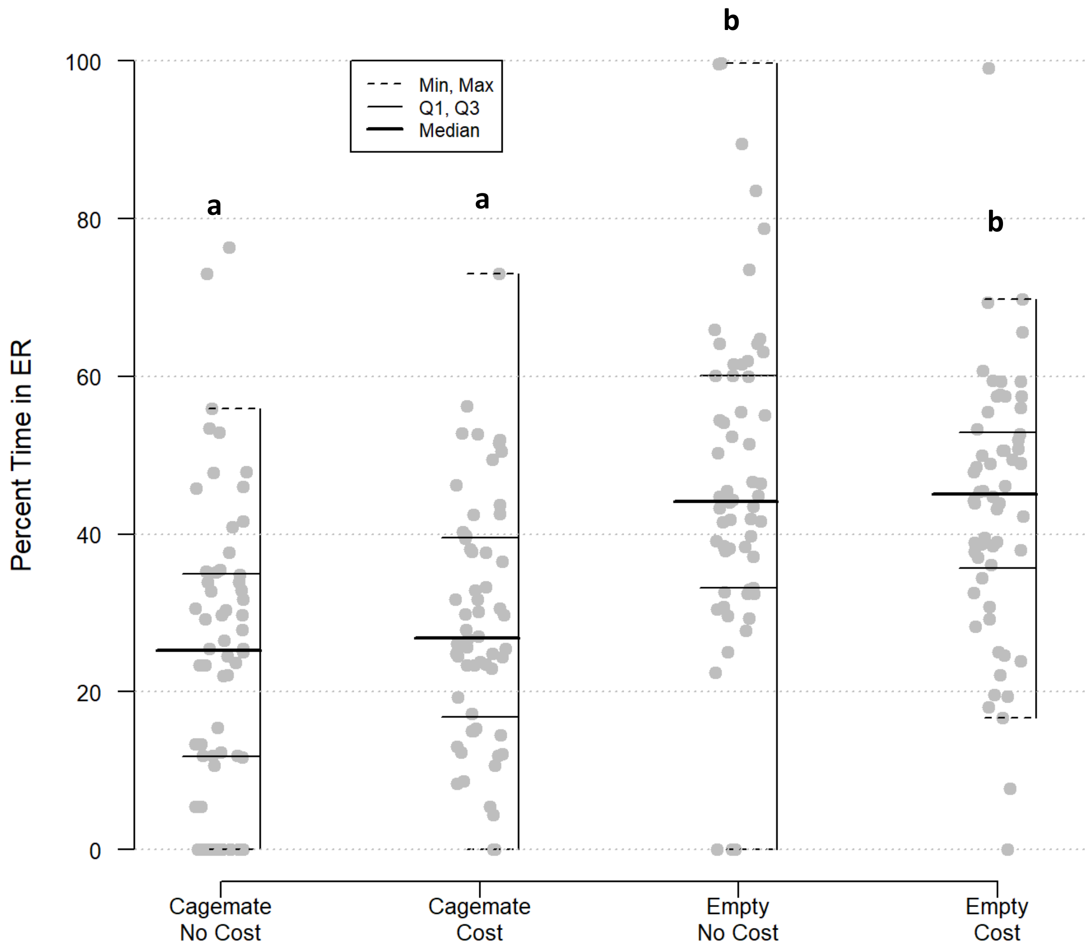

Figure 3.5 Strip chart with boxplot statistics of percent time spent in escape area before door-opening when the restrainer contained a cagemate and no cost was present, when the restrainer contained a cagemate and a cost was present, when the restrainer was empty and no cost was present, and when the restrainer was empty and a cost was present. Clusters labeled with different letters are statistically different from one another at the $p<0.01$ level. Note: In cases where the free rat did not open the restrainer door, researcher opened the door half-way at the 25-minute mark.

IV. Discussion

i. Interpretation of Results

Rats are highly motivated to help others in distress even when a cost is present. While the introduction of a cost decreased helping behavior, the reduction in opening the restrainer door to release a trapped cagemate was not significant when a cost was present4, suggesting an associated cost only mildly hinders helping behavior but fails to override the motivation to release a trapped cagemate. Rats demonstrated an aversion to the cold-water barrier as evidenced by the drastic decrease in door-opening when the restrainer was empty as compared to when the restrainer contained a trapped cagemate. This finding indicates that the distress of another is a salient motivator for costly helping behavior.

When tested in a modified T-Maze to gauge the degree to which rats weigh associated costs and benefits when exposed to approach-avoidance conflicts, rats preferentially engaged in high-risk, high reward options over low-risk, low-reward options (Friedman et al., 2015). Crossing the cold-water barrier likely provided a higher-risk condition that inhibited opening the door to an empty restrainer, potentially considered a reward of low value. However, releasing a trapped cagemate may be considered a reward of high value, thus prompting engagement in high-risk behavior.

The frequency of door-opening for an empty restrainer was initially high when it was not coupled with a cost; however, the rate dropped off as trials progressed. Conversely, free rats continued to open the door to release a trapped cagemate until trials were discontinued, independent of the presence of the cost. Rats may enjoy the motor mastery associated with opening the restrainer door (Bartal et al., 2016); however, it is not enough to sustain motivation to continue the behavior, but the act of releasing a trapped cagemate may provide enough of a reward to promote continued engagement in the behavior.

Rats introduced to an arena with a trapped cagemate spent significantly less time in the escape area before door-opening compared to those where the restrainer was empty. Exposure to brightly lit open spaces can be anxiety-provoking in a nocturnal prey species like the rat and could motivate individuals to seek refuge (La-Vu, Tobias, Schuette, & Adhikari, 2020). Thus, free rats may have been motivated to pursue a safe alternative to mollify anxiety induced by the brightly lit open space of the experimental arena when the restrainer was empty. However, the presence of a distressed cagemate prompted a level of sympathic concern or empathic perspective-taking that overcame any experienced personal distress that would motivate self-directed behavior.

Consistent with previous findings, when a cagemate was trapped in the restrainer, free rats showed increased activity levels as compared to when the restrainer was empty (Bartal et al., 2014). Increased activity levels are suggestive of heightened affective arousal, a necessary component motivating helping behavior (Bartal et al., 2016), as a shared emotional state between witness and demonstrator provides the foundation for an empathetic response (de Waal, 2008; Preston & de Waal, 2002a).

ii. Addressing Alternative Explanations

1. Pre-Trial Training

The incorporation of the pre-trial training protocol presents a possible explanation for the door-opening demonstrated in our study. While rats learned to open a restrainer door without prior training in previous studies (Bartal et al., 2011, 2014, 2016), most rats (11 of 14) did not learn to open the restrainer door used in this study during pilot experiments. Although the restrainer door was designed based on the configurations from Bartal et al. (2011, supplemental methods), the rats used in the study demonstrated difficulty in learning how to open the door. Anecdotally, many of the rats that did not open the door for a trapped cagemate moved frantically around the restrainer, biting at different parts and spent the majority of the trials in close proximity to the restrainer, despite having access to an escape area.

Because in this paradigm door-opening is indicative of helping behavior, and thus empathy, when free rats fail to open the door for their trapped cagemates it suggests a lack of empathy. However, when the measured behavior lies outside the typical behavioral repertoire of the study subject, training individuals prior to experimentation may be necessary to ensure knowledge of required skillset (Blystad et al., 2019; Tomek et al., 2019).

It cannot be discounted that the reinforcement, in the form of a food reward offered when the restrainer door was opened during the training period, potentially carried over through the experiment and consequently underlies the rate of occurrence during trials. However, we would then expect to see no difference in door-opening whether the restrainer was empty or contained a trapped cagemate, not the significant difference exhibited. It is possible that the reinforcement conferred during training maintained door-opening for the first couple trials, but as trials progressed, door-opening significantly declined when the restrainer was empty but remained consistently high when a cagemate was trapped. It is possible that the food reinforcement from the training period was replaced by the social reinforcement when a free rat opened the door to a trapped cagemate. The social contact allowed by the releasing of a trapped cagemate could have been equally or more rewarding than the initial food reinforcement and allowed for continuance of the door-opening behavior; thus, future studies could incorporate an adjacent release area, similar to the one used in Bartal et al. (2011), that inhibits social contact following release.

2. Pursuit of Social Contact

Pursuit of social contact is often offered as an alternative explanation for door-opening behavior in rodent empathy studies utilizing the HBT (Hiura et al., 2018; Schwartz et al., 2017; Silberberg et al., 2014)) and may be suggested as the driving force behind the door-opening seen in this study. While others have offered evidence to counter this claim (Bartal et al., 2011, 2016; Cox et al., 2020; Sato et al., 2015), Vieira Sugano et al. (2022) have recently provided the most compelling evidence to oppose this argument. When compared to the control group, dooropening was significantly lower when free rats were tested with trapped rats treated with metyrapone, a drug that inhibits the production of corticosterone, and thus displayed no signs of distress. This suggests pursuit of social contact does not provide sufficient motivation for door-opening and a shared affective state may be an important element provoking helping behavior. However, door-opening was consistently high when free rats were tested with trapped rats that were anesthetized. Furthermore, after opening the door to an immobilized rat, many free rats subsequently pulled the individual out and away from the restrainer (Vieira Sugano et al., 2022). Despite lacking an affective state suggestive of experienced danger, the presence of an unconscious rat may be in and of itself distressing for an observer as it is potentially at odds with expected behavior. Vieira Sugano et al. (2022) suggest the free rats' response is indicative of cognitive dissonance; however, the possibility that the behavior is similar to the corpse management behavior seen in social insects is not discussed. Though, this is unlikely as rats have been shown to discriminate between live and dead conspecifics via olfactory cues (Carr, Landauer, & Sonsino, 1981; Pinel, Gorzalka, & Ladak, 1981).

3. Motivated by Personal Distress

Personal distress is often elicited when bearing witness to another individual in distress. This automatic affective state-matching is considered to be at the core of all empathetic responses (de Waal, 2007, 2008; Preston & de Waal, 2002a). On its own, personal distress provoked by another's distress can elicit other-oriented behavior or self-directed behavior, both intended to alleviate the witness's own negative emotional state (Batson, 1987; Batson, Fultz, & Schoenrade, 1987; Eisenberg et al., 1989). Other-oriented behaviors are expected to arise only when easier self-benefitting options are absent (Batson, 1987; Eisenberg et al., 1989).

If rats find the distress calls of conspecifics unpleasant, releasing a trapped cagemate may serve as a means to stop these vocalizations; thus, door-opening is enacted for pursuit of personal comfort rather than concern for another (Blystad, 2021; Hiura et al., 2018; Lavery & Foley, 1963; Preobrazhenskaya and Simonov, 1974; Silberberg et al., 2013). The addition of the escape area provided an opportunity to determine whether personal distress or a more complex empathetic response motivated the helping behavior demonstrated in this study. If door-opening was executed to alleviate personal distress by eliminating the aversive distress cues of the trapped cagemate, fleeing to the escape area where these distress cues are greatly diminished provides a quicker and simpler way to achieve this goal, especially when a cost is present. Conversely, rats tested with trapped cagemates exploited the escape area significantly less than those tested with an empty restrainer which indicates motivations for releasing a trapped cagemate went beyond what was necessary to relieve personal distress and suggests concern for another as a motivation to elicit behavior.

Emotional regulation is often considered a necessary component of more complex forms of empathy, such as sympathic concern and empathic perspective-taking, where a witness to distress has a dampened emotional response compared to that of the demonstrator (de Waal, 2008; Perez-Manrique & Gomila, 2018). This blunted emotional response may be necessary as an overly heightened state of anxiety may inhibit the success of helpful acts (Bartal et al., 2016). However, since all empathetic responses are built upon shared emotions between witness and demonstrator, any behavior aimed at diminishing the distress of another can be seen as at least partially motivated for self as the result will ultimately improve the affective state of both parties (Preston & de Waal, 2002b).

iii. Application of Research & Future Directions

While the behaviors and neuroendocrine mechanisms involved in an empathetic response are receiving attention, the communicative antecedents to helping behavior remain scarcely investigated (Blystad, 2019). Understanding the communication between individuals prior to the dispense of aid may provide further insights into the contextual appraisal that precedes its performance. The ultrasonic vocalizations (USVs) of rats are used to infer affective state as calls can be categorized by frequency based on the contexts and neural recruitment associated with calls (ie. Burgdorf et al., 2000, 2001, 2008; Blanchard et al., 1991; Knutson et al., 2002; Panksepp & Burgdorf, 2003; Brudzynski, 2013; Wintik & Brudzynski, 2001). Rats emit 22kHz calls, ranging from 18-32 kHz, when in distress and are suggestive of a negative affective state, while 50kHz calls, ranging from 35-70 kHz, are emitted in anticipation of and while receiving a reward and are suggestive of a positive affective state (Burgdorf, Knutson, & Panksepp, 2000; Knutson, Burgdorf, & Panksepp, 2002; Litvin et al., 2007; Panksepp & Burgdorf, 2000). When exposed to playbacks of 22 kHz and 50 kHz USVs of conspecifics, rats demonstrate affective and physiological state-matching reflective of the caller (L. Kaufmann et al., 2022; Sadananda et al., 2008; Saito, Yuki, Seki, Kagawa, & Okanoya, 2016).

However, to date, only three studies on rodent helping behavior have analyzed USVs, finding trapped rats emit distress calls prior to release (Bartal et al., 2011), trapped rats produce significantly more distress calls when help from their partner was pharmacologically reduced (Cox et al., 2022), and hungry rats emit 50 kHz call to induce food sharing in social partners (N. I. Paulsson & Taborsky, 2022). Together these results indicate the importance of USVs in eliciting aid from observers.

iv. Concluding Remarks

There is an abundance of anecdotal evidence documenting animals providing costly helping behavior (cetaceans: (Caldwell & Caldwell, 1966; Kuczaj et al., 2015; Lilly, 1963); elephants: (Bates et al., 2008; Douglas-Hamilton et al., 2006; Hart, Hart, & Pinter-Wollman, 2008); great apes: (Goodall, 1990; Preston & de Waal, 2002a)); however, controlled studies investigating this phenomenon in large-brained mammals are constrained by ethical limitations, making rodents a viable study system. Exploring both the proximate and ultimate mechanisms supporting helping behavior should include the effects of cost on its expression as providing aid to others typically involves associated costs (Eisenberg & Miller, 1987; Fehr & Fischbacher, 2003)and the presence of a cost may hinder helping behavior when the risk is deemed too high (Batson et al., 1983; Shotland & Stubbins, 1983). Determining the contexts and antecedents promoting engagement in costly helping behavior and the neuroendocrine mechanisms facilitating such behavior will enhance our understanding of empathy, especially as costly helping behavior is unlikely to exist without an emotional investment in another's welfare (de Waal, 2008). Rodent empathy studies employing the HBT will continue to provide opportunities to investigate lingering unknowns surrounding empathy on both the behavioral and cellular levels.

CHAPTER IV

THE COMMUNICATIVE ANTECEDENTS TO HELPING BEHAVIOR IN RATS

I. Introduction

The social complexity hypothesis for communicative complexity predicts animals that interact with many different conspecifics, particularly those of varying age and rank, require a social repertoire that includes assorted signals to maintain relationships of varying degrees and transfer information regarding the physical environment (Freeberg, Dunbar, & Ord, 2012). As interactions with others increases, the transfer of socially acquired information also increases, thus natural selection should favor individuals readily able to understand the emotions of others and garner information from observed affective states. The communicative antecedents of an empathic response contribute to the strength of the emotional reaction and may determine the subsequent behaviors of observers.

When studied in other animals, empathy is often tested by measuring how one responds to the distress or pain of another (ie. Church, 1959; Bartal et al., 2011, 2014, 2016, 2021; Breton et al., 2022; Sato et al., 2015; Burkett et al., 2016; Cox et al., 2020, 2022; Lu et al., 2018; Li et al., 2018; Plotnik et al., 2014; Douglas-Hamilton et al., 2006). As ethical regulations have mostly constrained inquiries into the empathic abilities of larger-brained mammals to observational studies (ie. Plotnik & de Waal, 2014; Douglas-Hamilton et al., 2006; Lily, 1963; Kuczaj et al., 2015; Cheng et al., 2018; Clay & de

Waal, 2013), rodent studies allow for investigations into empathy where socio-environmental contexts and neural manipulations can be tested for their influence on the expression of empathy and associated behaviors (Ben-Ami Bartal et al., 2014; Burkett et al., 2016; Havlik et al., 2020; Schneeberger et al., 2012; Yamagishi, Lee, et al., 2020; Yamagishi, Okada, et al., 2020).

The helping behavior test (HBT), first introduced by Bartal et al. (2011), and others of similar design (ie. Cox et al., 2020; Sato et al., 2015; Yamagishi et al., 2020) have been utilized to determine if rats display concern for others and demonstrate a primitive form of empathic perspective-taking via the performance of targeted helping behavior. Rats repeatedly demonstrate conduct suggestive of targeted helping by working to release a conspecific from distressing conditions where the subsequent helping behavior is dependent on the affective state of the observer (Bartal et al., 2016) and the demonstrator (Vieira Sugano et al., 2022), the age of observer (Breton et al., 2022), in group membership of demonstrator (Bartal et al., 2014; Breton et al., 2022), presence of bystanders (Havlik et al., 2020), and the observer's prior experience with the stressor (Sato et al., 2015). The flexibility seen in rodent helping behavior can be manipulated and utilized when exploring the antecedents of empathic responses to further elucidate the factors that contribute to prosocial behavior.

Despite a growing number of studies reporting on helping behavior in rats, the antecedents to helping behavior and the communicative factors that facilitate its expression remain scarcely investigated in the rodent empathy model (Blystad, 2019). Given the extensive literature on the vocal communication of rats, audio recordings can be incorporated into rat empathy studies to determine the communicative factors that facilitate successful bouts of targeted helping behavior. Ultrasonic vocalizations (USVs) emitted by rats are reliably produced in response to specific contexts, correlated to distinct brain region activation, and can be used to make inferences regarding the affective state of the caller (Burgdorf et al., 2008; Brudzynski, 2013; Knutson et al., 2002).

Two main categorizes of ultrasonic vocalizations are recognized in juvenile and adult rats: 22 kHz USVs and 50 kHz USVs.22 kHz USVs include vocalizations ranging from 18-32 kHz and are emitted in response to stress and pain (Ben-Ami Bartal et al., 2011; Blanchard et al., 1992; Blanchard et al., 1991; Calvino et al., 1996; Litvin et al., 2007; Tonoue, Ashida, Makino, & Hata, 1986) and are indicative of a negative emotional state (Brudzynski, 2007, 2009, 2014; Jelen, Soltysik, & Zagrodzka, 2003). 22 kHz USVs are often categorized as alarm calls in the literature (Blanchard et al., 1991; Karwicka et al., 2021; Litvin et al., 2007); however, a fear conditioned experiment demonstrated rats emitted 22 kHz USVs at similar rates when alone as when in the presence of either anesthetized or active conspecifics suggesting 22 kHz USVs are not emitted with intent to inform colony members of impending danger and may be better described as distress calls than alarm calls (Wöhr & Schwarting, 2008b). 50 kHz USVs are vocalizations ranging from 35-70 kHz and are emitted in anticipation of or while experiencing both social and non-social rewards (Burgdorf et al., 2000; Burgdorf, Knutson, Panksepp, & Ikemoto, 2001; Knutson, Burgdorf, & Panksepp, 1998, 1999; Knutson et al., 2002; Panksepp & Burgdorf, 2000). The production of 50 kHz USVs is suggestive of a positive emotional state analogous to excitement (Knutson et al., 1999) and may be a primitive form of human laughter (Panksepp & Burgdorf, 2000); thus, ‘positive calls’ and 50 kHz USVs will be used interchangeably henceforth.

To better understand the role of rewards in facilitating and maintaining helping behavior, the current study measured 50 kHz USVs emitted before and after the opening of a restrainer door when the restrainer was empty and when it contained a cagemate. We anticipated significantly more 50 kHz USVs would be produced when free rats opened the door to release a trapped cagemate than when free rats opened the door to an empty restrainer suggesting helping others is a rewarding event for rats.

When a cagemate was trapped in the restrainer, we anticipated that significantly more 22 kHz USVs would be produced than when the restrainer was empty. Furthermore, we expected there to be a positive correlation between 22 kHz call rate and door-opening. We expected all trapped rats to be similarly distressed by confinement in the restrainer; however, 22 kHz call rates were used as a proxy to measure the trapped rats’ distress levels and could be compared to determine if interpersonal variation in distress levels existed. If trapped rats did display differences in stress levels, we would expect rats with higher 22 kHz call rates to receive help more frequently and at shorter latencies than those with lower 22 kHz call rates.

To explore whether personal distress or concern for the welfare of another motivates helping behavior, an escape area where a free rat could find reprieve from the stress associated with a brightly lit open space and the distress cues of a trapped cagemate was connected to the experimental arena via an easily accessed tunnel. Time spent in the escape area prior to door-opening was analyzed to determine if a relationship between the rate of 22 kHz USVs and the time spent in the escape area existed. We predicted time spent in the escape area would have no correlation with 22 kHz call rate suggesting rats would forgo opportunities to relieve their own personal distress to open the restrainer door, but only when the restrainer contained a trapped cagemate.

II. Methods

i. Animals

Seventy adult female Sprague-Dawley rats, aged 63-70 days old, were procured from Charles River (Portage, MI). Upon arrival, rats were housed in pairs. Rats were kept on a 12-hour light/dark schedule and had ad libitum access to food and water. Their use in the experiment detailed below was approved by the Institutional Animal Care and Use Committee at the University of Louisville.

ii. Restrainer

The Plexiglas rodent restrainer (25cm x 8.75cm x 7.5cm, Harvard Apparatus, was outfitted with a modified door that could only be opened from the outside. The restrainer door had two panels, one panel reached the bottom of the restrainer preventing the trapped rat from opening the door, and the second was slightly shorter which allowed for free rats to prod with their snouts to knock the door up and open. The shaft of the restrainer has several openings facilitating communication between trapped and free rats.

iii. Experimental Arena

Trials were conducted in a 150cm x 50 cm clear Plexiglas arena that was lifted 5 cm off the ground by six steel legs. (**Figure 4.1**). The rodent restrainer was placed at one end of the arena. The other end of the arena provided access to a 3-foot Plexiglas tunnel that led to into an escape area. The escape area was a 50cm x 50cm black Plexiglas box with a blackout curtain draped over the top. In the escape area, visual distress cues given of the trapped cagemate were entirely blocked while auditory distress cues were diminished. The arena, restrainer, tunnel, and escape area were cleaned with Peroxiguard after every trial.

iv. Handling & Boldness Measures

Upon arrival, rats were housed in pairs, and were given 14 days to acclimate to their new home cage and become familiar with their cagemate. Starting on day 15, pairs were assessed for boldness each day for three days. To measure boldness within a pair, the researcher opened the cage lid halfway and timed the latency until each rat lifted their upper body and placed paws on edge of cage. Latencies were averaged across the three days and the individual with the shorter average latency was assigned the role as the free rat and the individual with the longer average latency was assigned the role as the trapped rat (Bartal et al., 2011). After each boldness measure and for an additional seven days after, rats were handled for 15 minutes a day to reduce handler anxiety. During handling sessions, each rat was picked up from cage and set in researcher's lap where it was gently stroked and lifted from lap and placed back down ten times. Following handling sessions, pairs of rats were released into the experimental arena for 30 minutes.

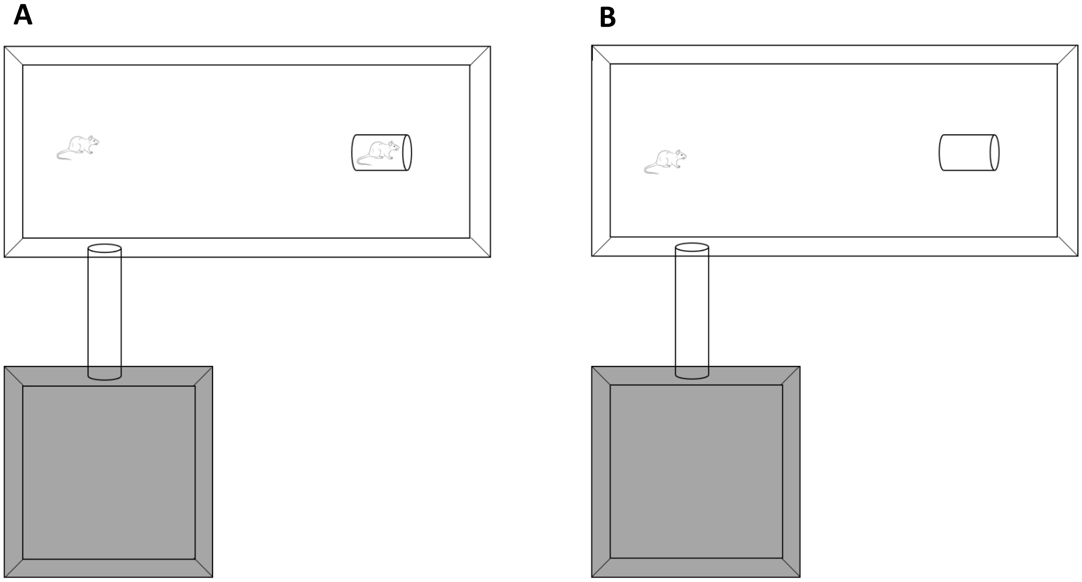

Figure 4.1 Experimental arena for treatments: free rat introduced to experimental arena where (A) their cagemate is trapped in the restrainer, or (B) the restrainer is empty. Restrainer is placed in the middle of the experimental arena prior to trial commencing. Free rats have access to dark escape area where distress cues of the cagemate are diminished.

v. Experimental Trials

Free rats were tested in one of the two treatments (n = 23):

(1) Cagemate treatment: the free rat's cagemate was trapped in the restrainer.

(2) Empty treatment: the restrainer was empty.

At the beginning of each trial, the free rat was placed at the end of the arena opposite of the restrainer. Each trial lasted 40 minutes and each free rat underwent six trials. If the free rat did not open the door by the 25th minute, the door was opened halfway (manually), allowing trapped rat to open door fully, to reduce learned helplessness in trapped rat (Seligman, 1972; Seligman and Beagley, 1975), but this was also done in empty restrainer conditions.

Trials were video recorded using a Canon z200 and activity levels were measured as cm/min using the animal tracking software ANY-maze (Stoelting, Wood Dale, IL, USA). Trials were audio recorded on a M500-384 ultrasonic microphone from Pettersson Elektronik (Uppsala, Sweden) using Audacity, an open-source audio recording software. Ultrasonic calls were detected using DeepSqueak, a high frequency detection and analysis software (Coffey et al., 2019).

vi. Ultrasonic Vocalizations

Ultrasonic vocalizations (USVs) were divided into two categories: 22 kHz USVs and 50 kHz USVs. 22 kHz USVs are vocalizations ranging in frequency from 18 to 32 kHz (Brudzynski, 2014; Calvino et al., 1996; Tonoue et al., 1986)) and 50 kHz USVs are vocalizations ranging in frequency from 35 and 80 kHz (Knutson et al., 1998; 1999, 2000; Panksepp & Burgdorf, 2000).

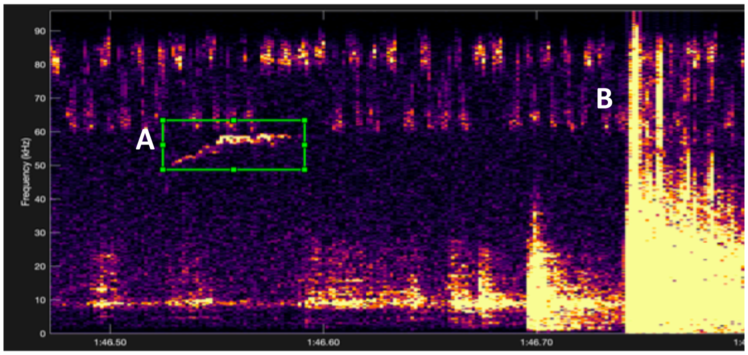

Figure 4.2 An example spectrogram from a cagemate trial. (A) Green box outlines an example of a 50 kHz USV. (B) Notes opening of restrainer door and the door falling to ground. Together this depicts a 50 kHz USV emitted milliseconds before a free rat opened the door to a restrainer containing a trapped cagemate.

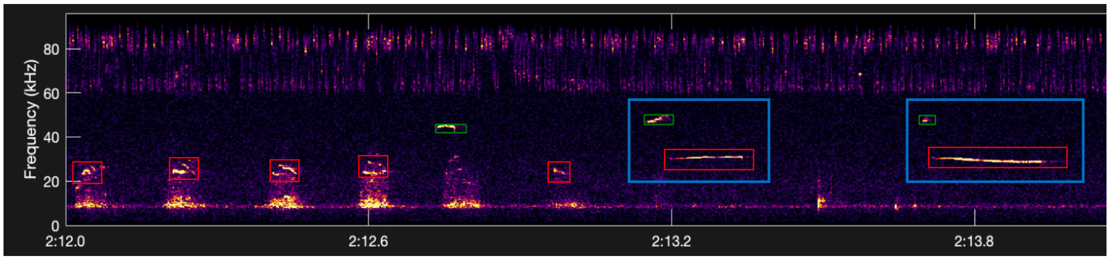

Figure 4.3 An example spectrogram with 22 kHz USVs and 50 kHz USVs emitted over 1.8 seconds. This abbreviated spectrogram is from a cagemate trial before the free rat opened the restrainer door to release their trapped cagemate. Red boxes outline 22kHz USVs and green boxes outline 50 kHz USVs. Blue boxes outline instances of vocal overlaps suggesting these 50 kHz USVs and 22 kHz USVs can be attributed to different sources.

vii. Statistical Analyses

All analyses were conducted in the open-source R software environment (R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. https://www.r-project.org/).

Linear mixed effects models were fit to evaluate 50 kHz call rates and 22 kHz call rates before and after door opening. The fixed effects in these models were a binary factor defining the presence of a cagemate, a binary factor defining before or after door opening, and their interaction. Logistic mixed effects models were used to examine the relationship between door opening, as the binary outcome, and call rates. The fixed effects were (1) call rate, for either 50 kHz USVs or 22 kHz USVs, (2) a binary factor defining the presence of a cagemate, and (3) their interaction. Random intercepts per rat were defined as the random effect for all models. The correlation between 22 kHz call rate and percent time spent in the escape area (ER) was calculated according to a nonparametric coefficient for clustered data (Lorenz et al., 2011).

III. Results

To determine if positive call rates (50kHz calls/min) varied in response to door-opening and the presence of a cagemate, positive call rates were compared before and after door-opening when the restrainer was empty and when the restrainer contained a cagemate (**Figure 4.4**). Overall, positive call rates were significantly higher before door-opening than after door-opening ($F = 23.4$, $p < 0.001$). This difference was likely driven by the significant difference in call rates between the cagemate group and the empty group ($F = 86.3$, $p < 0.001$). When a cagemate was trapped in the restrainer, positive call rates before door-opening ($F = 22.7$, $p < 0.001$) and after door-opening were significantly greater in the cagemate treatment than in the empty treatment ($F = 14.5$, $p < 0.001$).

The relationship between 50 kHz call rates before door-opening and door-opening frequencies was examined (**Figure 4.5**). Overall, 50 kHz call rates were positively associated with door-opening, as a one-unit increase in the 50 kHz call rate was associated with an increase by a factor of 1.23 (1.10, 1.38) in the odds of the free rat opening the door. When a cagemate was trapped in the restrainer, 50 kHz call rates were positively associated with door-opening where one unit increase in 50 kHz call rate was associated with an increase by a factor of 1.15 (1.03, 1.28) in the odds of the free rat opening the door. When the restrainer was empty, a relationship between 50 kHz call rates and door-opening was not detected (1.67 (0.95, 2.93)), in part a function of the low 50 kHz call rates among rats that did not open the door. The association between 50 kHz call rate and door-opening was not significantly different for the cagemate treatment and the empty treatment ($X^2= 2.44$, $p = 0.12$).

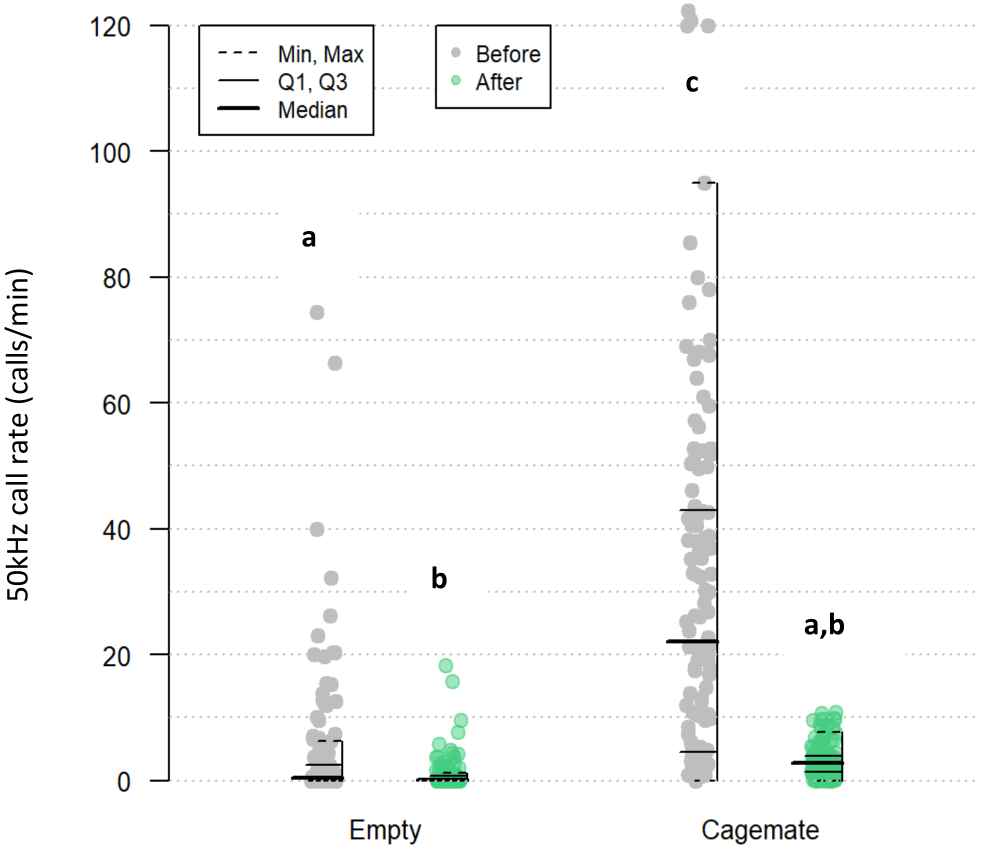

Figure 4.4 50 kHz call rates (calls/min) before and after door-opening for Empty and Cagemate treatments. Green circles represent 50 kHz call rates before door-opening and gray circles represent 50 kHz call rats after door-opening. Clusters labeled with different letters are statistically different from one another at the $p<0.01$ level. Note: In trials where the free rat did not open the door, researcher opened door halfway at the 25th minute, in the cagemate treatment this allowed the trapped rat to free themselves.

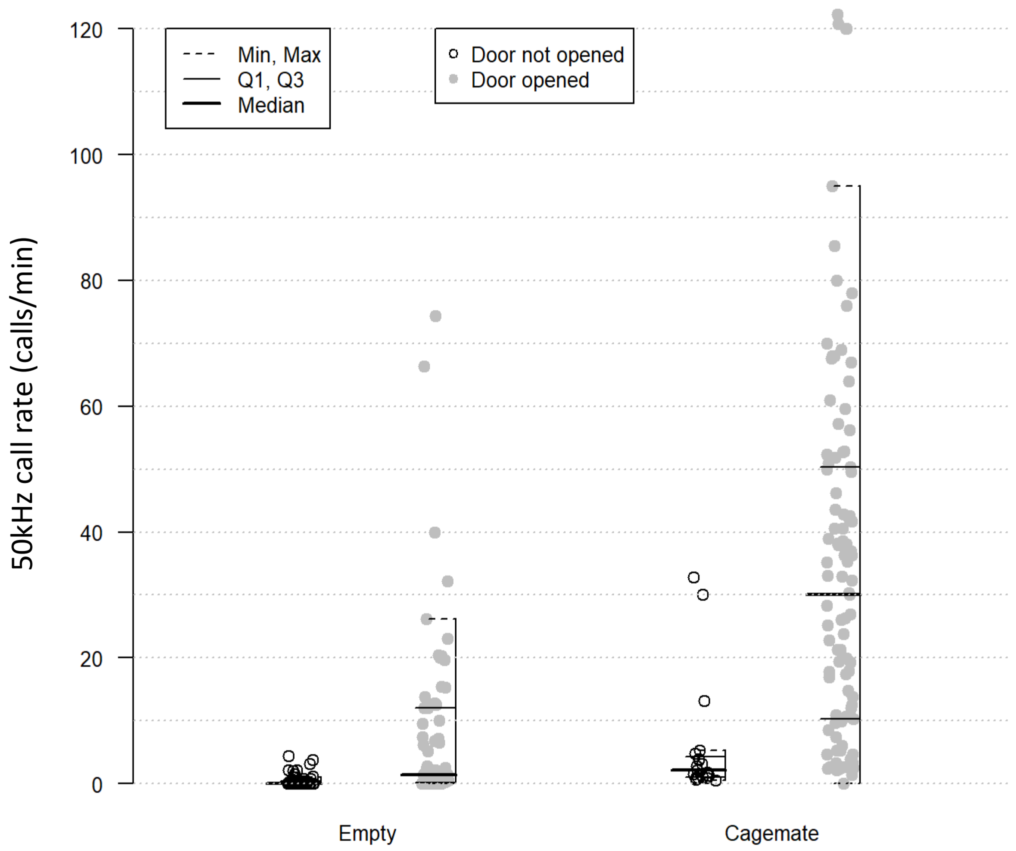

Figure 4.5 Scatterplot depicting 50 kHz call rates (calls/min) for the empty treatment and the cagemate treatment. Gray circles represent trials where free rats opened the restrainer door, and open circles represent trials where the free rat did not open the restrainer door.

To determine if 22 kHz call rates (calls/min) varied in response to door-opening and confinement of a cagemate, 22 kHz call rates were compared before and after door-opening when the restrainer was empty and when the restrainer contained a cagemate (**Figure 4.6**). Overall, 22 kHz call rates were significantly higher before door-opening than after door-opening ($F = 175.4$ $p < 0.001$). This difference was likely driven by the significant difference between the cagemate treatment and the empty treatment ($F = 281.9$, $p < 0.001$). When a cagemate was trapped in the restrainer, 22 kHz call rates before door-opening were significantly greater than when the restrainer was empty ($F = 93.58$, $p < 0.001$). 22 kHz call rates after door-opening were statistically similar when a cagemate was trapped in the restrainer and the restrainer was empty ($F = 3.54$, $p = 0.065$).

The relationship between 22 kHz call rates before door-opening and door-opening frequencies was examined (**Figure 4.7**). Overall, 22 kHz call rates were positively associated with door-opening, as a one-unit increase in the 22 kHz call rate corresponded with an increase in the odds of door opening by a factor of 1.07 (1.02, 1.11). When a cagemate was trapped in the restrainer, 22 kHz call rates were positive associated with door-opening where one unit increase in the 22 kHz call rate was associated with an increase in the odds of the free rat opening the door by a factor of 1.05 (1.01, 1.09). When the restrainer was empty, the odds ratio was poorly estimated due to the low magnitude and minimal variation in the distress calls from rats that did not open the door, 24.8 (1.54, 399.5).

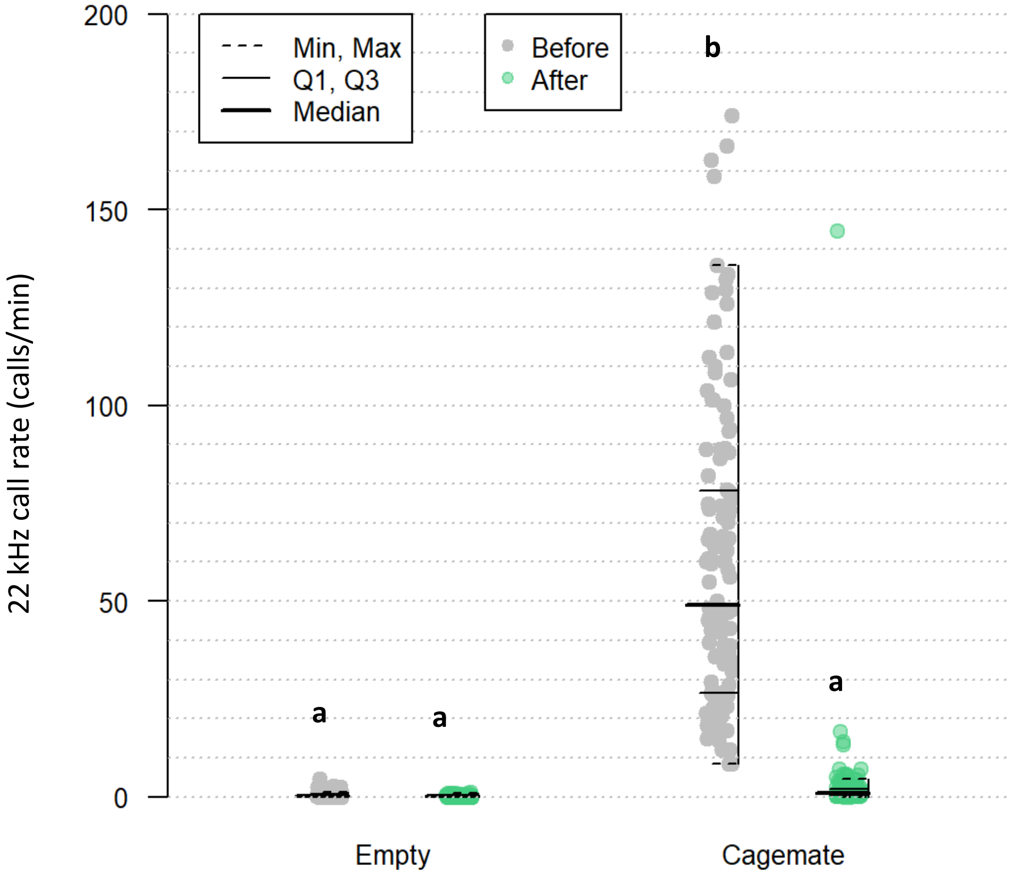

Figure 4.6 22 kHz call rates (calls/min) before and after door-opening for Empty and Cagemate treatments. Green circles represent distress call rates before door-opening and gray circles represent distress call rats after door-opening. Clusters labeled with different letters are statistically different from one another at the p<0.01 level. Note: In trials where the free rat did not open the door, researcher opened door halfway at the 25th minute, in the cagemate treatment this allowed the trapped rat to free themselves.

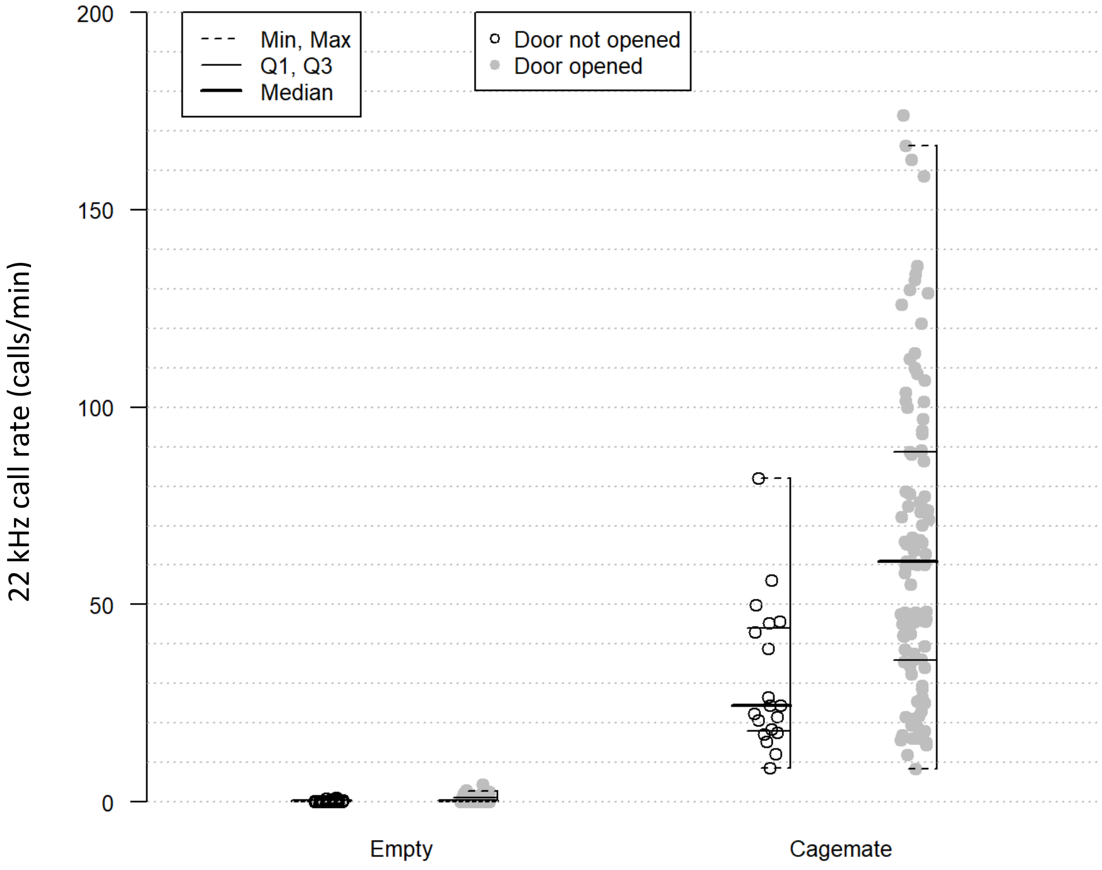

Figure 4.7 Scatterplot depicting 22 kHz call rates (calls/min) before door-opening for the empty treatment and the cagemate treatment. Gray circles represent trials where free rats opened the restrainer door, and open circles represent trials where the free rat did not open the door. Note: In trials where the free rat did not open the door, researcher opened door halfway at the 25th minute, in the cagemate treatment this allowed the trapped rat to free themselves.

22 kHz call rate and percent time spent in the escape area (ER) prior to door-opening were negatively correlated over all (clustered Spearman correlation = -0.47 (-0.58, -0.35)). This was largely a function of rats in the empty treatment spending more time in the ER and giving minimal 22 kHz calls. Due to the low rate of 22 kHz calls among free rats in the empty treatment, an analysis focused on free rats in the cagemate treatment was conducted. No correlation between 22 kHz calls and time spent in the ER for rats in the cagemate treatment was found (Spearman correlation coefficient = -0.16 (-0.37, 0.06)).

Considering 22 kHz USVs were relatively rare in the empty condition and after door-opening in the cagemate condition, and 50 kHz call rates were significantly greater in the cagemate treatment as compared to the empty treatment and before door-opening as compared to after door-opening, only 22 kHz call rates and 50 kHz call rates before door-opening in the cagemate treatment are considered in remaining analyses. Neither 22 kHz call rates (**Figure 4.9**) (slope = 2.28, $p > 0.15$) nor 50 kHz calls rates (**Figure 4.10**) (slope = 1.148, $p > .29$) differed significantly across trials.

Prior to the door being opened, the 22 kHz call rate was significantly greater than the 50 kHz call rate when a cagemate was trapped in the restrainer, with an average of 30.0 (14.1, 46.0) more calls per minute ($p < 0.001$) (**Figure 4.11**).

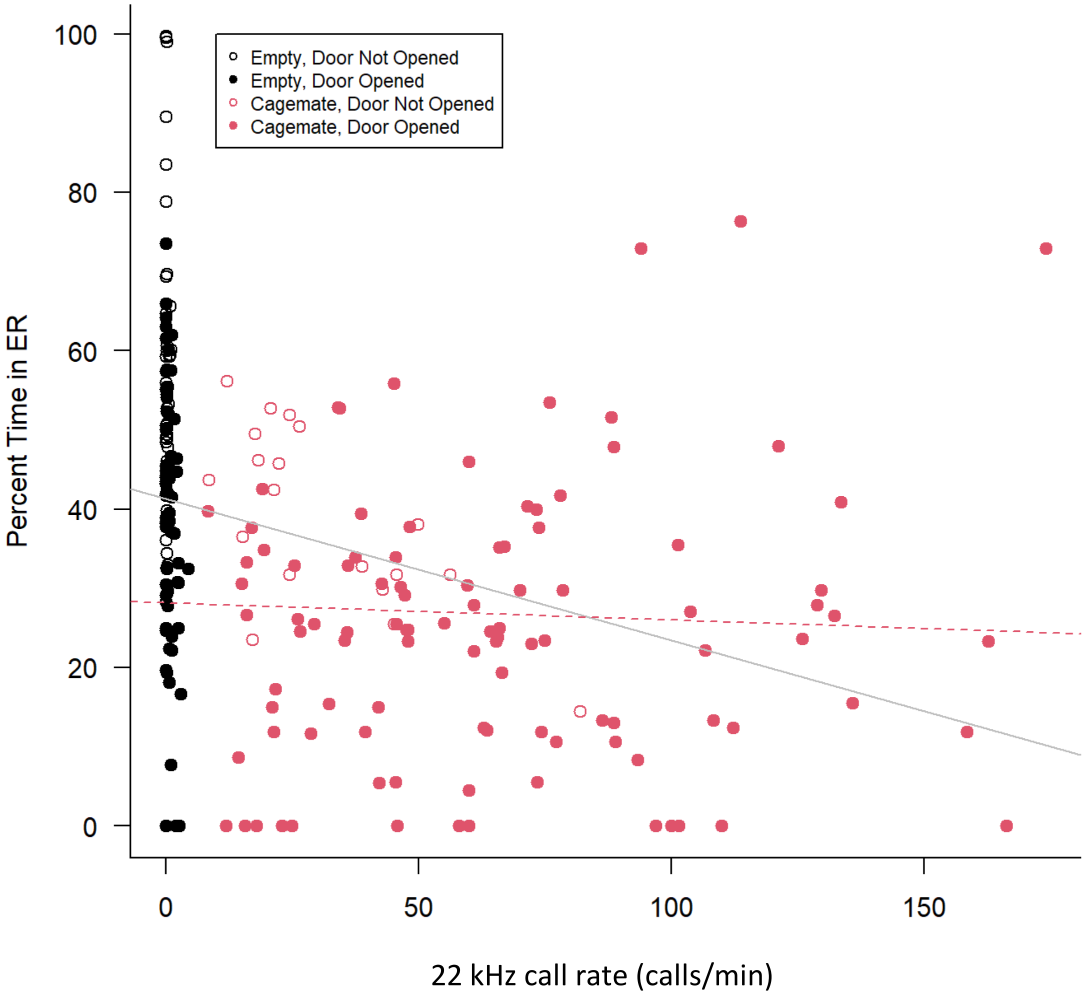

Figure 4.8 Solid black line represents the line of best fit for all observations. The dashed red line represents the line of best fit for cagemate-only where no correlation is found between 22 kHz call rate and proportion of time spent in escape area (ER) before door-opening.

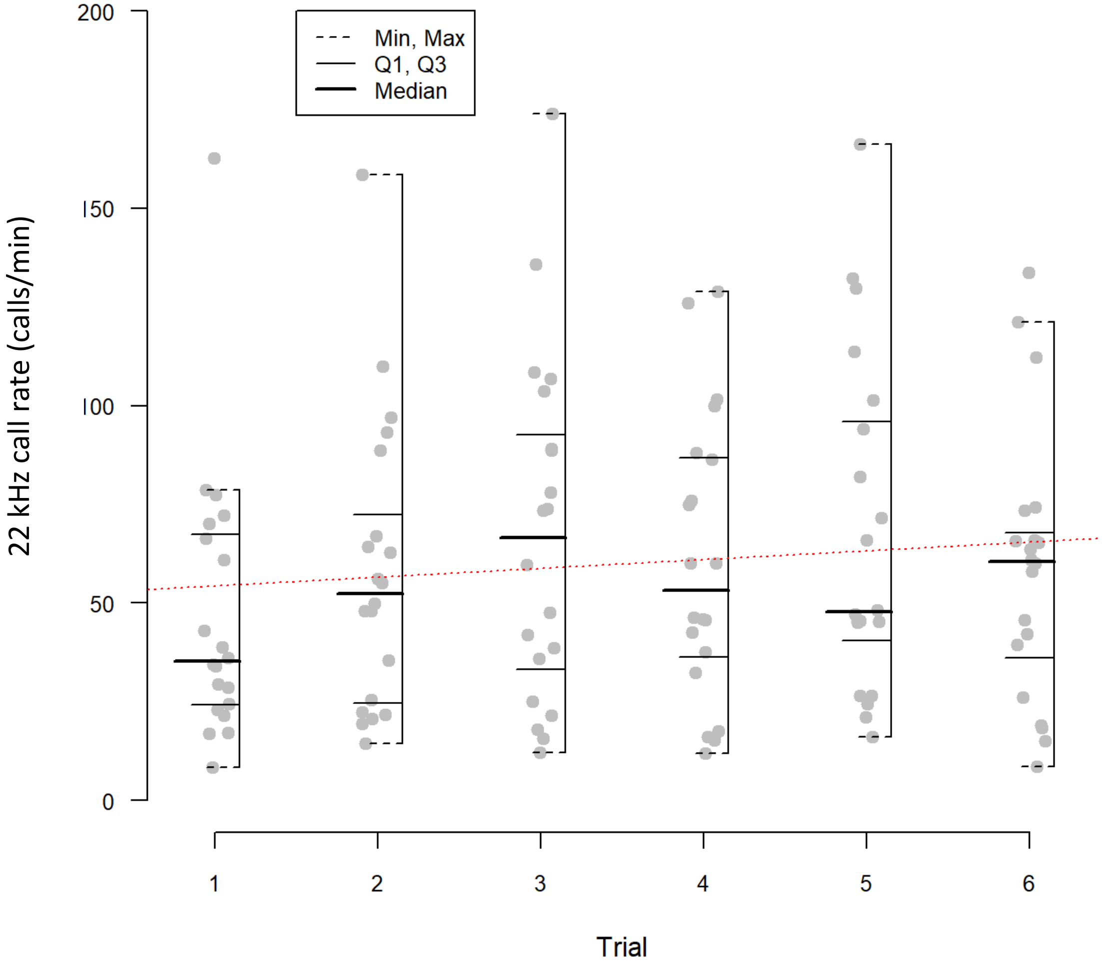

Figure 4.9 Scatterplot of 22 kHz call rates (calls/min) prior to door-opening in the cagemate treatment across all six trials. Red dotted line indicates line of best fit (slope = 2.28). 22 kHz call rates did not differ significantly between trials.

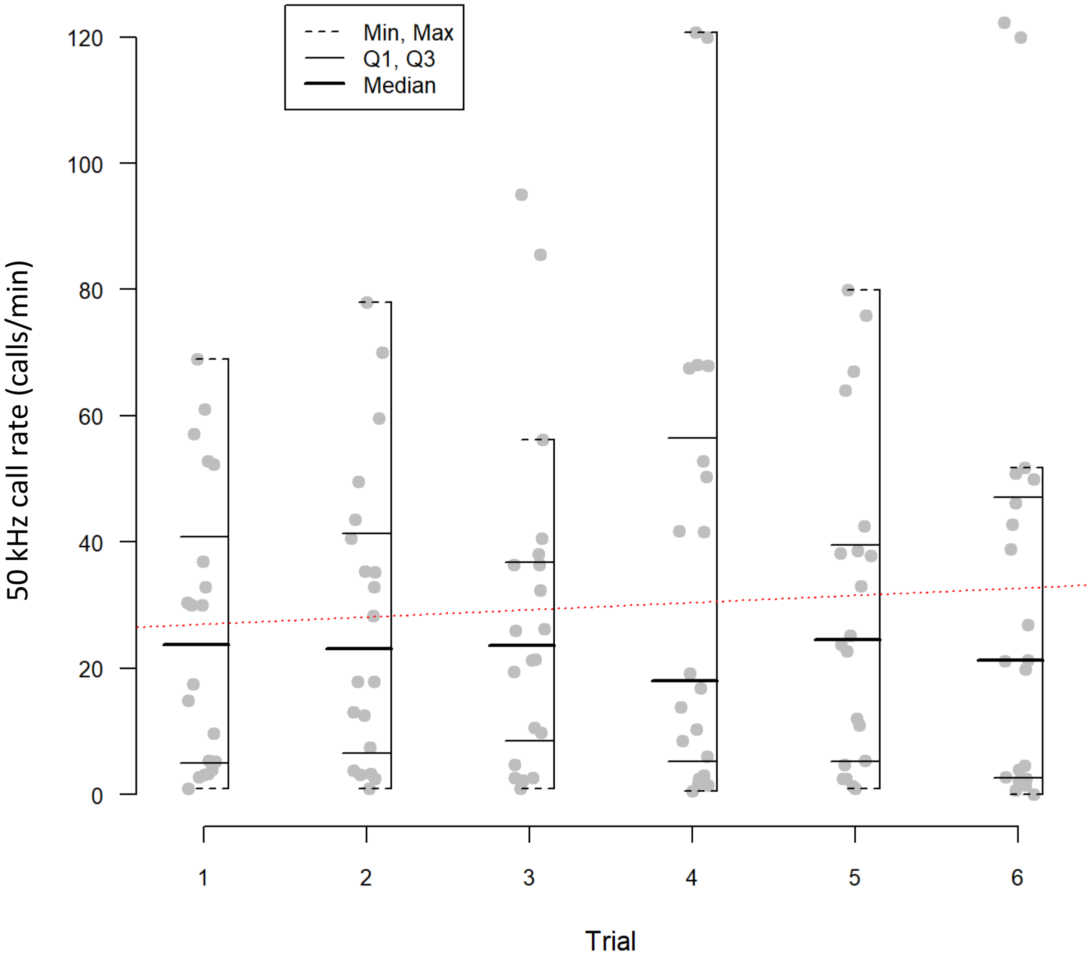

Figure 4.10 Scatterplot of 50 kHz call rates (calls/min) prior to door-opening in the cagemate treatment across all six trials. Red dotted line indicates line of best fit (slope = 1.148). 50 kHz call rates did not differ significantly between trials.

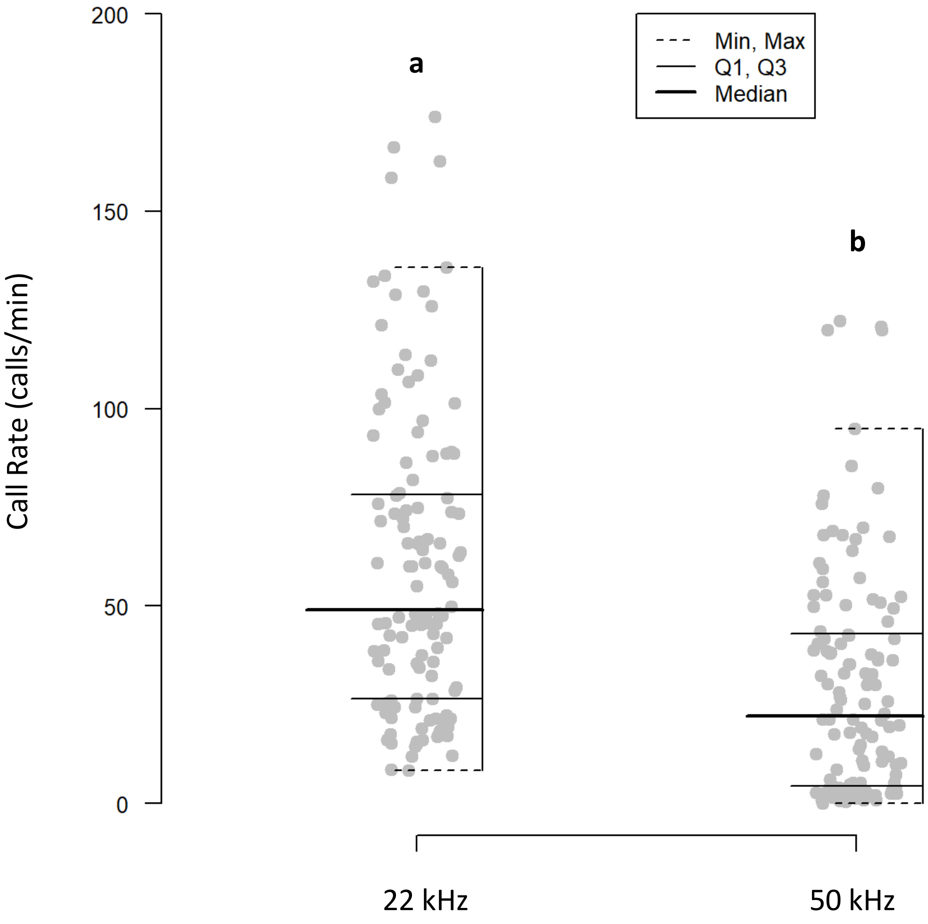

Figure 4.11 Scatterplot of a) 22 kHz call rates and b) 50 kHz call rates (calls/min) before door-opening in the cagemate treatment. Clusters labeled with different letters are statistically different from one another at the $p<0.01$ level.

IV. Discussion

This work demonstrates that both 22 kHz USVs and 50 kHz USVs are emitted prior to release during the helping behavior test (HBT). The communicative antecedents to helping behavior have been scarcely investigated in rodent models of empathy; thus, examining the prevalence of distinct USVs produced prior to providing aid may elucidate motivations and other factors facilitating prosocial action (Blystad, 2019; Hiura et al., 2018). While 22 kHz USVs were emitted at a higher rate than 50 kHz USVs prior to door-opening in the cagemate treatment, suggesting the distress of the trapped cagemate was the impetus for door-opening behavior, production of both USV call types suggests communicative complexities related to prosocial behavior that warrant further exploration.

22 kHz USVs

Vocalizations indicative of distress were likely initially favored by natural selection as their production enhanced the ability of mothers to respond appropriately to the needs of offspring (MacLean, 1985). 22 kHz USVs are considered the adult counterpart to the 40 kHz USVs of rat pups produced to provoke retrieval behavior in mothers (Hofer & Shair, 1978; Wöhr & Schwarting, 2008a, 2008b). The retention of signals that function to elicit concern in others highlights their role in prompting other-oriented behavior (Blanchard et al., 1991; Brudzynski, 2014). 22 kHz USVs are produced when a rat is under stress or in discomfort, such as after contact with a predator (Blanchard et al., 1991; 1992), in response to acute (Tonoue et al., 1986) and chronic pain (Calvino et al., 1996), and confinement to a restrainer (Bartal et al., 2011) and have recently been recognized as vocal signals homologous to human crying (Brudzynski, 2019).

22 kHz call rates were unsurprisingly almost completely absent from empty treatment trials and showed significant reduction following door-opening in trials from the cagemate treatment suggesting confinement in a restrainer is a distressing event that provokes the production of salient cues intended to elicit helping behavior in others. Furthermore, confinement in the restrainer remained stress-inducing evident by consistent production of 22 kHz USVs throughout all trials. Considering 22 kHz call rates in trials where the free rat released their trapped cagemate were more than double that of trials where the free rat did not open the door potentially indicates that those emitting higher rates of 22 kHz USVs signaled greater distress and thus in return received higher rates of help akin to the increased food-sharing of rats exposed to doubled rates of 50 kHz calls (Paulsson & Taborsky, 2022).

The induction of physiological, behavioral and affective state-matching when observing another in distress is well documented in rats and other animals (Gonzalez-Liencres, Juckel, Tas, Friebe, & Brüne, 2014; Y. Han et al., 2020; L. Kaufmann et al., 2022; Langford et al., 2006; Pérez-Manrique & Gomila, 2022; J. M. Plotnik & de Waal, 2014; Romero et al., 2013) and although Bartal et al. (2011) discerned the trapped rats were the source of the 22 kHz USVs, we cannot rule out the possibility that free rats contributed to the 22 kHz USVs in the cagemate treatment as DeepSqueak does not allow for distinguishing the source of the USVs. If we assume free rats did substantially contributed to the 22 kHz call rate, then the significantly higher rate of 22 kHz USVs in trials where the free rat opened the door to release their cagemate than those where the free rat did not open the door suggests reciprocal vocalizing may facilitate affective state-matching necessary to facilitate helping behavior.

Personal distress provoked when witnessing another in pain or stress can elicit other-oriented behavior with the intention of quelling one’s own subjugation to an aversive stimulus (Eisenberg & Eggum, 2009; Eisenberg et al., 1989). The inclusion of an escape alternative allowed free rats to quickly and easily alleviate their personal distress; thus, if the empathic abilities of rats were limited to emotional contagion, they would likely exploit this escape option when a cagemate was trapped and emitting distress cues. Conversely, rats tested with a trapped cagemate spent significantly less time in the escape area prior to door-opening than those tested with an empty restrainer. Furthermore, 22 kHz call rate and percent time spent in the escape area were negatively correlated further suggesting the distress cues of a trapped cagemate stimulate concern for others promoting helping behavior as opposed to personal distress which would promote escape behavior or freezing.

50 kHz USVs

50 kHz USVs are indicative of a positive affective state analogous to excitement (Knutson et al., 1998; Simola & Brudzynski, 2018) and are emitted in anticipation of or while experiencing a reward, such as before and during social play (Knutson et al., 1998), prior to trained electrical brain stimulation of reward centers (Knutson et al., 2000), and in response to tickling (Panksepp & Burgdorf, 2000).

Despite the greater prevalence of 22 kHz USVs prior to door-opening, the production of 50 kHz USVs prior to door-opening highlight the complexity of the social communication and affective influence preceding helping behavior in rats. To the best of our knowledge, this study is the first to demonstrate the production of 50 kHz USVs by rats subjected to the HBT and provides the foundation to prompt further exploration into the role of these USVs in empathically motivated behavior. 50 kHz USVs were emitted at a significantly higher rate prior to door-opening than after door-opening, especially when the restrainer contained a trapped cagemate. Unlike 22 kHz USVs, 50 kHz USVs are produced across a variety of contexts, both social and non-social, and while they are typically associated with the anticipation of a reward (Brenes Sáenz & Schwarting, 2015; Burgdorf et al., 2000; Knutson et al., 1999) the role of these 50 kHz USVs in this study could be many.

The production of 50 kHz USVs are preceded by increased dopamine activity in the nucleus accumbens (Simola & Brudzynski, 2018) and can be exogenously inhibited via administration of a dopamine antagonist (Wintink & Brudzynski, 2001) demonstrating the link between 50 kHz USVs and the dopaminergic reward system (Brudzynski, 2007). Thus, rats may find releasing a trapped cagemate rewarding, consistent with research suggesting helping others is a rewarding event that promotes positive affect in actors (Alden & Trew, 2013; Nelson, Layous, Cole, & Lyubomirsky, 2016). Considering the role of dopamine in motivating behavior, the emittance of 50 kHz USVs prior to door-opening could signal intent to perform incentivizing behavior (Berridge, 2007); however, we consider alternative functions of the 50 kHz USVs produced prior to door-opening.

Alternative Explanations for production of 50 kHz USVs prior to door-opening

I. 50 kHz USVs emitted by trapped rat:

As the source of USVs cannot be identified, it cannot be ruled out that the trapped rat is contributing to the higher 50 kHz call rate found in the cagemate treatment when compared to the empty treatment. Trapped rats may emit 50 kHz USVs in anticipation of being released; however, in cases where the free rat did not open the door, the researcher entered the experimental room and opened the restrainer door halfway allowing the trapped rat to release themselves, therefore, we would expect the rates of 50 kHz USVs to be the same in cagemate treatments when the free rat opened the door and when the free rat opened the door. Conversely, the median 50 kHz call rate prior to door-opening was almost 15 times greater in trials where the free rat released their trapped cagemate than when door-opening was facilitated by the researcher.

II. 50 kHz USVs facilitate social coordination:

50 kHz calls may aid in social coordination where these USVs are reciprocally emitted to exchange information with others and establish contact (Wöhr, Houx, Schwarting, & Spruijt, 2008). The highly social and intelligent dolphin incorporates vocal communication, opting for whistles and clicks, when coordinating behavior between individuals (Jaakkola, Guarino, Donegan, & King, 2018; Marulanda et al., 2021). Comparable to dolphins relying on acoustic signals as murky waters may impede synchronizing behavior using body language cues, the nocturnal rat has similar visual limitations that likely contributed to the evolution of complex vocalizations to aid in navigating social interactions, especially when cooperation is required to overcome challenges (Schweinfurth, 2020). Trials from the cagemate treatment where the free rat opened the door to release their cagemate had significantly higher rates of 50 kHz USVs than those where the free rat did not open the restrainer door; potentially increased

reciprocating 50 kHz calls produced prior to door-opening served to establish a common goal between the pair and facilitated door-opening by the free rat. This type of vocal communication may be important in promoting prosocial behaviors and potentially provides evidence of a primitive form of empathic perspective-taking (EPT) as EPT has been shown to enhance the accuracy and success of coordinated actions (Dale, Marshall-Pescini, & Range, 2020; Novembre, Mitsopoulos, & Keller, 2019) furthering support of the existence of 'primal empathy' in rodents (Panksepp & Panksepp, 2013).

III. Social buffering via 50 kHz USVs:

Karwicka et al. (2021) recorded the USVs of rat pairs when one witnessed their cagemate endure electric foot shocks and found all trials were marked by high prevalence of 22 kHz calls; however, only trials where the witness was warned of their cagemate's impending shock showed high levels of 50 kHz calls, as well. These 50 kHz calls, particularly those greater than 75 kHz, may be evidence of social buffering. Social buffering functions to soothe distressed individuals and aid in enduring adverse conditions (Cohen & Wills, 1985; Morrison et al., 2021). Playbacks of 50 kHz calls produce positive affect in listener rats (Sadananda et al., 2008) and social buffering has been shown to reduce stress in both male and female rats (Ishiyama & Brecht, 2016; Kiyokawa, Hiroshima, Takeuchi, & Mori, 2014). This interpretation would provide evidence of consolation in rats, where individuals act to provide comfort to another in effort to reduce their distress, suggesting rats possess empathic abilities beyond emotional contagion.

IV. 50 kHz USVs emitted in anticipation of social contact:

50 kHz USVs have been documented in a variety of scenarios preceding and during social interactions: male rats increase their production of 50 kHz calls before introduction to a female (Bialy, Rydz, & Kaczmarek, 2000), during copulation (Thomas & Barfield, 1985) and prior to social interactions (C. J. Burke, Markovina, Pellis, & Euston, 2021); both juveniles and adults initiate bouts of rough and tumble play by emitting 50 kHz calls (C. Burke, Kisko, Pellis, & Euston, 2017; C. Burke, Kisko, Swiftwolfe, Pellis, & Euston, 2017; Himmler, Kisko, Euston, Kolb, & Pellis, 2014), and reciprocal 50 kHz vocalizing between partners may help to maintain affiliative actions and help protect against development of aggression during play (C. Burke, Kisko, Pellis, et al., 2017)thus 50 kHz calls can be produced in anticipation of social encounters or utilized to enhance social coordination between individuals. Although multiple studies have demonstrated pursuit of social contact does not drive helping behavior in rats (Bartal et al., 2011; Cox et al., 2020; Vieira Sugano et al., 2022), we must consider the subsequent social interaction following restrainer release as a possible reinforcing agent. However, if this were the case, we would expect to see similar rates of 50 kHz USVs emitted prior to door-opening in all cagemate trials, independent of who opened the restrainer door. Future work could compare 50 kHz USVs preceding reunions between cagemates after innocuous separation and following reunions after release from restrainer.

V. Begging for release via 50 kHz USVs:

Begging behavior in adults has been documented in chimpanzees (Silk, Brosnan, Henrich, Lambeth, & Shapiro, 2013), orangutans (Pelé, Dufour, Thierry, & Call, 2009), vampire bats (Carter & Wilkinson, 2013, 2016), and rats (Paulsson & Taborsky, 2021, 2022), where unrelated individuals signal their need for help where begging is, as expected, preferentially directed toward reciprocal partners (Trivers, 1971). To better understand the role of 50 kHz USVs in prosocial behavior, Paulsson and Taborsky (2022) monitored food donation behavior by rats when exposed to 50 kHz USV playbacks. When calls were manipulated to playback at double the rate, food-sharing with a partner significantly increased. This indicates 50 kHz USVs can be used as begging signals and increased intensity provokes increased aid. However, so far, these begging calls have only been measured in regards to food acquisition (N. Paulsson & Taborsky, 2021; N. I. Paulsson & Taborsky, 2022; Schweinfurth & Taborsky, 2018). Future studies could compare the acoustic structure of 50 kHz USVs emitted by food-deprived rats involved in food-sharing opportunities to those emitted by rats trapped rats testing in the HBT.

Concluding Remarks

The production of 22 kHz and 50 kHz USVs by rats subjected to the HBT denotes the communicative complexity of rodent prosocial behavior contributing to the mounting evidence that rats potentially possess empathic capacities beyond emotional contagion. USVs have been reliably linked to affective state and neural correlates (Brudzynski, 2007, 2009, 2013; Burgdorf et al., 2000; Burgdorf et al., 2008; Sadananda et al., 2008; Simola & Brudzynski, 2018); however, the variety of contexts inducing USV production, the diversity of subtypes, and the heterogeneity in acoustic structures of USVs, particularly 50 kHz USVs, emphasizes the need for further research on the intricacies of USV emission to understand the role of communication in promoting empathically motivated prosocial behavior (Burgdorf et al., 2008; Burgdorf, Panksepp, & Moskal, 2011; C. Burke, Kisko, Swiftwolfe, et al., 2017; Simola & Costa, 2018; Wöhr, 2018;

Wöhr & Schwarting, 2013). Understanding the factors involved in eliciting helping behavior in rats may aid in elucidating the evolution of empathy and the proximate mechanisms facilitating its expression (Seffer, Schwarting, & Wöhr, 2014). The addition of USV analysis as a tool to study motivations of and antecedents to helping behavior can further advance translational research designed to enhance understanding of social disorders.

CHAPTER V

GENERAL DISCUSSION & FUTURE DIRECTIONS

This collection of experiments contributes to the existing literature utilizing the rodent model of empathy by providing additional contexts under which rats will act to help a distressed cagemate. Research investigating the empathic abilities of smaller-brained mammals and the contexts that promote associated behaviors serve to explore complex prosocial behaviors in a simple system and fill gaps in knowledge regarding the evolution of empathy and the proximate mechanisms involved. Consistent with the results of previous experiments utilizing the helping behavior test (HBT), rats were motivated to help distressed cagemates by opening the door to the restrainer confining the individuals (Ben-Ami Bartal et al., 2011, 2014, 2016, 2021; Carvalheiro et al., 2019; Cox et al., 2020, 2022; Havlik et al., 2020; Breton et al., 2022; Vieira Sugano et al., 2022). The results of the current studies offer novel findings documenting motivation to help a conspecific despite the presence of an associated cost and the opportunity to escape and demonstrate the communicative complexity potentially facilitating the help provided.

The Russian Nesting Doll of empathy depicts emotional contagion at the core of all empathic responses and denotes the most primitive form of empathy (de Waal, 2008). Critics skeptical of the empathic complexity afforded to rats suggest personal distress, via emotional contagion, drives the helping behavior repeatedly demonstrated (Lavery &

Foley, 1963; Rice, 1964; Preobrazhenskaya & Simonov, 1974; Jentsch & Ringbach, 2014; Hiura et al., 2018; Blystad, 2021). Theory predicts that the presence of an escape option will abolish helping behavior motivated by personal distress (Batson, 1987; Eisenberg et al., 1989). To test if concern for self or other promotes the helping behavior previously demonstrated in rats, the current studies conducted trials in a Plexiglas arena that was approximately 100 cm longer than those utilized in previous studies and connected a 3-foot tunnel to the opposite end of the arena that housed the restrainer. This tunnel led to a darkened escape area where a free rat could escape and presumably ameliorate the distress induced by observing the trapped rat. The increased length of the arena and the incorporation of the tunnel to separate the escape area from the arena served to create more distance between the restrainer and the escape area with the aim of attenuating the distress cues of the trapped rat when the free rat was in the escape area. Despite the opportunity to easily alleviate their own distress, rats forewent the escape option and opted to liberate their trapped cagemate an overwhelming amount of time, suggesting the distress of the trapped cagemate was the motivating factor driving the helping behavior exhibited.

Helping another occasionally requires the actor to incur an associated cost (Eisenberg & Miller, 1987; Fehr & Fischbacher, 2003), and both theory and empirical evidence suggest the presence of a cost should temper the performance of helping behavior (Batson et al., 1983; Schneeberger et al., 2012; Shotland & Stebbins, 1983; Wagner & Wheeler, 1969); thus, implementing a cold-water barrier, a known aversive condition for rats (Sato et al., 2015), that had to be crossed in order to reach the restrainer served as the associated cost required to help release a trapped cagemate. While the presence of a cost slightly diminished helping behavior, the decline observed was entirely dependent on two rats; one never opened the door and the other only did so once. Further research is necessary to discern the magnitude of the trend as the current study was limited by a small sample size.

The last installment of this collection of experiments provided novel information lending to the complex vocal communication preceding the release of a trapped cagemate. To the best of our knowledge, the experiment provided the first analyses of 50 kHz ultrasonic vocalizations (USVs) produced when rats were exposed to the HBT. The social complexity hypothesis for complex communication posits that selection will favor sophisticated means for relaying diverse information in species exploiting robust social structures where individuals are interacting with many conspecifics, especially those of different ages and ranks (Freeberg et al., 2012). In the wild, rats live in colonies often numbering over 100 individuals composed of juveniles and adults of both sexes (Schweinfurth, 2020). Much like their large-brained counterparts recognized for their complex empathic capacities, rats produce a variety of signals to convey information to others and display affective arousal in response to the emotions of others.

The contexts that elicit the emission of 22 kHz USVs are consistently associated with negative affect and the structure and length of these USVs are relatively stereotypical, showing little variation (Brudzynski & Ociepa, 1992; Brudzynski & Holland, 2005). Thus, it was unsurprising that the rate of 22 kHz USVs were significantly greater during trials when a cagemate was trapped in the restrainer and drastically declined upon their release. In addition to the 22 kHz USVs produced prior to door-opening in the cagemate treatment, an abundance of 50 kHz USVs were produced. While

50 kHz USVs were emitted at a significantly higher rate prior to door-opening when a cagemate was released, they were also produced in the empty treatment, especially in trials where the free rat opened the restrainer door. Unlike 22 kHz USVs, 50 kHz USVs are produced in response to a variety of contexts and show diversity in their length and acoustic structure (Wöhr et al., 2008; Wright, Gourdon, & Clarke, 2010), 50 kHz USVs are often indicative of positive affect (Brudzynski, 2007; Burgdorf et al., 2000; Burgdorf et al., 2001; Burgdorf et al., 2011; Panksepp & Burgdorf, 2000); however, these calls have also been implicated in social coordination ((C. Burke, Kisko, Pellis, et al., 2017; Wöhr, 2018; Wöhr & Schwarting, 2008b); thus, the function of the 50 kHz USVs emitted prior to door-opening remains unclear and highlights the importance of teasing apart the structural variation of these vocalizations to understand the information they carry and their associated affective states and neural recruitment as these calls may be used as a proxy for positive affect.

The findings revealed by the experiments conducted for this dissertation prompt subsequent questions that remain unanswered. Firstly, what magnitude of a cost would be needed to inhibit helping behavior; and once that threshold was found, are there factors regarding the distressed party that could override the decline caused by the cost. For example, a visual predatory simulation coupled with urine from a predator could be used to provide a more salient cost associated with helping. Following a similar protocol to Paulsson & Taborsky (2021), playbacks of conspecific 22 kHz USVs could expose rats to higher-than-normal rates to determine if doubled or tripled distress call rates could promote risky helping behavior. In human studies, the severity of an individual's situation determines how willing people are to help as costs to doing so increase

(Cialdini, Brown, Lewis, Luce, & Neuberg, 1997; Darley & Batson, 1973). Furthermore, the role of 22 kHz USVs, and potentially that of 50 kHz USVs, in soliciting aid could be investigated to determine if other cues indicating distress are salient enough to evoke helping behavior by testing free rats with trapped conspecifics that have been devocalized.

Triver's (1971) Theory of Reciprocal Altruism predicts aid will be preferentially dispensed towards cooperative partners, whereas the reputation of defectors will deter individuals from helping individuals previously demonstrating an unwillingness to reciprocate. However, it remains unknown how observers may respond to those unable to return the favor in the future, such as the elderly and those impaired physically or mentally. Anecdotes of other animals providing aid to those upon their death (Douglas-Hamilton et al., 2006; Park et al., 2013) and the social integration of a developmentally delayed female rhesus monkey in a captive population (de Waal, 1996) suggest empathically motivated behaviors likely adopted motivational autonomy (de Waal, 2008) and thus one's inability to provide symmetrical benefits to the actor will not deter the performance of helping behavior, especially in cases where social bonds are strong.

The HBT first introduced by Bartal et al. (2011) has been altered multiple times and the results from these studies have provided evidence of the complexity and flexibility of the helping behavior performed by rats while also informing the involvement of multiple brain regions and neurotransmitters (Ben-Ami Bartal et al., 2011, 2014, 2016, 2021; Breton et al., 2022; Carvalheiro et al., 2019; Cox et al., 2020, 2022; Havlik et al., 2020; Vieira Sugano et al., 2022). The experiments detailed in this dissertation were conducted with intent to further understand the empathic abilities of rats in an effort to expand our understanding of the evolution of empathy and provide additional information for neuroendocrine studies to build from to discern the neural correlates of empathically motivated behavior.

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Voted 'Student Champion' 2021-2022
Bill Furnish Award for Excellence in Teaching Biology 2022-2023

2017- 2018 **Adjunct Professor**, Department of Biology, Winthrop University, SC.
Introduction to Biology for non-majors, lecture & lab

2015- 2017 **Graduate Teaching Assistant**, Department of Biology, Winthrop University, SC.
Botany Lab, Introduction to Biology Lab (majors and non-majors)

2014- 2015 **Veterinary Assistant**, Greenbrier Emergency Animal Hospital, Charlottesville VA.

2013 **Wildlife Rehabilitation Intern**, Wildlife Center of Virginia, Waynesboro, VA.

2011- 2013 **Research Assistant,** University of South Carolina, PI: Dr. Austin Hughes
Data collection and collaboration on long-term zoo experiment

Publications

Dugatkin, L.A., **Driscoll, C.** 2021. Empathy in nonhumans: a brief overview. *Periodicum Biologorum*, 123 (1-2): 1-5.

Hughes, A.L., **Driscoll, C.** 2014. Being in the thick of things: context-dependent network centrality in a captive flock of American flamingos. *Journal of Ethology,* 32: 83-90.

Hughes, A.L., Cauthen, J., **Driscoll, C.** 2014. Testing for behavioral lateralization in observational data: a Monte Carlo approach applied to neck-looping in American flamingos. *The Wilson Journal of Ornithology,* 126: 345-352.

Hughes, A.L., Raynes, A., **Driscoll, C.,** Babbler, J. 2013. Behavioral correlates of post-breeding weight change in a captive flock of American flamingos (*Phoenicopterus ruber ruber*). *Zoo Biology*, 32: 204-209.

Grants & Awards

2023	Travel Grant, UofL, Graduate Network in Arts & Sciences	$250
2022	Research Grant, The Dan and Margaret Carper Foundation	$5,500
2021	Student Research Grant, Animal Behavior Society	$1,500
2021	Summer Research Opportunity Program, UofL	$400

2020	Mentored Undergraduate Research & Creative Activities Grant, UofL College of Arts & Sciences	$1,500
2020	Research Grant, UofL, Graduate Network in Arts & Sciences	$248
2020	Research & Creative Activities Grant, UofL College of Arts & Sciences	$490.56
2020	Research Grant, UofL Graduate Student Council	$496
2019	Research Grant, UofL Biology Graduate Student Association	$125
2019	Research & Creative Activities Grant, UofL College of Arts & Sciences	$1,997
2019	Research Grant, UofL Executive Vice President for Research & Innovation	$2,999.20
2019	Research Grant, UofL Graduate Network in Arts & Sciences	$250
2019	Research Grant, UofL Graduate Student Council	$488
	Total Funding Awarded:	**$16,243.76**

Presentations

2023 **UofL Biology Department Seminar Series** (Louisville, KY)

2023 **Indiana University Animal Behavior Conference** (Bloomington, IN)

2023 **Graduate Student Regional Research Conference** (Louisville, KY)

2022 **International Society for Behavioral Ecology Council** (Stockholm, Sweden)

2022 **Indiana University Animal Behavior Conference** (Bloomington, IN)

2022 **UofL Biology Awards Day** (Louisville, KY)

2022 **Graduate Student Regional Research Conference** (Louisville, KY)

2021 **Graduate Student Regional Research Conference** (Louisville, KY)
First Place: Outstanding Graduate Student Oral Presentation

2021 **Sigma Xi Student Research Showcase** (virtual)

University Service & Leadership

2021-2023 **Poster Judge**, Undergraduate Arts & Research Showcase, UofL

2021-2022 **Outreach Chair**, Biology Graduate Student Association, UofL

2021 **Mentor**, Summer Research Opportunity Program, UofL

2020- Pres **Graduate Peer Mentor**, Department of Biology, UofL

2020- 2021 **Secretary**, Biology Graduate Student Association, UofL

2019- Pres **Undergraduate Research Mentor**, Department of Biology, UofL
- *Jeraan Fernando (UofL) —Does personal distress or empathetic perspective-taking motivate helping behavior in rats?*
- *Swarnima Mukherjee (India) – Does a perceived cost influence helping behavior in rats?*

2019- 2020 **Fundraising Chair**, Biology Graduate Student Association, UofL

Community Outreach

2021 **Judge**, Genesis Poster Competition, Animal Behavior Society Conference

2020- Pres **Presenter**, Skype a Scientist
Connects scientists with teachers, classrooms, & other groups worldwide

2020 **Mentor**, Central Highschool Science Fair, Louisville, KY