In search of Prosocial Behaviors in Rodents: Paradigms, Behavioral Analyses, and Mediating Factors

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Dissertation submitted to the University of Ottawa

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Clinical Psychology

School of Psychology

Faculty of Social Sciences

University of Ottawa

Acknowledgment

Many people in academia made this dream possible and this journey so rich:

To my supervisor, Hélène: thank you for allowing this twenty-something to step into your office with big dreams. Thank you for your trust, the countless hours you spent reading manuscripts, and for your valuable teachings.

To my thesis committee: Anne, Andra, and Karen: thank you for your guidance and professionalism, but especially for being as excited and curious about this thesis as I was; you never made learning feel boring or intimidating.

Thank you to my external examiner, Dr. Inbal Ben-Ami Bartal, for your significant work in the field of rodent prosociality, for inspiring this dissertation from the start, and for being part of its revision. Thank you for advocating for animal welfare and changing views on how rodent research should be conducted.

To everyone who crossed my path during my training in clinical psychology, thank you for teaching me that empathy is one of the most important strengths I have, and that the world needs more of it.

I could not write a thesis on behavioral neuroscience and rodents without acknowledging all the living organisms that have gifted and continue to gift us with the power of knowledge.

On a personal note, I am deeply grateful for my most important friend, Véronique: nothing is more powerful than two girls who have known each other since preschool. Through good times and bad, you have always believed in me. Thank you for your infinite support and the much-needed laughs.

Although you cannot read, to Bailey, the most perfect, sassy Beagle in the world: thank you for the emotional support cuddles, the mental health walks, and for brightening the days of everyone who crosses your path.

Finally, my deepest gratitude goes to my partner in research and in life: my fiancé and soon-to-be husband, Joey. Thank you for making life a true adventure. From building operant boxes in a dark (probably haunted) basement to going through graduate school together, and now stepping into the ‘real’ world, I look forward to a lifetime of more fun and adventure with you. Thank you for being a part of my thesis and for allowing me to be a part of yours; learning alongside you is a privilege I will never take for granted.

Abstract

From sharing resources to caring for offspring, prosociality allows animals to survive. In humans, mental health challenges affecting sociability are often associated with reduced quality of life. Despite years of research aimed at understanding the behavioral and neural mechanisms of prosociality in humans, non-human primates, and other social mammals (including rodents), little is known about the specific processes involved. Consequently, it is crucial to standardize the assessment of prosocial behaviors in rodents to promote the development of valid experimental paradigms and methodologies. Such research will enhance understanding of prosociality and its implications for mental and physical health challenges.

The first study of this thesis used a quasi-experimental design to examine two strains of adolescent rats, Long-Evans (n = 8) and Sprague-Dawley (n = 12), in a modified double operant box paradigm that allowed paired rats to share food rewards. Both strains were randomly assigned to either a pretrained or a naive group. To assess vicarious learning, dyads exchanged roles during the second phase of the experiment. Performance for all groups (Long-Evans versus Sprague-Dawley; Original Actors versus Vicarious Observers; Pretraining versus No Pretraining) and transitional probabilities of behavioral sequences were analyzed. The findings indicated that pretraining mediated behavioral responses, whereas vicarious learning had minimal influence on task performance. This initial groundwork highlighted the pivotal role of variables such as pretraining, behavioral analysis, and task contingency in mediating prosocial behaviors.

Building on these observations, Study Two characterized current findings on prosocial paradigms in rodents through a scoping review. A research librarian devised an extensive search strategy encompassing five databases—APA PsycInfo, Embase, MEDLINE, Scopus, and Web of Science—covering the period from January 2000 to 2021. Subsequently, a semi-supervised machine learning technique utilizing ASReview was employed to update the search and gather studies published from 2021 to 2023. In total, 80 articles were included. The results were as follows: (1) Three categories of tasks were identified (i.e., cooperation, helping, and sharing tasks). Rodents demonstrated the ability to perform prosocial actions in all three categories; (2) notable discrepancies were observed in reported methodologies, such as the omission of animal characteristics, housing conditions, and experimental protocols, (3) behavioral analyses were identified as crucial for studying prosociality in rodents, yet many studies overlooked their inclusion. Finally, (4) important mediating factors were identified as critical determinants in the assessment of prosociality in rodents, including sex, age, strain, housing, familiarity, food restriction, aggression, and dominance. These findings collectively provide insights for future studies into the influence of mediating factors and highlight the significance of behavioral analyses in the expression of prosocial behaviors in rodents.

Considering the observations from Study Two, Study Three presented a conceptual framework that (1) reframed prosociality as a set of complex behaviors emerging in response to environmental determinants that cannot be reduced to a single set of data, (2) highlighted important methodological considerations, mediating variables, and behavioral analyses that influence prosocial behaviors, and (3) introduced a decision tree as a dynamic element within this framework to guide researchers. The conceptual framework and decision tree provide a robust foundation for the continued use of existing models and the development of new paradigms. Integrating this conceptual framework into research practices will contribute to the

advancement of knowledge in the field of rodent prosociality and foster greater confidence in the validity and reproducibility of study findings.

Taken together, these three studies make a substantial contribution to the literature by highlighting crucial factors involved in rodent prosociality, including mediating variables and behavioral analyses. Moreover, the development of a conceptual framework marks a pivotal advancement in the field, leading to a deeper understanding of rodent prosociality and its underlying behavioral mechanisms. This foundational work paves the way for future research on prosociality impairments observed in mental health disorders, neurodevelopmental conditions, and neurological impairments.

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List of Abbreviations

ACC= Anterior cingulate cortex

AI= Anterior insula

ANOVA= Analysis of variance

ANS= Autonomic nervous system

APA= American Psychiatric Association

ASD= Autism spectrum disorder

BLA= Basolateral amygdala

CVA= Cerebrovascular accident

dmPFC= dorsomedial prefrontal cortex

EE= Enriched environment

HPA axis= Hypothalamic–pituitary–adrenal axis

IL= Infralimbic cortex

PAM= Perception-action mechanism

PFC= Prefrontal cortex

TBI= Traumatic brain injury

TOM= Theory of mind

USVs= Ultrasonic vocalizations

vmPFC= ventromedial prefrontal cortex

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CHAPTER ONE

General introduction

From an evolutionary perspective, social behaviors allow animals to survive, communicate, and share resources (O'Connell & Hofmann, 2011). Even before Darwin's published collection entitled *The Expression of the Emotions in Man and Animals* in 1872 (Darwin & Prodger, 1998), Charles Bell had proposed a comprehensive analysis of muscular expressions in human faces (*The Anatomy of Expression,1806* in Gilman, 1984), while the French neurologist Duchenne de Boulogne (1862) had characterized discrete emotions conveyed by facial expressions (Darwin & Prodger, 1998). Darwin, however, was the first scientist to extensively explore facial expressions and emotions in animals (Darwin & Prodger, 1998). This foundational work underscores the complexity of social behaviors that are crucial for survival and interaction. Such behaviors observed in humans and many animals include reproduction (Knop et al., 2017), maternal and paternal care (Alves et al., 2020; Saltzman et al., 2017), dominance and aggression (Murlanova et al., 2022; Fulenwider et al., 2022), responses to social novelty and social recognition (de la Zerda et al., 2022; Cavigelli et al., 2011), as well as vicarious observation (Keum & Shin, 2019), and social play (Vanderschuren et al., 2016). Empathy and prosociality also form essential components of this repertoire (Decety et al., 2012).

Empathy can be described as feeling, understanding, and sharing the emotional states of others (Chen, 2018; Decety & Holvoet, 2021). The word comes from the German *Einfühlung*, first used in 1873 by philosopher Robert Vischer, which translates to "in feeling" (Jahoda, 2005). The term was further promoted by German philosopher Theodor Lipps (Jahoda, 2005) and was later translated by Edward Bradford Titchener into the word "empathy" at the beginning of the 20th century (Titchener, 2014). Since then, many theories have attempted to understand and define the bases of empathic expression in humans, including the Russian-doll model, the top-down versus bottom-up approach, and the theory of mind (TOM).

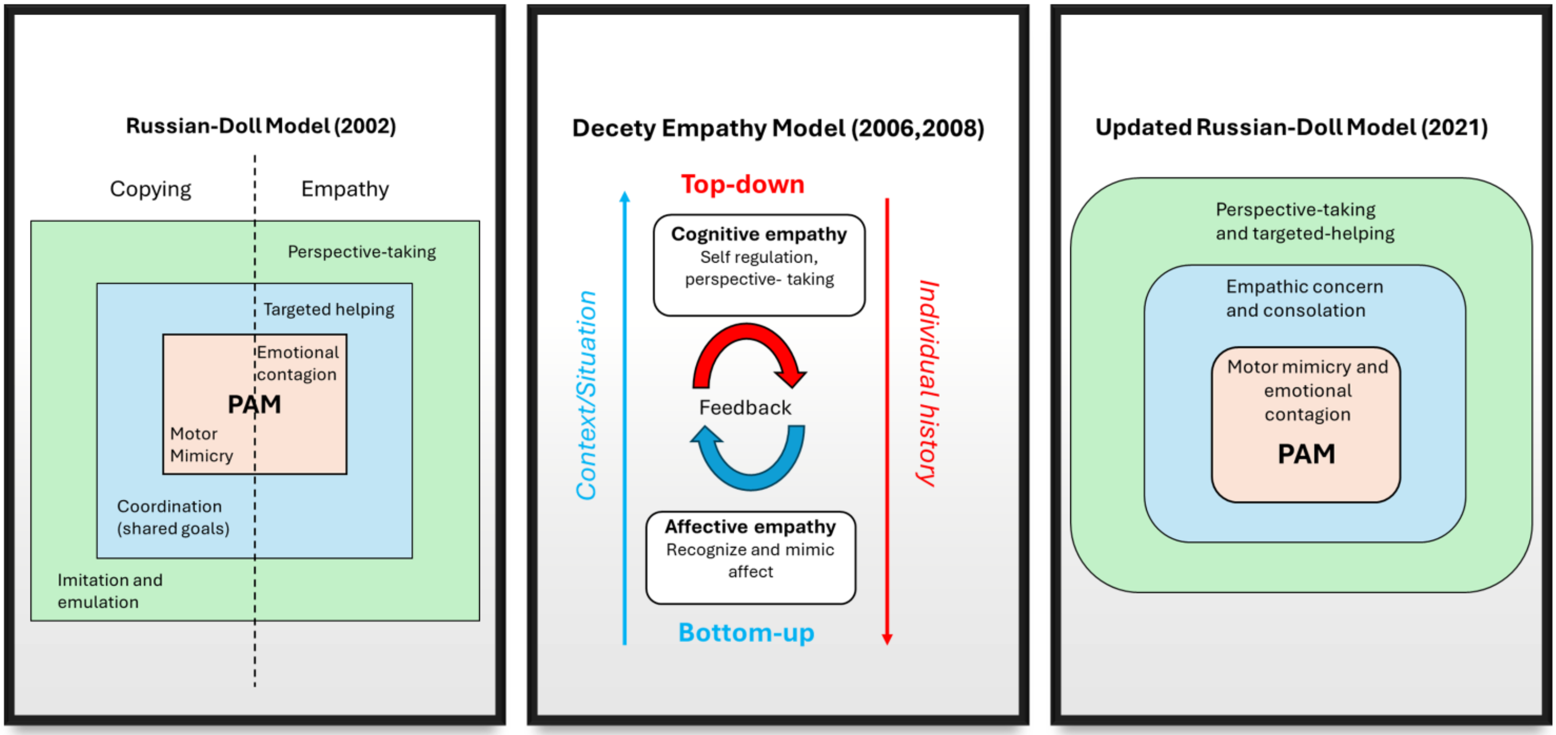
1.1 Russian-doll model

In 2002, de Waal proposed the Russian-doll model to classify and organize distinct types of empathic expressions in humans (Preston & de Waal, 2002a; de Waal, 2007). He suggested that imitation and empathy are distinct concepts: motor mimicry, coordination, and true imitation fall under the imitation category (also called “copying” or affective empathy), while emotional contagion, sympathetic concern, and perspective-taking are part of the empathy category (also referred to as cognitive empathy). At the core of the model is the perception-action mechanism (PAM), defined as the ability of an observer to access the subjective state of another (Preston & de Waal, 2002b; see Figure 1). In an updated version, Michalec and Hafferty (2021) presented this model as a continuum, ranging from the most “primitive” to the most “advanced” forms of empathy. PAM remains central to the theory, encompassing motor mimicry and emotional contagion, followed by empathic concern and consolation. Finally, perspective-taking and targeted helping represent the most cognitively advanced forms of empathy (Michalec & Hafferty, 2021).

1.2 The top-down versus bottom-up approach

Decety (2006, 2008) proposed a model suggesting that empathy involves both bottom-up and top-down information processing (see Figure 1). According to this model, affective empathy refers to emotion sharing and emotional contagion, which rely on bottom-up processed information (e.g., perceiving someone in pain, which triggers empathy in others; Decety & Jackson, 2006; Decety & Meyer, 2008). In contrast, cognitive empathy—defined as the ability to understand and take the perspective of another person—requires a top-down approach. This model suggests that both mechanisms (top-down and bottom-up) are essential to empathy. The bottom-up mechanism allows individuals to recognize and mimic emotions, while the top-down mechanism supports self-regulation and perspective-taking, enabling individuals to understand the viewpoints of others (Decety & Meyer, 2008).

Figure 1. Human Models of Empathy: Theories from 2002 to 2021.



Note. **A) Russian-Doll Model (2002):** Images adapted on the work of Preston, S. D., & de Waal, F. B. M. (2002). Empathy: Its ultimate and proximate bases. The Behavioral and Brain Sciences, 25(1), 1–20; discussion 20-71. https://doi.org/10.1017/s0140525x02000018 and de Waal, F. B. M. (2007). The "Russian doll" model of empathy and imitation. In On being moved: From mirror neurons to empathy (pp. 49–69). John Benjamins Publishing Company. https://doi.org/10.1075/aicr.68.06waa. **B) Decety Empathy Model (2006, 2008):** Image based on the work of Decety, J., & Meyer, M. (2008). From emotion resonance to empathic understanding: A social developmental neuroscience account. Development and Psychopathology, 20(4), 1053–1080. https://doi.org/10.1017/S0954579408000503. **C) Updated Russian-Doll Model (2021)**: Image adapted from the work of Michalec, B., & Hafferty, F. W. (2022). Challenging the clinically-situated emotion-deficient version of empathy within medicine and medical education research. Social Theory & Health, 20(3), 306–324. https://doi.org/10.1057/s41285-021-00174-0.

1.3 The theory of mind

The theory of mind (TOM) refers to a uniquely human ability to understand the intentions, emotions, desires, beliefs, and thoughts of others (Carlson et al., 2013; Blakemore & Decety, 2001; Krupenye & Call, 2019). This ability is influenced by spoken language and cultural background, which play a crucial role in understanding social cues and perceiving others' emotions and intentions, which are both necessary for successful social interactions (Aival-Naveh et al., 2019; Díaz, 2022; Blakemore & Decety, 2001; Byom & Mutlu, 2013). Developmental research has shown that children as young as two years old begin to adopt the principles of TOM (Slaughter, 2015). While the basic concepts of TOM and PAM in relation to human empathy share similarities, TOM’s core principle is linked to the ability to attribute mental states to others—understanding that others have different thoughts and feelings than oneself (Carlson et al., 2013). In contrast, PAM refers to the ability to understand another’s subjective state and respond to it, as seen in emotional contagion and targeted helping (Preston & de Waal, 2002b).

1.4 Empathy: necessity, deficits, and consequences

From an evolutionary perspective, empathy benefits group living and species survival (Decety et al., 2016; Sivaselvachandran et al., 2018). Researchers suggest that individuals with higher levels of empathy demonstrate greater survival fitness, allowing them to pass these traits on to their lineage (Decety et al., 2012). Indeed, witnessing fear or pain can signal danger to others, even without direct experience of a specific, potentially life-threatening situation (Skversky-Blocq et al., 2021).

As social beings, humans require interactions and support to survive and thrive in their environments (Holt-Lunstad, 2021; Coan & Sbarra, 2015). According to self-determination theory, humans have three universal needs for adequate functioning: autonomy, competence, and relatedness (Deci & Ryan, 2000). Relatedness refers to the desire to feel connected to others and receive their support (Deci & Ryan, 2000). In this context, deficits in empathy can significantly impair an individual's ability to recognize, understand, and respond to the emotions of others. This can lead to difficulties in forming meaningful relationships and negatively impact quality of life (Roth et al., 2019; Patrick et al., 2007). Individuals with empathy impairments often experience reduced social support and increased isolation, resulting in a decline in overall quality of life (Guariglia et al., 2023) and a higher risk of mental health and physical disorders (Wang et al., 2018; Hakulinen et al., 2016).

1.4.1 Autism spectrum disorder

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social interaction and communication across various contexts (American Psychiatric Association [APA], 2013). These deficits manifest as difficulties in developing, maintaining, and understanding relationships, as well as challenges in nonverbal communication and social-emotional reciprocity (APA, 2013).

To assess social interaction in rodents, two commonly used paradigms are the social interaction test and the three-chamber apparatus. These models evaluate social approach, novelty, and social recognition/memory (Jabarin et al., 2022; Kondrakiewicz et al., 2019). They are also frequently used to assess anxiety-like behaviors, based on the premise that anxious rodents will display fewer social novelty behaviors, such as sniffing and approaching a new conspecific (Rex et al., 2004; Shirenova et al., 2023). Interestingly, genetic mouse models of

ASD have demonstrated a reduced ability to experience emotional contagion of pain, a phenomenon also observed in children with ASD (Martin et al., 2022; Helt et al., 2020). However, despite the use of these various paradigms, high levels of variability and a lack of reproducibility hinder the scientific community's ability to reach a consensus on an ecological model of ASD (Kas et al., 2014).

1.4.2 Mental health disorders

Anxiety disorders are the most prevalent mental health disorders, characterized by excessive fear and worry that significantly impact normal functioning (APA, 2013; Beesdo-Baum & Knappe, 2014; Stein et al., 2017; Yang et al., 2021). A meta-analysis revealed that individuals with higher levels of anxiety tend to experience greater emotional contagion and affective empathy (e.g., feeling for others), but also struggle with understanding emotions in others (i.e., cognitive empathy; Nair et al., 2024). Stress and fear-based models, such as emotional contagion and fear conditioning, are often used to investigate emotional contagion and anxiety in rodents. Fear and stress seem to negatively impact prosocial decision-making (Karakilic et al., 2018), while the presence of a conspecific acts as a buffer against stress (i.e., social buffering; Kiyokawa et al., 2018; Hostinar et al., 2014; Nakamura et al., 2016; Ishii et al., 2016). Research has also shown that lower levels of anxiety correlate with increased prosocial actions, such as opening a door to rescue a conspecific (Yüksel et al., 2019; Ben-Ami Bartal et al., 2016).

Schizophrenia encompasses a wide range of symptoms, including delusions, hallucinations, disorganized speech, catatonic behavior, and negative symptoms (i.e., avolition or diminished emotional expression; APA, 2013). Studies indicate that schizophrenia is associated with deficits in both affective and cognitive empathy (Bonfils et al., 2016; Pijnenborg et al., 2013). So far, rodent models have primarily focused on social isolation to mimic the social withdrawal commonly seen in schizophrenia (Powell & Swerdlow, 2023; Wilson & Koenig, 2014).

Depression is characterized by a significantly low mood and a loss of interest in previously enjoyed activities (APA, 2013). In humans, depression may correlate with difficulties in recognizing emotions in others (i.e., affective empathy; Yan et al., 2021; Thoma et al., 2011). In rodents, while various behavioral tests exist to simulate depressive symptoms (e.g., tail suspension test, forced swimming test, sucrose preference test, open-field test, and learned helplessness; Yan et al., 2021; Silveira & Joca, 2023), studies investigating specific features of depression and empathy are still limited.

A personality disorder consists of long-term patterns of behavior and inner experiences that significantly deviate from cultural norms and cause impairment or distress (APA, 2013). Borderline, antisocial, and narcissistic personality disorders are the most studied in relation to empathy (Baskin-Sommers et al., 2014; Dinsdale & Crespi, 2013; Campos et al., 2022). It has been suggested that borderline personality disorder affects cognitive empathy (Ripoll et al., 2013), while antisocial and narcissistic personality disorders negatively impact affective empathy (Baskin-Sommers et al., 2014; Campos et al., 2022). To date, rodent models that capture the characteristics of personality disorders are still in development (Corniquel et al., 2019; Hernandez-Lallement et al., 2018).

1.4.3 Dementia and Parkinson’s disease

Dementia is a neurodegenerative disorder that includes several subtypes, such as Alzheimer’s disease, vascular dementia, and frontotemporal dementia (Cao et al., 2020). Common symptoms of dementia include loss of empathy and progressive deficits in social functioning (Piguet et al., 2011; Pasquini et al., 2020; Fischer et al., 2019; Bartochowski et al., 2018). In humans, cognitive empathy is particularly negatively affected (Vernay et al., 2016; Eslinger et al., 2011), while emotional contagion (i.e., affective empathy) seems to be heightened (Sturm et al., 2013; Hua et al., 2018). In an Alzheimer’s disease model, mice showed enhanced emotional contagion, whereas those in a frontotemporal dementia model exhibited a decreased response to the distress of a conspecific (Phillips et al., 2023; Choi & Jeong, 2017).

Parkinson’s disease is another neurodegenerative disorder that leads to deficits in cognitive empathy (Coundouris et al., 2020). Recently, researchers have begun investigating non-motor symptoms—such as mood changes, cognitive decline, and sleep disturbances—due to their significant impact on quality of life (Sveinbjornsdottir, 2016; Politis et al., 2010; Lindgren & Dunnett, 2012). However, rodent models of Parkinson’s disease have not yet explored these non-motor symptoms, including mood changes and empathy (Zhang et al., 2022; Vingill et al., 2018).

1.4.4 Traumatic brain injuries and cerebrovascular accidents

Empathy deficits are commonly reported in cases of traumatic brain injuries (TBI; de Sousa et al., 2010; Wearne et al., 2020). Researchers suggest that these impairments may be linked to difficulties in facial recognition often seen after a TBI. The inability to recognize emotions in facial expressions can hinder the capacity to perceive, understand, and feel the emotions of others (Neumann et al., 2012; de Sousa et al., 2010). Several rodent models of TBI exist, including the weight drop model, piston-driven model, and rotational injury model, all designed to simulate brain injury (Bodnar et al., 2019). Deficits in emotional contagion and observational fear learning (i.e., affective empathy) have been documented, as well as issues with social recognition (Bahader et al., 2024; Runyan et al., 2021). Interestingly, rescue behavior following TBI in rats showed no significant difference compared to sham controls (Hosgorler et al., 2020).

A cerebrovascular accident (CVA) occurs when blood flow is compromised, damaging tissue in a specific part of the brain; Campbell et al., 2019). Symptoms following a CVA may include impairments in both affective and cognitive empathy (Yeh & Tsai, 2014; Nijsse et al., 2019; Leigh et al., 2013; Adams et al., 2019). These impairments can manifest as challenges in perspective-taking, interpreting non-verbal cues, and recognizing faces (Yeh & Tsai, 2014; Leigh et al., 2013). In rodents, the middle cerebral artery occlusion model is commonly used due to its reliability and similarity to human strokes (Fluri et al., 2015; Sommer, 2017). Social contact appears to improve stroke recovery in rats and mice, while social isolation leads to poorer outcomes (Shinozuka et al., 2020; Venna et al., 2014; O’Keefe et al., 2014). However, researchers have yet to investigate empathy in relation to stroke using rodent models.

1.5 The bridge between human empathy and rodent prosociality

Empathy is frequently regarded as a uniquely human ability (Decety & Holvoet, 2021) due to (1) its definition is closely linked to an internal state (i.e., emotions), and (2) the advanced cognitive skills that enable humans to comprehend the intentions, emotions, desires, beliefs, and thoughts of others (i.e., Theory of Mind; Carlson et al., 2013; Blakemore & Decety, 2001). Consequently, studying such phenomena in animals poses significant challenges and relies heavily on subjective assessments of internal states. However, investigating prosociality in animals could serve as an initial step towards understanding what drives an animal to assist another. Prosociality is defined as actions taken to benefit others or enhance their well-being (Decety et al., 2016). By examining prosocial behaviors in rodents, researchers can objectively assess specific actions aimed at helping or benefiting others. In the animal kingdom, prosociality is as crucial for survival as empathy is for humans; engaging in prosocial behaviors fosters cooperation and resource sharing (Decety et al., 2016; Karakilic et al., 2018).

1.5.1 Existing rodent paradigms of prosociality

The first documented rodent studies utilizing a prosocial model date back to the 1950s, beginning with Church's research published in 1959, followed by Greene's work in 1969. Both studies involved tasks in which a rat could alleviate the pain of a conspecific (i.e., foot shock) by pressing a lever. While the results yielded mixed findings, they paved the way for further exploration. At the beginning of the 21st century, there was a noticeable increase in research focused on the more primal aspects of prosociality, such as emotional contagion (e.g., through fear or pain; Knapska et al., 2010; Langford et al., 2006; Langford et al., 2010). In 2011, Ben-Ami Bartal and colleagues published a landmark study demonstrating that rats could open a door to free a restrained conspecific. Since then, the field of rodent prosociality has been rapidly expanding.

1.5.1.1 Aversive models

Aversive models are experimental paradigms that incorporate one or more aversive elements, inducing stress, pain, and/or fear in animals. Examples include electric foot shocks, forced swimming, and restraint devices. These models have been favored for studying prosocial behaviors due to the observable distress exhibited by rodents. In such paradigms, a frightened or stressed rodent displays specific behaviors that can be quantified such as freezing—characterized by a complete cessation of movement—and distressed calls, assessed through ultrasonic vocalizations (Fendt et al., 2021).

Emotional contagion and fear conditioning. Emotional contagion, or sympathetic concern, is the tendency to mimic or express the emotions displayed by another individual (Hatfield et al., 2014; Pérez-Manrique & Gomila, 2018, 2022). These paradigms are considered aversive due to the nature of the stimuli (e.g., electric foot shocks, aversive sounds, tail pinches) that trigger a fear response in the animal. A commonly used apparatus is the double operant box, in which one subject receives electric foot shocks while the other observes (Cruz et al., 2020; Pérez-Manrique & Gomila, 2022). This model enables researchers to record and analyse fear expression and behavioral responses of the observing animal (Pérez-Manrique & Gomila, 2022). A meta-analysis by Hernandez-Lallement and colleagues (2022) found that rats and mice can exhibit similar levels of emotional contagion, as indicated by increased freezing responses.

Fear conditioning involves a rodent associating a conditioned stimulus (e.g., a sound or context) with an aversive unconditioned stimulus (e.g., a congener’s distress; Kim et al., 2019). Fear conditioning is similar to emotional contagion in that both involve a demonstrator (i.e., the animal receiving the shocks) and an observer. In fear conditioning, the study focuses on the animal's response (i.e., freezing) to the conditioned stimulus after being exposed to the demonstrator (Kim et al., 2019; Jones & Monfils, 2018). For example, Bruchey and colleagues (2010) exposed a rat to a tone followed by a mild electric shock. Once the rat began to show freezing behavior upon hearing the tone, it was exposed to a naive congener. Results indicated that the naive rat also froze at the sound, suggesting that fear of the stimulus can be transmitted

(Bruchey et al., 2010). While these tasks allow for the study of a rat's response to a congener's distress, they do not involve a concrete action and may not fully represent prosociality as defined in the literature (i.e., actions taken to benefit others or enhance their well-being; Decety et al., 2016).

Harm prevention task. Harm prevention tasks, while similar to emotional contagion and fear conditioning, require an action to alleviate harm to a conspecific. Hernandez-Lallement and colleagues published this task in 2020, in which a rat could choose between a lever that produced harm (i.e., foot shock) to a conspecific in an adjacent compartment while also delivering a sucrose pellet, and a lever that provided only a food reward for the actor without causing shock to the conspecific. They found that male and female rodents decreased the number of lever presses when it caused harm to a conspecific, choosing the reward-only lever more often than the shock-delivering one (Hernandez-Lallement et al., 2020). Interestingly, they showed that this harm aversion decreased when the difference in value between the levers was too high—deciding between a harmful lever that provided three pellets to the actor but delivered a shock to the victim versus a lever providing one pellet to the actor and no shock to the victim. This task was later replicated by Hess and colleagues (2023), who found that female rats tended to deliver more shocks to the conspecific to receive a food reward than male rats. While Hernandez-Lallement's experiment delivered a food reward regardless of the chosen lever, this later paradigm increased the cost for the actor rat by offering a choice between a lever that delivered a reward and a shock, or a lever that delivered neither. Although this model has shown promising results, it is still recent and requires further replication to fully explore its potential for studying prosocial behaviors in rodents.

Rescuing or freeing task. Ben-Ami Bartal and colleagues (2011) were the first to study prosociality in rodents by using an experimental paradigm involving a rat learning to open the door of a restrainer to free a trapped conspecific. Although this task is less aversive than ones using electric foot shock, a level of aversiveness remains due to the stress and fear experienced by the trapped rodent (Ben-Ami Bartal et al., 2011; Cox & Reichel, 2021). Variants of this task include scenarios in which a rat is trapped in a water-filled area, since rats typically dislike immersion in water (Sato et al., 2015; Morris, 1981). Mice also show a similar inclination to act for the benefit of others; a study by Ueno and colleagues (2019a) demonstrated the willingness of mice to chew through a paper lid to free a conspecific.

1.5.1.2 Limitations of aversive models

Stress as a confounding variable. Aversive models are based on the premise that a stressed or scared animal will elicit a response from a conspecific. However, aversive conditions can induce significant stress in both the demonstrator and the observer (Peen et al., 2021). The stress system, which comprises the hypothalamic–pituitary–adrenal (HPA) axis and the autonomic nervous system (ANS; Atrooz et al., 2021), acts in tandem to reestablish homeostasis (Zamora-González et al., 2013). When activated by stress, this system leads to a wide range of behavioral and physiological changes (Atrooz et al., 2021). For instance, the ANS is responsible for physiological responses to stress, such as changes in temperature and heart palpitations (Inagaki et al., 2004; Van Bogaert et al., 2006). Research has shown that elevated levels of stress correlate with decreased helping behaviors, such as less door opening to free a trapped conspecific (Ben-Ami Bartal et al., 2016). More recently, Karakilic and colleagues (2018) demonstrated that the intensity of stress (defined as low and high-intensity foot shocks) influences the prosocial responses displayed by rats, with lower stress levels associated with increased rescuing behavior and higher stress levels resulting in decreased helping behavior.

Animal welfare. According to the Canadian Council on Animal Care (CCAC), the Institutional Animal Care and Use Committee (United States), and the Federation of Laboratory Animal Science Associations (FELASA; Europe), animal research should focus on the 3Rs (replacement, reduction, and refinement) to minimize harm and maximize animal welfare (Fenwick et al., 2009; Curzer et al., 2016; Cheluvappa et al., 2017). Replacement refers to avoiding or substituting the use of animals with alternatives such as computers, technologies, or smaller organisms (e.g., invertebrates) whenever feasible (Fenwick et al., 2009). Reduction involves strategies or methodologies that allow for the use of fewer animals while maximizing the data and information obtained from each one. Refinement encompasses modifications to experimental procedures aimed at minimizing distress and pain (Fenwick et al., 2009). Therefore, employing aversive models that cause pain and/or distress to rodents complicates the application of the 3Rs and hinders efforts to maximize animal welfare.

Survival instinct. Aversive models imply that one animal experiences distress or pain while another observes and/or can take action to alleviate that distress, as seen in emotional contagion and freeing models. Although these tasks allow for observable behaviors (e.g., freezing), they raise important questions about the distinction between prosociality and survival instinct. While prosociality benefits species survival and group cohesion (e.g., helping, caring for others, sharing resources; Decety et al., 2016; Sivaselvachandran et al., 2018), survival instinct can act to confound the interpretation of results when testing prosociality in a laboratory setting (e.g., survival versus prosocial responses). In aversive models, observing a conspecific in pain or distress may trigger this survival instinct, leading to avoidance of the same stimulus, as evidenced by freezing during emotional contagion or fear conditioning tasks. To effectively translate findings to situations experienced by humans, it is crucial to develop models that can distinguish between survival responses and prosocial behaviors. Furthermore, aversive models are limited to negative states (e.g., fear, pain) and do not allow for the study of other forms of prosociality seen in humans (e.g., sharing, cooperation). Thus, to better understand the cerebral mechanisms involved in all types of prosociality and eventually translate this knowledge to humans, it is important to further investigate non-aversive models.

1.5.1.3 Non-aversive models

Non-aversive models refer to experimental paradigms that do not induce distress, fear, or pain in animals. These models offer several advantages over aversive ones: they promote animal welfare by eliminating pain or fear, are less stressful for the animals, and reduce the confounding variable associated with instinctive survival responses. Additionally, non-aversive models can study other forms of prosociality seen in humans, such as sharing and cooperation.

Imitation and mimicry tasks. In rodents, imitation and mimicry are most studied using the observation of specific behaviors like yawning and scratching (Moyaho et al., 2015; Yu et al., 2017). This phenomenon is associated with mirror neurons, a group of neurons that activate when an action is both performed and observed (Kilner & Lemon, 2013). Mirror neurons may play a significant role in prosociality by enabling the interpretation of nonverbal body cues and facilitating learning through observation (e.g., vicarious learning; Corradini & Antonietti, 2013; Rana et al., 2022). Interestingly, rats and mice display similar levels of yawning and itch contagion (Moyaho et al., 2015; Yu et al., 2017). The classification of socially contagious behaviors as prosocial remains debated, as some researchers argue that prosociality involves benefiting another individual, while socially contagious behaviors focus solely on observation, similar to emotional contagion (Rana et al., 2022; Meyza et al., 2017).

Prosocial choice task. Hernandez-Lallement and colleagues (2015) first introduced the prosocial choice test. This task utilizes a double T-maze with four compartments, compelling an actor rat to choose between a ‘selfish’ option (a single reward) and a ‘sharing’ option (a mutual reward) that benefits both rats. The actor rat can either eat the single reward alone or select the ‘both reward’ option to share food with a conspecific through a perforated wall. Choosing the mutual reward option allows the actor rat to enjoy its reward in the presence of another rat while maintaining physical separation (i.e., via the perforated wall; Hernandez-Lallement et al., 2015). Results showed that rats chose the ‘both-reward’ option more often than the selfish one when paired with a partner, but not when paired with a toy rat (i.e., control condition; Hernandez-Lallement et al., 2015).

Prisoner’s dilemma. The prisoner’s dilemma is an experimental task where rats are placed in divided compartments and must choose between pressing a cooperative lever or a defective one during repeated trials. This choice can lead to either a shared reward or no reward at all (Wood et al., 2016; Schneeberger et al., 2012; Delmas et al., 2019). For instance, Wood and colleagues (2016) designed a prisoner’s dilemma with three possible scenarios: 1) both rats refrain from pressing the lever, resulting in each receiving a food pellet; 2) both rats press the lever, leading to no reward; or 3) one rat presses the lever while the other does not, yielding five food pellets for the responding rat and no reward for the other. Research using this paradigm has shown that rats are willing to withhold their responses to achieve mutual rewards (Wood et al., 2016; Delmas et al., 2019; Donovan et al., 2020).

Cooperation learning tasks. Cooperation learning tasks involve paired rodents that must learn to coordinate their actions to achieve a mutual reward (Avital et al., 2016; Conde-Moro et al., 2019; de Carvalho et al., 2018). These tasks typically utilize an operant box paradigm, requiring both partners to learn specific actions (e.g., lever pressing, lever pulling, nose poking) to secure a shared benefit, such as a food reward. Research indicates that rodents can learn to coordinate their actions to achieve a mutual reward (Avital et al., 2016; Conde-Moro et al., 2019; de Carvalho et al., 2018).

Generalized and direct reciprocity. Generalized reciprocity is often studied through the repeated donation game, where an actor rat decides whether to share a food reward with a conspecific after interacting with multiple partners who display varying degrees of helpfulness (Rutte & Taborsky, 2007). In contrast, direct reciprocity focuses on the immediate decision of a rat to reciprocate help after experiencing either a generous or selfish partner (Kettler et al., 2021; Freidin et al., 2017). Both paradigms allow researchers to investigate if rodents remember previously helpful partners and whether they are more prosocial towards these partners than towards those who were unhelpful (Rutte & Taborsky, 2007). Findings indicate that rodents can demonstrate both direct and generalized reciprocity by matching the quantity of help previously provided (Kettler et al., 2021; Engelhardt & Taborsky, 2024).

1.5.1.4 Limitations of non-aversive models

Motivation versus prosociality. While non-aversive models offer several advantages, one significant limitation is related to the rewarding component of these models. Non-aversive paradigms often incorporate elements like food, social contact, play, or novelty, which could act as mediating factors. For example, an actor rat that frees a conspecific from a restrainer may receive social contact in return, serving as a reward that motivates the rat to continue the behavior (Silberberg et al., 2014; Blystad, 2019; Hachiga et al., 2018). Cox and Reichel (2020) found that rats still engage in helping behavior even when social contact is not possible, indicating the need for further research to clarify the role of social contact as a mediating variable in prosocial behaviors. Models that either prevent social contact or provide rewards independently of the prosocial action could address this limitation, as demonstrated in the prosocial choice test. In this test, actor rats receive a food reward in both options (i.e., choosing between a single or mutual reward) while maintaining no physical contact between the rats (Hernandez-Lallement et al., 2015).

Training time. Non-aversive models typically require longer training and experimental session durations compared to aversive models. This extended time commitment can increase needed resources, staff, and overall time from researchers, potentially impacting the study timeline—especially when investigating specific life stages, such as adolescence or older adulthood. For instance, Festucci and colleagues (2020) employed a sharing paradigm in which rats learned to press a lever to deliver food to a conspecific, totaling 12 days of training followed by 12 days of experimental sessions (Festucci et al., 2020). Similarly, Raz (2013) utilized a cooperation task that required 14 days of habituation, followed by 4 days of experimental sessions (Raz, 2013). In contrast, aversive tasks often have shorter experimental durations; for example, Han and colleagues (2020) conducted an emotional contagion task involving foot shocks, with the entire experiment lasting only 5 days (Han et al., 2020a). While non-aversive models present many advantages, longer training periods and experimental timelines can pose limitations due to increased resource requirements. Additionally, prolonged sessions may influence findings if rodents perform learned actions primarily related to extended training or in anticipation of a reward.

1.5.2 Limitation of existing studies: assessment of behaviors

Rodent models allow researchers to assess behaviors in a controlled environment, where rodents display various body cues in response to their surroundings and social interactions. (Ebbesen & Froemke, 2021). In social contexts, rats and mice display a range of communicative behaviors, including ultrasonic vocalizations (USVs; Simola & Granon, 2019), olfactory signaling and sniffing (Deschênes et al., 2012; Sullivan et al., 2015), grooming (Keller et al., 2022; Wu et al., 2021), play fighting, and rough-and-tumble play (Pellis et al., 2022; Pellis et al., 2023). Other behaviors include huddling (e.g., lying close together; Wilson, 2017) and dominance displays (e.g., boxing, submissive postures, aggressive grooming; Schweinfurth, 2020). The assessment of these behaviors, in addition to observing specific actions, can deepen our understanding of prosociality in rodent models. While aversive tasks provide visual cues of fear, stress, or pain (e.g., freezing, squinting, ear retraction; Ebbesen & Froemke, 2021; Singewald & Holmes, 2019), body language in non-aversive contexts remains underexplored in rodents (Ebbesen & Froemke, 2021). To address this, non-aversive models often incorporate specific actions (e.g., lever pressing, nose poking, entering compartments) that facilitate objective observation. Recent studies indicate that rodents exhibit distinct body language during positive emotional states, such as jumping (Ishiyama & Brecht, 2016) and ear blushing (Finlayson et al., 2016). Additionally, USVs can reflect both negative and positive emotional states, with rats and mice producing ultrasonic frequencies ranging from 30 to 110 kHz (Premoli et al., 2023; Holy & Guo, 2005). Research indicates that 50-kHz USVs and 22-kHz USVs are respectively associated with positive and negative affect (Engelhardt et al., 2018).

Behavioral analysis has been largely overlooked in the field of behavioral neuroscience (Krakauer et al., 2017). Assessing behaviors during prosocial tasks is essential for a comprehensive understanding of this concept. Not only does this enrich research findings, but it also enhances animal welfare through refinement strategies that maximize data collection (e.g., using statistical methods, video recording, or USVs).

1.6 Thesis objectives

Rodent models can offer important insights into the behavioral and cerebral mechanisms underlying prosociality, with the objective of eventually translating this knowledge to benefit human conditions. Currently, rodent paradigms on prosociality lack proper standardization. This research program includes three distinct studies with the following objectives: (1) to assess a sharing task using two strains of rats while investigating the potentially mediating role of pretraining sessions on prosocial behaviors, (2) to conduct a scoping review specifically on prosocial models to highlight current findings and gaps in methodologies, and (3) to develop a conceptual framework on rodent prosociality to increase the reproducibility and validity of prosocial models in rodents.

Chapter Two includes Study One, published in *Animal Behavior and Cognition* (2022). Using a quasi-experimental design, this study employed a novel sharing paradigm to investigate prosocial behavior in two strains of adolescent rats. The goals were (1) to characterize baseline prosocial behaviors in a non-aversive task, (2) to investigate the impact of potential mediating factors on prosociality (e.g., pretraining sessions, strain, vicarious learning), and (3) to promote the use of advanced statistical methods (e.g., transitional probabilities analysis) to refine analyses of the rats' behavioral interactions. This study addresses the first research objective stated above.

Chapter Three includes the findings of Study Two, which have been published in *PLOS ONE* (2024). This scoping review aimed to characterize current findings regarding prosocial paradigms in rodents and highlight gaps in reporting and mediating factors, addressing the second objective of this thesis.

Chapter Four includes Study Three, which developed a conceptual framework that aimed to (1) reframe prosociality as a set of complex behaviors emerging in response to environmental determinants that cannot be reduced to a single set of data, (2) highlight important methodological considerations, mediating variables, and behavioral analyses that influence prosocial behaviors, and (3) present a decision tree as a dynamic element within this conceptual framework to offer guidance to researchers. This study addresses the third objective stated above. Finally, Chapter Five provides a summary of the key results of this research program in relation to the existing literature. Limitations and major contributions for each study are also discussed.

CHAPTER TWO

Study One

Prosocial decision making in an operant box paradigm promotes visual communication and complex behavioral sequences in adolescent rat dyads

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Published in *Animal Behavior and Cognition*

Find publication: https://doi.org/10.26451/abc.09.01.05.2022

Abstract

The adolescent period is marked by intense social play behavior in rats, shown to influence social, cognitive, and emotional processes. The goal of this study was to assess the ability of adolescent rats to display prosocial behaviors through a sharing task and to learn prosociality from vicarious observation. The paradigm involved a pretraining phase, using a two-chamber operant box with two reward differentiated levers on the actor side, providing one and two sucrose pellets respectively upon pressing. Dyads in which actors were not exposed to pretraining sessions acted as controls. The prosocial phase ensued, in which an easy lever pressed dispensed one pellet while a hard lever pressed dispensed one reward to both the actor and observer in the adjacent chamber. Actor and observer rats then switched roles enabling vicarious learning assessment. Findings revealed pretraining to be critical for behavior and task contingency in adolescent rats. Complex behavioral sequences marked by increased visual communication between dyads were observed. Despite the diversity of behaviors, observer rats failed to learn prosocial behaviors. This study shows pretraining to act as a key element promoting behavioral interactions; the thorough behavioral analysis performed highlights the ability for adolescent rats to display a richness of behaviors when paired with a congener. Another interesting finding was the ability for rats to learn prosocial behaviors, but the inability to learn such behaviors by observation. These findings call for further studies to understand prosocial behaviors in rodents and their ability to learn such behaviors from a congener.

Introduction

Research on prosocial behaviors in rats is growing within the fields of comparative psychology and social neuroscience. The concept of prosocial behavior in rodents is commonly referred to as any helping behavior towards a congener to improve their well-being (e.g., freeing a restrained congener, sharing a food reward; Ben-Ami Bartal et al., 2014, Cronin, 2012; Hernandez-Lallement et al., 2015). Many factors can influence the likelihood of rodents behaving in a prosocial manner, such as developmental stages, strain, the paradigm used, and the training components (Meyza et al., 2017). Although the use of aversive tasks (i.e., water aversive task, fear conditioning paradigms) has initially predominated in studies assessing prosocial behaviors in rodents (Church, 1959; Greene, 1969; Lee et al., 2018; Meyza et al., 2017; Sato et al., 2015; Yusufishaq & Rosenkranz, 2013), the last decade has seen an emergence of paradigms using sharing or freeing tasks (Sivaselvachandran et al., 2018). In this context, the study conducted by Ben-Ami Bartal and colleagues (2011) represents a hallmark. These authors were the first to show a rat's motivation to free a cagemate from a restrainer, even when no physical contact was possible once the rat was free. Moreover, in around half of situations in which a choice between freeing the cagemate or receiving palatable chocolate chips had to be made, rats decided to first free the congener and share the food reward (Ben-Ami Bartal et al., 2011). In the same vein, Hernandez-Lallement and colleagues (2015) used a double T-Maze to conceptualize a prosocial choice task in which rats could make a choice between attribution of a single or a mutual food reward by entering a specific maze compartment (i.e., the own-reward compartment, or the both-reward compartment). Rats chose the both-reward compartment when paired with a partner, but not a rat toy, showing that rats preferred the mutual reward with another conspecific (Hernandez-Lallement et al., 2015). Vicarious learning is defined as a behavior acquired through the observation of a conspecific's action and its consequences (Schaik et al., 2016; Zentall, 2016). Similar to prosocial models, vicarious learning has mostly been studied using aversive paradigms (Meyza et al., 2017). Recently, research has moved towards models limiting the use of aversive stimuli (e.g., electric shocks). A study by Yamada and Sakurai (2018) proposed a paradigm in which a rat placed in the middle of a Barnes' maze observed its congener performing the task (i.e., finding a black box allowing it to escape from aversive light). The observer rats were able to find the box faster and escape more quickly than the rats that had not previously observed a congener (Yamada & Sakurai, 2018). It is established that rodents can display prosocial behaviors and learn different tasks from the observation of a congener (Ben-Ami Bartal et al., 2011; Hernandez-Lallement et al., 2015; Meyza et al., 2017; Yamada & Sakurai, 2018). However, abilities of rodents to learn prosocial behaviors through vicarious observation remain undetermined.

To date, studies have focused on characterizing prosociality during adulthood, and other developmental periods have been neglected. Considering the well-known and significant brain reorganization taking place during adolescence that is marked by behavioral changes, heightened brain development, and cognitive maturation (Caballero et al., 2016; Walker et al., 2017), it is surprising that few studies have assessed prosocial behavior and associated communication patterns in adolescent rats. Adolescence is a period of remodeling of the brain architecture in which hormones play a role along with experience (Sisk & Zehr, 2005). The prefrontal cortex, which undergoes a prolonged course of maturation well after puberty, is exquisitely vulnerable in the adolescent period as well as the interconnected amygdala, hippocampus, and mesolimbic/nigrostriatal systems (Caballero et al., 2016), all of which might influence prosocial behaviors. Furthermore, the adolescent period is characterized by intense social play behaviors, which is of particular interest when studying prosocial behaviors (Manduca et al., 2014), and in which rapid growth in learning capacity and adaptability has been described (Dahl et al., 2018). For instance, social play in adolescent rats, which consists of rough-and-tumble play, is known to induce positive vocalization and play an important role in emotional, social, and cognitive development (Achterberg et al., 2019; Lampe et al., 2019). Adolescent rats deprived of social interactions show increased anxiety and depressive-like behaviors (Burke et al., 2017), which supports the idea that social contact is crucial for healthy neurobehavioral maturation.

Studies assessing prosocial behaviors have indicated benefits of using a pretraining phase to familiarize rats to the environment and the rewards associated with a specific action (e.g., lever press; Oomen et al., 2013). Brady and Floresco (2015) showed that 10 to 20 sessions are sufficient in operant procedures to familiarize rats with the levers and associated rewards. The reward provided during pretraining can vary, with some using a fixed ratio of one reward/reinforcement per lever press (Brady & Floresco, 2015) while others recommend the use of different lever-reward contingencies to facilitate lever discrimination, and later test the willingness of the actor to work harder towards reward delivery to a congener in the prosocial phase (Horner et al., 2013). Although pretraining influences learning in operant tasks, its contribution to the development of communication patterns in rat dyads has not been explored. Communication and behavioral interactions can be considered key elements in understanding prosocial behaviors in rat dyads (Martin et al., 2014). These can be studied with ultrasonic vocalizations, which can unravel information about the emotional state of rats such as positive affect (Engelhardt et al., 2018; Łopuch & Popik, 2011; Seffer et al., 2014) or statistical tools like discriminant analyses (O’Connor, 1999). Results from Ben-Ami Bartal and colleagues (2011) highlight the importance of studying behaviors in addition to task performance. Rats that opened the restrainer were considered prosocial based on the performed task (i.e., opening of the restrainer) in conjunction with previously observed behavioral interactions (circling the restrainer, contacting the trapped cagemate through the holes in the restrainer; Ben-Ami Bartal et al., 2011). It is paramount to pursue the assessment of behaviors in addition to task performance to better understand prosociality in rodents.

Different rat strains have been tested in prosocial paradigms, including Long-Evans (Atsak et al., 2011; Hernandez-Lallement et al., 2015) and Sprague-Dawley rats (Ben-Ami Bartal et al., 2011; Lee et al., 2018). Although both strains are known to be social and perform well in operant tasks (Ku et al., 2016), studies have suggested behavioral differences between these strains. Pigmented rats (e.g., Long-Evans) are known to perform better in visual and in lever press tasks (Andrews et al., 1995; Gökçek-Saraç et al., 2015), and to be more active during daytime than albino rats (e.g., Sprague-Dawley; Stryjek et al., 2013). In contrast, Sprague-Dawley rats engage more frequently and for longer periods of time with a novel congener during a social dyad task than Long-Evans rats (Ku et al., 2016). Strain-specific responses remain poorly characterized, although such assessments may help establish genomic changes mediating prosocial, biochemical, and behavioral responses.

The main objective of this study was to characterize baseline prosocial behaviors in adolescent Long-Evans and Sprague-Dawley male rats using an operant box paradigm. As part of this endeavor, we determined the role of a pretraining phase in shaping the actor rats' prosocial responses (i.e., reward sharing). An additional goal of this study was to assess vicarious learning and determine if being exposed to prosocial behaviors once in the observer role influence behavior upon becoming the actor. Finally, this study is the first to use transitional probabilities analysis to determine the influence of prosocial behavior on communication patterns and behavioral responses (complexity and repertoire of behavioral sequences) observed in adolescent rat dyads.

Methodology

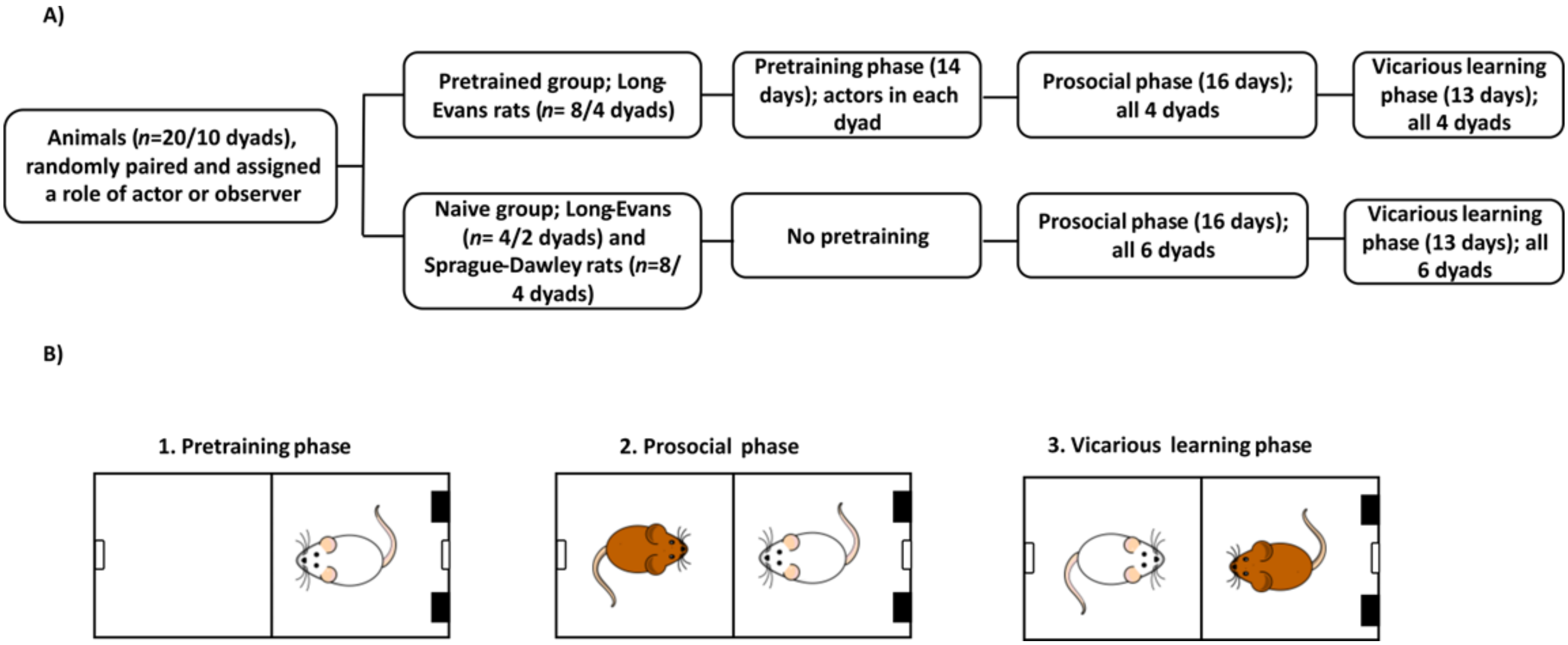
Ethic statement

All experimental procedures were approved by the University of Ottawa Animal Care Committee (Protocol Review Group) and met the guidelines put forth by the Canadian Council on Animal Care.

Animals and housing

Male Long-Evans and Sprague-Dawley rats (n = 20) were obtained from Charles River Laboratory (Québec, Canada). Rats arrived at the animal care facility at post-natal day (PND) 23 and 28 for the pretrained and naive (not exposed to pretraining) group, respectively (see Figure 1A for group repartition). Considering ethical reasons (e.g., use of a minimal number of animals), assessment of strain differences on prosocial behaviors was limited to the naive group only. Upon arrival, rats of each strain were housed in pairs in a temperature/humidity-controlled room (22ºC / 60% humidity level) under a 12h dark/light cycle (lights on at 7 AM) with ad libitum access to food (Teklad Global 18% Protein Diet manufactured by Envigo) during the 2 days of habituation. Daily handling began on day 3 of arrival. Starting on day 5, rats were food restricted at 85-90% of free feeding body weight. Rats were regularly weighed, and all showed adequate weight gain as the experiment progressed. Upon testing initiation, Long-Evans and Sprague-Dawley body weights ranged between 200-225 g and 108-200 g, respectively. Testing of the pretrained and naive rats was initiated on PND 30 and PND 35, respectively. Food rewards used during the testing consisted of 45 mg chocolate flavored sucrose tablets (Test Diet, USA).

Figure 1. Phases of the Experiment.

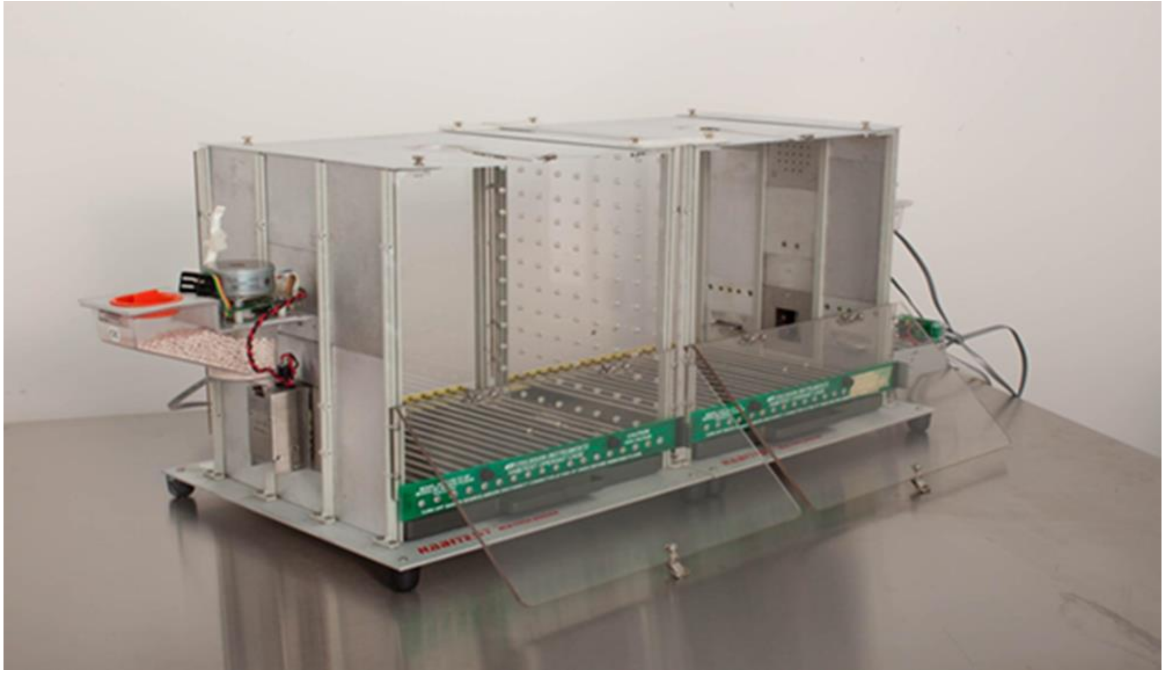


Note. A) The three conditions (pretraining, prosocial and vicarious phases) included testing sessions that lasted 60 min. Testing of the pretrained and naive rats was initiated on PND 30 and PND 35, respectively. B) 1- The pretraining phase enabled an actor rat to learn the reward contingencies associated with the two levers. Actor rats in the naive group were not exposed to this phase. 2- The prosocial phase assessed the willingness of an actor rat (right side) to press a hard lever delivering one pellet to itself and one to the congener (prosocial behavior) or select the easy lever delivering one pellet to itself. 3- Following the prosocial phase, actor and observer rats switched compartments enabling the assessment of vicarious learning in naive and pretrained observer rats.

Apparatus

Two Coulbourn Instruments Habitest™ operant boxes (30 cm x 25 cm x 30 cm) were modified as to our protocol's needs (see Figure 2). Graphic State 3.03™ from Coulbourn Instruments was used as the data acquisition software.

Figure 2. Modified Coulbourn Operant Box.



Note. Modified Coulbourn InstrumentsⓇ Habitest™ operant box with two compartments separated by a perforated acrylic sheet. On the right side of the box (the actor side), two levers, cue lights and a feeder were present while on the left side (the observer side), only a feeder was present.

Procedure

Pretraining phase. Only Long-Evans actor rats (n = 4) that were part of the pretrained group were exposed to pretraining. Rats were placed in the right-hand side chamber compartment (left side remaining empty) with accessibility to the two levers, the easy lever (35 g of pressure) delivering one pellet and the hard lever (75 g of pressure) delivering two pellets. The hard lever was identified by a flashing cue light placed above it, while the easy lever was identified by a steady light. Levers were changed regularly with their associated light stimuli once every 5 days of testing to prevent location preference. The pretraining phase lasted a total of 14 days, following the recommendation of Brady and Floresco (2015), who stated that a pretraining phase should last between 10 to 20 days. After the 14-day pretraining period, all rats reached the minimum criterion of 90 presses on each lever per session, confirming that sufficient exposure to the levers had occurred for all rats. Actor rats from the naive group were not exposed to any form of pretraining.

Prosocial phase. During this phase, the lever settings remained the same as during the pretraining. All dyads of rats (n = 10 dyads) participated in this phase, which lasted 16 days for both groups. The duration of this phase was based on the pretraining and previous studies (Ben-Ami Bartal et al., 2014; Tomek et al., 2019). The prosocial phase introduced an observer rat (the actor's cagemate), which was placed in the compartment adjacent to the actor, with sole access to a feeder. The actor's task remained identical, except that a press on the hard lever now provided one pellet to itself and one pellet to the observer, while the easy lever dispensed a pellet to the actor only.

Vicarious learning phase. The vicarious learning phase was meant to assess how well the observer performed after witnessing the prosocial phase. This phase used the same experimental protocol, except that the observer rat exchanged roles with their previous assigned actor rat (the actor is now in the other compartment, witnessing the task). This third phase lasted 13 days for both groups (Bem et al., 2018; Carlier & Jamon, 2006).

Behavioral recording

All sessions were recorded, the frequency and duration of behaviors were coded by two graduate students using the Boris behavioral coding software (Friard & Gamba, 2016). To assess the intercoder reliability, four sessions (equal to four hours of video) were randomly selected, coded, and analyzed. The intercoder reliability for all variables was ranging from .81 to .91, using the time-unit Kappa in the GSEQ 5.1 software, translating to a strong level of agreement (Quera et al., 2007). In total, six behaviors were observed and quantified (see Table 1 for behavior description). The behavioral recordings were used to assess and compare the behavior frequency and transition in the actor and observer rats.

Table 1. Operational Definition of Analyzed Behaviors.

Behavior	**Action**	**Operational Definition**
Communication	Mutual	Both rats stand in contiguity of the acrylic walls, side by side or facing each other, rearing, or standing on four paws, excluding grooming.
Looking	Mutual	Both rats directly looking at each other from a distance of the acrylic wall.
Prosocial decision making	Actor	The actor approaches the easy lever, actively explores it (i.e., touching with nose or paws, sniffing, looking), and decides to press the hard lever.
Selfish decision making	Actor	The actor goes towards the hard lever, actively explores it (i.e., touching with nose or paws, sniffing, looking), and decides to press the easy lever.
*Fetching hard lever reward	Observer	When the actor presses the hard lever, the observer goes looking into his feeder. *Only when following a prosocial decision making.
*Fetching easy lever reward	Observer	When the actor presses the easy lever, the observer goes looking into his feeder. *Only when following a selfish decision making.

Note. Behavioral data logging enabled comparison of behavioral responses and characterized behavioral sequences in pretrained and naive rat dyads. *Fetching behaviors enabled the assessment the observers' understanding of the task. A rat that goes towards its own feeder when its congener presses the hard lever could indicate an understanding that a reward pellet will also be delivered to itself. However, a rat that goes towards its feeder when the easy lever was press can indicate that it did not understand the function of levers and thus the task itself.

Statistical analysis

All statistical analyses were performed using IBM SPSS (version 23). Data were analyzed using a one-way multivariate analysis of variance (MANOVA) for lever press (easy and hard presses being two separate measures), differences between groups, and the frequencies of behaviors. For each of the MANOVA analysis, the mean of each rat was analyzed thus removing the factor of variability. A strain comparison of performance (easy and hard lever presses) and behavior frequencies were assessed using independent samples t-tests. Homogeneity of variance was verified using the Levene's test. When significant, the Welch t Test Statistic was used. Data are presented as mean ± SEM. Statistical significance was set to $p < .05$.

Behavioral sequences between groups were analyzed using transitional probability analysis with an alpha level of .05 (O'Connor, 1999). The discriminant analysis suit developed by O'Connor (1999) produces a series of statistical analyses that allows the identification of behavioral sequences and the comprehension of its asymmetrical distribution. Within the application, there is the transitional probability analysis, which produces a likelihood ratio of behavioral sequences (e.g., There is an "X%" chance that behavior "A" will be followed by behavior "B") accompanied by Z scores, significance levels, and descriptive statistics. Based on the observed frequencies of the monitored behaviors, this analysis compares real observations with expected behavioral transitions that assumes no interdependency between behaviors. It produces a statistical significance level between behaviors and their transitions indicating if the likelihood ratio is valid and accurate. For additional information on likelihood ratio calculations and transitional probability analysis, see O'Connor (1999).

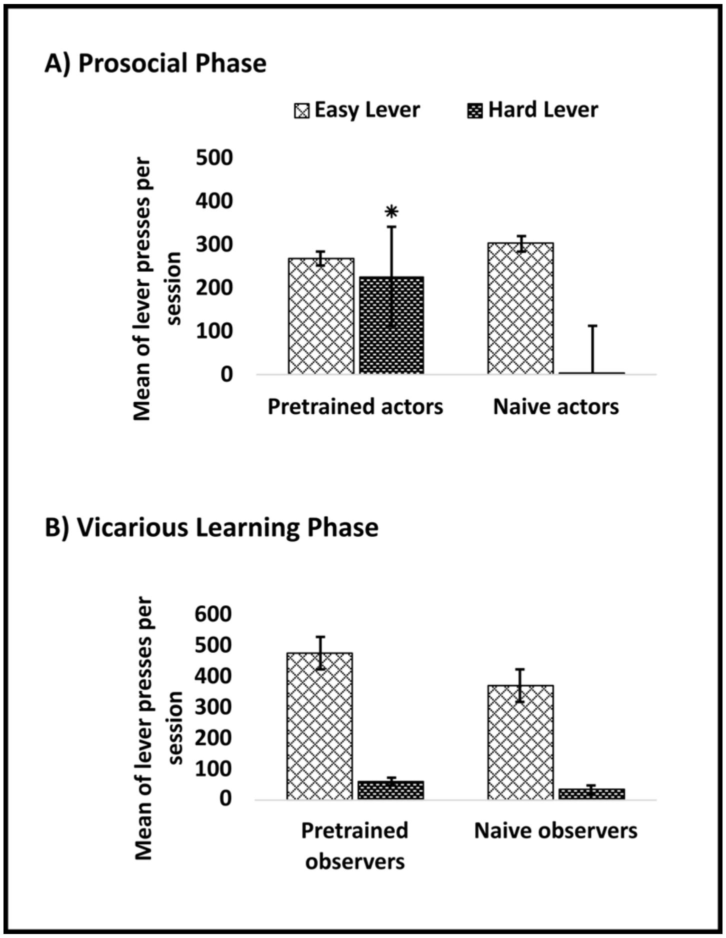
Results

Group comparisons

A one-way MANOVA assessed two separate measures of performance: the mean of lever presses on the easy and hard levers. Groups were the following: 1) pretrained actors, 2) naive actors, 3) pretrained observers and 4) naive observers. For the visual representation of the data, see Figure 3A and B.

Preliminary assumption checking revealed that data was normally distributed, as assessed by a Normal Q-Q plot. There was one univariate outlier (removed from the dataset), as assessed by boxplot and eight multivariate outliers assessed by the Mahalanobis distance ($p < .001$). After analysis with and without the eight outliers, they were kept in the data as the decision rule did not affect the result of the one-way MANOVA. There were linear relationships, as assessed by scatterplot and no multicollinearity between the variables ($r = .272$, $p < .001$). There was no homogeneity of variance-covariance matrices, as assessed by Box's M test ($p < .001$). Because this assumption was not fulfilled, a Pillai’s Trace effect was used instead of a Wilks' Λ. Additionally, since the Levene’s test of equality of variances was significant ($p < .05$), a lower alpha level of .025 ($p < .025$) was used. Considering a small sample size, a power analysis was conducted using G*Power (version 3.1.9.4) for the MANOVA analysis (O’Keefe, 2007). A statistical power of 0.733 ($\beta = 0.733$) was achieved using a conservative alpha value of .025 ($\alpha =.025$).

Figure 3. Pretraining Modulates Prosocial Choices but Does Not Affect Vicarious Learning.



Note. A) Pretrained actors pressed more on the hard lever, thus choosing to share a reward with their congener more often compared to naive rats. B) Exposure to the prosocial phase had no impact on the behaviors of the observer rats once becoming actors. *denotes significant difference from naive actors, $p < .05$.

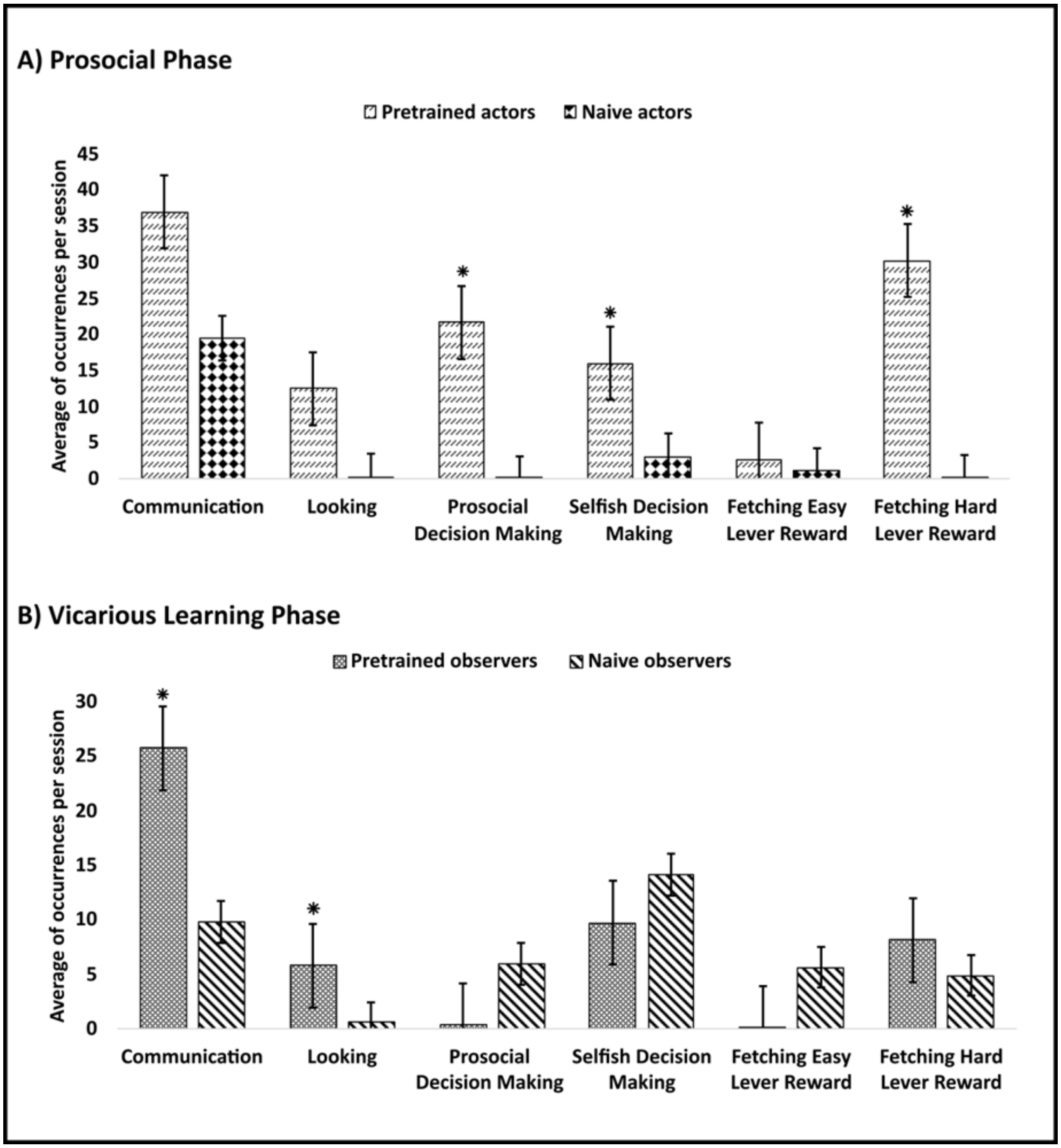
The descriptive statistics of lever presses indicated that, for the prosocial phase, pretrained actors pressed more on the hard lever (M = 227.06, $\sigma\bar{x}$ = 17.95, 95% CI [191.71, 262.41]) than naive actors (M = .53, $\sigma\bar{x}$ = 14.66, 95% CI [-28.33, 29.39]), which pressed more on the easy lever (M = 303.38, $\sigma\bar{x}$ = 37.42, 95% CI [229.71, 377.05]) than pretrained actors (M = 269.93 $\sigma\bar{x}$ = 45.83, 95% CI [179.71, 360.16]). Testing vicarious learning, observers from both pretrained and naive groups showed no differences in lever presses, both groups making significantly more easy lever presses (M = 475.92, $\sigma\bar{x}$ = 50.85, 95% CI [375.82, 579.02]; M = 371.02, $\sigma\bar{x}$ = 41.79, 95% CI [288.76, 453.28], respectively) than hard lever presses (M = 58.98,

$\sigma\overline{x}$ = 19.92, 95% CI [19.76, 98.19]; M = 31.92, $\sigma\overline{x}$ = 16.37, 95% CI [-0.30, 64.14], respectively). Results showed that groups differed in terms of hard lever presses (F(3, 285) = 34.882, $p = .013$; partial $\eta 2$ = .269) and easy lever presses (F(3, 285) = 3.645, $p < .001$; partial $\eta 2$ = .037). Games-Howell post-hoc tests showed that in the prosocial phase, the pretrained actors made significantly more hard lever presses compared to the naive actors ($p < .001$, 95% CI [148.87, 304.18]). No differences were observed between the pretrained and the naive actors in easy lever presses ($p = .911$, 95% CI [-164.91, 98.01]; see Figure 3A). For the vicarious learning phase, Games-Howell post-hoc tests showed no significant differences on the hard lever presses ($p = .761$, 95% CI [-45.54, 99.66]) and the easy lever presses ($p = .473$, 95% CI [-83.95, 293.75]) between pretrained and naive observers (see Figure 3B).

Behavioral analysis

Behavior frequencies

Figure 4. Pretraining Promotes Interaction and Communication Behaviors in Adolescent Rat Dyads.



Note. A) During the prosocial sessions, pretrained actors made significantly more prosocial decision making than naive actors. Notably, observer rats in pretrained dyads reached their feeders significantly more often following hard lever presses by the actor, suggesting an understanding of the lever contingencies. B) Exposure to prosocial behavior did not influence prosocial behavior of observer rats. However, rats from pretrained dyads communicated and looked at each other more often during vicarious testing sessions than rats from naive dyads. *denotes significance, $p < .05$.

A one-way MANOVA was used to determine the effect of a pretraining phase on behavior frequencies, treating the number of observations as units. See Figure 4A and B for visual representation of the data. Six behaviors were assessed (Communication, Looking, Prosocial decision making, Selfish decision making, Fetching hard lever reward, Fetching easy lever reward; see Table 1 for operational definition of these behavioral responses). For detailed descriptive statistics of all coded behavior, see Table 2.

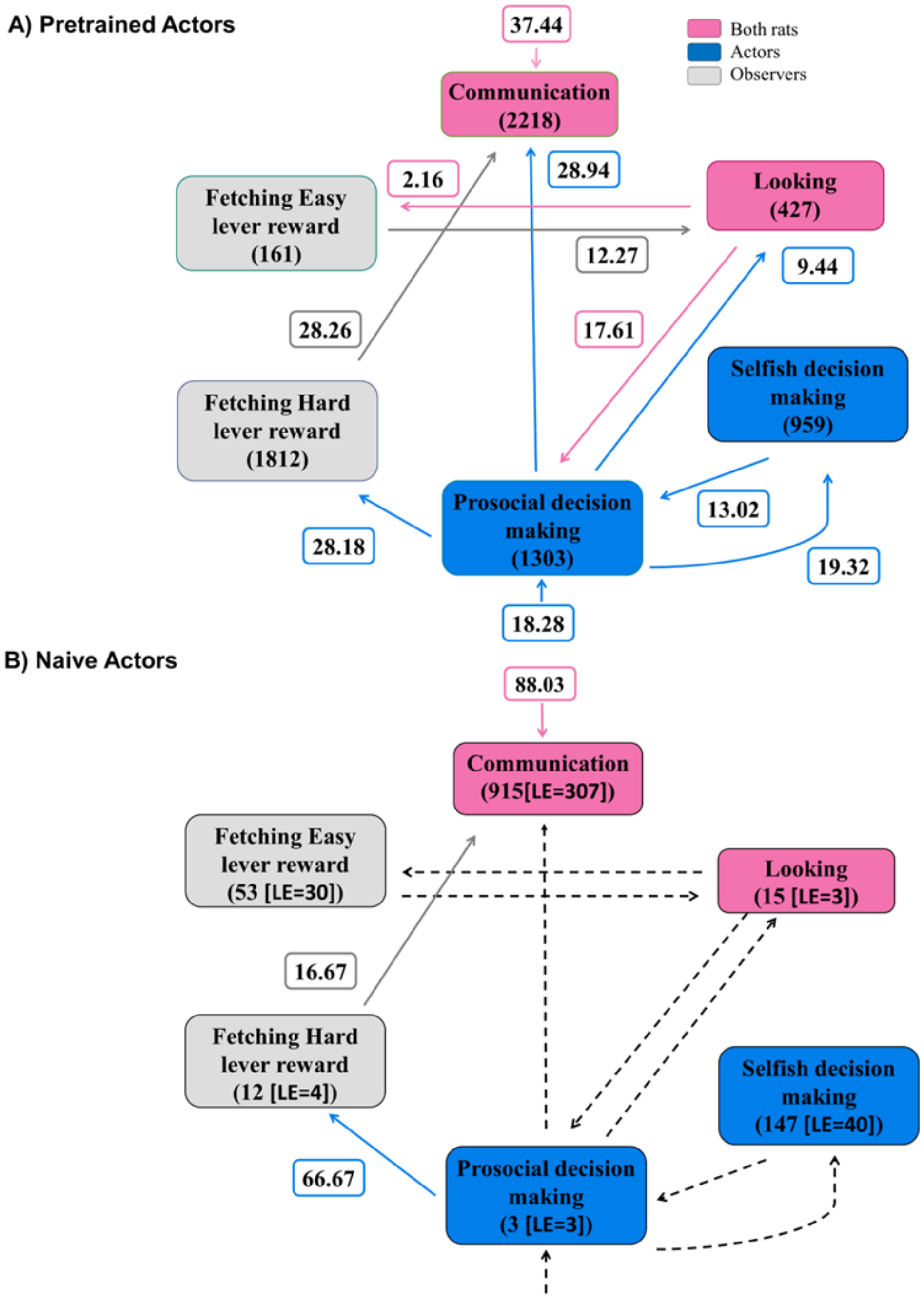
Table 2. Descriptive Statistics of All Coded Behaviors.

Behavior	**Group**	**Frequency**	**Mean**	**Std. Error**	**95% CI Lower Bound**	**95% CI Upper Bound**
Communication – Total duration	Pretrained actors	NA	213.31	18.69	176.40	250.22
	Naive actors	NA	258.07	21.11	216.37	299.78
	Pretrained observers	NA	190.59	31.59	128.20	252.97
	Naive observers	NA	80.20	23.48	33.82	126.57
Communication – Total of Occurrences	Pretrained actors	2218	36.96	1.70	33.60	40.32
	Naive actors	915	19.46	1.92	15.67	23.26
	Pretrained observers	542	25.81	2.87	20.13	31.48
	Naive observers	372	9.78	2.13	5.56	14.01
Looking – Total duration	Pretrained actors	NA	12.21	1.47	9.30	15.12
	Naive actors	NA	0.80	1.66	-2.48	4.09
	Pretrained observers	NA	14.84	2.49	9.92	19.76
	Naive observers	NA	0.87	1.85	-2.78	4.52
Looking – Total of occurrences	Pretrained actors	427	7.20	0.82	5.57	8.82
	Naive actors	15	0.31	0.93	-1.52	2.15
	Pretrained observers	122	5.81	1.39	3.05	8.56
	Naive observers	22	0.57	1.03	-1.46	2.62
Prosocial Decision Making	Pretrained actors	1303	21.71	2.93	15.92	27.51
	Naive actors	3	0.06	3.31	-6.48	6.61
	Pretrained observers	7	0.33	4.96	-9.46	10.13
	Naive observers	226	5.94	3.68	-1.33	13.23
Selfish Decision Making	Pretrained actors	959	15.98	2.29	11.45	20.50
	Naive actors	147	3.12	2.59	-1.98	8.24
	Pretrained observers	204	9.71	3.87	2.06	17.36
	Naive observers	537	14.13	2.88	8.44	19.81
Fetching Hard Lever Reward	Pretrained actors	1812	30.20	2.33	25.58	34.81
	Naive actors	12	0.25	2.64	-4.96	5.47
	Pretrained observers	171	8.14	3.95	0.33	15.94
	Naive observers	38	4.89	2.93	-0.90	10.69
Fetching Easy Lever Reward	Pretrained actors	161	2.68	0.98	0.74	4.62
	Naive actors	53	1.12	1.10	-1.06	3.31
	Pretrained observers	2	0.09	1.65	-3.18	3.37
	Naive observers	214	5.63	1.23	3.19	8.06

Preliminary assumption checking revealed that data were normally distributed, as assessed by a Normal Q-Q plot. There were no univariate outliers in the data, as assessed by inspection of a boxplot for values greater than 1.5 box-lengths from the edge of the box and 11 multivariate outliers assessed by the Mahalanobis distance ($p < .001$). After analysis with and without these 11 outliers, they were kept in the data as they did not affect the results of the one-way MANOVA. There were linear relationships, as assessed by scatterplot and no multicollinearity ($r < .90$, $p < .001$). There was no homogeneity of variance-covariance matrices, as assessed by Box's M test ($p < .001$). Because this assumption was not fulfilled, a Pillai's Trace effect was used instead of a Wilks' Λ. Additionally, since the Levene's test of equality of variances was significant ($p < .05$), a lower alpha level of .025 ($p < .025$) was used. Analysis revealed significant difference in behavioral responses between groups ($p < .020$). Games-Howell post-hoc tests showed that there was a significant difference between pretrained and naive dyads ($p < .024$) for all analyzed behaviors. More precisely, for the prosocial phase, pretrained dyads communicated more often ($p < .001$), looked at each other more often and for longer periods of time ($p < .001$) and had more prosocial decision making ($p < .021$) than naive dyads (see Figure 4A). As for the vicarious learning phase, the naive dyads communicated less and for shorter periods of time ($p < .003$) and looked less often and for shorter periods of time at each other compared to pretrained dyads ($p < .001$; see Figure 4B).

Transitional probability of behavioral responses. Transitional probability analyses were performed using O'Connor's SEQGROUPS SPSS syntax program (O'Connor, 1999) between naive and pretrained dyads. Data logging of six behaviors during the 16 sessions of the prosocial phase (i.e., Communication, Looking, Prosocial decision making, Selfish decision making, Fetching hard lever reward, Fetching easy lever reward). See Figure 5 A and B for a visual representation of the data. An assumption to perform the transitional probability analysis is a confirmation of behavioral interdependence in each of the groups. The analysis revealed that the likelihood ratio chi-square values reached significance for the pretrained actors (χ2 (25, 7205) = [83.3], p < .001), naive actors (χ2 (25, 1157) = [221.67], p < .001), pretrained observers (χ2 (25, 572) = [403.86], p < .001) and naive observers (χ2 (25, 1556) = [1160.41], p < .001), indicating an interdependence in the transition of behaviors within the naive and the pretrained groups. Incidentally, the likelihood ratio indicates the percentage of which a behavior might follow another (e.g., X% that the behavior “A” follows behavior “B”) but is not indicative of the number of occurrences of the targeted behavior. For instance, the naive group displayed 915 communication behaviors over the 16 testing sessions, and in this group, there was an 88% probability that a communication behavior was followed by another communication. In contrast, the pretrained group communicated 2218 times over the 16 sessions but this group showed only a 37% probability for the same behavior (see Figure 5). In this context, the transitional probability analysis examined sequences of behavioral responses from the actor to the observer rat, and vice versa. Analyses of behavioral responses of the pretrained dyads in the prosocial phase revealed a 37.44% (z = 7.34; p < .001) probability that communication would be followed by a second communication behavior. Moreover, the probability for a prosocial decision being followed by a communication was 28.94% (z = -2.22; p = .02) and 28.18% for that behavior being followed by the observer fetching a reward in its feeder (z = 2.20; p = .02), suggesting that the observers associated a hard lever press by the actor with a reward pellet gain. Following fetching behavior, the probability for a communication between dyads was 28.26% (z = -3.51; p < .001; see Figure 5). As for the naive actors in the prosocial phase, the transitional probability of communication following any other behavior was very low (Looking: z = -1.96; p = .04, Prosocial Decision Making: z = - 2.03; p = .04, Selfish Decision Making: z = -8.57; p < .001, Fetching Easy Lever Reward: z = -8.62; p < .001, Fetching Hard Lever Reward: z = - 4.08; p < .001) except for a communication being followed by another communication (88.03%; z = 13.52; p < .001). Moreover, communication was rarely preceded by either the prosocial (0.11%; z = -2.03; p = .0420) or selfish decision making (8.52%; z = -8.578; p < .001). After a prosocial decision making, the probability for the observer to go towards its feeder was higher (66.67%; z = 11.23; p < .001), suggesting that observers associated a hard lever press by the actor with a reward. The transitional probability also revealed many behavior omissions, such as the probability that a prosocial decision being followed by a communication was completely absent from the behavior sequences (p < .001; see Figure 5 for all behavior omissions).

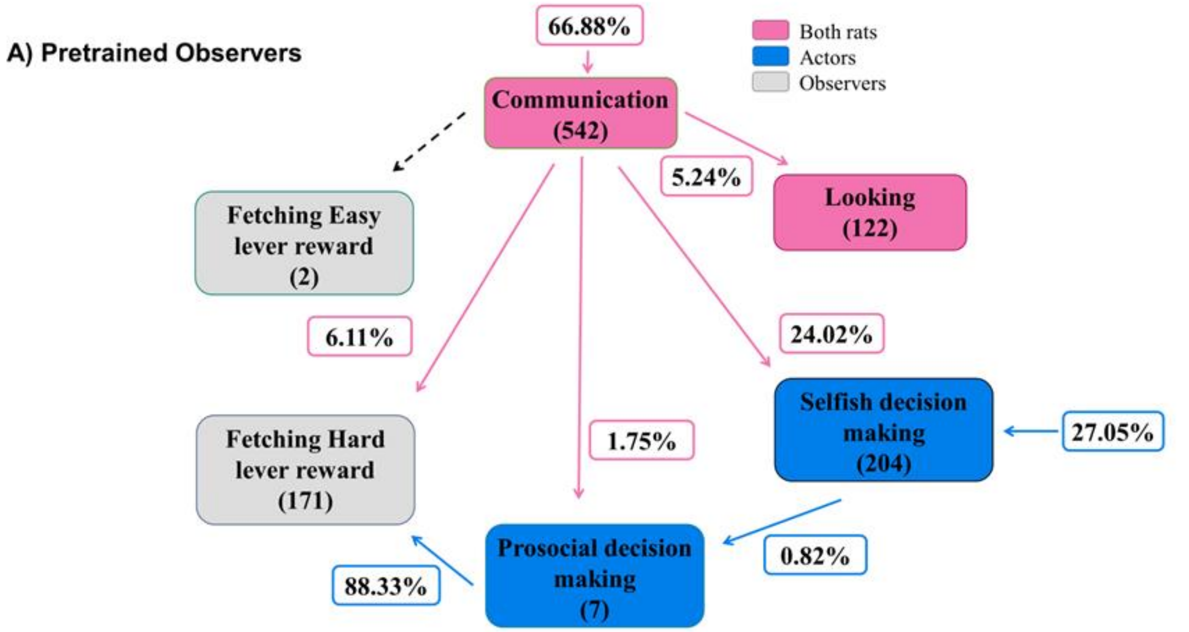
Figure 5. Transitional Probabilities in Behavioral Responses in Pretrained vs Naive Actors (Prosocial Phase).

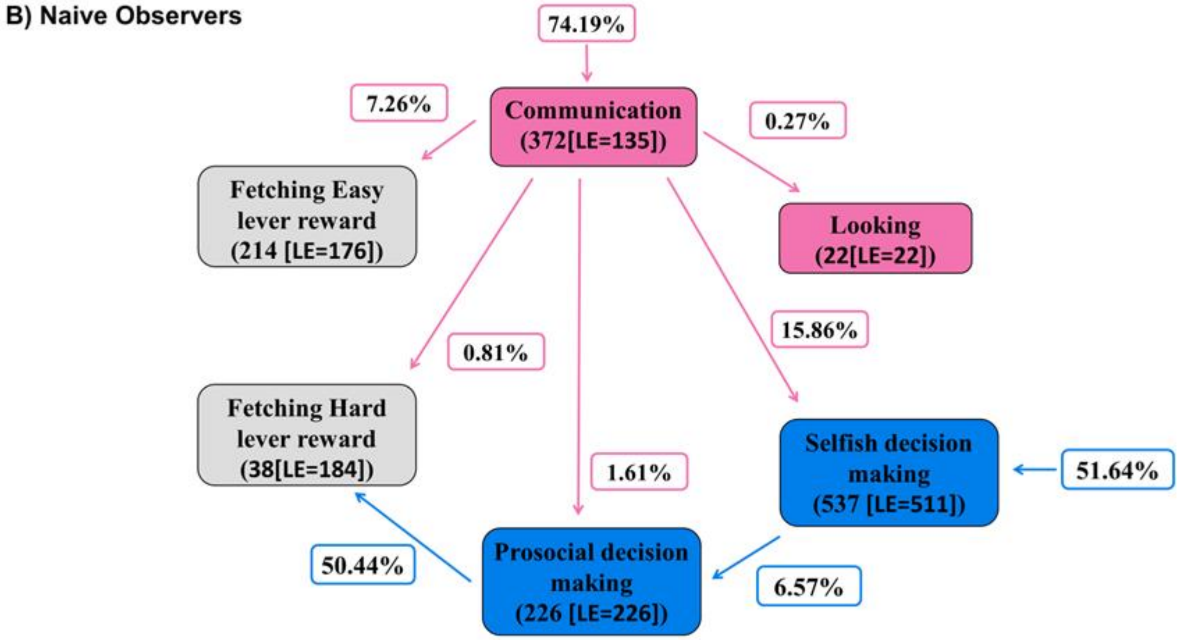


Note. Pretrained actors (A) showed behavioral diversity and complex behavioral sequences compared to naive actors (B). This is manifest through significant differences in frequencies of the logged behaviors (indicated in parentheses) and by arrows indicating probabilities (in %) for one behavior to be related to another one (e.g., fetching hard lever being followed by communication). Dotted lines indicate the absence of behavioral relationships in the naive dyads. LE = Long-Evans.

As for the vicarious phase (see Figure 6A and B), there was a probability of 83.33% (z = 4.84; p < .001) that the pretrained observers would retrieve their reward after the actor made a prosocial decision by pressing the hard lever. This could suggest that the observers understood the relationship between a hard lever press and the delivered reward of one sucrose pellet (see Fig. 6A). For the group of naive observers, the probability for a communication to be followed by another communication was elevated (74.19%, z = 26.12; p < .001; see Figure 6B). However, there were very low probabilities for communication between naive dyads to be followed by any other monitored behaviors (Fetching easy lever reward: 7.26%; z = - 4.17, p < .001; Fetching hard lever reward: 0.81%; z = -7.59; p < .001; Looking: 0.27%; z = -2.14, p = .03; Prosocial decision making: 1.61%; z = -8.10, p < .001; Selfish decision making: 15.86%; z = -8.67, p < .001). Following a selfish decision making, the probability of pressing the easy lever again was elevated (51.64%; z = 5.60; p < .001) compared to the probability of a prosocial decision making – i.e., pressing on the hard lever (6.57%; z = -3.50; p < .001). These findings indicate that the responses of the observers once becoming actors were not influenced by their association with a pretrained or naive actor. Our findings did not support vicarious learning.

Figure 6. Transitional Probabilities in Behavioral Responses in Pretrained vs Naive Observers (Vicarious Phase).


A) Pretrained Observers


B) Naive Observers

Note. Pretrained observers (A) and naive observers (B) both showed fewer behavioral diversity than rats in the prosocial phase (Figure 5 A and B). (B) Long-Evans did the entirety of the prosocial decision making, and nearly all of the selfish decision making, showing more activity The collected data than Sprague-Dawley. Dotted lines indicate the absence of behavioral relationships in the dyads. LE = Long-Evans.

Strain differences. An independent-samples t-test was run to determine if there were differences between Long-Evans (LE) and Sprague-Dawley's (SD) performance in the naive groups. The data was normally distributed, as assessed by a Normal Q-Q plot and there were five outliers as assessed by inspection of a boxplot. After inspection, these 5 outliers were removed from the data due to unusually high lever presses. The assumption of homogeneity of variances was rejected, as assessed by Levene's test for equality of variances (p < .001). Therefore, the Welch t Test Statistic was used. Sprague-Dawley rats pressed more on the easy lever than Long-Evans rats did (M = 383.93, $\sigma\bar{x}$ = 438.89, 95% CI [303.71, 464.14]; M = 233.46, $\sigma\bar{x}$ = 225.08, 95% CI [175.53, 291.38], respectively). As for the hard lever, no differences were found between Long-Evans and Sprague-Dawley rats (M = 6.64, $\sigma\bar{x}$ = 20.75, 95% CI [1.30, 11.98]; M = 4.40, $\sigma\bar{x}$ = 15.80, 95% CI [1.51, 7.28], respectively). A significant difference was found in easy lever presses between the LE and the SD, t(167) = -2.89, p < .001, 95% CI [29.29, 271.64] but not in hard lever presses, t(167) = .703, p = .37, 95% CI [-3.87, 8.35].

An independent-samples t-test further examined differences between naive LE and SD's behavioral responses. The data was normally distributed, as assessed by a Normal Q-Q plot and there was no outlier as assessed by inspection of a boxplot. The assumption of homogeneity of variances was rejected, as assessed by Levene's test for equality of variances (p < .001). Therefore, the Welch t Test Statistic was used. Analyses showed no differences between the strains for the mean occurrences of communication behaviors t(57.35) = .469, p = .641, 95% CI [-3.14, 5.06], or mean communication duration t(83) = .005, p = .996, 95% CI [-72.59, 72.97]. There were also no differences in occurrences of looking, t(29.97) = 1.98, p = .056, 95% CI [-0.01, 1.38], but a difference was found in looking duration, t(35.41) = 2.08, p = .045, 95% CI [0.02, 2.07], LE looking at each other for longer time periods than SD rats. Furthermore, LE engaged in more prosocial and selfish decision making compared to SD rats, t(27.04) = 2.11, p = .043, 95% CI [0.25, 16.10]; t(27.81) = 3.30, p = .003, 95% CI [6.60, 28.09], respectively. As for observer rats, LE approached feeders more frequently when the actor pressed the hard and the easy levers than SD rats, t(27.04) = 2.29, p = .030, 95% CI [0.70, 12.37]; t(28.25) = 2.75, p = .010, 95% CI [1.60, 10.96], respectively.

Long-Evans' activity does not impact the naive group. An independent-samples t-test further examined differences between Pretrained and Naive LE's behavioral responses. The data were normally distributed, as assessed by a Normal Q-Q plot and there was no outlier as assessed by inspection of a boxplot. The assumption of homogeneity of variances was rejected, as assessed by Levene's test for equality of variances (p < .001). Therefore, the Welch t Test Statistic was used. Analyses comparing pretrained and naive LE rats revealed that exposure to pretraining sessions led to increased behavioral responses (communication t(74) = 5.33, p < .001, 95% CI [11.08, 24.47]; Looking: t(74) = 5.34, p < .001, 95% CI [4.38, 9.63]; Prosocial decision making: t(74) = 4.77, p < .001, 95% CI [12.50, 30.55]; Selfish decision making: t(74) = 5.03, p < .001, 95% CI [8.13, 18.82]; Fetching hard lever reward: t(74) = 8.83, p < .001, 95% CI [23.16, 36.73]). Only fetching the easy lever reward was not affected t(74) = 0.35, p = .727, 95% CI [-3.79, 5.41]. Consistently, analyses showed pretrained LE rats to press more on both levers compared to naive LE rats (Easy: M = 269.93, $\sigma_{\bar{x}}$ = 37.43, 95% CI [260.45,279.40], M =162.31, $\sigma_{\bar{x}}$ = 27.04, 95% CI [149.06, 175.55], [t(94) = 2.33, p = .022]; Hard: M = 227.06, $\sigma_{\bar{x}}$ = 29.42, 95% CI [219,61, 234,50], M = 0.65, $\sigma_{\bar{x}}$ = 0.36, 95% CI [0.47, 0.82], [t(94) = 7.69, p < .001]).

Discussion

Using a non-aversive protocol, this study demonstrates the contribution of pretraining to increase occurrences of behaviors associated to prosociality in an operant paradigm and its impact to regulate behavioral interactions between adolescent rat dyads. This study is the first to show that pretraining is associated with increased interactions between actor and observer rats, which influence goal directed behaviors in the actor rats. Our findings failed to support vicarious learning in observer rats of the pretrained or naive groups, suggesting that observation even in a rewarding context was not sufficient to foster behavioral emulation of prosocial behaviors. Consistent with previous studies (Andrews et al., 1995; Gökçek-Saraç et al., 2015; Stryjek et al., 2013), Long-Evans rats show increased activity and displayed more prosocial behaviors compared to Sprague-Dawley rats. This study helps to clarify the role of behaviors in prosociality, more specifically the role of communication patterns in a dyad based paradigm. Considering that the adolescent period is characterized by many cerebral and behavioral changes, as well as intense social play, it is an interesting period to assess prosociality in rat dyads (Dahl et al., 2018). Marked by significant brain plasticity, adolescent exposure to different environmental settings (enrichment or stress) has been shown to influence adult responses (both behavioral and biochemical responses; Cotella et al., 2019). This paradigm benefits from sensitivity observed at the adolescent period, which is likely to promote further understanding of brain processes contributing to prosocial behavior. This study demonstrates that adolescent exposure to pretraining can impact behavioral responses in late adolescence/early adulthood, likely impacting neurochemical responses. These findings pave the way for future research on prosocial behaviors in adolescent rats and possible consequences on cerebral development and adulthood.

Pretraining strongly influences actor’s prosocial-like behavioral expression

Our findings indicate that lever discrimination acquired during the pretraining phase is an essential element in developing delayed prosocial responses towards a congener. This is evident through elevated hard lever presses in the pretrained compared to the naive groups, a response associated with significantly increased rewards provided to observer rats in pretrained compared to non-trained dyads. While the naive group was composed of two strains (see strain differences section below), when pretrained Long-Evans were compared to naive Long-Evans only (i.e., excluding Sprague-Dawley rats from analyses), results showed that pretrained Long-Evans pressed more on both levers and engaged in more behaviors than their naive congeners. These findings further support the pretraining to act as an essential element in prosocial responses. Looking at the behavioral sequences, our findings support different behavioral interactions in naive and pretrained dyads. Results from transitional probabilities revealed that pretrained rats displayed a more complex set of behaviors compared to the naive dyads, marked by increased communication that could influence fetching behavior and associated with increased probability of recurrent prosocial hard lever press (see Figure 5A and B). Indeed, the pretrained actors' decision-making process was marked by more frequent prosocial decision making (i.e., an initial approach towards an easy lever ending in a hard lever press). Moreover, the transitional probability analysis also supports prosocial decision making to initiate periods of communication that promoted more interactions (e.g., communication, looking) in the pretrained actors (see Figure 5A). This may suggest that a form of feedback may have happened after the prosocial decision making. Rats can communicate their positive affect such as a prosocial affiliative call and appetitive state at a frequency of 50 kilohertz (Engelhardt et al., 2018; Seffer et al., 2014). Indeed, Łopuch and Popik (2011) found that rats tend to communicate ultrasonically in a paired operant task using the 50 kilohertz frequency. Based on these findings, it is probable that the observed interaction involved a form of ultrasonic communication destined to convey their positive or cooperative feelings to one another. Finally, the observed behaviors in the pretrained actors appears related to pretraining, and the fact that these actors remained in a familiar environment in which they had been exposed to the levers' function and differential reward attributes (Horner et al., 2013).

Interestingly, naive actors had lower behavior frequencies and more rigid behavior sequences compared to pretrained rats (see Figure 5B). Communication followed by any other behaviors was also lower according to the transitional probability analysis. Furthermore, naive actors had three missing relationships between behaviors, all surrounding communication, which were present in the pretrained group (see Figure 5A and B). These missing relationships can inform us that the naive actors had fewer behaviors and significantly lower communication, which according to results presented above, could be related to the lack of a pretraining phase and thus help explain the discrepancy between the pretrained and naive rats task performances.

Our observations strongly support task contingencies learned during the pretraining phase (e.g., familiarization of the rats to the operant box and the lever press-associated rewards) to play a crucial role in the observation of learned prosocial behaviors using an operant task. Specifically, the 14-day pretraining session (using a double reward upon a hard lever press as recommended by Brady and Floresco, 2015) helped discriminate the two levers and acted to minimize generalization between the pretraining and the prosocial phase (Horner et al., 2013; Oomen et al., 2013). Other studies assessing prosociality in rats using aversive conditions also support the importance of repeated exposure to a setting and control gained over a situation as a key factor influencing prosocial behavior– an 'actor' rat is indeed shown to need a series of exposures to a trapped congener for it to gain the ability to rescue the congener rat (Ben-Ami Bartal et al., 2011). Thus, prosociality requires familiarity to express itself. Our findings further demonstrate that prosocial responses are accompanied by singular behavioral responses between the rat dyads. These findings support the contextual setting to significantly influence an animal's disposition to attend to a congener.

Pretraining had no impact on vicarious learning

Our findings failed to confirm that rats observing a pretrained congener performing a task would subsequently learn to display prosocial behaviors by vicarious observation. Interestingly, rats from the vicarious learning phase demonstrated an understanding of the task contingencies as shown by the ability to press on both levers (see Figure 3B) and by appropriately timed reward retrieval (fetching hard lever reward) (see Figure 4A). While rats seemed to understand task conditions through vicarious learning, as seen in previous literature (Ben-Ami Bartal et al., 2011; Hernandez-Lallement et al., 2015; Meyza et al., 2017; Yamada & Sakurai, 2018), a novel finding of this study is the lack of prosocial behaviors displayed by the pretrained and naive observers (see Figure 6). Our results support the ability of rats to display prosocial behaviors, but only in specific scenarios in which rats are directly exposed to task contingencies such as lever pressing, door opening or entering a specific compartment (Ben-Ami Bartal et al., 2011; Hernandez-Lallement et al., 2015). In contrast, although observer rats showed task contingency understanding through fetching behaviors and lever presses, they failed to display the same level of overall behaviors compared to the actors that received the pretraining. These results could indicate that vicarious learning of a prosocial task is not enough to trigger a prosocial response in rats and that pretraining is a necessity for it to be triggered.

Whereas our findings indicate the inability for rats to learn prosocial behaviors by vicarious observation, behavior frequencies revealed that observers that learned from pretrained rats communicated and looked at each other more often and for longer periods of time compared to naive observers (see Figure 4B). Furthermore, naive observers' results revealed that the probability of any behavior following a communication was reduced compared to pretrained observers, and communication frequencies and duration were significantly lower than pretrained observers (see Figure 6). These results may be related to learning from naive actors (i.e., that did not have a pretraining phase), having been exposed to reduced learning opportunities, which hindered their performance.

Interestingly, behavioral analysis of the observer during the prosocial phase showed that it was able to anticipate reward. When the pretrained actor pressed on the hard lever, the probability for the observer to fetch its reward in the feeder was 28% versus 1.70% following an easy lever press, indicating that the pretrained observers learned to discriminate the levers' dispensed rewards (see Figure 5A). A similar response was noted in the naive dyads, although the small number of hard lever presses in this condition render interpretation more hazardous. Therefore, although animals showed some understanding of the task contingencies, witnessing a pretrained or naive actor did not transfer to prosocial responses. This may indicate that the observation of a pretrained actor is not sufficient to learn a task, and that being actively exposed to a pretraining phase is necessary to generate prosocial responses.

Long-Evans display more diverse set of behaviors

Long-Evans and Sprague-Dawley rats have commonly been used in social/behavioral paradigms (Ben-Ami Bartal et al., 2011; Hernandez-Lallement et al., 2015). In this study, independent t-tests supported strain differences in the number of easy presses in the naive group. Our findings indicate that Sprague-Dawley rats pressed more on the easy lever than Long-Evans. This appears related to Long-Evans rats showing overall greater activity than Sprague-Dawley, translating in reduced time spent pressing the easy lever and increased time interacting with the congener and exploring the hard lever. Indeed, analyses of behavior frequencies support the idea that Long-Evans rats engage in more behaviors than Sprague-Dawley, spending longer periods looking at each other and more decision making towards both levers. Additionally, Long-Evans were more active, displaying higher frequencies of behaviors than Sprague-Dawley rats (e.g., engaging in more prosocial decision making, observers going more often to their feeders). These results contrast Ku and colleagues' (2016) findings supporting juvenile Sprague-Dawley rats to engage more frequently with a congener and for longer periods of time compared to Long-Evans rats. This difference could partly be explained by the fact that pigmented (Long-Evans) rats tend to be more active during daytime than albino rats (Sprague-Dawley, Stryjek et al., 2013). Therefore, our results support research on Long-Evans' aptitude and their suitability for operant paradigms (Andrews et al., 1995) and confirm our hypothesis of enhanced performance of Long-Evans compared to Sprague-Dawley using the proposed model.

Limitations and future directions

The data support the learning of prosocial responses to be accompanied by singular communication patterns likely regulated through discrete biochemical changes. Similar to Hernandez-Lallement et al. (2015), our study has defined prosociality through behavioral responses expressed in a social environment in which one party acts in a helping manner towards a congener. Using a paradigm assessing the presence of prosocial behaviors in an environmental context where choices were dichotomous (prosocial versus selfish; prosocial responses involved an extra effort), our findings support pretraining as essential to prosocial responses, and vicariant learning sessions as insufficient to promote prosocial choices. As such, one hypothesis brought forward by this study is the role of a pretraining phase as one possible explanation for the expression of prosocial behaviors and not necessarily indicative of prosociality itself. It is important to note that a limiting factor and unknown mediator of performance surrounding the role of the pretraining phase is the possible extinction phase that may have occurred with the hard lever due to phase-dependent differentiated rewards for the hard lever. Additional work is required to further characterize biobehavioral relationship regulating prosocial behaviors and vicarious learning in rodents. Thus, a study including males and females is warranted to further validate this model. Subsequent studies would also benefit from including recording of the rats' ultrasonic vocalization, a limiting factor in our analyses. This would help interpret the nature of the interaction between the actors and observers. Many studies have explored ultrasonic communication in rats and have found it to be a reliable metric to assess socio-emotional states (Seffer et al., 2014). Furthermore, future studies should consider a study in which all groups would be exposed to a pretraining phase, best controlling for familiarity with the context and task contingencies. This will allow a more in-depth analysis of behaviors and performance. Lastly, we found levers to act as a limiting physical factor as the pressure needed for the hard lever was set at 75 grams. When testing rats during the adolescence and early adulthood, it is important to consider the pressure needed to trigger a response relative to their weight. It can thus be hard for the younger rat to maintain regular presses. As the rat gains weight, the pressure to body weight ratio diminishes and may no longer influence press rate. As explored by Oomen and colleagues (2013), a touch screen could be an alternative to the physical levers and thus is worth exploring in future studies.

References

Achterberg, E. J. M., van Swieten, M. M. H., Houwing, D. J., Trezza, V., & Vanderschuren, L. J. M. J. (2019). Opioid modulation of social play reward in juvenile rats. *Neuropharmacology*, *159*, 107332. https://doi.org/10.1016/j.neuropharm.2018.09.007

Andrews, J. S., Jansen, J. H. M., Linders, S., Princen, A., & Broekkamp, C. L. E. (1995). Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. *Physiology & Behavior*, *57*(4), 785–790. https://doi.org/10.1016/0031-9384(94)00336-X

Atsak, P., Orre, M., Bakker, P., Cerliani, L., Roozendaal, B., Gazzola, V., Moita, M., & Keysers, C. (2011). Experience Modulates Vicarious Freezing in Rats: A Model for Empathy. *PLOS ONE*, *6*(7), e21855. https://doi.org/10.1371/journal.pone.0021855

Bem, T., Jura, B., Bontempi, B., & Meyrand, P. (2018). Observational learning of a spatial discrimination task by rats: Learning from the mistakes of others? *Animal Behaviour*, *135*, 85–96. https://doi.org/10.1016/j.anbehav.2017.10.018

Ben-Ami Bartal, I. B.-A., Decety, J., & Mason, P. (2011). Helping a cagemate in need: Empathy and pro-social behavior in rats. *Science (New York, N.Y.)*, *334*(6061), 1427–1430. https://doi.org/10.1126/science.1210789

Ben-Ami Bartal, I., Rodgers, D. A., Bernardez Sarria, M. S., Decety, J., & Mason, P. (2014). Pro-social behavior in rats is modulated by social experience. *eLife*, *3*, e01385. https://doi.org/10.7554/eLife.01385

Brady, A. M., & Floresco, S. B. (2015). Operant Procedures for Assessing Behavioral Flexibility in Rats. *Journal of Visualized Experiments : JoVE*, *96*, 52387. https://doi.org/10.3791/52387

Burke, A. R., McCormick, C. M., Pellis, S. M., & Lukkes, J. L. (2017). Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. *Neuroscience and Biobehavioral Reviews*, *76*(Pt B), 280–300. https://doi.org/10.1016/j.neubiorev.2017.01.018

Caballero, A., Granberg, R., & Tseng, K. Y. (2016). Mechanisms contributing to prefrontal cortex maturation during adolescence. *Neuroscience and Biobehavioral Reviews*, *70*, 4–12. https://doi.org/10.1016/j.neubiorev.2016.05.013

Carlier, P., & Jamon, M. (2006). Observational learning in C57BL/6j mice. *Behavioural Brain Research*, *174*(1), 125–131. https://doi.org/10.1016/j.bbr.2006.07.014

Church, R. M. (1959). Emotional reactions of rats to the pain of others. *Journal of Comparative and Physiological Psychology*, *52*(2), 132–134. https://doi.org/10.1037/h0043531

Cotella, E. M., Gómez, A. S., Lemen, P., Chen, C., Fernández, G., Hansen, C., Herman, J. P., & Paglini, M. G. (2019). Long-term impact of chronic variable stress in adolescence versus adulthood. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *88*, 303–310. https://doi.org/10.1016/j.pnpbp.2018.08.003

Cronin, K. A. (2012). Prosocial behaviour in animals: The influence of social relationships, communication and rewards. *Animal Behaviour*, *84*(5), 1085–1093. https://doi.org/10.1016/j.anbehav.2012.08.009

Dahl, R. E., Allen, N. B., Wilbrecht, L., & Suleiman, A. B. (2018). Importance of investing in adolescence from a developmental science perspective. *Nature*, *554*(7693), 441–450. https://doi.org/10.1038/nature25770

Engelhardt, K.-A., Schwarting, R. K. W., & Wöhr, M. (2018). Mapping trait-like socio-affective phenotypes in rats through 50-kHz ultrasonic vocalizations. *Psychopharmacology*, *235*(1), 83–98. https://doi.org/10.1007/s00213-017-4746-y

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. *Methods in Ecology and Evolution*, *7*(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Gökçek-Saraç, Ç., Wesierska, M., & Jakubowska-Doğru, E. (2015). Comparison of spatial learning in the partially baited radial-arm maze task between commonly used rat strains: Wistar, Sprague-Dawley, Long-Evans, and outcrossed Wistar/Sprague-Dawley. *Learning & Behavior*, *43*(1), 83–94. https://doi.org/10.3758/s13420-014-0163-9

Greene, J. T. (1969). Altruistic behavior in the albino rat. *Psychonomic Science*, *14*(1), 47–48. https://doi.org/10.3758/BF03336420

Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., & Kalenscher, T. (2015). Rats prefer mutual rewards in a prosocial choice task. *Frontiers in Neuroscience, 8*. https://doi.org/10.3389/fnins.2014.00443

Horner, A. E., Heath, C. J., Hvoslef-Eide, M., Kent, B. A., Kim, C. H., Nilsson, S. R. O., Alsiö, J., Oomen, C. A., Holmes, A., Saksida, L. M., & Bussey, T. J. (2013). The touchscreen operant platform for testing learning and memory in rats and mice. *Nature Protocols, 8*(10), 1961–1984. https://doi.org/10.1038/nprot.2013.122

Ku, K. M., Weir, R. K., Silverman, J. L., Berman, R. F., & Bauman, M. D. (2016). Behavioral Phenotyping of Juvenile Long-Evans and Sprague-Dawley Rats: Implications for Preclinical Models of Autism Spectrum Disorders. *PLOS ONE*, *11*(6), e0158150. https://doi.org/10.1371/journal.pone.0158150

Lampe, J. F., Ruchti, S., Burman, O., Würbel, H., & Melotti, L. (2019). Play like me: Similarity in playfulness promotes social play. *PLOS ONE*, *14*(10), e0224282. https://doi.org/10.1371/journal.pone.0224282

Lee, J., Russo, A. S., & Parsons, R. G. (2018). Facilitation of fear learning by prior and subsequent fear conditioning. *Behavioural Brain Research*, *347*, 61–68. https://doi.org/10.1016/j.bbr.2018.03.008

Łopuch, S., & Popik, P. (2011). Cooperative behavior of laboratory rats (Rattus norvegicus) in an instrumental task. *Journal of Comparative Psychology*, *125*(2), 250–253. https://doi.org/10.1037/a0021532

Manduca, A., Campolongo, P., Palmery, M., Vanderschuren, L. J. M. J., Cuomo, V., & Trezza, V. (2014). Social play behavior, ultrasonic vocalizations and their modulation by morphine and amphetamine in Wistar and Sprague-Dawley rats. *Psychopharmacology*, *231*(8), 1661–1673. https://doi.org/10.1007/s00213-013-3337-9

Martin, L. J., Tuttle, A. H., & Mogil, J. S. (2014). The interaction between pain and social behavior in humans and rodents. In *Behavioral neurobiology of chronic pain* (pp. 233–250). Springer-Verlag Publishing/Springer Nature. https://doi.org/10.1007/7854_2014_287

Meyza, K. Z., Bartal, I. B.-A., Monfils, M. H., Panksepp, J. B., & Knapska, E. (2017). The roots of empathy: Through the lens of rodent models. *Neuroscience and Biobehavioral Reviews*, *76*(Pt B), 216–234. https://doi.org/10.1016/j.neubiorev.2016.10.028

O'Connor, B. P. (1999). Simple and flexible SAS and SPSS programs for analyzing lag-sequential categorical data. *Behavior Research Methods, Instruments, & Computers*, *31*(4), 718–726. https://doi.org/10.3758/BF03200753

O'Keefe, D. J. (2007). Brief Report: Post Hoc Power, Observed Power, A Priori Power, Retrospective Power, Prospective Power, Achieved Power: Sorting Out Appropriate Uses of Statistical Power Analyses. *Communication Methods and Measures*, *1*(4), 291–299. https://doi.org/10.1080/19312450701641375

Oomen, C. A., Hvoslef-Eide, M., Heath, C. J., Mar, A. C., Horner, A. E., Bussey, T. J., & Saksida, L. M. (2013). The touchscreen operant platform for testing working memory and pattern separation in rats and mice. *Nature Protocols*, *8*(10), 2006–2021. https://doi.org/10.1038/nprot.2013.124

Quera, V., Bakeman, R., & Gnisci, A. (2007). Observer agreement for event sequences: Methods and software for sequence alignment and reliability estimates. *Behavior Research Methods*, *39*(1), 39–49. https://doi.org/10.3758/BF03192842

Sato, N., Tan, L., Tate, K., & Okada, M. (2015). Rats demonstrate helping behavior toward a soaked conspecific. *Animal Cognition*, *18*(5), 1039–1047. https://doi.org/10.1007/s10071-015-0872-2

Seffer, D., Rippberger, H., Schwarting, R. K. W., & Wöhr, M. (2015). Pro-social 50-kHz ultrasonic communication in rats: Post-weaning but not post-adolescent social isolation leads to social impairments—phenotypic rescue by re-socialization. *Frontiers in Behavioral Neuroscience*, *9*. https://doi.org/10.3389/fnbeh.2015.00102

Sisk, C. L., & Zehr, J. L. (2005). Pubertal hormones organize the adolescent brain and behavior. *Frontiers in Neuroendocrinology*, *26*(3–4), 163–174. https://doi.org/10.1016/j.yfrne.2005.10.003

Sivaselvachandran, S., Acland, E. L., Abdallah, S., & Martin, L. J. (2018). Behavioral and mechanistic insight into rodent empathy. *Neuroscience & Biobehavioral Reviews*, *91*, 130–137. https://doi.org/10.1016/j.neubiorev.2016.06.007

Stryjek, R., Modlińska, K., Turlejski, K., & Pisula, W. (2013). Circadian Rhythm of Outside-Nest Activity in Wild (WWCPS), Albino and Pigmented Laboratory Rats. *PLOS ONE*, *8*(6), e66055. https://doi.org/10.1371/journal.pone.0066055

Tomek, S. E., Stegmann, G. M., & Olive, M. F. (2019). Effects of heroin on rat prosocial behavior. *Addiction Biology*, *24*(4), 676–684. https://doi.org/10.1111/adb.12633 van Schaik, C., Graber, S., Schuppli, C., & Burkart, J. (2017). The Ecology of Social Learning in Animals and its Link with Intelligence. *The Spanish Journal of Psychology*, *19*, E99. https://doi.org/10.1017/sjp.2016.100

Walker, D. M., Bell, M. R., Flores, C., Gulley, J. M., Willing, J., & Paul, M. J. (2017). Adolescence and Reward: Making Sense of Neural and Behavioral Changes Amid the Chaos. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *37*(45), 10855–10866. https://doi.org/10.1523/JNEUROSCI.1834-17.2017

Yamada, M., & Sakurai, Y. (2018). An observational learning task using Barnes maze in rats. *Cognitive Neurodynamics*, *12*(5), 519–523. https://doi.org/10.1007/s11571-018-9493-1

Yusufishaq, S., & Rosenkranz, J. A. (2013). Post-weaning Social Isolation Impairs Observational Fear Conditioning. *Behavioural Brain Research*, *242*, 142–149. https://doi.org/10.1016/j.bbr.2012.12.050

Zentall, T. R. (2016). Reciprocal altruism in rats: Why does it occur? *Learning & Behavior*, *44*(1), 7–8. https://doi.org/10.3758/s13420-015-0201-2

CHAPTER THREE

Study Two

In search of prosociality in rodents: a scoping review

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Published in *PLOS ONE*

Find publication: https://doi.org/10.1371/journal.pone.0310771

Abstract

Studying prosociality in rodents can provide insight into brain mechanisms potentially related to neurodevelopmental disorders known to impact social behaviors (e.g., autism spectrum disorder). While many studies have been published suggesting promising models, current knowledge remains scattered, including potential factors mediating prosocial behaviors in rodents. Prosocial behavior is characterized by an action done to benefit another or promote their well-being. The goal of this scoping review is to characterize current findings regarding prosocial paradigms in rodents, highlight current gaps in reporting, and identify factors shown to be important in mediating prosocial responses in rodents. Five databases were consulted in search of relevant studies published between 2000 and 2020 (APA PsycInfo, Embase, MEDLINE, Scopus, Web of Science). An update using a semi-supervised machine learning approach (ASReview) was then conducted to collect studies from 2021-2023. In total, 80 articles were included. Findings were the following: (1) Three categories of prosocial paradigm were extracted: cooperation, helping, and sharing tasks, (2) Rodents showed the ability to perform prosocial actions in all three categories, (3) Significant gaps in reported methodologies (e.g., failure to report animals' characteristics, housing conditions, and/or experimental protocol) and mediating factors (e.g., sex, strain, housing, food restriction) were found, and (4) Behaviors are determinant when investigating prosociality in rodents, however many studies omitted to include such analyses. Together these results inform future studies on the impact of mediating factors and the importance of behavioral analyses on the expression of prosocial behaviors in rodents.

Introduction

While there has been extensive research in humans [1], investigating prosociality in animals can present many challenges as their internal state can only be assumed and interpreted [2]. Rodents, prominently rats and mice, are often used because of their sociability (e.g., social interaction seeking, hierarchical structure) [3] and practicality (reduced costs and resources compared to non-human primates) [4]. Aversive conditions such as pain or fear have been interpreted in rats and mice through operationalized nonverbal cues (e.g., freezing behaviors) and related tasks (e.g., emotional contagion). However, there has not yet been a standardized way to measure positive affect through behavioral indicators [2]. As such, prosocial tasks can be useful since they require an explicit action (e.g., pressing a lever, nose poking). Prosocial behavior is characterized by an action done to benefit another or promote their well-being [2]. Acting in a way that is prosocial is considered important to the survival of many species, such as a social group cooperating for resources [2,5,6]. Understanding prosociality in animals is crucial to gain deeper insights into endogenous mechanisms related to neuropsychiatric disorders that are characterized by social impairments (e.g., autism spectrum disorder, schizophrenia, personality disorders) [4,7,8].

Rodent prosocial paradigms

Research examining prosociality in rodents began with the use of observational tasks, such as fear conditioning and emotional contagion paradigms [9-11]. These aversive models involve two animals, one receiving a painful stimulus (e.g., electric foot shock, tail pinch) while the other observes [12-14]. While these paradigms can be useful to study observational learning and the ability to respond to the distress of another conspecific, these models primarily focus on examining survival responses (i.e., learning from the environment and others to avoid danger/an aversive situation) while offering limited insights into prosocial responses [14,15]. It is an important distinction to be made as the primary incentive driving these behaviors differ, the former being a survival response while the latter involves a higher level of decision-making [16]. In 2011, Ben-Ami Bartal and colleagues published a groundbreaking study in which rats learned to open a door to free a conspecific from a restraining device [17]. Since then, minimally aversive paradigms have been implemented, including other variations of freeing task (e.g., freeing a conspecific from a pool of water), and a prosocial choice task, in which a rat can choose between eating a reward on its own (own-reward compartment) or eating it with a conspecific, who also receives a reward (both-reward compartment) [18, 19]. The development of non-aversive paradigms becomes imperative as welfare is gaining importance in animal studies [20]. Moreover, many highlighted the possibility that the absence of an aversive variable (i.e., foot shock), eliminating the 'survival instinct' factor, could better represent prosocial behaviors [16]. To date, objective assessments of non-aversive models have been challenging [21,22], and some have raised questions as to whether recorded observations truly represent prosociality rather than the simple desire of a reward (i.e., social contact, food) [23,24]. Attempts to address this issue have included preventing social contact between rodents using a wire mesh [25] or freeing a conspecific in a separate compartment [26]. Other studies have included quantitative metrics, such as discriminant analysis of behaviors [27] or recording of ultrasonic vocalizations [17] to help determine prosociality beyond task performance.

Mediating factors in the expression of prosociality in rodent

Prosociality being a complex behavior, multiple mediating factors have been suggested as potentially impacting rodents' expression of prosocial behaviors [27,28]. Such factors include individual characteristics (i.e., sex, age, strain) [27-29], dominance and social hierarchy [30,31], familiarity [17, 31], characteristics of the paradigm (i.e., reward, task contingency, social contact) [32,33], housing conditions [34], and stress (i.e., handling and experimental procedures such as surgeries) [35,36]. For example, rodents that are housed in pairs tend to develop a role of dominance or submission, which are determined by numerous factors, including the sex and weight of the animal [37]. Furthermore, stress levels have a direct impact on dyadic relationships, with the stressed rat being more submissive [38]. Moreover, rodents tend to display more prosocial behaviors with other familiar animals versus strangers [12], and with same-strain congeners [17]. Interestingly, adolescent rats tend to be prosocial with ingroup and outgroup members, whereas adult rats tend to display prosociality with ingroup members only [29]. These examples show that various mediating factors can play a significant role in the expression of prosocial behaviors in rodents.

Research objectives

Although interesting studies have been published suggesting promising paradigms [7, 17, 19], current knowledge remains scattered, including potential factors mediating prosocial behaviors in rodents. Therefore, the goal of this scoping review is to characterize current findings regarding prosocial paradigms in rodents, highlight current gaps in reporting, and identify factors shown to be important in mediating prosocial responses in rodents. Furthermore, the review sought to characterize and define categories of paradigms often used in the field of prosocial research (cooperation, helping, sharing). In this review, the focus is placed on prosocial tests, which are defined as tasks that include a concrete action toward a congener for individual or mutual benefit. As such, the following search strategy is based solely on articles using a task requiring two or more rodents and a specific action.

Methodology

The methodology of this review was registered in PROSPERO (registration number: CRD42022335883). We followed the Preferred Reporting Items for Systematic reviews and Meta-Analyses guidelines (PRISMA) protocol and flowchart. Covidence (Veritas Health Innovation) was used to manage references.

Search strategy

The original search strategy sought to identify studies on prosociality in rodents. A research librarian (PRL) with experience in planning reviews drafted, developed, and implemented a search strategy to find pertinent published articles in APA PsycInfo (Ovid), Embase (Ovid), MEDLINE (Ovid), Scopus and Web of Science (Clarivate). The strategy was informed by ones conducted in previous reviews on rodents, mice, and rats [39-41] and on prosociality [42-45]. A draft strategy, which included subject headings and keywords, was developed for APA PsycInfo (Ovid) by the research librarian and feedback was obtained from other review team members. The strategy was also peer-reviewed by another librarian following the Peer-Review of Electronic Search Strategy guideline [46]. The initial searches were executed on January 26, 2021. The search did not use any database limits other than a date restriction, limiting results to those published since 2000. The complete search strategy is available in Appendix 1.

Citations found through the database searches were imported into Covidence, an online tool used to manage various steps of a review's screening phases. A total of 24485 published articles between January 1st, 2000, and December 31st, 2020, were found. Duplicate references were identified and removed once imported into Covidence. Additional duplicates were identified and excluded while screening references. After duplicate removal, 12434 articles were left for abstract screening (Fig 1). All inclusion and exclusion criteria can be found in Table 1 and all information to be extracted from each study can be found in Table 2.

Figure 1. PRISMA Flow Diagram.

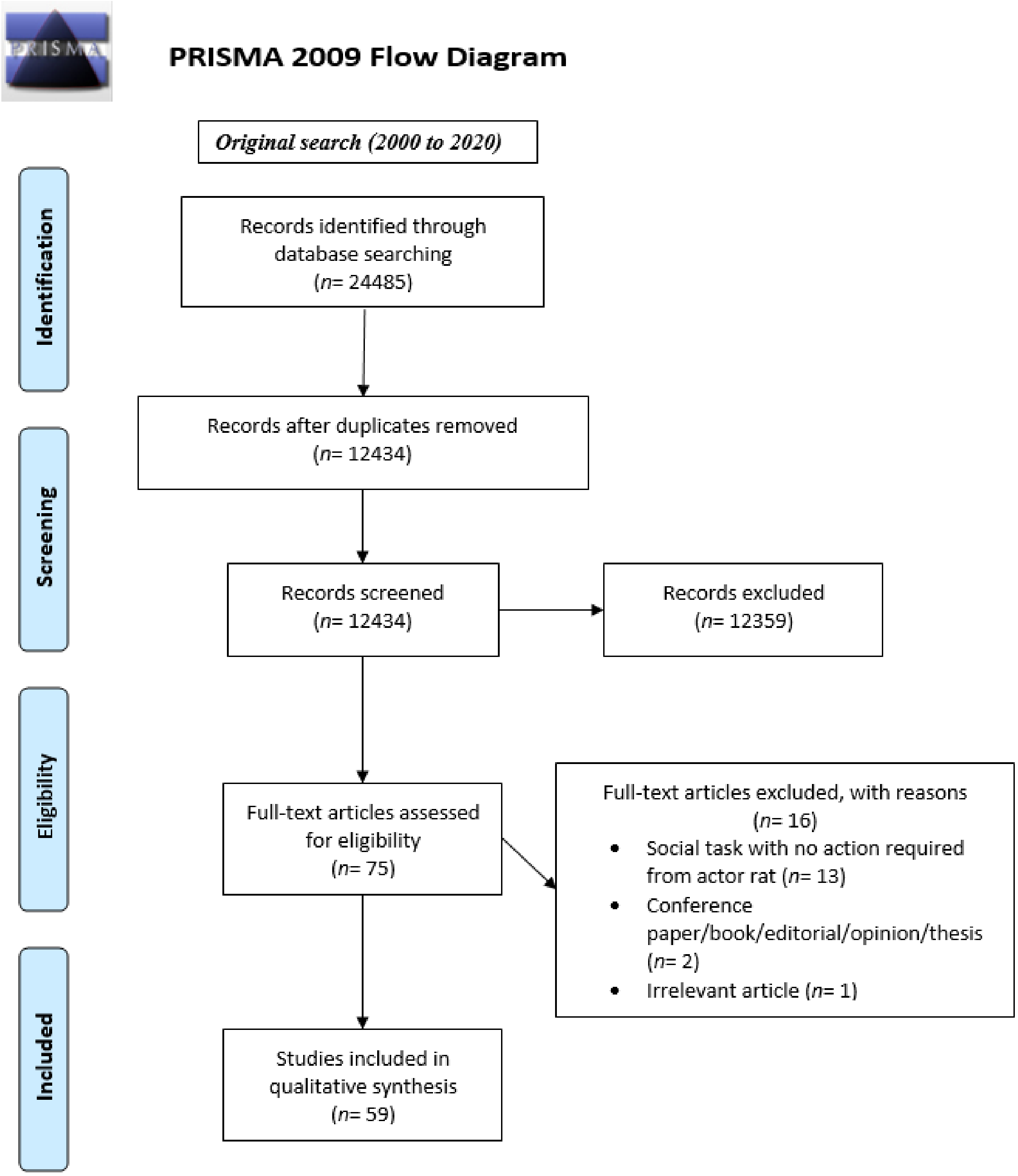


Table 1. Inclusion and Exclusion Criteria for the Scoping Review.

Inclusion criteria	**Exclusion criteria**
Rats	Animals other than rodents (mole rats, guinea pigs, primates, humans, etc.)
Mice	Study not published in English
Study published in English	Study published before 2000
Study published between 2000-2020	Conference paper, thesis, opinion/editorial paper, or books
The task includes two or more animals (including inanimate control or conditions)	Scoping review, systematic review, or meta-analysis
The task includes an action for at least one rat (no passive tasks)	Social task that requires no action from rats (social interaction test, open field, novelty tasks – no prosocial components)
	Pregnancy or maternal/paternal studies
	Study Erratum
	Irrelevant studies
	Study not found

Table 2. Details to be Extracted from Each Included Study.

Category	**Extracted data**	**Details**
Article journal	• Title • Authors names • Date of publication • Journal • Open access • Definition of the task	• Open access: Is the article published in an open access journal? yes or no • Definition of the task: Report any definitions that authors have used to refer to prosociality or the task

Animals	● Ethical note ● Type of rodent ● Total # of subjects ● Total of males/females ● Age at arrival ● Strain ● Weight at arrival ● Food restriction ● Water restriction	● Ethical note: Including the name of the animal care committee, the license number if applicable and the followed guidelines ● Type of rodent: Rats or mice ● Total of males/females: In case only one sex was tested, 'NA' is indicated for the non-relevant sex ● Food and water restriction: Yes or no, - if yes, precision is given (e.g., 85% of free feeding body weight)
Housing conditions	● Number of animals per cage ● Relationship ● Dark-light cycle ● Enriched environment	● Number of animals per cage: Modalities of animal housing – ex: Were the animals housed in pairs or more? If housed with more than one individual, specify whether the animal is housed with its experimental partner ● Relationship: Are the animals' littermates or unrelated? ● Dark-light cycle: Indicate if the investigators specified the light/dark cycle (e.g., 12h, 16h, reversed) ● Enriched environment: Did the investigators use an enriched environment (yes or no). If yes, details if available are specified (description of the environment)
Methodology	● Operant paradigm ● Total of rats involved ● Groups ● Description of the task ● Type of task ● Computerized task ● Aversive or non-aversive ● Sensory paradigm (can rodents see/hear/smell each other) ● Duration of task (in minutes) ● Total of testing days ● Pretraining/habituation ● Control Group	● Operant paradigm: Precise the selected operant paradigm (e.g., prosocial choice task, prisoner's dilemma, freeing task, repeated donation game, etc.) ● Groups: Precise the group repartition ● Description of the task: Summary of the selected task ● Type of task: Cooperation, sharing, freeing, etc. ● Computerized task: Does the paradigm use an automated or computerized system? (e.g., operant box, video recording, ultrasonic vocalization, etc.) ● Aversive or non-aversive: Does the paradigm involve an aversive component (e.g., pain, fear, distress); if so, for one or both rats? (yes or no) ● Sensory elements: Do authors mention if rodents can see, hear, or smell each other during the experimental task? (yes or no) ● Pretraining/Habituation: Is there a mention of a habituation or pretraining phase? (if yes, describe)

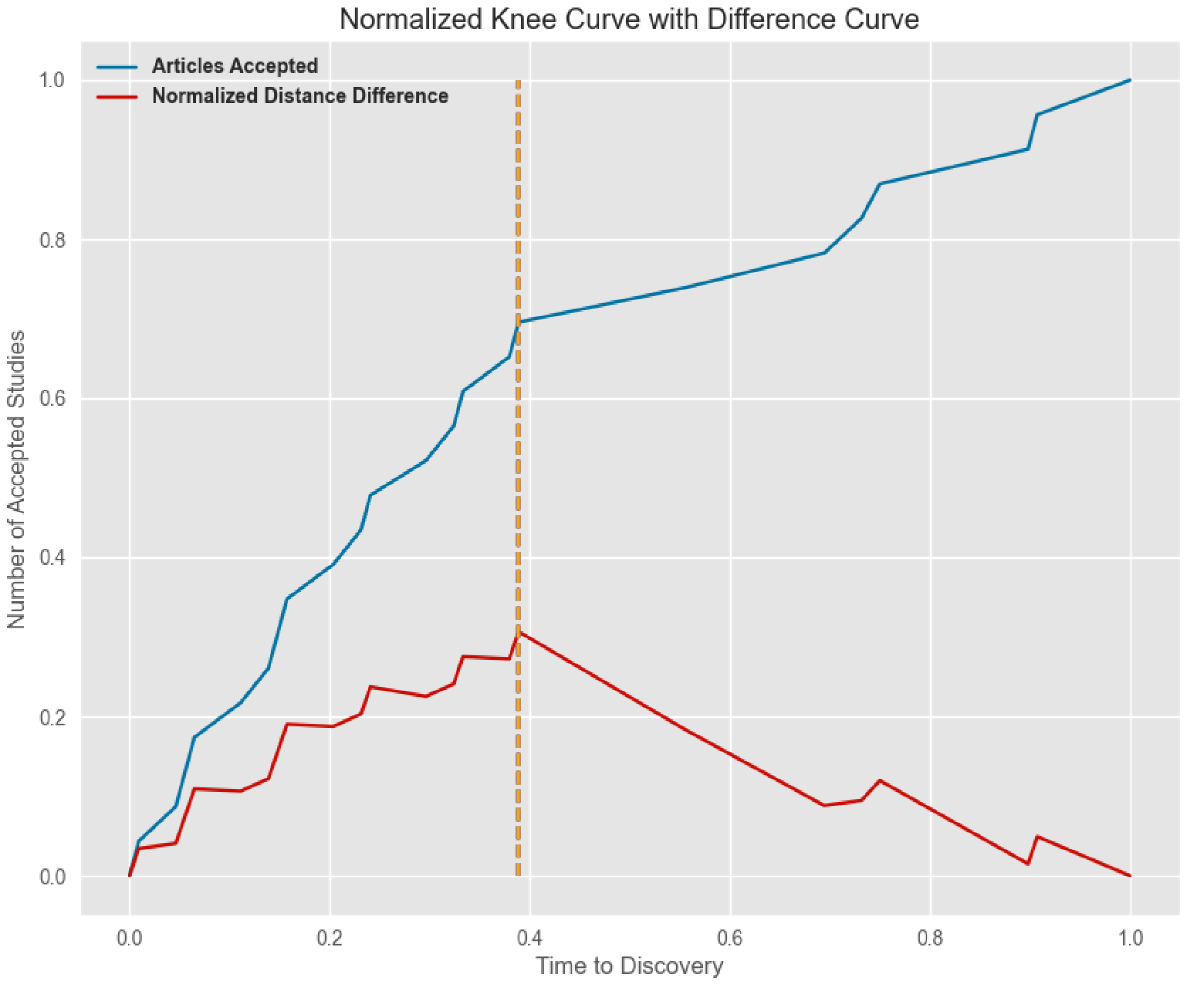
		● <u>Control group</u>: Is a control group included? (if yes, describe)
Experimental interventions	● Drugs ● Surgery ● Diet ● Stress ● Other	● <u>Experimental interventions</u>: Describe any use of drugs, diet, stress variables, surgery or other tests done during the experiment (e.g., open field, elevated plus maze, etc.)
Results	● Performance index ● Behavioral analyses ● Sex differences ● Age differences ● Strain differences	● <u>Performance index:</u> How did the study quantify the action? (e.g., lever presses, door openings, etc.) ● <u>Behavioral analyses</u>: Did the study use any metrics to analyze rodents' behaviors? (e.g., video recording, ultrasonic vocalization) if yes, summarize the results. ● <u>Sex differences:</u> If the study used both sexes, summarize sex differences' results if applicable ● <u>Age differences:</u> Summarize any results regarding age differences if applicable ● <u>Strain differences:</u> Summarize any results regarding strain differences if applicable

Update using ASReview

An update from January 1, 2021, to January 17th, 2023, was performed by means of a semi-supervised machine learning approach. The software used for this purpose, ASReview [47] calculates the relevancy of studies based on a trained semi-supervised machine learning algorithm. The same search strategy and search phrases were used to establish the updated database. A total of 4071 articles were found. After duplicate removal, 2001 articles were left for abstract screening (see Fig 2). Using the open-source ASReview software, the semi-supervised machine learning model was trained (by either accepting or rejecting studies) by the two principal authors using the original database of 12432 articles. The original dataset (the training data) was then imported into the ASReview software for the review update. The ASRreview software presents relevant articles to the researchers early in the screening process, and depending on the stopping rule, allows the researchers to end the screening process earlier than other methods such as manual screening [47]. The use of such methods allows for faster screening and higher accuracy in the identification of relevant studies [47]. When conducting a scoping review with ASReview, settings need to be chosen by the researcher based on the needs and goals of the study: 1) The algorithm for feature extraction, 2) classifying algorithm, 3) balancing strategy, 4) Querying strategy. Chosen settings were the following: 1) the SentenceBert algorithm in conjunction with 2) a logistic regression for its classifier [48,49], 3) a double dynamic resampling strategy to prevent model overfitting, and 4) a mixed querying strategy consisting of a 95% maxed matching and a 5% random study presentation.

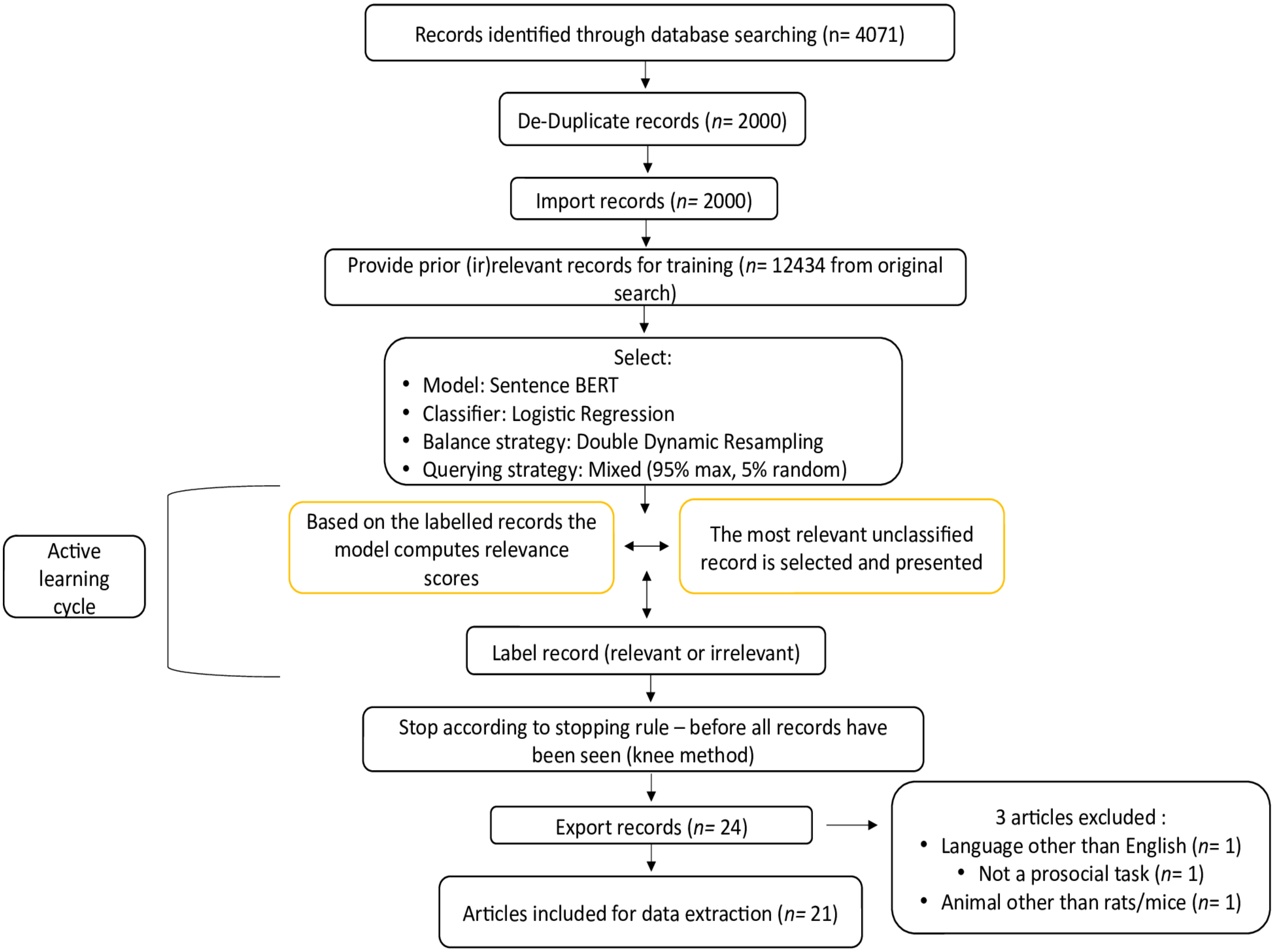
A knee detection algorithm was actively applied throughout the screening process to identify an appropriate stopping point. Within the screening process, a stopping point is when most of the potentially relevant studies have been identified and accepted, and the probability of unscreened studies to contain a relevant study is low [50]. A sensitivity of 5 was selected, (the default being a value of 1), to be more conservative in the stopping time, thus increasing the probability of accepting relevant studies [50]. The increasing concave curve option was chosen to visually represent the rising number of screened studies. For detailed explanation of the Kneedle algorithm and parameters, see the original paper [50]. Once the knee was detected by the Kneedle algorithm, screening continued for a short time to confirm that the initial knee was correct [50]. The location of the knee was at the 17th accepted article (time of discovery: 43 articles; Fig 2). In total, 128 articles were screened, representing 6.39% of the 2001 articles. Amongst these, 21 articles were included for data extraction (Fig 3).

Figure 2. Stopping rule: knee curve.



Note. The knee is located at the 17th accepted article (time of discovery: 43 articles) which is represented by the dotted line. In total, 128 articles were screened (represented by the blue line) and 21 were accepted for data extraction.

Figure 3. ASReview flow diagram.



Risk of bias tool

This review used an adapted version of SYRCLE risk of bias tool for animal studies to assess the methodological quality of included studies [51]. Two independent reviewers (two principal authors) assessed the articles, and any disagreement was resolved through consensus-oriented discussion or by consulting a third party (principal investigator) [51]. SYRCLE is a tool designed for randomized control trial (RCT) studies. Therefore, different aspects pertaining to RCT designs were not evaluated in this review, considering that many of the included studies could be defined as observational studies rather than RCTs.

Statistical analyses

Descriptive statistics (e.g., averages, percentages) of the extracted data were performed to identify key information (e.g., number of rats, sex, strain). When studies used similar paradigms (i.e., freeing task, food sharing task), descriptive statistics were used to compare and discuss them. Key components of every paradigm were extracted and described using qualitative comparisons (e.g., task, rewards, operant chamber, etc.).

Results

The 80 included articles (*n*= 59 from original search) were organized into 3 categories (i.e., cooperation, helping, or sharing task) and classified as either using an aversive (*n*= 38) or a non-aversive (*n*= 42) testing paradigm (see Table 3). An aversive task is characterized by inclusion of an aversive element causing distress or discomfort to the animals, such as a restraining device, water exposure, electric shocks, or tail pinches. All details regarding the data extraction for each included article can be found in supplementary data (Appendix 2).

The first category combines all cooperation tasks, such as the Prisoner's dilemma task and the cooperation learning tasks (*n*= 16). The cooperation tasks involved two rodents that were trained to give a mutual response (lever presses, running through a maze) in a certain time interval to get a mutual reward. Another variation of cooperation tasks involved the Prisoner's dilemma, in which both rodents have to decide to either cooperate or defect by pressing on a lever within a certain time interval.

The second category combines all helping tasks, including freeing tasks and tasks that prevent harm to a conspecific (*n*= 37). Helping tasks involved a rodent trapped in a restrainer tube or a soaked area while another conspecific could press a lever to open the door and free the animal. Another variant involved a rodent receiving electric shock while a conspecific could opt to press a lever to stop the shocks or to get a reward for itself.

The third and final category combines all sharing tasks, such as the prosocial choice task and the repeated donation game (*n*= 27). The latter is characterized by two rodents that can press a lever or pull a stick to give each other food rewards, while the prosocial choice task gives to a rodent the possibility to eat a food reward in an *own-reward* compartment or in a *both-reward* compartment (providing a reward to itself and a conspecific).

Table 3. Included Articles.

Study	**Type of rodent**	**Type of task**	**Aversive or non-aversive**
Original search (2000 to 2020)			
Avital, Aga-Mizrachi & Zubedat, 2016 [52]	Rats	Cooperation (learning task)	Non aversive
Bartal, Decety & Mason, 2011 [17]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Bartal, Rodgers, Sarria, Decety, & Mason, 2014 [53]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Bartal, Shan, Molasky, Murray, Williams, Decety & Mason, 2016 [54]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Blystad, Andersen & Johansen, 2019 [32]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Carvalheiro, Seara-Cardoso, Mesquita, de Sousa, Oliveira, Summavielle & Magalhães, 2019 [55]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Conde-Moro, Rocha-Almeida, Sánchez-Campusano, Delgado-García & Gruart & 2019 [56]	Rats	Cooperation (learning task)	Non aversive
Cox & Reichel, 2020 [26]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Daghestani, Selim, Abd-Elhakim, Said, Abd-Hameed, Khalil & El-Tawil, 2017 [57]	Rats	Helping (freeing task – tube)	Aversive (restraint)
de Carvalho, Dos Santos, Regaço, Barbosa, Da Silva, de Souza & Sandaker, 2018 [58]	Rats	Cooperation (learning task)	Non aversive
Delmas, Lew & Zanutto, 2019 [59]	Rats	Cooperation (Prisoner’s dilemma)	Non aversive
Dolivo & Taborsky 2015 (A) [60]	Rats	Sharing (repeated donation game)	Non aversive
Dolivo & Taborsky 2015 (B) [61]	Rats	Sharing (repeated donation game)	Non aversive
Donovan, Ryan & Wood, 2020 [62]	Rats	Cooperation (Prisoner’s dilemma)	Non aversive
Festucci, Buccheri, Cerniglia, Paciello, Cimino, Curcio & Adriani, 2020 [63]	Rats	Sharing task (repeated donation game)	Non aversive
Fontes-Dutra, Nunes, Santos-Terra, Souza-Nunes, Bauer-Negrini, Hirsch, Green, Riesgo, Gottfried & Bambini-Junio, 2019 [64]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Gerber, Schweinfurth & Taborsky, 2020 [65]	Rats	Sharing task (repeated donation game)	Non aversive
Hachiga, Schwartz, Silberberg, Kearns, Gomez & Slotnick, 2018 [66]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Han, Yoon, Shin, Um & Ko, 2020 [67]	Mice	Cooperation (learning task)	Non aversive
Havlik, Sugano, Jacobi, Kukreja, Jacobi & Mason, 2020 [68]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Hernandez-Lallement, van Wingerden, Marx, Srejic	Rats	Sharing (prosocial choice task)	Non aversive

& Kalenscher, 2015 [19]			
Hernandez-Lallement, van Wingerden, Schäble & Kalenscher, 2016 [69]	Rats	Sharing (prosocial choice task)	Non aversive
Hernandez-Lallement, Attah, Soyman, Pinhal, Gazzola & Keysers, 2020 [70]	Rats	Helping (not harming)	Aversive (electric shocks)
Hosgorler, Koc, Kizildag, Canpolat, Argon, Karakilic, Kandis, Guvendi, Ates, Arda & Uysal, 2020 [71]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Kandis, Ates, Kizildag, Camsari, Yuce, Guvendi, Koc, Karakilic, Camsari & Uysal, 2018 [72]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Karakilic, Kizildag, Kandis, Guvendi, Koc, B. Camsari, M. Camsari, Ates, Arda & Uysal, 2018 [6]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Kentrop, Kalamari, Danesi, Kentrop, van IJzendoorn, Bakermans-Kranenburg, Joëls & van der Veen, 2020 [73]	Rats	Sharing (lever presses)	Non aversive
Kozma, Kassai, Ernyey & Gyerytán, 2019 [74]	Rats	Cooperation (learning task)	Non aversive
Li & Wood, 2017 [75]	Rats	Sharing (repeated donation game)	Non aversive
Lopuch & Popik, 2011 [25]	Rats	Cooperation (learning task)	Non aversive
Márquez, Rennie, Costa & Moita, 2015 [76]	Rats	Sharing (prosocial choice task)	Non aversive
Oberliessen, Hernandez-Lallement, Schäble, van Wingerden, Seinstra & Kalenscher, 2016 [77]	Rats	Sharing (prosocial choice task)	Non aversive
Raz, 2013 [78]	Rats	Cooperation (learning task)	Non aversive
Rutte & Taborsky, 2007 [79]	Rats	Sharing (lever presses)	Non aversive
Rutte & Taborsky, 2008 [80]	Rats	Sharing (repeated donation game)	Non aversive
Sato, Tan, Tate & Okada, 2015 [18]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Schmid, Schneeberger & Taborsky, 2017 [81]	Rats	Sharing (stick pulling)	Non aversive
Schneeberger, Dietz & Taborsky, 2012 [82]	Rats	Sharing (repeated donation game)	Non aversive
Schönfeld, Schäble, Zech & Kalenscher, 2020 [83]	Rats	Sharing (prosocial choice task)	Non aversive
Schwartz, Silberberg, Casey, Kearns & Slotnick, 2017 [84]	Rats	Helping (freeing task - soaked area)	Aversive (water)
Schweinfurth & Taborsky, 2016 [85]	Rats	Sharing (repeated donation game)	Non aversive
Schweinfurth & Taborsky, 2017 [86]	Rats	Sharing (repeated donation game)	Non aversive
Schweinfurth & Taborsky, 2018 (A) [87]	Rats	Sharing (repeated donation game)	Non aversive
Schweinfurth & Taborsky, 2018 (B) [88]	Rats	Sharing (repeated donation game)	Non aversive
Schweinfurth & Taborsky, 2018 (C) [89]	Rats	Sharing (stick pulling)	Non aversive

Schweinfurth, Aeschbacher, Santi & Taborsky, 2019 [90]	Rats	Sharing (repeated donation game)	Non aversive
Schweinfurth & Taborsky, 2020 [91]	Rats	Cooperation (Prisoner's dilemma)	Non aversive
Silberberg, Allouch, Sandfort, Kearns, Karpel & Slotnick, 2014 [24]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Silva, H. Silva, Lima, Meurer, Ceppi & Yamamoto, 2020 [92]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Tomek, Stegmann & Olive, 2019 [93]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Tomek, Stegmann, Leyrer-Jackson, Piña & Olive, 2020 [94]	Rats	Helping (freeing task – tube)	Aversive (restraint)
Tsoory, Youdim & Schuster, 2012 [95]	Rats	Cooperation (learning task)	Non aversive
Ueno, Suemitsu, Murakami, Kitamura, Wani, Matsumoto, Okamoto & Ishihara, 2019 (A) [96]	Mice	Helping (freeing task – tube)	Aversive (restraint)
Ueno, Suemitsu, Murakami, Kitamura, Wani, Matsumoto, Okamoto & Ishihara, 2019 (B) [97]	Mice	Helping (freeing task – tube)	Aversive (restraint)
Viana, Gordo, Sucena & Moita, 2010 [98]	Rats	Cooperation (Prisoner's dilemma)	Aversive (tail pinches)
Wood, Kim & Li, 2016 [99]	Rats	Cooperation (Prisoner's dilemma)	Non aversive
Yamagishi, Okada, Masuda & Sato, 2020 (A) [100]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Yamagishi, Lee & Sato, 2020 (B) [101]	Rats	Helping (freeing task – soaked area)	Aversive (water)
Yüksel, Ates, Kizildag, Yüce, Koç, Kandis, Güvendi, Karakilic, Gümüs & Uysal, 2019 [102]	Mice	Helping (freeing task – soaked area)	Aversive (water)

Updated search (2021 to 2023)	**Type of rodent**	**Type of task**	**Aversive or non-aversive**
Asadi, Khodagholi, Asadi, Kamsorkh, Kaveh & Maleki, 2021 [103]	Rats	Helping (freeing task - soaked area)	Aversive (water)
Bartal, Breton, Sheng, Long, Chen, Halliday, Kenney, Wheeler, Frankland, Shilyansky, Deisseroth, Keltner & Kaufer, 2021 [104]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Breton, Eisner, Gandhi, Musick, Zhang, Long, Perloff, Hu, Pham, Lalchandani, Barraza, Kantor, Kaufer & Bartal, 2022 [29]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Conde-Moro, Rocha-Almeida, Gebara, Delgado-Garcia, Sandi & Gruart, 2022 [105]	Rats	Cooperation (learning task)	Non-aversive
Cox, Kearns, Woods, Brown, Brown & Reichel, 2022 [106]	Rats	Helping (freeing task - soaked area)	Aversive (water)
Cox, Brown, Woods, Brown, Kearns & Reichel, 2022 [107]	Rats	Helping (freeing task - soaked area)	Aversive (water)
de Carvalho, dos Santos, Regaço, Couto, de Souza & Todorov, 2020 [108]	Rats	Cooperation (learning task)	Non-aversive
Gachomba, Esteve-Agraz, Caref, Maroto, Bortolozzo-	Rats	Sharing (prosocial choice task)	Non-aversive

Gleich, Laplagne & Márquez, 2022 [30]			
Heslin & Brown, 2021 [109]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Joushi, Taherizadeh, Esmaeilpour & Sheibani, 2022 [110]	Rats	Sharing (prosocial choice task)	Non-aversive
Kalamari, Kentrop, Danesi, Graat, van Ijzendoorn, Bakermans-Kranenburg, Joëls & van der Veen, 2021 [111]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Misiolek, Klimczak, Chrószcz, Szumiec, Bryksa, Przyborowicz, Parkitna & Harda, 2023 [33]	Mice	Sharing (prosocial choice task)	Non-aversive
Paulsson & Taborsky, 2021 [112]	Rats	Sharing (repeated donation game)	Non-aversive
Scheggia, La Greca, Maltese, Chiacchierini, Italia, Molent, Bernardi, Coccia, Carrano, Zianni, Gardoni, Di Luca & Papaleo, 2022 [31]	Mice	Sharing (nose poking)	Non-aversive
Schweinfurth, 2021 [113]	Rats	Sharing (repeated donation game)	Non-aversive
Segura, Clavijo & Bouzas, 2019 [126]	Rats	Cooperation (learning task)	Non-aversive
Sen, Kara, Koyu, Simsek, Kizildag & Uysal, 2021 [35]	Rats	Helping (freeing task - soaked area)	Aversive (water)
Shima, Kawabata-Iwakawa, Onishi, Jesmin & Yoshikawa, 2022 [114]	Mice	Helping (freeing task - soaked area)	Aversive (water)
Subhadeep, Srikumar, Shankaranarayana & Kutty, 2022 [115]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Wan, Kirkman, Jensen & Hackenberg, 2021 [116]	Rats	Helping (freeing task - tube)	Aversive (restraint)
Wu, Cheng, Liang, Lee & Yen, 2023 [117]	Rats	Helping (freeing task - tube)	Aversive (restraint)

Bias assessment

Results from the bias assessment can be found in Table 4. More details on risk of bias by category of task can be found in the discussion.

Table 4. Risk of bias assessment - Results (*n*= 80 included articles).

Bias	Low risk	Unclear	High risk
Sequence generation	44 (55%)	13 (16.25%)	23 (28.75%)
Baseline characteristics	39(48.75%)	4 (5%)	37 (46.25%)
Allocation concealment	2 (2.5%)	74 (92.5%)	4 (5%)
Random housing	50 (62.5%)	10 (12.5%)	20 (25%)
Blinding (assessment)	4 (5%)	70 (87.5%)	6 (7.5%)
Random outcome assessment	53 (66.25%)	9 (11.25%)	18 (22.5%)
Blinding (outcome)	13 (16.25%)	65 (81.25%)	2 (2.5%)
Attrition bias	51 (63.75%)	12 (15%)	17 (21.25%)
Selective outcome reporting	1 (1.25%)	61 (76.25%)	18 (22.5%)
Other source of bias	28 (35%)	24 (30%)	28 (35%)

Prosocial paradigms: definitions

Of the 80 included articles, 69 provided a definition to describe the action of helping, sharing, or cooperating. Using word clouds, which is described as a collection of words depicted in different sizes according to the mode with more frequent terms being highlighted [118], a definition for each task (cooperation, helping and sharing) was extracted (Fig 4; see Appendix 2 for detailed definitions).

Figure 4. Word clouds of prosocial tasks – definition.

Cooperation tasks

Helping tasks

Sharing tasks

Combined tasks

From the world clouds, the following definitions were extracted 1) Cooperation task: Combined or coordinated behavior of 2 or more individuals for mutual benefit or to benefit the recipient (with or without a disadvantage for the donor); 2) Helping task: Action that provides benefit or help to another, and 3) Sharing task: Task in which two or more individuals alternate in helping each other.

Animals

In total, 7 articles reported using mice and 73 used rats. The selected mouse strain included C57BL/6 (5), Shank2 and 4 (1), and Balb-c mice (1). Regarding rats, strains included Sprague-Dawley (28), Wild type Norway (16), Long-Evans (11), Wistar (14), Lister Hooded (3), and a mix of strain (Long-Evans/Sprague-Dawley (3), and Long-Evans/Lister Hooded (1)). One study failed to specify the tested rat strain. On average, the included articles used 47 rodents. Six studies did not specify the sex or the included number of females and males. Forty-two studies used males only, 19 used females only, and 13 included both sexes. From all the articles including females, one study specified the animals to have been ovariectomized, while another specified doing vaginal smears after each experimental session. Both studies assessed male and female rats. Most studies (*n*= 50) used adult rodents, 6 used adolescents, 2 used older rats and 1 study used a combination of adolescent and adult rats. Fifteen studies did not specify the age of the rodents, and 37 studies failed to report the animals' weight. Sixty-five studies mentioned maintaining ad libitum water intake, with 6 studies restricting water intake throughout the testing period and 9 omitting such mention. As for food restriction, 50 studies maintained ad libitum food intake, 22 mentioned restricted food intake (between 85 and 90% of free-feeding body weight), and 8 omitted this information. A majority of studies (*n*= 63) did not specify if the animals were littermates or unrelated, while 4 specified rodents to be unrelated and 13 mentioned animals to be littermates.

Housing conditions

Housing conditions were the following: paired (*n*= 39), individual (*n*= 2), group housing ranging from 4 to 10 animals per cage (*n*= 34), a mix of single and paired housing (*n*= 3), and not specified (*n*= 2). Forty-four studies used a 12h light/dark cycle with lights on in the morning (i.e., experimental sessions conducted during light phase) while 27 used a reversed 12h light/dark cycle with lights on in the evening (i.e., experimental sessions conducted during the dark phase). Three studies used a reverse 14:10 light/dark cycle (i.e., testing sessions occurring during the dark phase), and 2 used a 16:8 light/dark cycle (i.e., testing sessions occurring during the light phase). Four studies did not specify the selected light/dark cycles. Although most studies (*n*= 70) were conducted using regular cage housing conditions, 10 studies used enriched environment housing [60, 73, 86-91, 110-113]. From these studies, 8 specified using cages enriched with paper and wood toys, a tunnel, a wooden shelter, or digging material, with some using a salt block. Two studies used two-story cages with the first floor containing running wheels, a shelter, woodblocks, a maze leading to a tube with food, and a ladder giving access to the second floor.

Apparatus

Twenty-nine studies reported using a computerized apparatus such as an operant box, digital counter and/or computer-controlled levers or doors. The remaining articles (*n*= 51) used manually operated apparatus (e.g., maze, open field). Thirty-four studies recorded behaviors, with 9 also recording ultrasonic vocalizations. Seventy articles specified that animals could see, hear, and smell each other during the testing sessions.

Type of reward/punishment

The articles using a cooperation task (*n*= 16) either provided a food reward (*n*= 9) such as food pellets, sucrose pellets or oat flakes, or a liquid reward (*n*= 7) such as liquid sucrose or water. Most studies selecting a helping task (*n*= 22) used social contact as a reward or a mix of food and social contact (*n*= 6). The remaining articles either used a mix of social contact as a reward and no social contact as a punishment (*n*= 1), heroin as a reward (*n*= 2), a mix of reward and punishment (in the form of food and foot shocks, *n*= 1), did not specify (*n*= 2) or did not use a reward (*n*= 3). All articles using a sharing task (*n*= 27) provided a food reward (e.g., food pellets, sucrose pellets, oat flakes, or pieces of banana).

Duration of the task

Most of the included articles reported the duration of the experimental task in minutes (*n* = 60), with the average length being 21.64 minutes. The rest of the studies (*n*= 20) quantified the experimental sessions by reporting the number of trials, coordinated lever press or reward deliveries. Sixty-nine studies reported the total duration of the experiment (in days), which lasted 14.5 days on average. The remaining 11 studies did not report duration.

Habituation and pretraining

In this review, habituation refers to the allotted time a rodent is placed in the novel environment with the possibility to freely explore it, while pretraining refers to the period allotted for rodents to learn specific actions (e.g., lever presses, nose poke, door opening). Of the 80 articles, 40 used a habituation period and 51 included a form of pretraining.

Of the 16 articles using a cooperation task, 6 used a habituation period ranging from 5 to 14 days, with a duration lasting from 10 to 30 minutes. Thirteen reported a form of pretraining consisting of learning how to climb a platform, press a lever, or roll a ball. All the studies used a specific criterion to determine when the pretraining was achieved (e.g., 25 responses per 20-min session).

Of the 37 articles using a helping task, 25 reported a period of habituation lasting between 2 to 14 days and ranging from 10 to 60 minutes. Fifteen articles reported a pretraining for which the duration to learn a specific action (i.e., door opening) varied from a single session (i.e., one day) up to 12 consecutive days (i.e., one session per day).

Of the 27 articles using a sharing task, 9 reported a period of habituation ranging from 1 to 2 days and lasting between 10 to 20 minutes. Twenty-three reported a pretraining period that was divided in two stages: (1) individual stage: initial exposure to learn how to pull/press/poke to obtain a reward, (2) paired stage: pairing the rodent with another conspecific to learn how to pull/press/poke to give a reward to the congener only. Most studies reported 11 days for individual training and 18 days for paired training.

Control group

In total, 58 articles reported using a control, the most popular ones being an empty compartment or a toy condition.

Experimental interventions

In total, 18 studies included an experimental manipulation using a specific drug, such as oxytocin (*n*= 5), heroin (*n*= 1), magnesium (*n*= 1), acetaminophen (*n*= 1), buprenorphine (*n*= 1), muscimol (*n*= 1), dimethyl sulfoxide (*n*= 1), honeybee venom (*n*= 1), 5-HT1A receptor agonist 8-OH-DPAT (*n*= 1). benzodiazepine (*n*= 2), AAV5-CamKIIa-mCherry Virus (*n*= 2) and Ibotenic acid (*n*= 1). Except for heroin, which was used as a reward, all studies used these drugs as an experimental variable.

Thirteen articles included an experimental manipulation requiring surgery such as electrodes (*n*= 2), cannulas (*n*= 4), intravenous catheters (*n*= 2), head trauma (*n*= 1), lesion in the basolateral amygdala (*n*= 1), ovariectomy (*n*= 1), viral brain injection (*n*= 1), and microelectrode with a telemetry sensor (*n*= 1).

Thirty-three articles included other tests such as the open field (*n*= 14), the elevated plus maze (*n*= 6), the dominance tube test (*n*= 4), the forced swim test (*n*= 2), the magnitude discrimination task (*n*= 2), the preference test (*n*= 2), the Rotarod performance test (*n*= 2), the kin discrimination test (*n*= 1), the hole-board test (*n*= 1), the social interaction test (*n*= 2), the self-grooming test (*n*= 1), a test of memory capacity (*n*= 1), the boldness test (*n*= 4), water or food competition test (*n*= 2), social conditioned place preference (*n*= 3), affective state discrimination (*n*= 1), maternal deprivation (*n*= 2), and observational fear conditioning (*n*= 2).

Performance index

Amongst the 27 articles using a sharing task, the most reported performance index was the total number of actions (e.g., stick pulling, lever presses, both-choice reward) enabling an animal to share with a congener. In the prosocial choice task (*n*= 9), all animals preferred the both-choice reward (giving a reward to the actor and partner rats) versus the individual-choice reward (only rewarding the actor). Two studies found that rats preferentially shared with a previously generous partner than a selfish one, suggesting a reciprocity effect [113, 35]. The other 18 studies using a sharing task (repeated donation game and sharing task using levers or sticks) used the frequency and latency of stick pulling or lever presses as the main performance index. Most studies (*n*= 17) found that rodents tended to perform more prosocial actions (share a food reward by pulling a stick or pressing a lever) to previously generous partners than defective ones.

The main performance index reported by studies using cooperation tasks was the number of successful coordinated actions (e.g., lever presses, maze running, nose poke, compartment entering). All studies using a prisoner's dilemma (*n*= 5) found that rodents cooperated more often than they defected (i.e., animals pressed on the cooperation lever more often than the defection lever), and 2 reported reduced likelihood for food restricted rats to cooperate compared to ad *libitum* fed congeners [98, 99]. They also tended to cooperate more with previously cooperating partners than defecting ones. All studies using a cooperation learning task (*n*= 11) found that rodents could learn and perform a coordinated task to receive a reward, and 2 identified visual cues as crucial to the success of coordinated behaviors [52, 25].

The main performance indexes for helping tasks were the frequency and latency of rescuing behavior (i.e., door opening). Most studies reported that rodents could learn to open a door to rescue a congener. The frequency of door openings significantly decreased in control conditions (i.e., empty restrainer, ball of yarn, toy). Four studies also used a food condition, allowing a rodent to open the restrainer door containing a food reward [17, 32, 96, 116]. Two of these studies reported that the food condition had the shortest latencies of door-opening [32, 96], and one study reported that rats would open the door of the restrainer containing the conspecific prior to opening the one containing the food reward [17]. Additionally, one study reported similar latencies in selecting social release or food [116]. Finally, one study also supported light-intensity exercise to increase release behavior [114].

Behavioral analyses

In total, 29 studies included behavioral analyses in their results. Studies using helping tasks most frequently reported behavioral analyses (*n*= 13) compared to sharing (*n*= 5), and co-operation tasks (*n*= 3).

In the helping paradigms, studies showed that rodents tended to stay close to the restrainer/soaked area while the trapped conspecific was inside, and then tended to enter the empty compartment after the freeing. Authors suggested that this behavior could be explained by a desire for social contact, which is often considered a reward in rescuing tasks [17, 53, 55, 70, 24, 96]. One study found that rats preferentially opted to play with a free conspecific (e.g., social opportunity) rather than liberate a trapped congener in an adjacent compartment [109]. Six studies reported distress/alarm calls (22 kHz) through ultrasonic vocalizations in restrained rats [17, 70]. One study reported no influence of enriched versus standard housing on emitted alarm calls [111]. One study also reported free rats to only emit alarm calls when a conspecific was placed in the restrainer, and not during the control condition (i.e., cotton ball in the restrainer) [115]. Finally, a study reported increased alarm calls from the target rat when their partner reduced helping behavior [107]. Other behavioral analyses revealed maternal care in early life to be associated with an ability to respond faster to a distressed conspecific [103]. In contrast, early life maternal deprivation was associated with a reduced motivation to free a trapped cagemate, although this result did not reach significance [111]. These two studies suggest early life experiences to play a role in regulating rodents’ prosociality.

Studies using a sharing task showed that rodents tended to modify their behaviors according to the partner’s action. For example, rats showed increased allogrooming and food donations to previously generous partners [88]. They also appeared sensitive to the food-seeking and social investigation of the recipient, which increased the prosocial actions [87]. A study using a direct reciprocity task revealed that rats pulled rewards differently for cooperators versus non-cooperators depending on the partner's aggressive behavior (e.g., aggressivity towards non-cooperators tended to increase sharing behaviors) [60]. Similarly, Gachomba and colleagues [30] found social hierarchy to contribute to prosocial choices. They found that dominant rats would acquire prosocial tendencies faster and more often selected the altruistic option compared to submissive counterparts. Scheggia and colleagues [31] also found dominant mice to show a preference for altruistic choices in a sharing task, while subordinate mice preferentially selected selfish actions. Two studies investigated the importance of sensory information. One study reported visual cues to have no impact on the latency of stick pulling, suggesting that visual information exchange is not required [60]. Another study supported the role of olfactory cues showing increased willingness of a rat to donate food when olfactory information from the partner was available [65]. Moreover, one study found that the number of non-prosocial male rats in an enriched environment was higher than rats in a standard housing, although large individual differences were observed amongst the animals [73]. Ultrasonic vocalizations recorded by one study revealed calls were more frequent when the actor failed to produce the sharing behavior to draw the partner's attention towards the reward (50 kHz) [87].

Finally, studies using a cooperation task showed a tendency for animals to synchronize their behaviors with their partners, such as waiting for the conspecific before performing an action (nose poke, platform climbing) [56, 74]. All studies (*n*= 3) also assessed behavioral profiles using the social dominance test. Two used the prisoner's dilemma and reported no effect of dominance status on pellets received or responses made [67, 99]. One study found that a natural hierarchy was installed amongst paired rats, in which one would become the leader of the dyad (e.g., the first to climb onto the platform) [105]. They also reported significantly reduced anxiety levels in the leader compared to the follower rat [105]. Finally, one study recorded ultrasonic vocalizations and reported that rodents produced ‘happy’ calls during cooperative behaviors and increased social interactions (50 kHz) [25].

Sex differences

Of the 80 included articles, 17 used both sexes and 8 amongst them investigated sex differences. Four studies did not find differences between males and females regarding task performance (i.e., freeing task, prisoner’s dilemma, sharing task) [73, 99, 101, 107]. Of the four studies that did find a sex difference, one reported elevated activity levels in females compared to male rats during a cooperation task, which could explain improved performance in the task (i.e., coordinating behavior with a congener in a maze to receive a reward) [52]. Another study observed a tendency for female rats to open the door and free a congener more frequently than males. However, this study used significantly less females (*n*= 6) than males (*n*= 24) [17]. Amongst the two studies using a sharing task, one reported female mice to show increased sharing towards familiar versus unfamiliar conspecifics [33], while the other one identified male as being more prosocial than female rats [31]. Finally, although the authors reported no sex differences in door opening, a study found that females emitted more alarm calls than male rats during a helping task [107].

Age differences

Only one study tested possible effects of rodents’ age by comparing responses of adolescent and adult rats in a helping task [29]. Findings indicated that adolescent rats released a trapped conspecific more consistently and quicker than adult counterparts. Moreover, adult rats selectively released ingroup rather than outgroup members, which was not the case for adolescent rats. This could potentially suggest that filiation (ingroup bias) develops over time to impact behavioral responses in adulthood [29].

Strain differences and familiarity

Of the 80 studies, 4 assessed strain differences and 3 investigated the impact of familiarity. One study assessed the strain familiarity of Sprague-Dawley rats in a helping paradigm (door opening) using Sprague-Dawley and Long-Evans rats, both in a familiar or unfamiliar (i.e., stranger rat) condition. The study showed that Sprague-Dawley rats did not open the door to free Long-Evans strangers. Rats also tended to help strangers from their own strain more often than strangers of a different strain, which suggests an in-strain bias [53]. Similarly, a study also indicated a propensity for rats to preferentially release conspecific of the same strain, supporting familiarity as a factor influencing prosociality [104]. Another study evaluated Wistar rats with different genotypes (Wild-Type and Heterozygous) in a repeated donation game. The authors observed no differences between the genotypes regarding task performance (i.e., number of pushes to share a food reward) [63]. Regarding familiarity, one study using a sharing task showed no impact of familiarity on rats' sharing behaviors [30]. A study assessing strain differences in Lister Hooded versus Long-Evans rats in a cooperation learning task found that Long-Evans took longer (i.e., in days) to complete the initial phase of the training (i.e., nose poke) compared to Lister Hooded rats. However, the slope of the learning curve was similar for both strains after this initial phase [74]. Finally, a study assessed two different types of mice (Shank2 and Shank3) in an autism spectrum disorder model, in which mice had to coordinate their running through a maze to receive a reward. The findings support Shank3 mice to display attenuated social cooperative behavior and reduced activity compared to Shank2 mice [67].

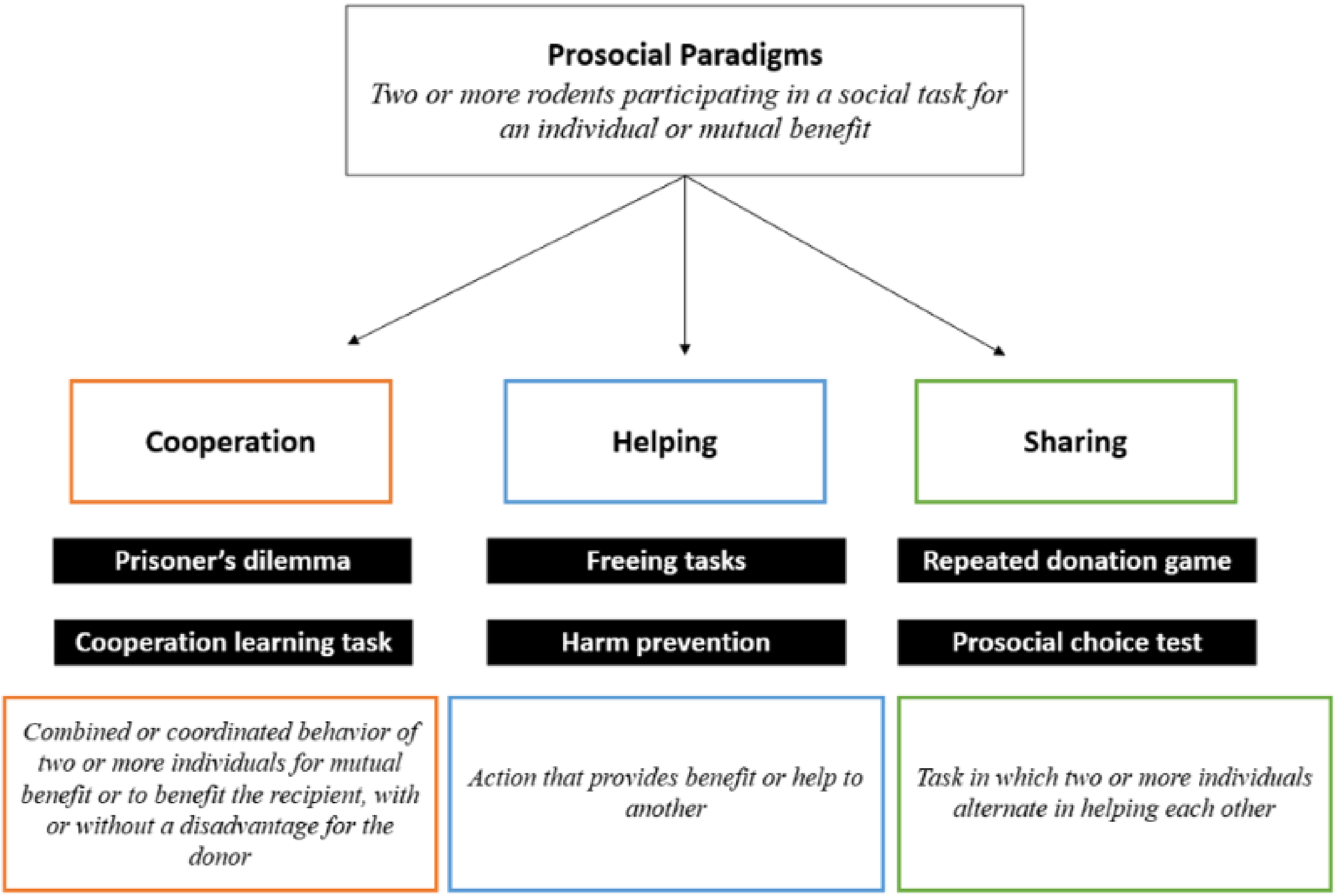
Discussion

This scoping review extracted and analyzed 80 articles with the aim of better characterizing prosocial paradigms in rodents, identifying potential gaps in reporting, and exploring mediating factors of prosociality. Initially, 59 articles published between 2000 and 2020 were included. An updated search conducted by semi-supervised machine algorithm included 21 articles published between 2021 and 2023. Results were the following: (1) Three categories of tasks were extracted: cooperation, helping, and sharing tasks, (2) Rodents performed prosocial actions in all three categories, (3) Significant gaps in reported methodologies (e.g., animals' characteristics, housing conditions, and/or experimental protocol) and mediating factors (e.g., sex, age, strain, housing) were identified, and (4) The incorporation of behavioral analyses provided valuable and pertinent insights into the prosocial behavior of rodents, complementing performance-based metrics. Nevertheless, numerous studies neglected to incorporate such analyses.

1. Prosociality in rodents: Three categories of tasks

While prosocial tasks have been diversified and broadly defined [119] the results of this review consistently noted three distinct tasks and their related definitions (cooperation, helping, and sharing tasks; see Fig 5).

Figure 5. Prosocial paradigms: Proposed definitions of related tasks.



Cooperation tasks

The definition for cooperation tasks (*combined or coordinated behaviors of two or more individuals for mutual benefit or to benefit the recipient, with or without a disadvantage for the donor*) highlights the importance of coordinated behaviors for the pursuit of benefit (i.e., for one recipient or both). The cooperation tasks involved two rodents that were trained to give a response (e.g., lever pressing, running through a maze) within a set time interval to obtain a mutual reward. Another variation involved the prisoner's dilemma, in which both rodents decided to either cooperate with mutual benefits or defect by pressing on a lever within a certain time interval to receive a ‘selfish’ reward.

This review found cooperation tasks to be the least commonly used prosocial paradigms, being selected by 20% of the screened articles (16/80). This is likely related to the extensive pretraining periods. Amongst screened studies, 81% (13/16) performed pretraining tasks requiring rodents to reach a specific criterion (i.e., 8 pulls within 7 minutes, 30 rewards in 10 minutes, pressing the lever ≥100 times/session for two consecutive days) prior to the experimental sessions. These training sessions required substantive investments (i.e., time and costs), with studies reporting pretraining sessions lasting from 18 to 20 days with a specific criterion needed to be reached by each rodent [85, 25]. While it is known that animals learn at a different pace [120-122], setting a specific criterion to be reached prior to moving onto the next stage ensures that all animals understand the task and its associated rewards [123, 124]. In this context, cooperation tasks tend to be considered of higher complexity in terms of required cognitive resources [80], hence rendering a pretraining period essential when using these types of paradigms. A drawback of extensive pretraining is related to some rodents taking much longer than others to learn, with some never reaching the set goal and being excluded from the study, which might create a reporting bias [125, 126].

Helping tasks

The helping paradigm was the most popular in this review, representing 46% of the total number of included studies (37/80). The popularity of this paradigm could be explained by the well-disseminated 2011 research findings by Ben-Ami Bartal and colleagues [17], in which rats opened a door to rescue a trapped congener. This study can be considered a pioneer in the assessment of helping behaviors in rodents, as all the articles included in this review were published subsequently. The definition for helping tasks (*action that provides benefit or help to another*) refers to a specific action that a rodent can do (or not) to explicitly benefit (e.g., giving a reward) or help (e.g., freeing from a restrainer) a congener. Specifically, helping tasks involves pressing a lever to free a congener trapped in a restraining device or a soaked area, or to prevent an electric shock to a congener (harm prevention). All studies using a helping paradigm included an aversive component (e.g., restraining device, soaked area or foot shock). This could be explained by the idea that aversive models trigger distress in animals, which is more explicitly defined and easily observed in rodents (e.g., freezing behavior) than behaviors emulating positive affect [21].

Of all the included studies, 68% of studies (25/37) using a helping paradigm used a habituation period and 40% (15/37) included a form of pretraining. The main goal of pretraining was to teach rodents to open the restrainer door, by either pulling/pressing a lever or breaking through a paper lid. Despite the minimal inclusion of pretraining sessions, the majority of studies reported the willingness of rodents to open the door to rescue a congener. This interesting finding could be influenced by the aversive/distressing component of the task (i.e., being restrained or trapped in a soaked area) possibly recruiting the rat’s survival instinct as a motivation to free a congener. This is concordant with findings from studies on fear contagion in rodents which found that when one conspecific is showing signs of distress (e.g., alarm calls, freezing behaviors) the other, although only witnessing the distressing situation, quickly displays the same fearful signs [127, 12]. This fear contagion allows rodents to respond appropriately to environmental threats [8].

Sharing tasks

The definition for sharing tasks (*tasks in which two or more individuals help each other in turn*) puts an emphasis on alternate actions to benefit one another. A commonly used sharing task involved two rodents that could press a lever or pull a stick to give each other food rewards. Another variation entailed a rodent having the option to consume a food reward in a solitary compartment or select an alternative compartment for a mutual reward (i.e., a prosocial choice task). Unlike cooperation tasks, sharing paradigms do not require coordinated behavioral responses between the two animals.

Twenty-seven studies (33.75%) used a sharing paradigm. Interestingly, this category was the only one to exclusively use non-aversive tasks. While sharing tasks had the lowest rate of habituation period (33%, 9/27), it also had the highest rate of pretraining sessions (85%, 23/27). Most studies opted for a pretraining in two stages: (1) An initial stage where the rodent would learn how to pull/press/poke to obtain a reward, (2) An ensuing paired stage in which the rat learned to pull/press/poke to give a reward to a conspecific. This pretraining in two steps allows the animal to (1) familiarize itself with the rewards (e.g., food pellet, sucrose water), (2) learn the levers' contingency, and (3) associate lever presses with donation to a congener. Pretraining seems essential, particularly for tasks that involve sharing, due to their inherent complexity. Indeed, rodents must learn the contingency of the presented options (e.g., levers, sticks) and associate this response with the impact on the congener (e.g., food sharing). This is consistent with previous research demonstrating the critical importance of pretraining for sharing tasks (Charron et al., 2022).

2. Rodents demonstrated prosociality in all three categories

Across all three task categories, rodents demonstrated the capacity to engage in prosocial behaviors. This finding indicates that all three tasks are suitable options for exploring prosocial behaviors in rats and mice, as they consistently yield meaningful results, as evidenced by the performance indexes.

All studies using a cooperation task supported rodents' ability to perform a coordinated action with a congener to obtain a mutual reward. Studies also found that rodents tended to cooperate more with previously cooperating partners than defecting ones. This indicates that rodents can remember previously cooperative partners and their intention to cooperate. This phenomenon called direct reciprocity is manifested through rodents adjusting the quality (e.g., giving the opportunity to share more palatable food rewards like bananas versus foods with lower levels of attractiveness such as carrots) of their help depending on the partner's previous help [61]. From an evolutionary perspective, this behavioral response matters for survival as a rodent is more susceptible to help a previously collaborative congener based on the premises that future help can be expected [80].

All studies using a helping task found rodents to be able to learn to rescue a congener. Acquisition of this competence is supported by the different control conditions, where the latency of door openings significantly increased when rodents had the possibility to 'rescue' a control (e.g., toy rat, a ball of yarn or open an empty restrainer) indicating that rodents can differentiate between a distressed congener and a control condition.

Similarly to cooperation tasks, studies using a direct reciprocity task reported that rats showed increased prosocial behaviors (e.g., sharing a food reward) to previously cooperative versus defective partners. This finding is consistent with observations of increased help reciprocity within rodent dyads using previously generous versus defecting partners. This supports the ability of rodents to recognize and remember helpful partners and the importance of such recognition in guiding prosocial responses, exemplified through increased allogrooming and food donations to previously sharing partners [79]. Rats also appeared sensitive to the food-seeking and social investigation of the recipient rat, a factor increasing the occurrences of prosocial actions.

3. Gaps in reporting and mediating factors of prosociality

This scoping review revealed many gaps in reported methodologies as revealed by the risk of bias assessment. For cooperation tasks, the highest risk found amongst the articles was the report on baseline characteristics: 61% of studies (8/13) were identified with a high risk. A high risk of bias was attributed if the baseline of the animals and groups was not comparable without any rational or methodological explanation for this approach. Studies failed to address the age difference between the tested groups (e.g., animals of varying age), selection of rodents with superior performance during pretraining, had varied housing conditions (e.g., with up to 5 rats housed per cage) and/or failed to identify and discuss previous exposure of some rodents to cognitive tests. Defining a study protocol prior to starting the experiment could have prevented these methodological issues [128]. While certain issues may be challenging to resolve due to logistical constraints or limited access to resources, it is crucial to acknowledge and address the potential impact of these choices to promote transparency and facilitate replication [128].

Regarding helping tasks, the highest risk of bias found amongst studies was the other sources of bias (46%, 17/37). Studies failed to report the results of tests previously conducted, mentioned cleaning testing apparatuses with a wet sponge (this being insufficient to remove previously tested rodent’s odors) [129], and failed to report methodological information regarding the coding of video recordings (e.g., manually, with software, how many researchers coded) and inter-coder reliability scores. Ideally, coders should be blind to the conditions and inter-coder reliability should always be assessed [130].

Sharing tasks showed the most elevated occurrence of ‘high risk’ flags. Many studies failed to report the methodology with sufficient details enabling replication (e.g., failed to specify the rats’ age or housing conditions, left a portion of the social task procedure unexplained, provided no information on the habituation or handling process, did not indicate the inter-coder reliability score or only used one coder, or failed to randomly assign the experimental groups and/or housing conditions). It is strongly recommended that authors follow the ARRIVE guidelines when designing animal studies, as well as using a guide for reporting animal studies, such as the one published by Grundy [131].

Impact of mediating factors on prosocial behaviors

Extracted data from the 80 included articles revealed potential mediating factors of prosociality in all 3 categories of tasks, including housing, aggression and dominance, strain and familiarity, food restriction, and sex differences. Although each factor did not include enough studies to draw strong conclusions, highlighting these trends may inform future studies investigating the expression of prosocial behaviors in rodents.

Housing

While many studies reported using an enriched environment (n= 10), only two investigated its potential impact on prosociality. One study reported enriched environment (EE) to be associated with an increased number of ‘non-prosocial’ male rats compared to standard housing, although large individual differences were observed amongst the animals [73]. Interestingly, the other study found that maternal separation impaired mutual reward preference in a prosocial choice task, but that exposure to an enriched environment prevented this impairment [110]. Although EE conditions can vary considerably between studies [132], it has been associated with beneficial effects on brain plasticity, cognition, and improved mental and physical health [34, 133]. Such housing conditions also promote animal welfare [133, 134]. Although limited, observations show that EE might have an impact on the expression of prosociality in rodents and that additional studies are needed.

Aggression and dominance

The impact of aggression and dominance was investigated by studies using cooperation and sharing tasks only. Of the 16 studies using cooperation tasks, 2 investigated dominance (using the dominance tube test). They both reported no effects of the dominance status on cooperative behaviors in male mice and rats, and in ovariectomized female rats [67, 99]. These results contrast findings from other studies showing dominant rats to display increased motivation for food rewards [135], or a correlation between dominance and social motivation [136]. This difference might be explained by females that were ovariectomized during the adolescence stage, which could have impacted results of the study [99]. Pubertal hormones could play a role in modulating aggression and dominance in female rodents [137], and pubertal ovariectomy is known to impact brain development [138], including preventing the development of social recognition abilities [139], impairments in spatial learning [140], and social investigation [141]. In contrast to cooperation tasks, 3 out of 27 studies using a sharing task found an impact of dominance and hierarchy on sharing behavior [30, 31]. Gachomba and colleagues [30] reported that dominant rats tended to acquire prosocial tendencies faster than submissive conspecifics. Behavioral analyses also revealed that submissive animals tended to increase proximity towards the focal (donor) rat and would follow them around the choice area, while dominant animals would respond to these cues by increasing their attention to the recipient through increased sniffing around the submissive animal [30]. Similarly, Scheggia and colleagues [31] found that prosocial actions were directed more frequently towards dominant actors, and that selfish decisions were more often made by submissive than dominant mice. Finally, one study found that rats pulled levers differently depending on the partner's aggressive behaviors. The authors suggested that cooperators can reduce helping behavior by showing aggression whereas non-cooperators might increase helping behaviors of their partners by being aggressive towards them [60]. Interestingly this study was the only one allowing physical contact in the apparatus. It is known that rats and mice establish a social hierarchy [142], and the possibility for physical contact increases the chances of aggressive and dominance behaviors [143]. As demonstrated in this review, aggression and dominance behaviors seem to have a mediating impact on prosociality in rodents and remain an important element for future studies to document.

Food restriction

Four studies investigated the impact of food restriction on prosocial behaviors. Three studies using a helping task included a food condition, where a rodent could open a restrainer containing food. Two of these studies found that the food condition (i.e., restrainer containing a food reward) had the shortest latency of door-opening [32, 96] whereas one study found that rats would open the door of the restrainer containing the conspecific first followed by the restrainer containing the food [17]. Interestingly, the two first studies used a food restriction paradigm while the third one used ad libitum fed rats. This finding is similar to the cooperation paradigm, in which studies found that ad libitum fed rodents cooperated more than food deprived ones [98, 99]. Food restriction in rodents can alter impulsiveness and the incentive value of food rewards [98, 144], which could explain the increased willingness to cooperate in satiated rats. Rodents are commonly maintained between 80-85% of free-feeding body weights to maximize motivation to participate and facilitate operant responding [99, 145, 146]. However, some have raised welfare concerns of such practice [145, 146], arguing that the individual food intake and adult body weight can significantly vary amongst animals [145] and that such practice does not account for strain, age, housing conditions, exercise opportunities, and so forth [145-147]. Finally, the circadian rhythm is known to regulate caloric intake, with rodents consuming 70 to 85% of their diet during the dark phase of the circadian cycle [145, 148]. Thus, the period at which restricted feeding is imposed is also important to consider, as it can influence daily food/calorie intake, and consequently impact task performance.

Strain and familiarity

Of the 7 studies investigating strain differences and the impact of familiarity on prosocial behaviors, 4 found performance differences amongst different strains and levels of familiarity. For example, rats seem to present an in-strain bias, with Sprague-Dawley not opening the door to free Long-Evans strangers [53]. Moreover, Sprague-Dawley tended to help strangers from their own strain, but not from a different one [53]. Another study supported the preference for rats to release conspecific of the same strain [104]. Interestingly, findings from Breton and colleagues [29] found that ingroup and outgroup bias seem to emerge during adulthood, with adolescent rats sharing as much with ingroup and outgroup members, but not adult rats. These findings suggest that the choice of strain, the age of the rodents, and the level of familiarity between the animals can play a role in the expression of prosocial behaviors and more research is needed to provide further insight in this mediating factor of prosociality.

Sex differences

Of the 80 included studies, 42 only used males (52.5%), 19 used only females (23.75%), 13 included both sexes (16.25%), and 6 did not specify (7.5%). These study samples are consistent with underrepresentation of females in the scientific literature. In neuroscience, only 20% of studies include both sexes, and for every research on females, there are five publications with males [149, 150]. One of the reasons mentioned for this discrepancy is related to the menstrual/estrous cycle and hormonal fluctuations, which could create variability amongst females [150]. The role of hormones such as estrogen, estradiol, and progesterone on the regulation of cognitive processes (e.g., memory, learning, decision-making) in females is well documented, particularly in relation to the abundance of receptors that respond to these hormones in the hypothalamus, pituitary gland, hippocampus, and prefrontal cortex [151, 152]. To date, sex-related differences have been widely studied in relation to hormonal secretion, which regulates various aspects of social behaviors, including social interactions, social learning, reproduction, and aggression [153, 154]. In this review, only one study specified doing vaginal smears after each experimental session to determine the levels of circulating sex hormones throughout the estrous cycle [73]. Interestingly, they found that females did not show an overall preference for the prosocial option regardless of their estrous cycle [73]. Mate choice also requires cognitive functions of social recognition and vicarious social learning, two behaviors partly regulated by estrogen secretion in females [153, 154]. Indeed, ovariectomized females show reduced social recognition, whereas the injection of estrogen alone or in combination with progesterone restores this cognitive function [155]. In this review, one study specified that females have been ovariectomized to control for hormone levels [99]. The authors found no sex differences regarding direct reciprocity. To date, examination of sex-related differences in rodents' prosociality remains preliminary and needs further investigation and replication [28]. As highlighted, not enough studies have investigated sex differences to enable clear conclusions on the effect of sex on prosocial behaviors.

4. Behaviors are determinant in rodents prosociality

Results from this scoping review revealed behavioral analyses during prosocial testing to provide a source for important refinement in the interpretation of collected observations.

However, many included articles failed to report such analyses. When performed, behavioral analyses included video recording of the task and audio recording of ultrasonic vocalizations.

Behavioral analyses

Of the 37 studies using a helping task, 15 also investigated behavioral patterns. In general, authors found that rodents tended to stay close to the restrainer or soaked area while the trapped conspecific was inside. They also displayed a tendency to enter the compartment once the congener was free, indicating a potential desire for social contact. This behavior could serve as a reward in this context, implying that the underlying motivation may be social interaction rather than the intention to help another [24, 53, 66, 97]. Interestingly, 5 articles prevented social contact from happening after the freeing action, and all showed that rodents could also learn to open the door [18, 70-72, 102]. These contrasting findings suggest that social contact might not be the only motivation driving rodents' helping behaviors. Hence, further research is warranted to define the influence of social contact on helping tasks. Regarding cooperation tasks, Conde-Moro and colleagues [56] used a paradigm in which rats had to simultaneously climb a platform to receive a reward. They found that the 'leader' rats would initiate climbing onto a first platform and wait for their partner to reach the same level prior climbing the second platform. This clearly demonstrates a specific behavioral pattern that took place during the cooperation task. Similarly, Kozma and colleagues [74] indicated rats to keep close body contact with their partner, possibly to influence or control the partner's action. These results show that analyzing rodents' behaviors can give access to a rich array of information on prosociality [27, 148]. Consequently, researchers limiting analyses to task performance might miss important data stemming from rodents' behavioral patterns [156, 157].

Ultrasonic vocalizations

Rodents communicate with each other using frequencies undetectable to the human ear [133]. Such communication can be investigated through the recording of ultrasonic vocalizations (USVs). It is known that rats tend to emit low frequency ‘alarm’ calls (∽22 kHz) when in distress [133] and high frequency ‘happy’ calls during play and mating [158]. Only one study amongst the 16 using a cooperation task performed such analyses. They found prosocial behavior to be positively correlated with the number of emitted ‘happy calls’ (∽50 kHz). The calls were recorded before and after a simultaneous nose-poke response, suggesting that the task might have been considered reinforcing (appetitive) for the rats [25]. While all studies using a helping task included an aversive component, only two recorded USVs [70, 115]. Aversive paradigms tend to trigger alarm calls, which alert the other conspecific and can motivate its action (i.e., opening a door) [159]. Indeed, Hernandez-Lallement and colleagues [70] found that rats would produce an alarm call when trapped or receiving electric shocks, probably to inform the free rat of its distress [159]. Similarly, Subdhadeep and colleagues [115] found that trapped rats would emit distress and ‘happy’ calls before and after liberation, respectively. This suggests that communication through USVs could be at the core of the rodents’ motivation to open the door. Finally, one study using a sharing paradigm measured USVs [87]. Similar to findings from helping tasks, alarm calls were more frequently emitted in situations where the actor rat failed to produce the awaited sharing behavior, or when the actor wanted to draw the partner’s attention to a shared reward. While the evidence remains limited, studies examining USVs within the three prosocial paradigms indicate that vocalizations play a pivotal role in the manifestation of prosocial behaviors.

Conclusion

Results from this scoping review revealed four important findings. Firstly, 3 categories of prosocial paradigms were extracted and defined. Secondly, findings from the 80 included articles showed rodents to be able to display prosocial behaviors in all 3 categories of tasks. Thirdly, significant gaps in reported methodologies, such as variations in animal characteristics, housing conditions, and experimental protocols, were identified, alongside potential mediating factors like sex, housing, aggression, dominance, strain, familiarity, age, and food restriction. Lastly, behavioral analyses unveiled significant behavioral patterns exhibited by rodents, which could potentially influence the expression of prosociality. While these findings demonstrate the ability for rats and mice to display prosocial behaviors in three categories of paradigm, this scoping review also informs future studies on the importance of behavioral analyses and mediating factors in the expression of prosociality in rodents. However, a limitation in studying prosocial behavior in rodents arises from the fact that many paradigms involve mutual benefits, such as the focal rodent also receiving a reward (either food or social interaction). This raises questions about the genuine motivation underlying prosocial behavior (e.g., are rodents engaging in tasks solely for a food reward or out of a desire to assist or benefit a congener?). While some reviewed studies have addressed this issue by restricting social contact or modulating food reward, further research is necessary to deepen our understanding of prosocial mechanisms in rodents. Consequently, integrating behavioral analyses alongside performance-based metrics for each task, as well as investigating further potential mediating factors involved in the expression of prosocial behaviors could be the next step to gain further insight into rodent prosociality.

References

*** Included articles**

1. Decety J, Holvoet C. The emergence of empathy: A developmental neuroscience perspective. Developmental Review. 2021 Dec 1;62:100999.

2. Decety J, Bartal IBA, Uzefovsky F, Knafo-Noam A. Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. Philosophical Transactions of the Royal Society B: Biological Sciences. 2016;371(1686):20150077. Doi: 10.1098/rstb.2015.0077.

3. Ellenbroek B, Youn J. Rodent models in neuroscience research: is it a rat race? Dis Model Mech. 2016;9(10):1079–87. Doi: 10.1242/dmm.026120.

4. Möhrle D, Fernández M, Peñagarikano O, Frick A, Allman B, Schmid S. What we can learn from a genetic rodent model about autism. Neuroscience & Biobehavioral Reviews. 2020; 109:29–53. Doi: 10.1016/j.neubiorev.2019.12.015.

5. de Waal FBM. Putting the altruism back into altruism: the evolution of empathy. Annu Rev Psychol. 2008; 59:279–300. Doi: 10.1146/annurev.psych.59.103006.093625.

*6. Karakilic A, Kizildag S, Kandis S, Guvendi G, Koc B, Camsari GB, et al. The effects of acute foot shock stress on empathy levels in rats. Behavioural Brain Research. 2018; 349:31–6. Doi: 10.1016/j.bbr.2018.04.043.

7. Cox SS, Reichel CM. Current rodent models for the study of empathic processes. Behavioural Pharmacology. 2021;32(2 & 3):96–111. Doi: 10.1097/FBP.0000000000000590.

8. Meyza K, Knapska E. What can rodents teach us about empathy? Current Opinion in Psychology. 2018; 24:15–20. Doi: 10.1016/j.copsyc.2018.03.002.

9. Anderson EE. The Effect of the Presence of a Second Animal upon Emotional Behavior in the Male Albino Rat. The Journal of Social Psychology. 1939;10(2):265–8. Doi: 10.1080/00224545.1939.9713365.

10. Church RM. Emotional reactions of rats to the pain of others. J Comp Physiol Psychol. 1959;52(2):132–4. Doi: 10.1037/h0043531.

11. Riess D. Vicarious conditioned acceleration: successful observational learning of an aversive Pavlovian stimulus contingency. J Exp Anal Behav. 1972;18(1):181–6. Doi: 10.1901/jeab.1972.18-181.

12. Gonzalez-Liencres C, Juckel G, Tas C, Friebe A, Brüne M. Emotional contagion in mice: the role of familiarity. Behav Brain Res. 2014; 263:16–21. Doi: 10.1016/j.bbr.2014.01.020.

13. Sivaselvachandran S, Acland EL, Abdallah S, Martin LJ. Behavioral and mechanistic insight into rodent empathy. Neuroscience & Biobehavioral Reviews. 2018; 91:130–7. Doi: 10.1016/j.neubiorev.2016.06.007.

14. Pérez-Manrique A, Gomila A. Emotional contagion in nonhuman animals: A review. WIREs Cognitive Science. 2022;13(1):560. Doi: 10.1002/wcs.1560.

15. Lahvis G. Make animal models more meaningful. Nature. 2017;543(7647):623. Doi: 10.1038/543623d.

16. Rault JL. Be kind to others: Prosocial behaviours and their implications for animal welfare. Applied Animal Behaviour Science. 2019; 210:113–23. Doi: 10.1016/j.applanim.2018.10.015.

*17. Bartal IBA, Decety J, Mason P. Empathy and pro-social behavior in rats. Science. 2011;334(6061):1427–30. Doi: 10.1126/science.1210789.

*18. Sato N, Tan L, Tate K, Okada M. Rats demonstrate helping behavior toward a soaked conspecific. Anim Cogn. 2015;18(5):1039–47. Doi: 10.1007/s10071-015-0872-2.

*19. Hernandez-Lallement J, van Wingerden M, Marx C, Srejic M, Kalenscher T. Rats prefer mutual rewards in a prosocial choice task. Frontiers in Neuroscience. 2015; 8:443. Doi: 10.3389/fnins.2014.00443.

20. Špinka M. Social dimension of emotions and its implication for animal welfare. Applied Animal Behaviour Science. 2012;138(3):170–81. Doi: 10.1016/j.applanim.2012.02.005.

21. Knapska E, Mikosz M, Werka T, Maren S. Social modulation of learning in rats. Learn Mem. 2010;17(1):35–42. Doi: 10.1101/lm.1670910.

22. Proctor HS, Carder G, Cornish AR. Searching for animal sentience: a systematic review of the scientific literature. Animals (Basel). 2013;3(3):882–906. Doi: 10.3390/ani3030882.

23. Panksepp JB, Lahvis GP. Rodent empathy and affective neuroscience. Neurosci Biobehav Rev. 2011;35(9):1864–75. Doi: 10.1016/j.neubiorev.2011.05.013.

*24. Silberberg A, Allouch C, Sandfort S, Kearns D, Karpel H, Slotnick B. Desire for social contact, not empathy, may explain “rescue” behavior in rats. Anim Cogn. 2014;17(3):609–18. Doi: 10.1007/s10071-013-0692-1.

*25. Łopuch S, Popik P. Cooperative behavior of laboratory rats (Rattus norvegicus) in an instrumental task. Journal of Comparative Psychology. 2011;125(2):250–3. Doi: 10.1037/a0021532.

*26. Cox SS, Reichel CM. Rats display empathic behavior independent of the opportunity for social interaction. Neuropsychopharmacol. 2020;45(7):1097–104. Doi: 10.1038/s41386-019-0572-8.

27. Charron V, Talbot J, Plamondon H. Prosocial decision making in an operant box paradigm promotes visual communication and complex behavioral sequences in adolescent rat dyads. Anim Behav Cogn. 2022;9(1):53–71. Doi: 10.26451/abc.09.01.05.2022.

28. Meyza KZ, Bartal IBA, Monfils MH, Panksepp JB, Knapska E. The roots of empathy: through the lens of rodent models. Neurosci Biobehav Rev. 2017;76(Pt B):216–34. Doi: 10.1016/j.neubiorev.2016.10.028.

*29. Breton JM, Eisner JS, Gandhi VS, Musick N, Zhang A, Long KLP, et al. Neural activation associated with outgroup helping in adolescent rats. iScience. 2022;25(6):104412. Doi: 10.1016/j.isci.2022.104412.

*30. Gachomba MJM, Esteve-Agraz J, Caref K, Maroto AS, Bortolozzo-Gleich MH, Laplagne DA, et al. Multimodal cues displayed by submissive rats promote prosocial choices by dominants. Curr Biol. 2022;32(15):3288-3301.e8. Doi: 10.1016/j.cub.2022.06.026.

*31. Scheggia D, La Greca F, Maltese F, Chiacchierini G, Italia M, Molent C, et al. Reciprocal cortico-amygdala connections regulate prosocial and selfish choices in mice. Nat Neurosci. 2022;25(11):1505–18. Doi: 10.1038/s41593-022-01179-2.

*32. Blystad MH, Andersen D, Johansen EB. Female rats release a trapped cagemate following shaping of the door opening response: Opening latency when the restrainer was baited with food, was empty, or contained a cagemate. PLOS ONE. 2019;14(10). Doi: 10.1371/journal.pone.0223039.

*33. Misiołek K, Klimczak M, Chrószcz M, Szumiec Ł, Bryksa A, Przyborowicz K, et al. Prosocial behavior, social reward and affective state discrimination in adult male and female mice. Sci Rep. 2023;13(1):5583. Doi: 10.1101/2022.08.19.504492.

34. Brenes JC, Lackinger M, Höglinger GU, Schratt G, Schwarting RKW, Wöhr M. Differential effects of social and physical environmental enrichment on brain plasticity, cognition, and ultrasonic communication in rats. Journal of Comparative Neurology. 2016;524(8):1586–607. Doi: 10.1002/cne.23842.

*35. Sen A, Kara AY, Koyu A, Simsek F, Kizildag S, Uysal N. The effects of chronic restraint stress on empathy-like behaviour in rats. Neurosci Lett. 2021; 765:136255. Doi: 10.1016/j.neulet.2021.136255.

36. Gouveia K, Hurst JL. Improving the practicality of using non-aversive handling methods to reduce background stress and anxiety in laboratory mice. Sci Rep. 2019 Dec 30;9(1):20305.

37. Fulenwider HD, Caruso MA, Ryabinin AE. Manifestations of domination: Assessments of social dominance in rodents. Genes, Brain and Behavior. 2022;21(3):e12731.

38. Timmer M, Cordero MI, Sevelinges Y, Sandi C. Evidence for a role of oxytocin receptors in the long-term establishment of dominance hierarchies. Neuropsychopharmacol. 2011;36(11):2349–56. Doi: 10.1038/npp.2011.125.

39. Campbell GJ, Senior AM, Bell-Anderson KS. Metabolic effects of high glycaemic index diets: a systematic review and meta-analysis of feeding studies in mice and rats. Nutrients. 2017;9(7):646. Doi: 10.3390/nu9070646

40. Himsworth CG, Parsons KL, Jardine C, Patrick DM. Rats, cities, people, and pathogens: a systematic review and narrative synthesis of literature regarding the ecology of rat-associated zoonoses in urban centers. Vector Borne Zoonotic Dis. 2013;13(6):349–59. Doi: 10.1089/vbz.2012.1195

41. Tractenberg SG, Levandowski ML, de Azeredo LA, Orso R, Roithmann LG, Hoffmann ES, et al. An overview of maternal separation effects on behavioural outcomes in mice: Evidence from a four-stage methodological systematic review. Neurosci Biobehav Rev. 2016;68:489–503. Doi: 10.1016/j.neubiorev.2016.06.021

42. Cheang R, Gillions A, Sparkes E. Do mindfulness-based interventions increase empathy and compassion in children and adolescents: a systematic review. J Child Fam Stud. 2019;28(7):1765–79. Doi: 10.1007/s10826-019-01413-9

43. Lorié Á, Reinero DA, Phillips M, Zhang L, Riess H. Culture and nonverbal expressions of empathy in clinical settings: A systematic review. Patient Educ Couns. 2017;100(3):411–24. Doi: 10.1016/j.pec.2016.09.018

44. Luberto CM, Shinday N, Song R, Philpotts LL, Park ER, Fricchione GL, et al. A systematic review and meta-analysis of the effects of meditation on empathy, compassion, and prosocial behaviors. Mindfulness (N Y). 2018;9(3):708–24. Doi: 10.1007/s12671-017-0841-8

45. Portt E, Person S, Person B, Rawana E, Brownlee K. Empathy and positive aspects of adolescent peer relationships: A scoping review. Journal of Child and Family Studies. 2020;29(9):2416–33. Doi: 10.1007/s10826-020-01753

46. McGowan J, Sampson M, Salzwedel DM, Cogo E, Foerster V, Lefebvre C. PRESS Peer Review of Electronic Search Strategies: 2015 Guideline Statement. J Clin Epidemiol. 2016;75:40–6. Doi: 10.1016/j.jclinepi.2016.01.021

47. van de Schoot R, de Bruin J, Schram R, Zahedi P, de Boer J, Weijdema F, et al. An open source machine learning framework for efficient and transparent systematic reviews. Nat Mach Intell. 2021 Feb;3(2):125–33.

48. Teijema JJ, Hofstee L, Brouwer M, Bruin J de, Ferdinands G, Boer J de, Vizan P, van den Brand S, Bockting C, van de Schoot R, Bagheri A. Active learning-based Systematic reviewing

using switching classification models: the case of the onset, maintenance, and relapse of depressive disorders. PsyArXiv. 2022. Doi: 10.31234/osf.io/t7bpd.

49. Ferdinands G, Schram R, de Bruin J, Bagheri A, Oberski DL, Tummers L, van de Schoot R. Active learning for screening prioritization in systematic reviews - A simulation study. Open Science Framework. 2020. Doi: 10.31219/osf.io/w6qbg.

50. Satopaa V, Albrecht J, Irwin D, Raghavan B. Finding a “Kneedle” in a Haystack: Detecting Knee Points in System Behavior. In: 2011 31st International Conference on Distributed Computing Systems Workshops [Internet]. 2011 [cited 2023 Sep 26]. p. 166–71. Available from: https://ieeexplore.ieee.org/document/5961514

51. Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE’s risk of bias tool for animal studies. BMC Medical Research Methodology. 2014;14(1):43. Doi: 10.1186/1471-2288-14-43.

*52. Avital A, Aga-Mizrachi S, Zubedat S. Evidence for social cooperation in rodents by automated maze. Sci Rep. 2016;6(1):29517. Doi: 10.1038/srep29517.

*53. Ben-Ami Bartal I, Rodgers DA, Bernardez Sarria MS, Decety J, Mason P. Pro-social behavior in rats is modulated by social experience. eLife. 2014; 3:01385. Doi: 10.7554/eLife.01385.

*54. Ben-Ami Bartal I, Shan H, Molasky NMR, Murray TM, Williams JZ, Decety J, Mason P. Anxiolytic treatment impairs helping behavior in rats. Frontiers in Psychology. 2016;7. Doi: 10.3389/fpsyg.2016.00850.

*55. Carvalheiro J, Seara-Cardoso A, Mesquita AR, de Sousa L, Oliveira P, Summavielle T, Magalhães A. Helping behavior in rats (Rattus norvegicus) when an escape alternative is present. Journal of Comparative Psychology. 2019; 133:452–62. Doi: 10.1037/com0000178.

*56. Conde-Moro AR, Rocha-Almeida F, Sánchez-Campusano R, Delgado-García JM, Gruart A. The activity of the prelimbic cortex in rats is enhanced during the cooperative acquisition of an instrumental learning task. Progress in Neurobiology. 2019; 183:101692. Doi: 10.1016/j.pneurobio.2019.101692.

*57. Daghestani MH, Selim ME, Abd-Elhakim YM, Said EN, El-Hameed NEA, Khalil SR, et al. The role of apitoxin in alleviating propionic acid-induced neurobehavioral impairments in rat pups: The expression pattern of Reelin gene. Biomedicine & Pharmacotherapy. 2017;93:48–56. Doi : 10.1016/j.biopha.2017.06.034.

*58. de Carvalho LC, dos Santos L, Regaço A, Barbosa TB, da Silva RF, de Souza D das G, et al. Cooperative responding in rats maintained by fixed- and variable-ratio schedules. Journal of the Experimental Analysis of Behavior. 2018;110(1):105–26. Doi: 10.1002/jeab.457.

*59. Delmas GE, Lew SE, Zanutto BS. High mutual cooperation rates in rats learning reciprocal altruism: The role of payoff matrix. PLOS ONE. 2019;14(1): e0204837. Doi: 10.1371/journal.pone.0204837.

*60. Dolivo V, Taborsky M. Cooperation among Norway Rats: The importance of visual cues for reciprocal cooperation, and the role of coercion. ethology. 2015;121(11):1071–80. Doi: 10.1111/eth.12421.

*61. Dolivo V, Taborsky M. Norway rats reciprocate help according to the quality of help they received. Biology Letters. 2015;11(2):20140959. Doi: 10.1098/rsbl.2014.0959.

*62. Donovan A, Ryan E, Wood RI. Cooperative responses in rats playing a 2 × 2 game: Effects of opponent strategy, payoff, and oxytocin. Psychoneuroendocrinology. 2020; 121:104803. Doi: 10.1016/j.psyneuen.2020.104803.

*63. Festucci F, Buccheri C, Cerniglia L, Paciello M, Cimino S, Curcio G, et al. A new paradigm for prosocial behavior and reciprocity, assessed in WT and HET rats for the DAT gene. Behavioural Brain Research. 2020; 393:112746. Doi: 10.1016/j.bbr.2020.112746.

*64. Fontes-Dutra M, Della-Flora Nunes G, Santos-Terra J, Souza-Nunes W, Bauer-Negrini G, Hirsch MM et al. Abnormal empathy-like pro-social behaviour in the valproic acid model of autism spectrum disorder. Behavioural Brain Research. 2019; 364:11–8. Doi: 10.1016/j.bbr.2019.01.034.

*65. Gerber N, Schweinfurth MK, Taborsky M. The smell of cooperation: rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task. Proceedings of the Royal Society B: Biological Sciences. 2020;287(1939):20202327. Doi: 10.1098/rspb.2020.2327.

*66. Hachiga Y, Schwartz LP, Silberberg A, Kearns DN, Gomez M, Slotnick B. Does a rat free a trapped rat due to empathy or for sociality? Journal of the Experimental Analysis of Behavior. 2018;110(2):267–74. Doi: 10.1002/jeab.464.

*67. Han KA, Yoon TH, Shin J, Um JW, Ko J. Differentially altered social dominance- and cooperative-like behaviors in Shank2- and Shank3-mutant mice. Molecular Autism. 2020;11(1):87. Doi: 10.1186/s13229-020-00392-9.

*68. Havlik JL, Vieira Sugano YY, Jacobi MC, Kukreja RR, Jacobi JHC, Mason P. The bystander effect in rats. Science Advances. 2020;6(28): eabb4205. Doi: 10.1126/sciadv.abb4205

*69. Hernandez-Lallement J, van Wingerden M, Schäble S, Kalenscher T. Basolateral amygdala lesions abolish mutual reward preferences in rats. Neurobiology of Learning and Memory. 2016; 127:1–9. Doi: 10.1016/j.nlm.2015.11.004.

*70. Hernandez-Lallement J, Attah AT, Soyman E, Pinhal CM, Gazzola V, Keysers C. Harm to others acts as a negative reinforcer in rats. Current Biology. 2020;30(6):949-961.e7. Doi: 10.1016/j.cub.2020.01.017.

*71. Hosgorler F, Koc B, Kizildag S, Canpolat S, Argon A, Karakilic A, et al. Magnesium Acetyl Taurate prevents tissue damage and deterioration of prosocial behavior related with vasopressin levels in traumatic brain injured rats. Turk Neurosurg. 2020;30(5):723–33. Doi: 10.5137/1019-5149.jtn.29272-20.1.

*72. Kandis S, Ates M, Kizildag S, Camsari GB, Yuce Z, Guvendi G, et al. Acetaminophen (paracetamol) affects empathy-like behavior in rats: Dose-response relationship. Pharmacology Biochemistry and Behavior. 2018; 175:146–51. Doi: 10.1016/j.pbb.2018.10.004.

*73. Kentrop J, Kalamari A, Danesi CH, Kentrop JJ, van IJzendoorn MH, Bakermans-Kranenburg MJ, et al. Pro-social preference in an automated operant two-choice reward task under different housing conditions: Exploratory studies on pro-social decision making. Developmental Cognitive Neuroscience. 2020; 45:100827. Doi: 10.1016/j.dcn.2020.100827.

*74. Kozma K, Kassai F, Ernyey AJ, Gyertyán I. Establishment of a rodent cooperation assay as a model of social cognition. Journal of Pharmacological and Toxicological Methods. 2019; 97:44–51. Doi: 10.1016/j.vascn.2019.03.003.

*75. Li G, Wood RI. Male rats play a repeated donation game. Physiology & Behavior. 2017; 174:95–103. Doi: 10.1016/j.physbeh.2017.03.010.

*76. Márquez C, Rennie SM, Costa DF, Moita MA. Prosocial choice in rats depends on food-seeking behavior displayed by recipients. Current Biology. 2015;25(13):1736–45. Doi: 10.1016/j.cub.2015.05.018.

*77. Oberliessen L, Hernandez-Lallement J, Schäble S, van Wingerden M, Seinstra M, Kalenscher T. Inequity aversion in rats, Rattus norvegicus. Animal Behaviour. 2016; 115:157–66. Doi: 10.1016/j.anbehav.2016.03.007.

*78. Raz S. Ameliorative effects of brief daily periods of social interaction on isolation-induced behavioral and hormonal alterations. Physiology & Behavior. 2013;116–117:13–22. Doi: 10.1016/j.physbeh.2013.03.009.

*79. Rutte C, Taborsky M. Generalized Reciprocity in Rats. PLOS Biology. 2007;5(7):e196. Doi: 10.1371/journal.pbio.0050196.

*80. Rutte C, Taborsky M. The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): direct vs generalised reciprocity. Behav Ecol Sociobiol. 2008;62(4):499–505. Doi: 10.1007/s00265-007-0474-3.

*81. Schmid R, Schneeberger K, Taborsky M. Feel good, do good? Disentangling reciprocity from unconditional prosociality. Ethology. 2017;123(9):640–7. Doi: 10.1111/eth.12636.

*82. Schneeberger K, Dietz M, Taborsky M. Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evol Biol. 2012;12(1):41. Doi: 10.1186/1471-2148-12-41.

*83. Schönfeld LM, Schäble S, Zech MP, Kalenscher T. 5-HT1A receptor agonism in the basolateral amygdala increases mutual-reward choices in rats. Sci Rep. 2020;10(1):16622. Doi: 10.1038/s41598-020-73829-z.

*84. Schwartz LP, Silberberg A, Casey AH, Kearns DN, Slotnick B. Does a rat release a soaked conspecific due to empathy? Anim Cogn. 2017;20(2):299–308. Doi: 10.1007/s10071-016-1052-8.

*85. Schweinfurth MK, Taborsky M. No evidence for audience effects in reciprocal cooperation of norway rats. Ethology. 2016;122(6):513–21. Doi: 10.1111/eth.12499.

*86. Schweinfurth MK, Taborsky M. The transfer of alternative tasks in reciprocal cooperation. Animal Behaviour. 2017; 131:35–41. Doi: 10.1016/j.anbehav.2017.07.007.

*87. Schweinfurth MK, Taborsky M. Norway rats (Rattus norvegicus) communicate need, which elicits donation of food. Journal of Comparative Psychology. 2018; 132:119–29. Doi: 10.1037/com0000102.

*88. Schweinfurth MK, Taborsky M. Reciprocal trading of different commodities in norway rats. Current Biology. 2018;28(4):594-599.e3. Doi: 10.1016/j.cub.2017.12.058.

*89. Schweinfurth MK, Taborsky M. Relatedness decreases and reciprocity increases cooperation in Norway rats. Proceedings of the Royal Society B: Biological Sciences. 2018;285(1874):20180035. Doi: 10.1098/rspb.2018.0035.

*90. Schweinfurth MK, Aeschbacher J, Santi M, Taborsky M. Male Norway rats cooperate according to direct but not generalized reciprocity rules. Animal Behaviour. 2019; 152:93–101. Doi: 10.1016/j.anbehav.2019.03.015.

*91. Schweinfurth MK, Taborsky M. Rats play tit-for-tat instead of integrating social experience over multiple interactions. Proceedings of the Royal Society B: Biological Sciences. 2020;287(1918):20192423. Doi: 10.1098/rspb.2019.2423.

*92. Silva PRR, Silva RH, Lima RH, Meurer YS, Ceppi B, Yamamoto ME. Are there multiple motivators for helping behavior in rats? Frontiers in Psychology. 2020;11. Doi: 10.3389/fpsyg.2020.01795.

*93. Tomek SE, Stegmann GM, Olive MF. Effects of heroin on rat prosocial behavior. Addiction Biology. 2019;24(4):676–84. Doi: 10.1111/adb.12633.

*94. Tomek SE, Stegmann GM, Leyrer-Jackson JM, Piña J, Olive MF. Restoration of prosocial behavior in rats after heroin self-administration via chemogenetic activation of the anterior insular cortex. Social Neuroscience. 2020;15(4):408–19. Doi: 10.1080/17470919.2020.1746394.

*95. Tsoory MM, Youdim MB, Schuster R. Social-cooperation differs from individual behavior in hypothalamic and striatal monoamine function: Evidence from a laboratory rat model. Behavioural Brain Research. 2012;232(1):252–63.

*96. Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Matsumoto Y, et al. Helping-like behaviour in mice towards conspecifics constrained inside tubes. Sci Rep. 2019;9(1):5817. Doi: 10.1038/s41598-019-42290-y.

*97. Ueno H, Suemitsu S, Murakami S, Kitamura N, Wani K, Takahashi Y, et al. Rescue-like behaviour in mice is mediated by their interest in the restraint tool. Sci Rep. 2019;9(1):10648. Doi: 10.1038/s41598-019-46128-5.

*98. Viana DS, Gordo I, Sucena É, Moita MAP. Cognitive and motivational requirements for the emergence of cooperation in a rat social game. PLOS ONE. 2010;5(1):e8483. Doi: 10.1371/journal.pone.0008483.

*99. Wood RI, Kim JY, Li GR. Cooperation in rats playing the iterated Prisoner's Dilemma game. Animal Behaviour. 2016; 114:27–35. Doi: 10.1016/j.anbehav.2016.01.010.

*100. Yamagishi A, Okada M, Masuda M, Sato N. Oxytocin administration modulates rats' helping behavior depending on social context. Neuroscience Research. 2020; 153:56–61. Doi: 10.1016/j.neures.2019.04.001.

*101. Yamagishi A, Lee J, Sato N. Oxytocin in the anterior cingulate cortex is involved in helping behaviour. Behavioural Brain Research. 2020; 393:112790. Doi: 10.1016/j.bbr.2020.112790.

*102. Yüksel O, Ateş M, Kızıldağ S, Yüce Z, Koç B, Kandiş S, et al. Regular Aerobic Voluntary Exercise Increased Oxytocin in Female Mice: The Cause of Decreased Anxiety and Increased Empathy-Like Behaviors. Balkan Med J. 2019;36(5):257–62.

*103. Asadi E, Khodagholi F, Asadi S, Mohammadi Kamsorkh H, Kaveh N, Maleki A. Quality of early-life maternal care predicts empathy-like behavior in adult male rats: Linking empathy to BDNF gene expression in associated brain regions. Brain Res. 2021; 1767:147568. Doi: 10.1016/j.brainres.2021.147568.

*104. Ben-Ami Bartal I, Breton JM, Sheng H, Long KL, Chen S, Halliday A, et al. Neural correlates of ingroup bias for prosociality in rats. Flagel SB, Wassum KM, editors. eLife. 2021;10: e65582. Doi: 10.7554/eLife.65582.

*105. Conde-Moro AR, Rocha-Almeida F, Gebara E, Delgado-García JM, Sandi C, Gruart A. Involvement of prelimbic cortex neurons and related circuits in the acquisition of cooperative learning by pairs of rats. bioRxiv; 20222;476162. Doi: 10.1101/2022.01.13.476162.

*106. Cox, S. S., Kearns, A. M., Woods, S. K., Brown, B. J., Brown, S. J., & Reichel, C. M. The role of the anterior insular during targeted helping behavior in male rats. Scientific Reports. 2022;12(1), 3315. Doi: 10.1038/s41598-022-07365-3.

*107. Cox SS, Brown BJ, Woods SK, Brown SJ, Kearns AM, Reichel CM. Neuronal, affective, and sensory correlates of targeted helping behavior in male and female Sprague Dawley rats. bioRxiv;2022.08.17.503412. Doi: 10.1101/2022.08.17.503412

*108. de Carvalho LC, Dos Santos L, Regaço A, Couto KC, de Souza D das G, Todorov JC. Cooperative responding in rats: II. Performance on fixed-ratio schedules of mutual reinforcement. J Exp Anal Behav. 2020;114(3):291–307. Doi: 10.1002/jeab.628.

*109. Heslin, K. A., & Brown, M. F. No preference for prosocial helping behavior in rats with concurrent social interaction opportunities. Learning & Behavior. 2021;49(4), 397–404. Doi: 10.3758/s13420-021-00471-8.

*110. Joushi, S., Taherizadeh, Z., Esmaeilpour, K., & Sheibani, V. Environmental enrichment and intranasal oxytocin administration reverse maternal separation-induced impairments of prosocial choice behavior. Pharmacology Biochemistry and Behavior. 2022;213, 173318. Doi: 10.1016/j.pbb.2021.173318.

*111. Kalamari A, Kentrop J, Hinna Danesi C, Graat EAM, van IJzendoorn MH, Bakermans-Kranenburg MJ, et al. Complex housing, but not maternal deprivation affects motivation to liberate a trapped cage-mate in an operant rat task. Front Behav Neurosci. 2021; 15:698501. Doi: 10.3389/fnbeh.2021.698501.

*112. Paulsson NI, Taborsky M. Reaching out for inaccessible food is a potential begging signal in cooperating wild-type norway rats, rattus norvegicus. Front Psychol. 2021; 12:712333. Doi : 10.3389/fpsyg.2021.712333.

*113. Schweinfurth MK. Cooperative intentions and their implications on reciprocal cooperation in Norway rats. Ethology. 2021;127(10):865–71. Doi: 10.1111/eth.13144.

*114. Shima T, Kawabata-Iwakawa R, Onishi H, Jesmin S, Yoshikawa T. Four weeks of light-intensity exercise enhances empathic behavior in mice: The possible involvement of BDNF. Brain Res. 2022; 1787:147920. Doi: 10.1016/j.brainres.2022.147920.

*115. Subhadeep D, Srikumar BN, Shankaranarayana Rao BS, Kutty BM. Ventral subicular lesion impairs pro-social empathy-like behavior in adult Wistar rats. Neurosci Lett. 2022; 776:136535. Doi: 10.1016/j.neulet.2022.136535.

*116. Wan, H., Kirkman, C., Jensen, G., & Hackenberg, T. D. Failure to Find Altruistic Food Sharing in Rats. Frontiers in Psychology. 2021;12. Doi : 10.3389/fpsyg.2021.696025.

*117. Wu WY, Cheng Y, Liang KC, Lee RX, Yen CT. Affective mirror and anti-mirror neurons relate to prosocial help in rats. iScience. 2023;26(1):105865. Doi: 10.1016/j.isci.2022.105865.

118. Mueller AC. Wordcloud [Internet]. 2023 [cited 2022 Sept 9]. Available from: https://github.com/amueller/wordcloud

119. Keum S, Shin HS. Rodent models for studying empathy. Neurobiology of Learning and Memory. 2016; 1(135):22–6. Doi: 10.1016/j.nlm.2016.07.022.

120. Matzel LD, Han YR, Grossman H, Karnik MS, Patel D, Scott N, et al. Individual differences in the expression of a "general" learning ability in mice. The Journal of Neuroscience. 2003;23(16):6423–33. Doi: 10.1523/JNEUROSCI.23-16-06423.2003.

121. Dalla C, Shors TJ. Sex differences in learning processes of classical and operant conditioning. Physiol Behav. 2009;97(2):229–38. Doi: 10.1016/j.physbeh.2009.02.035.

122. Tsakanikos E, Reed P. Individual differences in proactive interference in rats (Rattus Norvegicus). Psychon Bull Rev. 2022;29(1):203–11. Doi: 10.3758/s13423-021-01998-7.

123. Brady AM, Floresco SB. Operant procedures for assessing behavioral flexibility in rats. J Vis Exp. 2015;(96):52387. Doi: 10.3791/52387.

124. Oomen CA, Hvoslef-Eide M, Heath CJ, Mar AC, Horner AE, Bussey TJ, et al. The touchscreen operant platform for testing working memory and pattern separation in rats and mice. Nat Protoc. 2013;8(10):2006–21. Doi: 10.1038/nprot.2013.124.

125. Kondrakiewicz K, Kostecki M, Szadzińska W, Knapska E. Ecological validity of social interaction tests in rats and mice. Genes, Brain and Behavior. 2019;18(1):e12525. Doi: 10.1111/gbb.12525.

126. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLOS ONE. 2009;4(11):e7824. Doi: 10.1371/journal.pone.0007824.

127. Atsak P, Orre M, Bakker P, Cerliani L, Roozendaal B, Gazzola V, et al. Experience modulates vicarious freezing in rats: A model for empathy. PLoS One. 2011;6(7):e21855. Doi: 10.1371/journal.pone.0021855.

128. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research*. J Cereb Blood Flow Metab. 2020;40(9):1769–77. Doi: 10.1177/0271678X20943823.

129. Hershey JD, Gifford JJ, Zizza LJ, Pavlenko DA, Wagner GC, Miller S. Effects of Various Cleaning Agents on the Performance of Mice in Behavioral Assays of Anxiety. Journal of the American Association for Laboratory Animal Science. 2018;57(4):335–9. Doi: 10.30802/AALAS-JAALAS-17-000161.

130. Winters S, Dubuc C, Higham JP. Perspectives: The looking time experimental paradigm in studies of animal visual perception and cognition. Ethology. 2015;121(7):625–40. Doi: 10.1111/eth.12378.

131. Grundy D. Principles and standards for reporting animal experiments in The Journal of Physiology and Experimental Physiology. J Physiol. 2015;593(12):2547–9. Doi: 10.1113/JP270818.

132. Toth LA, Kregel K, Leon L, Musch TI. Environmental enrichment of laboratory rodents: The answer depends on the question. Comparative Medicine. 2011;61(4):314–21. PMID: 22330246.

133. Seffer D, Schwarting RKW, Wöhr M. Pro-social ultrasonic communication in rats: Insights from playback studies. Journal of Neuroscience Methods. 2014; 234:73–81. Doi: 10.1016/j.jneumeth.2014.01.023.

134. Peters SM, Pothuizen HHJ, Spruijt BM. Ethological concepts enhance the translational value of animal models. European Journal of Pharmacology. 2015; 759:42–50. Doi: 10.1016/j.ejphar.2015.03.043.

135. Davis JF, Krause EG, Melhorn SJ, Sakai RR, Benoit SC. Dominant rats are natural risk takers and display increased motivation for food reward. Neuroscience. 2009;162(1):23–30. Doi: 10.1016/j.neuroscience.2009.04.039.

136. Kunkel T, Wang H. Socially dominant mice in C57BL6 background show increased social motivation. Behav Brain Res. 2018; 336:173–6. Doi: 10.1016/j.bbr.2017.08.038.

137. Schulz KM, Molenda-Figueira HA, Sisk CL. Back to the future: The organizational-activational hypothesis adapted to puberty and adolescence. Horm Behav. 2009;55(5):597–604. Doi: 10.1016/j.yhbeh.2009.03.010.

138. Juraska JM, Willing J. Pubertal onset as a critical transition for neural development and cognition. Brain Research. 2017; 1654:87–94. Doi: 10.1016/j.brainres.2016.04.012.

139. Yoest KE, Henry MG, Velisek HA, Veenema AH. Development of social recognition ability in female rats: Effect of pubertal ovarian hormones. Hormones and Behavior. 2023; 151:105347. Doi: 10.1016/j.yhbeh.2023.105347.

140. Qu N, Wang L, Liu ZC, Tian Q, Zhang Q. Oestrogen receptor α agonist improved long-term ovariectomy-induced spatial cognition deficit in young rats. Int J Neuropsychopharmacol. 2013;16(5):1071–82. Doi: 10.1017/S1461145712000958.

141. Tang AC, Nakazawa M, Romeo RD, Reeb BC, Sisti H, McEwen BS. Effects of long-term estrogen replacement on social investigation and social memory in ovariectomized C57BL/6 mice. Horm Behav. 2005;47(3):350–7. Doi: 10.1016/j.yhbeh.2004.10.010.

142. Wang F, Kessels HW, Hu H. The mouse that roared: neural mechanisms of social hierarchy. Trends in Neurosciences. 2014;37(11):674–82. Doi: 10.1016/j.tins.2014.07.005.

143. Crawley JN. Designing mouse behavioral tasks relevant to autistic-like behaviors. Mental Retardation and Developmental Disabilities Research Reviews. 2004;10(4):248–58. Doi: 10.1002/mrdd.20039.

144. Uslaner JM, Robinson TE. Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice - mediation by enhanced incentive motivation? Eur J Neurosci. 2006;24(8):2345–54. Doi: 10.1111/j.1460-9568.2006.05117.x.

145. Toth LA, Gardiner TW. Food and water restriction protocols: physiological and behavioral considerations. Journal of the American Association for Laboratory Animal Science. 2000;39(6):9–17. PMID: 11487246.

146. Dietze S, Lees KR, Fink H, Brosda J, Voigt JP. Food deprivation, body weight loss and anxiety-related behavior in rats. Animals. 2016;6(1):4. Doi: 10.3390/ani6010004.

147. Rowland NE. Food or fluid restriction in common laboratory animals: balancing welfare considerations with scientific inquiry. Comp Med. 2007;57(2):149–60. PMID: 17536615.

148. Asher G, Sassone-Corsi P. Time for Food: The intimate interplay between nutrition, metabolism, and the circadian clock. Cell. 2015;161(1):84–92. Doi: 10.1016/j.cell.2015.03.015.

149. Beery AK. Inclusion of females does not increase variability in rodent research studies. Curr Opin Behav Sci. 2018; 23:143–9. Doi: 10.1016/j.cobeha.2018.06.016.

150. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev. 2014; 40:1–5. Doi: 10.1016/j.neubiorev.2014.01.001.

151. Hussain D, Shams W, Brake W. Estrogen and memory system bias in females across the lifespan. Translational Neuroscience. 2014;5(1):35–50. Doi: 10.2478/s13380-014-0209-7.

152. McEwen BS, Akama KT, Spencer-Segal JL, Milner TA, Waters EM. Estrogen effects on the brain: actions beyond the hypothalamus via novel mechanisms. Behav Neurosci. 2012;126(1):4–16. Doi: 10.1037/a0026708.

153. Choleris E, Galea LAM, Sohrabji F, Frick KM. Sex differences in the brain: Implications for behavioral and biomedical research. Neurosci Biobehav Rev. 2018; 85:126–45. Doi: 10.1016/j.neubiorev.2017.07.005.

154. Ervin KSJ, Lymer JM, Matta R, Clipperton-Allen AE, Kavaliers M, Choleris E. Estrogen involvement in social behavior in rodents: Rapid and long-term actions. Horm Behav. 2015; 74:53–76. Doi: 10.1016/j.yhbeh.2015.05.023.

155. Spiteri, T., & Ågmo, A. Ovarian hormones modulate social recognition in female rats. Physiology & Behavior. 2009;98(1), 247–250. Doi: 10.1016/j.physbeh.2009.05.001.

156. Kolu TC. Commentary: Behavior analysis and behavioral neuroscience. Frontiers in Human Neuroscience. 2016;10. Doi: 10.3389/fnhum.2016.00256.

157. Schlinger HD. Behavior analysis and behavioral neuroscience. Front Hum Neurosci. 2015; 9:210. Doi: 10.3389/fnhum.2015.00210.

158. Burgdorf J, Kroes RA, Moskal JR, Pfaus JG, Brudzynski SM, Panksepp J. Ultrasonic vocalizations of rats (Rattus norvegicus) during mating, play, and aggression: Behavioral concomitants, relationship to reward, and self-administration of playback. J Comp Psychol. 2008;122(4):357–67. Doi: 10.1037/a0012889.

159. Simola N, Granon S. Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. Neuropharmacology. 2019; 159:107420. Doi: 10.1016/j.neuropharm.2018.11.008.

CHAPTER FOUR

Study Three

Exploring rodent prosociality: a conceptual framework

Valérie Charron, Joey Talbot, and Hélène Plamondon

Abstract

Prosociality is a behavior characterized by actions performed for the benefit or well-being of others. Recent studies have corroborated parallels in brain activation patterns between rodents and humans during prosocial behaviors. These findings have the potential to advance our understanding of social impairments observed in neurodevelopmental disorders, brain injuries, neurological conditions, and mental health disorders. However, a consensus regarding prosocial paradigms in rodents remains scattered. This conceptual framework aims to: (1) reframe prosociality as a set of complex behaviors emerging in response to environmental determinants that cannot be reduced to a single set of data, (2) highlight important methodological considerations, mediating variables, and behavioral analyses that influence prosocial behaviors, and (3) present a decision tree as a dynamic element within this conceptual framework to offer guidance to researchers. The conceptual framework and decision tree are concise and straightforward, providing a robust foundation for the ongoing utilization of current models and the creation of novel paradigms. The integration of this conceptual framework into research practices will contribute to the advancement of knowledge in the field of rodent prosociality and foster greater confidence in the validity and reproducibility of study findings.

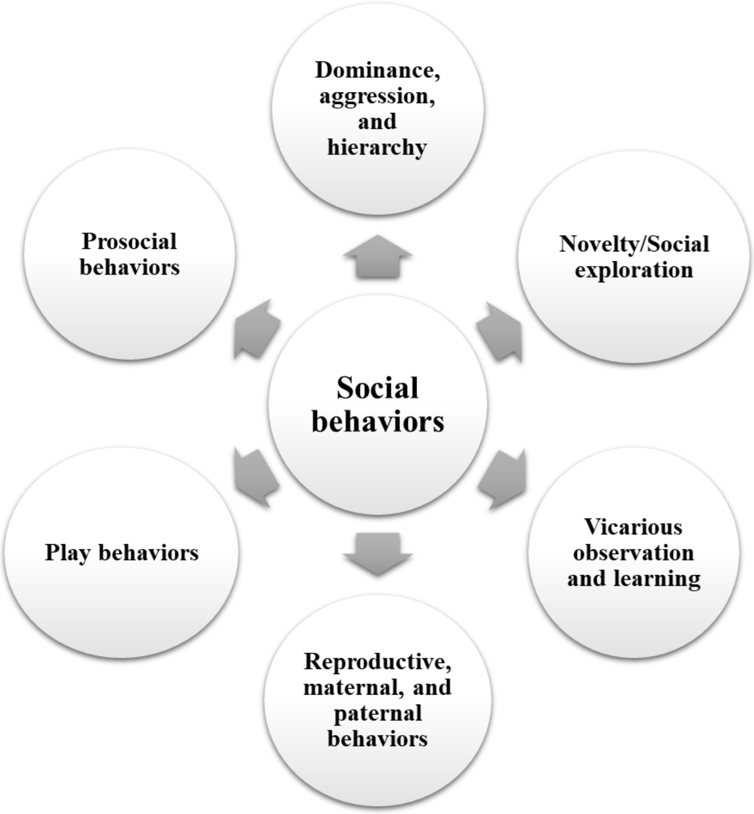
1. Introduction

Mammals, including humans, primates, and rodents, display diverse behaviors aimed at protecting and maintaining species survival [1]. Social behaviors, communication, and interactions are crucial for the maintenance of social organization and ensure the species' contextual adaptability [2]. Such behaviors have been studied in many animals, ranging from humans [3, 4] to non-human primates [5, 6], rodents [7], and fishes [8]. Animal models have been particularly valuable for examining social organization and hierarchies amongst different species (e.g., mole rats, prairie voles, non-human primates) [7, 9, 10] and the underlying cerebral mechanisms involved in social behaviors and deficits [11-13].

1.1 Social behaviors in rodents and humans

Studies utilizing mice and rats have shown valuable in investigating social behaviors, given that rodents are social animals [14]. Such research offers cost-effective options that require minimal resources compared to the complexities involved in studying non-human primates [15]. To date, the study of ‘animal social behavior’ has encompassed multiple social actions, including reproduction, aggression, and resource sharing [16, 17] (see Figure 1).

Figure 1. Sociality and its studied behaviors.



Notably, research in the field of rodent social behaviors examines dominance and aggression due to the significant role social hierarchy plays in regulating resource access, reproductive behavior, and overall well-being, thereby ensuring the survival of the species [18-20]. In rodents, dominance is frequently assessed through the observation of aggressive behaviors within pairs (e.g., biting, chasing) and defensive responses (e.g., freezing, lying down, standing upright with raised paws), or by using dominance paradigms such as the tube test [19]. From an evolutionary standpoint, aggression and dominance help animals and humans establish social hierarchies while securing control and priority over vital resources [19, 20, 21].

Rodents typically exhibit a natural inclination towards seeking social interaction and communication; therefore, research focusing on novelty and social interaction can help determine the presence of social deficits [22-24]. Communication across species, both verbal and non-verbal, is highly influenced by vicarious observation and learning [25, 26]. Observing fear or pain in a conspecific promotes the communication of valuable information to others [25, 26]. These communication strategies are vital for the survival of the species because vicariously learning about potential dangers and threats, such as predators or discomfort, enables observers to avoid or defend themselves from similar situations [27]. In humans, various factors influence social exploration and novelty seeking, including environmental variables (e.g., structure, stability, and predictability of the environment) [28, 29], individual characteristics (e.g., prior experience and knowledge, cognitive capacity, demographics) [28, 30, 31], and social contingencies (e.g., availability of information, competitiveness, mutual exploration) [28, 29, 32]. Social contingencies can predict humans' tendency to explore the unknown, influenced by the actions and behaviors of others [28, 33].

Similarly, reproductive and parental care are inherently associated with social behaviors that have been conserved through evolution and are oriented towards species survival [34]. In both humans and animals, maternal care is pivotal for fostering social and cognitive development, as well as the overall well-being of offspring [35-37]. Additionally, paternal care plays a significant role; fathers' social behaviors can influence the mother-infant relationship and impact the development of the offspring [38, 39]. Research also indicates that parental attitudes geared towards enhancing the survival and well-being of infants (e.g., increased attachment-related rewards and heightened anxiety regarding the child's safety) can trigger alterations in specific brain networks (e.g., the reward circuit) and affect hormonal secretion (e.g., oxytocin) in offspring [40, 41].

Other interactions are strongly associated with specific developmental stages. For instance, social play behaviors are prominently displayed by rodents during the juvenile and adolescent periods, but these behaviors tend to decrease as rodents transition into adulthood [42, 43]. These behaviors typically involve two animals engaging in rapid pushing and grabbing actions, commonly referred to as boxing, play-fighting, or rough-and-tumble play [42, 44]. Research employing isolation experiments has revealed the significance of play behaviors in the social, cognitive, and emotional development of rodents [43, 44]. Moreover, such behaviors are essential for species survival, given their highly rewarding nature and their role in fostering the development of communication skills amongst individuals [44]. In humans, engaging in social play behaviors has demonstrated numerous benefits for the social, emotional, and cognitive growth of children [45-47]. Interacting with peers through play is essential for acquiring vital social abilities such as communication, language, sharing, friendship, cooperation, and conflict resolution [45].

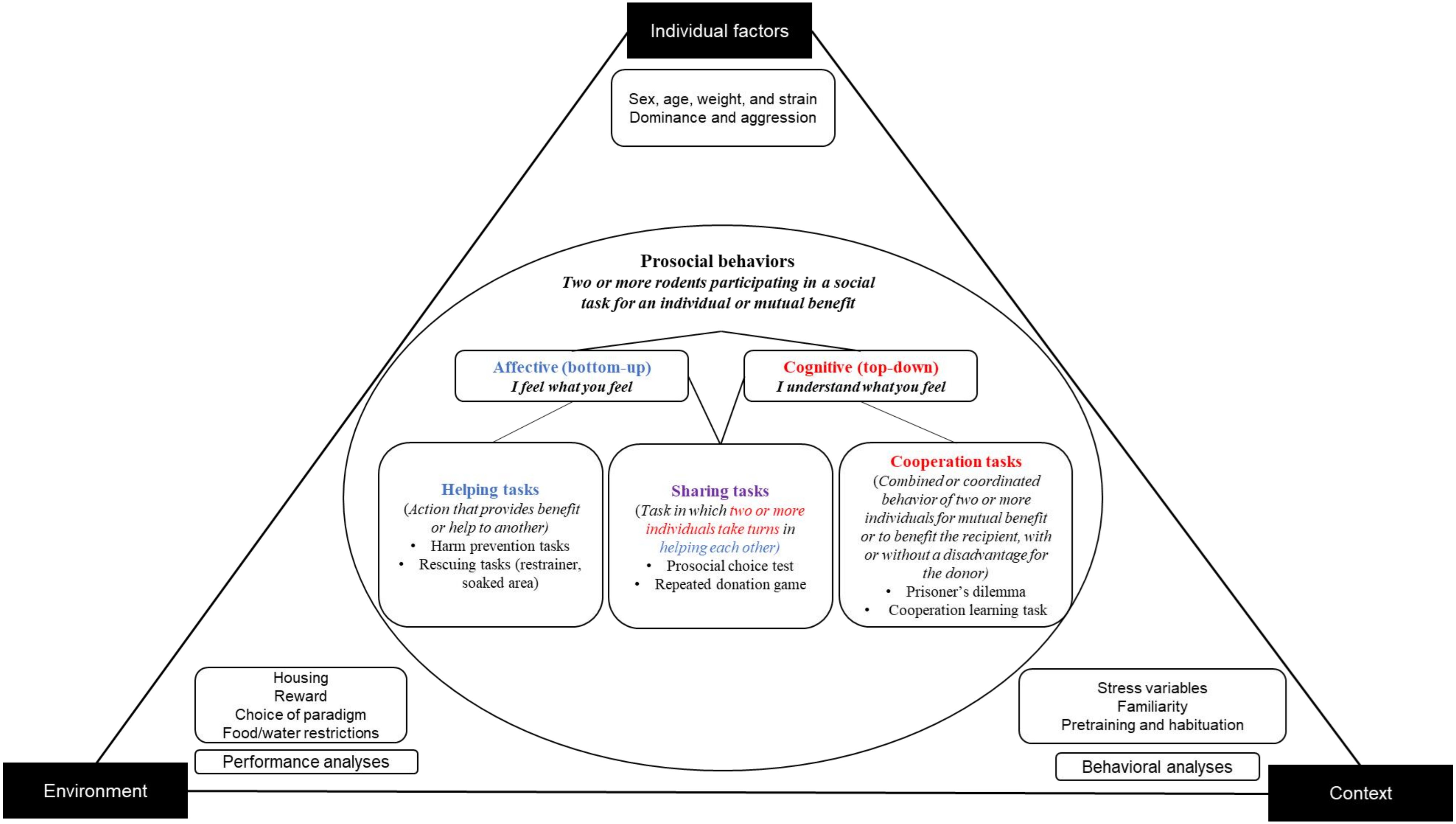
1.2 Prosocial behaviors

Prosocial behavior represents a category that encompasses many other behaviors classified and studied under the commonly used term "social behavior" [48] (see Figure 1). While there are similarities between the terms prosociality and empathy, the latter infers a capacity to feel and understand the suffering of others and to respond with compassion and help [49]. In this context, empathy is frequently regarded as an attribute exclusive to humans [49], despite emerging research on non-human primates suggesting the presence of comparable abilities [50]. This conceptual framework centers on prosocial behaviors, which involve actions undertaken to benefit another individual or enhance their well-being [48, 51]. Engaging in prosocial behaviors facilitates cooperation and resource sharing, both integral components for the survival of social species [48]. The investigation of prosociality in rodents originated in the 1960s when researchers observed how rodents responded to a conspecific experiencing electric shocks [52, 53]. In 2011, Ben-Ami Bartal and colleagues revisited this inquiry by introducing a paradigm wherein a rat could open a door to release a trapped conspecific. This study sparked a series of similar investigations [54, 55] and led to the development of various paradigms, including cooperation [56], direct reciprocity [57], and prosocial choice tasks [58]. For decades, researchers have delved into the neurobiology of human prosociality, aiming to gain a deeper understanding of the underlying cerebral mechanisms and associated impairments observed in various conditions such as mental health disorders (e.g., mood disorders, personality disorders), neurological conditions (e.g., traumatic brain injury, dementia, stroke), neurodevelopmental disorders (e.g., autism spectrum disorder), and brain injuries [59-64]. However, investigating prosociality deficits in humans often necessitates brain imaging studies or postmortem analyses [65], both of which are resource-intensive methods [66]. Consequently, rodent models offer insights into the cerebral mechanisms and circuits involved in prosocial behaviors, with the potential for knowledge translation to humans [67].

2. Conceptual framework

A conceptual framework is a 'network' or 'plane' of interconnected concepts that collectively provide a thorough understanding of a phenomenon [68]. Without such a framework, a field of research can quickly become a random collection of results lacking structure [69]. This conceptual framework builds upon previous research [51, 70], identifying gaps in the investigation of prosociality in rodents (see Figure 2). It aims to (1) reframe prosociality as a set of complex behaviors that emerge in response to environmental determinants, requiring multiple sets of observations for a comprehensive analysis, (2) highlight important methodological considerations, mediating variables, and behavioral analyses influencing prosocial behaviors in rodents, and (3) present a decision tree as a dynamic element to guide researchers. This framework will enhance the ecological validity of future studies by facilitating a more realistic representation of the complex social dynamics and environmental contexts in which prosocial behaviors occur. Additionally, its emphasis on standardized definitions and methodological rigor will promote replicability, providing clear guidelines for researchers and enhancing the reliability and consistency of research outcomes. Ultimately, integrating this conceptual framework into research practices will advance knowledge in the field of rodent prosociality and foster greater confidence in the validity and reproducibility of findings.

Figure 2. Conceptual framework of prosocial behaviors: definitions, tasks, and methodological considerations.



2.1 Affective aspects of prosocial behaviors

In this conceptual framework, the affective aspects of prosociality refer to the bottom-up information processing involved in prosocial tasks. This foundation is based on the theoretical construct of affective empathy, which suggests the ability to feel and share the emotional experiences of others [71, 72]. Bottom-up information processing is defined as the process of taking sensory information and using it to form a coherent understanding [73]. This approach suggests that rodents gather information from their peers, such as distress signals conveyed through verbal and nonverbal cues (i.e., bottom-up processing). They then process this information and adjust their behavior accordingly, such as by helping to alleviate the distress of their peer (i.e., top-down processing). It is argued that helping and harm prevention tasks are integral to the affective aspects of prosociality (see Figure 2). This is supported by the utilization of similar tasks to investigate each concept, and the comparable patterns of brain activation observed in response to these tasks (see Figure 4).

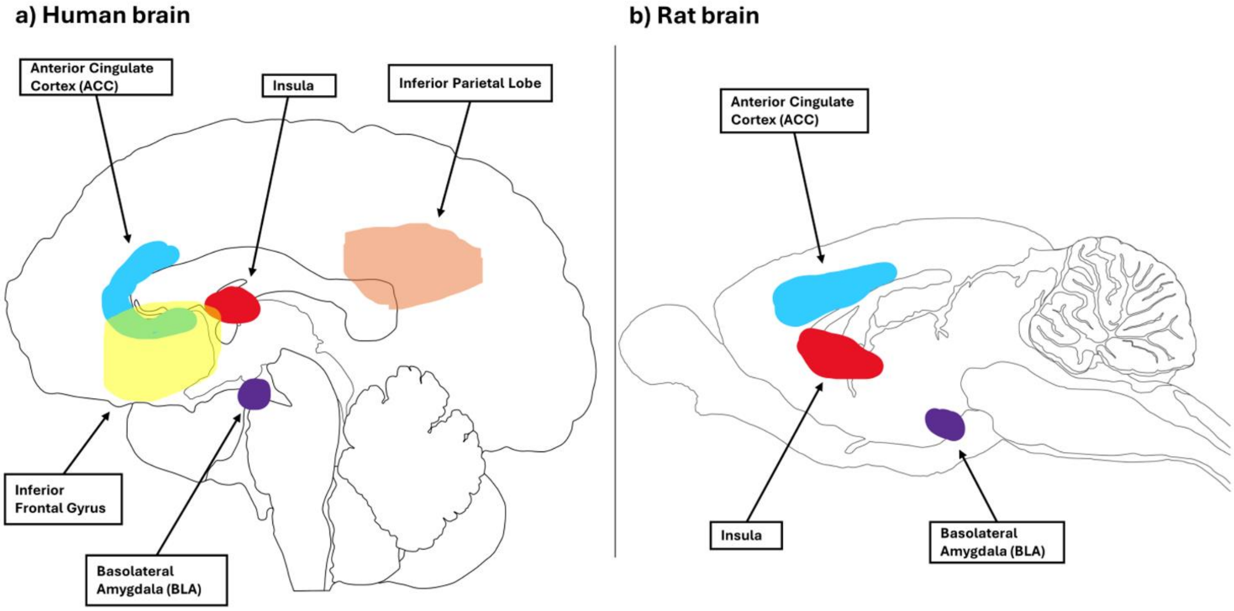
2.1.1 Existing paradigms and similarities

Helping tasks consist of a rodent presented with the possibility of opening a door (e.g., lever press, nose poke) to free a conspecific from an aversive environment (e.g., restraining device, soaked area) [54, 74]. Harm prevention tasks involve one rodent exposed to an adverse stimulus (e.g., electric foot shock), while another rodent can take action to terminate this stimulus (e.g., via lever pressing or nose poking) [75]. These paradigms share various similarities: (1) an aversive component is always involved, and (2) a rodent can mitigate the distress caused by the aversive stimulus.

2.1.2 Similar cerebral activation pathways supporting affective aspects of prosociality

The human emotional contagion brain network encompasses regions such as the inferior frontal gyrus (IFG), inferior parietal lobule (IPL), insula, and anterior cingulate cortex (ACC) [76, 77]. Findings from rodent studies suggest the involvement of the ACC [16, 78, 79, 80] and the insula [79-81]. Although the inferior frontal gyrus [81] and inferior parietal lobule [81, 82] may also play a role, further research is needed to confirm the involvement of these brain regions in rodents' affective prosociality. Additionally, the basolateral amygdala (BLA) appears to be implicated in both rodents [74, 83] and humans [84]. In humans, the BLA is crucial for the expression of fear responses [85] and fear-related memory [86], while fear conditioning activates BLA-projecting ACC neurons in rodents [83, 87, 88] (see Figure 3).

Figure 3. Brain regions involved in affective aspects of prosocial responses in humans and rats.



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2.2 Cognitive aspects of prosocial behaviors

Cognitive aspects of prosociality are regulated through top-down information processing. Current literature associates the expression of cognitive prosociality with the ability to understand the feelings of others [71, 72]. Top-down processing requires the use of prior experiences, knowledge, and cognition to interpret external information [73]. In this conceptual framework, top-down processing related to prosociality involves understanding a situation or task (top) and responding in a way that benefits a conspecific (down). Tasks in this category encompasses the prisoner's dilemma and cooperation learning tasks.

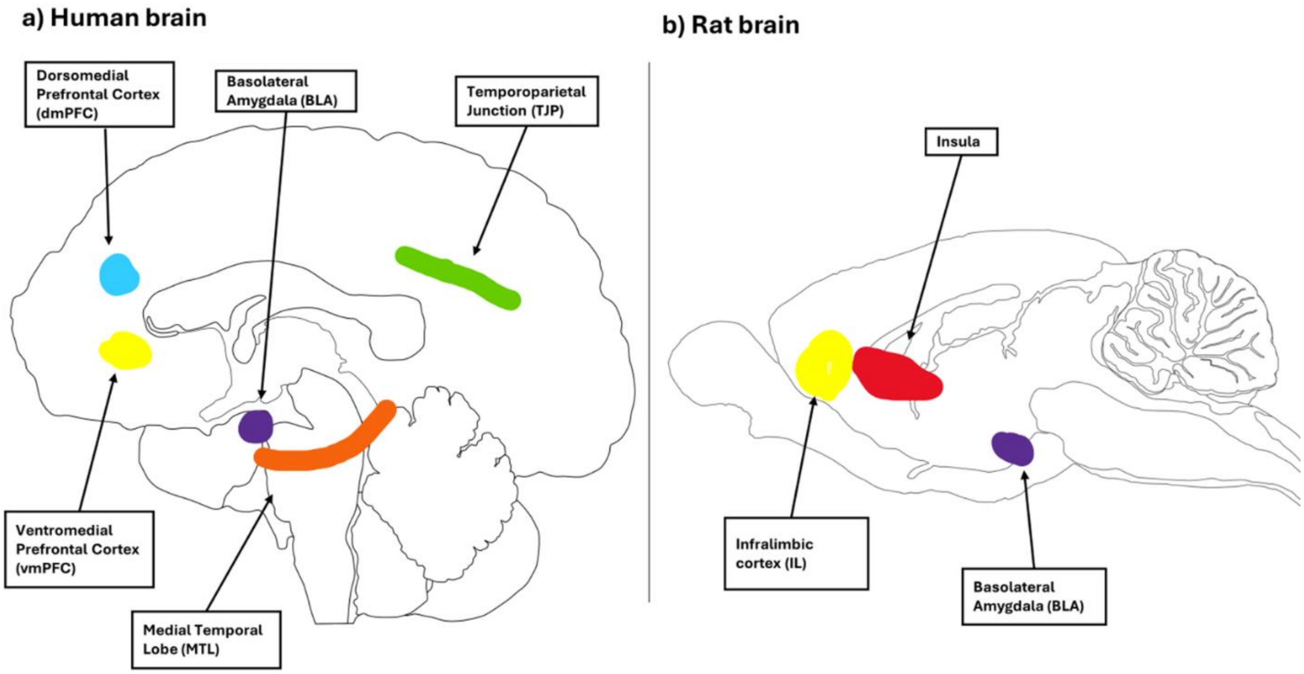
2.2.1 Existing paradigms and shared similarities in assessing cognitive prosociality

Cognitive aspects of prosociality are commonly assessed using tasks requiring conditioning sessions or the learning of a specific action (e.g., pressing a lever) [89]. While a learning component can be present in affective prosocial tasks (e.g., a rat learning how to open a door before the freeing task) [74], cognitive tasks necessitate that rodents acquire a higher level of knowledge or understanding of the situation to successfully perform an action in response to a conspecific's experience. Cooperation tasks are organized into two main categories: the prisoner's dilemma and cooperation learning tasks. The prisoner's dilemma occurs in divided compartments, in which rodents on both sides can choose between a cooperative or defective lever over repeated trials, resulting in either a mutual reward or a punishment [90-92]. Cooperation learning tasks involve two rats or mice that must learn to coordinate their actions to obtain a mutual reward [93-95]. Typically employing an operant box paradigm, these tasks require both partners to learn a coordinated action (e.g., lever pressing, lever pulling, or nose poking) to receive a mutual benefit (e.g., food reward).

2.2.2 Similar cerebral activation pathways supporting cognitive aspects of prosociality

Both human and animal studies support the involvement of the prefrontal cortex in the cognitive aspects of prosocial tasks (see Figure 4). Specifically, four subregions have garnered attention: the temporoparietal junction (TPJ), the medial temporal lobe (MTL), and the ventromedial and dorsomedial prefrontal cortices (vmPFC and dmPFC, respectively), the latter two comprising the infralimbic cortex (IL) in rodents [76, 94, 96]. Additionally, the insular cortex has emerged as significant for social decision-making and the integration of external sensory stimuli in rodents, which are key elements of cognitive tasks [97, 98]. The BLA is also highlighted as an important region for cognitive prosociality in both humans and rodents [99, 100]. Lesions to the BLA in humans impair social learning in a trust game [99], while projections to the BLA show activation during a rodent cooperation task [101].

Figure 4. Brain regions involved in cognitive aspects of prosocial responses in humans and rats.



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2.3 Affective and cognitive aspects in prosocial paradigms

As stated in the literature, bottom-up and top-down information processing are interrelated, providing constant feedback to an individual [102]. While earlier sections have emphasized the affective or cognitive dimensions of experimental tasks assessing prosocial behavior, the subsequent section suggests an additional task category that could facilitate the testing of both information processing. Based on the findings presented above, a behavioral task integrating both affective and cognitive facets of prosocial behavior should entail: 1) a helping component that benefits or helps another, 2) an action that needs to be learned (e.g., lever pressing/pulling, nose poking), and 3) two rodents that can alternate roles in the task. To fulfill this requirement, the conceptual framework introduces a third category of prosocial paradigms termed "sharing tasks." Examples of such tasks encompass the prosocial choice test [58] and the repeated donation game [103, 104].

2.3.1 Prosocial choice test

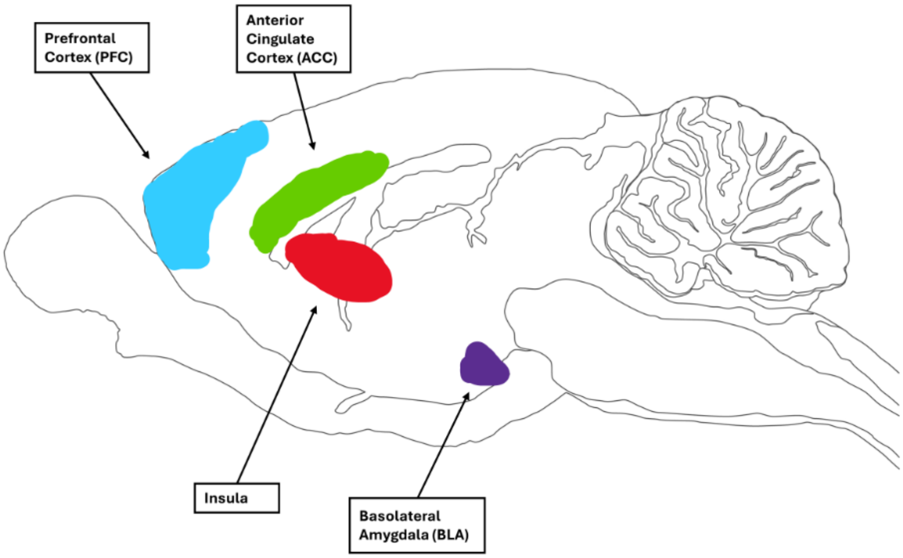
Hernandez-Lallement and colleagues (2015) were the first to publish findings using the prosocial choice test. This task involves a double T-maze containing four compartments, in which a rat designated as the actor can opt to consume a solitary reward in an individual compartment or select a "both reward" alternative. In the latter case, a food reward is simultaneously provided to both the actor rat and a conspecific rat, each positioned in separate compartments divided by a perforated wall. This paradigm forces the rat to choose between a "selfish" option (single reward) and a "sharing" option (mutual reward). Moreover, the mutual reward option also allows the actor rat to eat its reward in the company of another rat, in the absence of possible physical contact [58].

2.3.2 Repeated donation game

Comparable to the prosocial choice test, the repeated donation game involves an actor rat (the donor) deciding whether to share a food reward with another rat (the responder) based on whether the responder previously exhibited helpful behavior [104]. Furthermore, this task delves into reciprocity, examining whether rodents remember past instances of helpfulness and whether they are inclined to display prosocial behaviors towards those who have been helpful compared to unhelpful responders [104]. These two sharing tasks are promising tools for studying the cognitive and affective aspects of prosociality. Cognitive aspects can be assessed through the learning process, wherein rodents must acquire the ability to press a lever in a specific compartment or adhere to a given contingency. Additionally, the decision of rodents to share a food reward can indicate the actor's understanding of how the conspecific, without access to the reward, would benefit from receiving it. Supporting the involvement of cognitive processes, research has demonstrated the role of the PFC in the prosocial choice task [105]. Similar to tasks involving cognitive and affective aspects of prosociality, sharing tasks also involve activation of the BLA. In rats, BLA lesions have been associated with impairments in mutual social preference in a prosocial choice task [100].

Sharing tasks also facilitate the examination of components associated with the affective aspects of prosociality. Although this type of prosociality is more often studied using aversive tasks (e.g., observing a conspecific in pain or distress), helping tasks are defined as actions that provide benefits or assistance to another [51]. In the case of sharing tasks, the act of sharing a food reward with a conspecific provides a benefit to another, representing an affective aspect of prosociality. Activation of the ACC is considered crucial for learning actions aimed at rewarding others (as opposed to oneself) and has been shown to contribute to the behavioral responses observed in sharing tasks [106]. Likewise, the insula, known to engage in processing shared negative and positive experiences, has been involved in both forms of prosocial behavior, suggesting a potential role in regulating sharing tasks (see Figure 5) [107].

Figure 5. Brain regions involved in a sharing task in rats.



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2.4 Prosocial tasks: general considerations and important variables

Many studies using prosocial tasks in rodents omit methodological details that could impact the expression of prosociality and, subsequently, affect outcomes [51]. Although further investigation is warranted to deepen our understanding of the factors mediating rodents' prosociality, the following section provides an overview of previously identified factors that should be considered when conceptualizing tasks to assess prosociality in rodents.

2.4.1 Individual characteristics

Individual characteristics must be considered when conceptualizing prosocial tasks and analyzing data, as they have been associated to meaningful inter-individual differences. The most prevalent factors to consider include sex, age, weight, strain, and dominance/aggression.

Sex. The sex gap in the scientific literature is well acknowledged, and this field of research is no exception. A previous scoping review reported that only 15% of studies examined both sexes [51]. A longstanding rationale for the sole inclusion of males pertains to the hormonal fluctuations related to estrous cyclicity in females, which could introduce uncontrolled variability in the collected data [108]. From an evolutionary perspective, it is theorized that females may have developed heightened prosocial responses to improve reproductive success and ensure the survival of their offspring [109]. Additional research is required to characterize the role of sex in rodent behavior, particularly prosociality. Therefore, it is strongly encouraged to include both males and females when conceptualizing tasks that assess prosocial responses.

Age. Age is a factor known to directly impact the display of social behaviors in rodents. In particular, studies involving adolescent rodents have emphasized the importance of social behavior during this developmental stage [110]. Social play has been identified as a crucial behavior for healthy brain development in both mice and rats [42, 110, 111]. Adolescent rats deprived of social interaction have exhibited cognitive and social deficits, including impairments in social interaction and memory, as well as difficulties in processing socially transmitted information [110, 112]. Although studies involving older rats are scarce, current literature suggests a decline in social cognition and motivation, as evidenced by reduced social contact initiated by adult compared to adolescent rats [113]. Consequently, age is a defining variable in assessing the expression of social behaviors in rodents and necessitates careful consideration.

Strain. Numerous studies have indicated the importance of strain selection when designing research methodology. Depending on the selected experimental paradigms, certain strains with limited visual acuity, like albino rats, may be less optimal due to the visual components integral to these tasks (e.g., lever presses, nose pokes, and touchscreen tasks) [114]. In addition, research has demonstrated that certain strains exhibit higher levels of activity, which could impact learning curves for specific tasks as well as overall task performance [115]. For example, a study using five strains of mice (i.e., C57BL/6J, DBA/2J, FVB/NJ, A/J, and B6129PF2/J hybrids) revealed that A/J mice displayed significantly less interest in spending time with a congener in a social novelty test compared to the other tested strains, which could be explained by the hypolocomotion displayed by the A/J mice [116]. In rats, the tendency of Sprague-Dawley to be more active is associated with improved performance in social tasks compared to Long-Evans and Wistar rat strains [115, 117]. From an evolutionary perspective, heightened prosocial behaviors are expected to occur amongst conspecifics of the same strain to facilitate reproduction and individual survival [118]. Ben-Ami Bartal and colleagues indeed observed that rats exclusively helped strangers from the same strain and did not provide assistance to members of other strains [119]. In this context, the selection of a strain becomes a crucial variable in studying prosociality in rodents, and findings pertaining to strains should be thoroughly examined before adopting a specific experimental design [119].

Weight, dominance, and aggression. Rats and mice are social animals that establish specific social hierarchies and roles within their groups, evident in both pair and group housing arrangements, as well as in dyadic tasks such as those observed in prosocial paradigms. Weight appears to be one of the factors influencing which rodent assumes the dominant role, with lighter rodents typically adopting submissive roles and heavier ones assuming dominance [19].

Research has shown that dominant rodents display more prosocial behaviors than submissive ones [105, 120]. Additionally, aggression can serve as a means of communication amongst congeners. For instance, Dolivo and Taborsky [56] showed that rats tended to display aggression towards non-sharing partners, possibly to increase prosocial behavior. These examples demonstrate the importance of considering factors such as weight, dominance, and aggression, as they can mediate the expression of prosociality in rodents.

2.4.2 Context

The context of the study certainly represents an influential factor that can vary widely across laboratories and affect data collection. Important related factors should therefore be carefully considered, including stress, familiarity, pretraining and habituation, and behavioral analyses.

Stress. When designing rodent studies, especially behavioral paradigms, stress is a factor that can significantly impact observations and collected data [121]. Different laboratory routines can introduce elements that affect the stress levels of the animals [122]. Despite efforts by ethical committees and laboratories to minimize stress, certain manipulations or procedures inherently induce stress or anxiety in animals. The potential impact of such procedures should be meticulously considered during data analysis and interpretation. For example, handling is a common procedure involving the manipulation of rodents to acclimate them to human touch [123, 124]. While certain handling techniques may have positive effects on animals, others are reportedly aversive. Research indicates that tail handling induces more stress in mice compared to alternative methods such as tunnel or cup handling [123]. Conversely, for rats, tickling has been shown to mitigate the stressful effects of handling [124].

Other laboratory procedures have also been shown to induce stress in animals. These include blood collection [125, 126], gavage [127], injections [128], and other invasive or painful procedures (e.g., surgeries) [129], all of which can elevate stress levels and influence both behavioral and physiological data. While these techniques are often necessary for research purposes, it is strongly advised that experimenters make efforts to minimize stress. Additionally, including a control group (e.g., a sham group for surgeries) can provide valuable insights into the impact of stressful conditions on the animals' well-being and the outcomes of the study.

Studies using females or both sexes often utilize vaginal smears to monitor the estrous cycle and control for potential hormonal fluctuations across its phases [130]. This technique enables the identification of all four stages of the estrous cycle, known to alter behavior, including heightened anxiety during the diestrus phase [131, 132]. However, vaginal smears themselves can induce stress [133, 134], introducing additional variability into the results. Thus, it is important to consider this factor when interpreting data from female rodents, particularly when comparing it to data collected from males [134]. Alternative methods for evaluating the estrous cycle are available, such as visual inspection [135-137], although these assessments remain partly subjective.

Finally, aversive paradigms (e.g., foot shock, soaked area) that induce pain, fear, or discomfort generate stress in animals, which can complicate data interpretation [138]. Interestingly, recent studies suggest that stress and prosocial behavior in rats exhibit a U-shaped curve relationship. This implies that a certain level of stress is necessary to motivate an actor rat to liberate a distressed conspecific, but excessively elevated levels of stress hinder the release of a congener [139, 140].

Familiarity. Literature suggests that mice and rats possess kin recognition abilities, defined as the assessment of relatedness [141]. Social animals, including humans, non-human primates, mice, and rats, typically form groups consisting of both related and unrelated individuals [142]. Interacting with and providing benefits to individuals of varying degrees of relatedness contributes to the survival and reproductive success of the species [143]. The level of familiarity amongst rodents is also a key factor to consider when examining prosocial behaviors. Research indicates that rodents are more inclined to respond to the pain of a familiar conspecific compared to an unfamiliar one [81, 144-146]. Additionally, rats demonstrate quicker cooperation and helping behaviors with familiar partners than with unfamiliar ones [103, 119], while mice tend to exhibit less aggression towards familiar conspecifics compared to strangers [147, 148]. The level of familiarity can be mitigated by housing conditions and habituation to the experimental apparatus [149] and should be explicitly addressed in studies employing prosocial paradigms.

Training. It is essential to provide training to the animals prior to experimental testing. This serves two primary purposes: first, it familiarizes rodents with the testing environment, thereby reducing potential stress from encountering novelty, and second, it ensures that the animals understand the task's requirements, such as lever presses or nose poking [150]. Prior research demonstrated that a pretraining session has a notable effect on the manifestation of prosocial behaviors in rats [70]. Specifically, rats that underwent pretraining exhibited a higher level of activity, resulting in an increased frequency of prosocial behaviors compared to those that did not receive pretraining [70]. While there is no unanimous agreement on what constitutes effective habituation or pretraining [150], it is crucial to recognize that the duration and depth of these preparatory phases significantly influence the quality of data gathered on prosocial behaviors. Experimental conditions should thus be carefully reported and considered in analyzing the data and discussing findings.

Behavioral analyses. Animal behavior is intricately related to context, implying that an animal's actions are influenced by the specific nature of its environment and situation [151]. In prosocial tasks, for instance, rodents have demonstrated a tendency to wait for a conspecific before executing an action, such as climbing a platform for a mutual reward in a cooperative task [94, 152]. Additionally, following instances of rescuing behavior, rodents often engage in social contact and venture into the vacant compartment [74, 119]. These examples highlight the importance of analyzing behaviors during prosocial tasks, as they can unveil crucial insights into the dyadic interaction between the two animals. While numerous tracking software options are available, Ethovision is widely recognized as a popular choice for recording animal behavior [153], despite its costly nature. However, reliance on manual coding can lead to low inter-rater reliability [154]. Amongst open-access manual tracking software, Behavioral Observation Research Interactive Software (BORIS) [155] is frequently utilized. Furthermore, open-access semi-automated or fully automated tracking software presents a promising avenue for minimizing human error [156]. Regarding behavioral analyses, examining individual behaviors can yield valuable insights, but prosocial tasks typically involve interaction between two subjects. Therefore, analyzing behaviors from both animals can yield more comprehensive findings [70]. Structural and discriminant analysis techniques enable the examination of behaviors from multiple individuals and the prediction of behavioral probabilities [157, 158].

2.4.3 Environment

The environment in which the experiment takes place can significantly influence prosociality data and conclusions. Variables affecting environmental conditions in studies evaluating prosocial behavior in rodents include housing, reward systems, choice of experimental paradigm, restrictions on food and water, and performance analyses.

Housing. Housing conditions are known to affect the behavioral performance of rodents [159]. An enriched environment is a housing setup designed to provide animals with increased cognitive, sensory, motor, and social stimulation compared to standard laboratory conditions [160]. Enrichment typically includes the addition of items to promote play, exercise, foraging, or nesting, and/or offering larger and more complex cages (i.e., with many levels) [161, 162]. Co-housing larger groups of animals is social enrichment [163]; this type of enrichment promotes social behaviors such as play and communication [162]. Recent studies indicate that providing an enriched environment enhances animal welfare and ecological validity, especially considering that wild rodents typically live in social groups [161, 164]. Research also suggests that an enriched environment influences prosocial behaviors of rodents. For instance, one study observed that animals housed in enriched conditions exhibited more door-opening behaviors but engaged in fewer interactions with the released conspecific compared to individually housed rats [165]. Another study suggested that an enriched environment might mitigate deficits resulting from maternal separation in rats [166]. Consequently, housing conditions significantly impact rodent prosocial behavior and, in turn, affect study outcomes, highlighting the importance of careful consideration and documentation. Nonetheless, further research is necessary to fully comprehend the effects of an enriched environment on prosocial behaviors in rodents.

Reward and Restriction. For rodents to efficiently learn a specific task, such as lever pressing, rewards are typically essential in shaping the desired behavior [167]. The choice of reward used in a prosocial paradigm may influence a rodent's motivation to engage in prosocial behavior. Dolivo and Taborsky [168] demonstrated that rats reciprocate based on the quality of the help they receive, whether it is a highly palatable food item (such as banana, leading to increased reciprocity) or a less desirable reward (like carrot, resulting in decreased reciprocity). These findings suggest that the palatability of a reward can impact the subjects' performance in a social task. Rats show a preference for sweet and fatty food items, whether solid (e.g., cereals, chocolate chips, sucrose pellets) or liquid (e.g., chocolate milk, strawberry milk, condensed milk, sucrose water) [169]. To enhance the motivation and palatability of a food reward, researchers commonly practice food restriction, which involves limiting access to food and water. However, this practice can raise animal welfare concerns [167, 170]. Interestingly, studies investigating cooperative behaviors between rodents found that food-restricted rats cooperated less than ad libitum-fed rats [90, 171]. Consequently, it is crucial to clearly specify the type of reward and the restriction schedule, as these factors can influence motivation to learn a specific task and thereby modulate an animal's prosocial response.

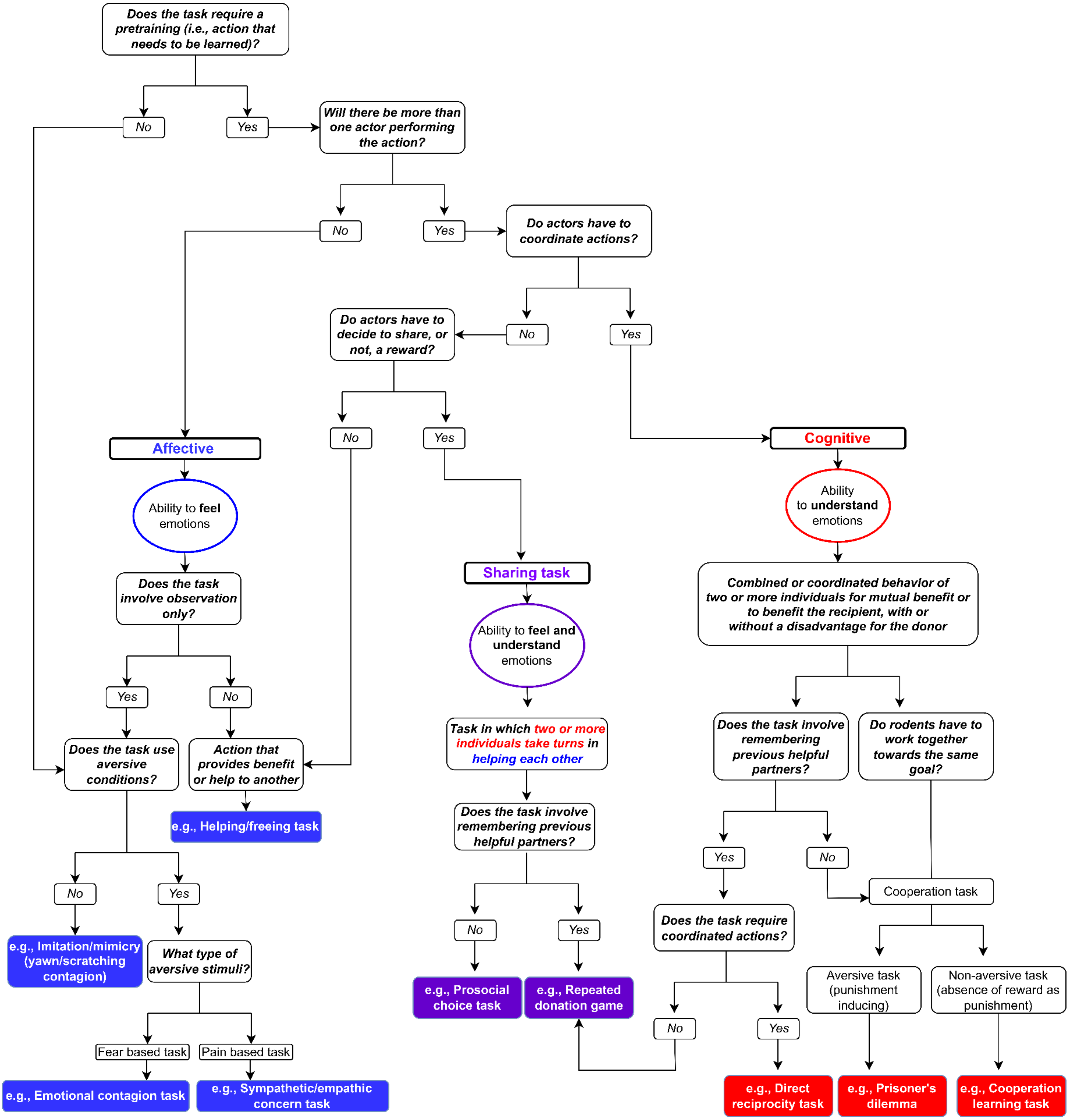
Another type of reward used in prosocial tasks involves social contact. For example, social contact is often permitted after a rodent frees a congener from a restrainer [172], or a rodent can consume a mutual food reward in the presence of a congener rather than alone [58]. This raises questions about the genuine motivation behind helping another individual versus the desire for social interaction [173]. Since rodents are inherently social creatures, social contact often motivates their behavior [174], making it challenging to distinguish between actions driven by the rewarding aspect of social interaction and those motivated by a prosocial desire to aid or share with a conspecific. Studies have demonstrated prosocial behavior occurring in the absence of social interaction [54, 74, 175], while others have reported conflicting results [176, 177]. Although these findings show promise, further research is necessary to establish the essential contingencies in prosocial behavior. In this regard, future studies should disclose the availability of social interaction throughout the experimental procedure to allow for a better characterization and discussion of its contribution to the expression of prosocial responses.

Performance analyses. Performance pertains to the quantity of prosocial actions executed by the animals (such as lever presses, nose poking, door opening, compartment choice, and lever pulling). As previously discussed, behavioral analyses are vital for enriching the insights obtained from task performance. While the frequency of lever presses on the 'sharing' lever can signify heightened prosociality, it is essential not to overlook the behavior exhibited by the rodent before and after the action. This comprehensive examination can provide a more nuanced and precise understanding of prosocial behavior.

3. Decision Tree

Figure 6 introduces a decision tree designed to facilitate the practical application of the conceptual framework. Its aim is to enhance the consistency and reproducibility of future studies, with the final step providing examples of specific tasks associated with each prosocial category. In addition to assisting in the selection of existing experimental paradigms, this decision tree can also aid researchers in developing new tasks by guiding them in categorizing and clearly defining experimental properties of the paradigm.

Figure 6. Decision Tree.



References

1. O'Connell LA, Hofmann HA. Genes, hormones, and circuits: An integrative approach to study the evolution of social behavior. Frontiers in Neuroendocrinology. 2011 Aug 1;32(3):320–35. Doi: 10.1016/j.yfrne.2010.12.004.

2. Ko J. Neuroanatomical Substrates of Rodent Social Behavior: The Medial Prefrontal Cortex and Its Projection Patterns. Front Neural Circuits. 2017;11:41. Doi: 10.3389/fncir.2017.00041.

3. Ebstein RP, Israel S, Chew SH, Zhong S, Knafo A. Genetics of Human Social Behavior. Neuron. 2010 Mar 25;65(6):831–44. Doi: 10.1016/j.neuron.2010.02.020.

5. Chang SWC, Brent LJN, Adams GK, Klein JT, Pearson JM, Watson KK, et al. Neuroethology of primate social behavior. Proceedings of the National Academy of Sciences. 2013 Jun 18;110;10387–94. Doi: 10.1073/pnas.1301213110.

6. Brent LJN, Lehmann J, Ramos-Fernández G. Social network analysis in the study of nonhuman primates: A historical perspective. Am J Primatol. 2011 Aug;73(8):720–30. Doi: 10.1002/ajp.20949.

7. Beery AK, Kaufer D. Stress, social behavior, and resilience: Insights from rodents. Neurobiology of Stress. 2015 Jan 1;1:116–27. Doi: 10.1016/j.ynstr.2014.10.004.

8. Qin M, Wong A, Seguin D, Gerlai R. Induction of Social Behavior in Zebrafish: Live Versus Computer Animated Fish as Stimuli. Zebrafish. 2014 Jun 1;11(3):185–97. Doi: 10.1089/zeb.2013.0969.

9. Holmes MM, Goldman BD. Social Behavior in Naked Mole-Rats: Individual Differences in Phenotype and Proximate Mechanisms of Mammalian Eusociality. Adv Exp Med Biol. 2021;1319:35–58. Doi: 10.1007/978-3-030-65943-1_2.

10. McGraw LA, Young LJ. The prairie vole: an emerging model organism for understanding the social brain. Trends Neurosci. 2010 Feb;33(2):103. Doi: 10.1016/j.tins.2009.11.006

11. Buwalda B, Geerdink M, Vidal J, Koolhaas JM. Social behavior and social stress in adolescence: a focus on animal models. Neurosci Biobehav Rev. 2011 Aug;35(8):1713–21. Doi: 10.1016/j.neubiorev.2010.10.004.

12. Moy SS, Nadler JJ. Advances in behavioral genetics: mouse models of autism. Mol Psychiatry. 2008 Jan;13(1):4–26. Doi: 10.1038/sj.mp.4002082.

13. Hernandez-Lallement J, van Wingerden M, Kalenscher T. Towards an animal model of callousness. Neuroscience & Biobehavioral Reviews. 2018 Aug 1;91:121–9. Doi: 10.1016/j.neubiorev.2016.12.029.

14. Beery AK, Holmes MM, Lee W, Curley JP. Stress in groups: Lessons from non-traditional rodent species and housing models. Neurosci Biobehav Rev. 2020 Jun;113:354–72. Doi: 10.1016/j.neubiorev.2020.03.033.

15. Bryda EC. The Mighty Mouse: The Impact of Rodents on Advances in Biomedical Research. Mo Med. 2013;110(3):207–11.

16. Meyza K, Knapska E. What can rodents teach us about empathy? Current Opinion in Psychology. 2018 Dec 1;24:15–20. Doi: 10.1016/j.copsyc.2018.03.002.

17.Seebacher F, Krause J. Physiological mechanisms underlying animal social behaviour. Philos Trans R Soc Lond B Biol Sci. 2017 Aug 19;372(1727):20160231. Doi: 10.1098/rstb.2016.0231

18. Murlanova K, Kirby M, Libergod L, Pletnikov M, Pinhasov A. Multidimensional nature of dominant behavior: Insights from behavioral neuroscience. Neuroscience & Biobehavioral Reviews. 2022 Jan 1;132:603–20. Doi: 10.1016/j.neubiorev.2021.12.015.

19. Fulenwider HD, Caruso MA, Ryabinin AE. Manifestations of domination: Assessments of social dominance in rodents. Genes Brain and Behavior. 2022 Mar;21(3):e12731. Doi: 10.1111/gbb.12731.

20. Choi J, Johnson DW, Johnson R. The Roots of Social Dominance: Aggression, Prosocial Behavior, and Social Interdependence. The Journal of Educational Research. 2011 Oct 1;104(6):442–54. Doi: 10.1080/00220671.2010.514689.

21. Holekamp KE, Strauss ED. Aggression and dominance: an interdisciplinary overview. Current Opinion in Behavioral Sciences. 2016 Dec 1;12:44–51. Doi: 10.1016/j.cobeha.2016.08.005.

22. Martínez-Torres S, Gomis-González M, Navarro-Romero A, Maldonado R, Ozaita A. Use of the Vsoc-maze to Study Sociability and Preference for Social Novelty in Rodents. Bio Protoc. 2019 Oct 20;9(20):e3393. Doi: 10.21769/BioProtoc.3393.

23. de la Zerda SH, Netser S, Magalnik H, Briller M, Marzan D, Glatt S, et al. Social recognition in laboratory mice requires integration of behaviorally-induced somatosensory, auditory and olfactory cues. Psychoneuroendocrinology. 2022 Sep;143:105859. Doi : 10.1016/j.psyneuen.2022.105859.

24. Cavigelli SA, Michael KC, West SG, Klein LC. Behavioral responses to physical vs. social novelty in male and female laboratory rats. Behav Processes. 2011 Sep;88(1):56–9. Doi: 10.1016/j.beproc.2011.06.006.

25.Martin LJ, Tuttle AH, Mogil JS. The interaction between pain and social behavior in humans and rodents. Curr Top Behav Neurosci. 2014;20:233–50. Doi: 10.1007/7854_2014_287.

26. Keum S, Shin HS. Neural Basis of Observational Fear Learning: A Potential Model of Affective Empathy. Neuron. 2019 Oct 9;104(1):78–86. Doi: 10.1016/j.neuron.2019.09.013.

27. Debiec J, Olsson A. Social Fear Learning: from Animal Models to Human Function. Trends in Cognitive Sciences. 2017 Jul 1;21(7):546–55. Doi: 10.1016/j.tics.2017.04.010.

28. Mehlhorn K, Newell BR, Todd PM, Lee MD, Morgan K, Braithwaite VA, et al. Unpacking the exploration–exploitation tradeoff: A synthesis of human and animal literatures. Decision. 2015;2(3):191–215. Doi: 10.1037/dec0000033.

29. Cohen JD, McClure SM, Yu AJ. Should I stay or should I go? How the human brain manages the trade-off between exploitation and exploration. Philos Trans R Soc Lond B Biol Sci. 2007 May 29;362(1481):933–42. Doi: 10.1098/rstb.2007.2098.

30. Lejarraga T. When experience is better than description: Time delays and complexity. Journal of Behavioral Decision Making. 2010;23(1):100–16. Doi: 10.1002/bdm.666.

31. Gonzalez C, Dutt V. Instance-based learning: Integrating sampling and repeated decisions from experience. Psychological Review. 2011;118(4):523–51. Doi: 10.1037/a0024558.

32. Phillips ND, Hertwig R, Kareev Y, Avrahami J. Rivals in the dark: How competition influences search in decisions under uncertainty. Cognition. 2014 Oct 1;133(1):104–19. Doi: 10.1016/j.cognition.2014.06.006.

33. Winet YK, Tu Y, Choshen-Hillel S, Fishbach A. Social exploration: When people deviate from options explored by others. Journal of Personality and Social Psychology. 2022;122(3):427–42. Doi: 10.1037/pspi0000350.

34. Knop J, Joëls M, van der Veen R. The added value of rodent models in studying parental influence on offspring development: opportunities, limitations and future perspectives. Current Opinion in Psychology. 2017 Jun 1;15:174–81. Doi: 10.1016/j.copsyc.2017.02.030.

35. Wang D, Levine JLS, Avila-Quintero V, Bloch M, Kaffman A. Systematic review and meta-analysis: effects of maternal separation on anxiety-like behavior in rodents. Transl Psychiatry. 2020 Jun 1;10(1):1–12. Doi: 10.1038/s41398-020-0856-0.

36. Alves RL, Portugal CC, Summavielle T, Barbosa F, Magalhães A. Maternal separation effects on mother rodents' behaviour: A systematic review. Neuroscience & Biobehavioral Reviews. 2020 Oct 1;117:98–109. Doi: 10.1016/j.neubiorev.2019.09.008.

37. Zimmerberg B, Sageser KA. Comparison of two rodent models of maternal separation on juvenile social behavior. Front Psychiatry. 2011;2:39. Doi: 10.3389/fpsyt.2011.00039.

38. Braun K, Champagne FA. Paternal influences on offspring development: behavioural and epigenetic pathways. J Neuroendocrinol. 2014 Oct;26(10):697–706. Doi: 10.1111/jne.12174.

39. Saltzman W, Harris BN, De Jong TR, Perea-Rodriguez JP, Horrell ND, Zhao M, et al. Paternal Care in Biparental Rodents: Intra- and Inter-individual Variation. Integrative and Comparative Biology. 2017 Sep 1;57(3):589–602. Doi: 10.1093/icb/icx047.

40. Feldman R. The adaptive human parental brain: implications for children's social development. Trends in Neurosciences. 2015 Jun 1;38(6):387–99. Doi: 10.1016/j.tins.2015.04.004.

41. Swain JE, Kim P, Spicer J, Ho SS, Dayton CJ, Elmadih A, et al. Approaching the biology of human parental attachment: Brain imaging, oxytocin and coordinated assessments of mothers and fathers. Brain Research. 2014 Sep 11;1580:78–101. Doi: 10.1016/j.brainres.2014.03.007.

42. Vanderschuren LJMJ, Trezza V. What the Laboratory Rat has Taught us About Social Play Behavior: Role in Behavioral Development and Neural Mechanisms. In: Andersen SL, Pine DS, editors. The Neurobiology of Childhood. Berlin, Heidelberg: Springer; 2014, p. 189–212.

43. Cox KH, Rissman EF. Sex differences in juvenile mouse social behavior are influenced by sex chromosomes and social context. Genes Brain Behav. 2011 Jun;10(4):465–72.

44. Vanderschuren LJMJ, Achterberg EJM, Trezza V. The neurobiology of social play and its rewarding value in rats. Neurosci Biobehav Rev. 2016 Nov;70:86–105.

45. Parker JG, Rubin KH, Erath SA, Wojslawowicz JC, Buskirk AA. Peer Relationships, Child Development, and Adjustment: A Developmental Psychopathology Perspective. In: Developmental Psychopathology. John Wiley & Sons, Ltd; 2015, p. 419–93.

46. Nicolopoulou A, Smith PK. Social Play and Social Development. In: The Wiley-Blackwell Handbook of Childhood Social Development. John Wiley & Sons, Ltd; 2022, p. 538–54.

47. Lee RLT, Lane S, Brown G, Leung C, Kwok SWH, Chan SWC. Systematic review of the impact of unstructured play interventions to improve young children's physical, social, and emotional wellbeing. Nursing & Health Sciences. 2020;22(2):184–96. Doi: 10.1111/nhs.12732.

48. Decety J, Bartal IBA, Uzefovsky F, Knafo-Noam A. Empathy as a driver of prosocial behaviour: highly conserved neurobehavioural mechanisms across species. Philos Trans R Soc Lond B Biol Sci. 2016 Jan 19;371(1686):20150077. Doi: 10.1098/rstb.2015.0077.

49. Riess H. The Science of Empathy. Journal of Patient Experience. 2017 Jun 1;4(2):74–7. Doi: 10.1177/2374373517699267.

50. Clay Z, Palagi E, de Waal FBM. Chapter 5 - Ethological Approaches to Empathy in Primates. In: Meyza KZ, Knapska E, editors. Neuronal Correlates of Empathy. Academic Press; 2018, p. 53–66.

51. Charron V, Talbot J, Labelle PR, Konkle ATM, Plamondon H. In search of prosociality in rodents: A scoping review. PLOS ONE. 2024 Nov 7;19(11):e0310771.

52. Church RM. Emotional reactions of rats to the pain of others. Journal of Comparative and Physiological Psychology. 1959;52:132–4. Doi: 10.1037/h0043531.

53. Greene JT. Altruistic behavior in the albino rat. Psychon Sci. 1969 Jan 1;14(1):47–8. Doi: 10.3758/BF03336420.

54. Sato N, Tan L, Tate K, Okada M. Rats demonstrate helping behavior toward a soaked conspecific. Anim Cogn. 2015 Sep 1;18(5):1039–47. Doi: 10.1007/s10071-015-0872-2.

55. Mason P. Lessons from helping behavior in rats. Current Opinion in Neurobiology. 2021 Jun 1;68:52–6. Doi: 10.1016/j.conb.2021.01.001.

56. Dolivo V, Taborsky M. Cooperation among Norway Rats: The Importance of Visual Cues for Reciprocal Cooperation, and the Role of Coercion. Ethology. 2015;121(11):1071–80. Doi: 10.1111/eth.12421.

57. Freidin E, Carballo F, Bentosela M. Direct reciprocity in animals: The roles of bonding and affective processes. International Journal of Psychology. 2017;52(2):163–70. Doi: 10.1002/ijop.12215.

58. Hernandez-Lallement J, van Wingerden M, Marx C, Srejic M, Kalenscher T. Rats prefer mutual rewards in a prosocial choice task. Frontiers in Neuroscience. 2015;8. Doi: 10.3389/fnins.2014.00443.

59. Trieu M, Foster AE, Yaseen ZS, Beaubian C, Calati R. Neurobiology of Empathy. In: Foster AE, Yaseen ZS, editors. Teaching Empathy in Healthcare: Building a New Core Competency. Cham: Springer International Publishing; 2019, p. 17–39.

60. Thoma P, Friedmann C, Suchan B. Empathy and social problem solving in alcohol dependence, mood disorders and selected personality disorders. Neuroscience & Biobehavioral Reviews. 2013 Mar 1;37(3):448–70. Doi: 10.1016/j.neubiorev.2013.01.024.

61. van der Zee E, Derksen JJL. Reconsidering Empathy Deficits in Children and Adolescents with Autism. J Dev Phys Disabil. 2020 Feb 1;32(1):23–39. Doi: 10.1007/s10882-019-09669-1.

62. Pick E, Kleinbub JR, Mannarini S, Palmieri A. Empathy In Neurodegenerative Diseases: A Systematic Review. Neuropsychiatr Dis Treat. 2019;15:3287–304. Doi : 10.2147/NDT.S225920.

63. Nijsse B, Spikman JM, Visser-Meily JM, de Kort PL, van Heugten CM. Social Cognition Impairments in the Long Term Post Stroke. Archives of Physical Medicine and Rehabilitation. 2019 Jul 1;100(7):1300–7. Doi: 10.1016/j.apmr.2019.01.023.

64. Vallat-Azouvi C, Azouvi P, Le-Bornec G, Brunet-Gouet E. Treatment of social cognition impairments in patients with traumatic brain injury: a critical review. Brain Inj. 2019;33(1):87–93. Doi: 10.1080/02699052.2018.1531309.

65. Hillis AE. Inability to empathize: brain lesions that disrupt sharing and understanding another's emotions. Brain. 2014 Apr;137(4):981–97. Doi: 10.1093/brain/awt317.

66. Keysers C, Gazzola V. Chapter 4 - Neural Correlates of Empathy in Humans, and the Need for Animal Models. In: Meyza KZ, Knapska E, editors. Neuronal Correlates of Empathy. Academic Press; 2018, p. 37–52.

67. Panksepp J, Panksepp JB. Toward a cross-species understanding of empathy. Trends Neurosci. 2013 Aug;36(8):10.1016/j.tins.2013.04.009. Doi: 10.1016/j.tins.2013.04.009.

68. Jabareen Y. Building a Conceptual Framework: Philosophy, Definitions, and Procedure. International Journal of Qualitative Methods. 2009 Dec 1;8(4):49–62. Doi: 10.1177/160940690900800406.

69. Berger-Tal O, Polak T, Oron A, Lubin Y, Kotler BP, Saltz D. Integrating animal behavior and conservation biology: a conceptual framework. Behavioral Ecology. 2011 Mar 1;22(2):236–9. Doi: 10.1093/beheco/arq224.

70. Charron V, Talbot J, Plamondon H. Prosocial decision making in an operant box paradigm promotes visual communication and complex behavioral sequences in adolescent rat dyads. Anim Behav Cogn. 2022 Feb 1;9(1):53–71. Doi: 10.26451/abc.09.01.05.2022.

71. de Waal FBM, Preston SD. Mammalian empathy: behavioural manifestations and neural basis. Nat Rev Neurosci. 2017 Aug;18(8):498–509. Doi: 10.1038/nrn.2017.72.

72. Young A, Khalil KA, Wharton J. Empathy for Animals: A Review of the Existing Literature. Curator: The Museum Journal. 2018;61(2):327–43. Doi: 10.1111/cura.12257.

73. McRae K, Misra S, Prasad AK, Pereira SC, Gross JJ. Bottom-up and top-down emotion generation: implications for emotion regulation. Social Cognitive and Affective Neuroscience. 2012 Mar 1;7(3):253–62. Doi: 10.1093/scan/nsq103.

74. Ben-Ami Bartal IBA, Decety J, Mason P. Helping a cagemate in need: empathy and pro-social behavior in rats. Science (New York, NY). 2011 Dec 12;334(6061):1427. Doi: 10.1126/science.1210789.

75. Hernandez-Lallement J, Attah AT, Soyman E, Pinhal CM, Gazzola V, Keysers C. Harm to Others Acts as a Negative Reinforcer in Rats. Current Biology. 2020 Mar;30(6):949-961.e7. Doi: 10.1016/j.cub.2020.01.017.

76. Shamay-Tsoory SG. The neural bases for empathy. Neuroscientist. 2011 Feb;17(1):18–24. Doi: 10.1177/1073858410379268.

77. Palagi E, Celeghin A, Tamietto M, Winkielman P, Norscia I. The neuroethology of spontaneous mimicry and emotional contagion in human and non-human animals. Neuroscience & Biobehavioral Reviews. 2020 Apr 1;111:149–65. Doi: 10.1016/j.neubiorev.2020.01.020.

78. Hernandez-Lallement J, Gómez-Sotres P, Carrillo M. Towards a unified theory of emotional contagion in rodents—A meta-analysis. Neuroscience & Biobehavioral Reviews. 2022 Jan 1;132:1229–48. Doi: 10.1016/j.neubiorev.2020.09.010.

79. Benassi-Cezar G, Carmona IM, Baptista-de-Souza D, Nunes-de-Souza RL, Canto-de-Souza A. Differential modulation of the anterior cingulate and insular cortices on anxiogenic-like responses induced by empathy for pain. Neuropharmacology. 2021 Jul 1;192:108413. Doi: 10.1016/j.neuropharm.2020.108413.

80. Ruzal K, Trachtenberg E, Kantor B, Flumin H, Roemer A, Crespo A, et al. Brain-wide activity-identity mapping of neural networks associated with prosocial motivation in rats. bioRxiv; 2023.12.10.570980. Doi: 10.1101/2023.12.10.570980.

81. Chen J. Empathy for Distress in Humans and Rodents. Neurosci Bull. 2018 Feb 1;34(1):216–36. Doi: 10.1007/s12264-017-0135-0.

82. Karakilic A, Kizildag S, Kandis S, Guvendi G, Koc B, Camsari GB, et al. The effects of acute foot shock stress on empathy levels in rats. Behavioural Brain Research. 2018 Sep 3;349:31–6. Doi: 10.1016/j.bbr.2018.04.043.

83. Allsop SA, Wichmann R, Mills F, Burgos-Robles A, Chang CJ, Felix-Ortiz AC, et al. Corticoamygdala Transfer of Socially Derived Information Gates Observational Learning. Cell. 2018 May 31;173(6):1329-1342.e18. Doi: 10.1016/j.cell.2018.04.004.

84. Paradiso E, Gazzola V, Keysers C. Neural mechanisms necessary for empathy-related phenomena across species. Current Opinion in Neurobiology. 2021 Jun 1;68:107–15. Doi : 10.1016/j.conb.2021.02.005.

85. Terburg D, Scheggia D, Triana del Rio R, Klumpers F, Ciobanu AC, Morgan B, et al. The Basolateral Amygdala Is Essential for Rapid Escape: A Human and Rodent Study. Cell. 2018 Oct 18;175(3):723-735.e16. Doi: 10.1016/j.cell.2018.09.028.

86. Hakamata Y, Mizukami S, Izawa S, Moriguchi Y, Hori H, Kim Y, et al. Basolateral Amygdala Connectivity With Subgenual Anterior Cingulate Cortex Represents Enhanced Fear-Related Memory Encoding in Anxious Humans. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging. 2020 Mar 1;5(3):301–10. Doi: 10.1016/j.bpsc.2019.11.008.

87. Jeon D, Kim S, Chetana M, Jo D, Ruley HE, Lin SY, et al. Observational fear learning involves affective pain system and Cav1.2 Ca2+ channels in ACC. Nat Neurosci. 2010 Apr;13(4):482–8. Doi: 10.1038/nn.2504.

88. Kim SW, Kim M, Shin HS. Affective empathy and prosocial behavior in rodents. Current Opinion in Neurobiology. 2021 Jun 1;68:181–9. Doi: 10.1016/j.conb.2021.05.002.

89. Preston SD, Waal FBM de. Empathy: Its ultimate and proximate bases. Behavioral and Brain Sciences. 2002 Feb;25(1):1–20. Doi: 10.1017/S0140525X02000018.

90. Wood RI, Kim JY, Li GR. Cooperation in rats playing the iterated Prisoner's Dilemma game. Animal Behaviour. 2016 Apr 1;114:27–35. Doi: 10.1016/j.anbehav.2016.01.010.

91. Schneeberger K, Dietz M, Taborsky M. Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evol Biol. 2012 Mar 29;12(1):41. Doi: 10.1186/1471-2148-12-41.

92. Delmas GE, Lew SE, Zanutto BS. High mutual cooperation rates in rats learning reciprocal altruism: The role of payoff matrix. PLOS ONE. 2019 Jan 2;14(1):e0204837. Doi: 10.1371/journal.pone.0204837.

93. Avital A, Aga-Mizrachi S, Zubedat S. Evidence for social cooperation in rodents by automated maze. Sci Rep. 2016 Jul 5;6(1):29517. Doi: 10.1038/srep29517.

94. Conde-Moro AR, Rocha-Almeida F, Sánchez-Campusano R, Delgado-García JM, Gruart A. The activity of the prelimbic cortex in rats is enhanced during the cooperative acquisition of an instrumental learning task. Progress in Neurobiology. 2019 Dec 1;183:101692. Doi: 10.1016/j.pneurobio.2019.101692.

95. de Carvalho LC, dos Santos L, Regaço A, Barbosa TB, da Silva RF, de Souza D das G, et al. Cooperative responding in rats maintained by fixed- and variable-ratio schedules. Journal of the Experimental Analysis of Behavior. 2018;110(1):105–26. Doi: 10.1002/jeab.457.

96. Kietzman HW, Gourley SL. How social information impacts action in rodents and humans: the role of the prefrontal cortex and its connections. Neuroscience & Biobehavioral Reviews. 2023 Apr 1;147:105075. Doi: 10.1016/j.neubiorev.2023.105075.

97. Rogers-Carter MM, Christianson JP. An insular view of the social decision-making network. Neuroscience & Biobehavioral Reviews. 2019 Aug 1;103:119–32. Doi: 10.1016/j.neubiorev.2019.06.005.

98. Daniel ML, Cocker PJ, Lacoste J, Mar AC, Houeto JL, Belin-Rauscent A, et al. The anterior insula bidirectionally modulates cost-benefit decision-making on a rodent gambling task. European Journal of Neuroscience. 2017;46(10):2620–8. Doi: 10.1111/ejn.13689.

99. Rosenberger LA, Eisenegger C, Naef M, Terburg D, Fourie J, Stein DJ, et al. The Human Basolateral Amygdala Is Indispensable for Social Experiential Learning. Current Biology. 2019 Oct 21;29(20):3532-3537.e3. Doi: 10.1016/j.cub.2019.08.078.

100. Hernandez-Lallement J, van Wingerden M, Schäble S, Kalenscher T. Basolateral amygdala lesions abolish mutual reward preferences in rats. Neurobiology of Learning and Memory. 2016 Jan 1;127:1–9. Doi: 10.1016/j.nlm.2015.11.004.

101. Conde-Moro AR, Rocha-Almeida F, Gebara E, Delgado-García JM, Sandi C, Gruart A. Involvement of prelimbic cortex neurons and related circuits in the acquisition of cooperative

learning by pairs of rats. bioRxiv; 2022, p. 2022.01.13.476162. Doi: 10.1101/2022.01.13.476162.

102. Decety J, Meyer M. From emotion resonance to empathic understanding: a social developmental neuroscience account. Dev Psychopathol. 2008;20(4):1053–80. Doi: 10.1017/S0954579408000503.

103. Li G, Wood RI. Male rats play a repeated donation game. Physiology & Behavior. 2017 May 15;174:95–103. Doi: 10.1016/j.physbeh.2017.03.010.

104. Rutte C, Taborsky M. Generalized Reciprocity in Rats. PLOS Biology. 2007 Jul 3;5(7):e196. Doi: 10.1371/journal.pbio.0050196.

105. Scheggia D, La Greca F, Maltese F, Chiacchierini G, Italia M, Molent C, et al. Reciprocal cortico-amygdala connections regulate prosocial and selfish choices in mice. Nat Neurosci. 2022 Nov;25(11):1505–18. Doi: 10.1038/s41593-022-01179-2.

106. Lockwood PL, O’Nell KC, Apps MAJ. Anterior cingulate cortex: A brain system necessary for learning to reward others? PLOS Biology. 2020 Jun 12;18(6):e3000735. Doi: 10.1371/journal.pbio.3000735.

107. Wu YE, Hong W. Neural basis of prosocial behavior. Trends in Neurosciences. 2022 Oct 1;45(10):749–62. Doi: 10.1016/j.tins.2022.06.008.

108. Prendergast BJ, Onishi KG, Zucker I. Female mice liberated for inclusion in neuroscience and biomedical research. Neurosci Biobehav Rev. 2014 Mar;40:1–5. Doi: 10.1016/j.neubiorev.2014.01.001.

109. Choleris E, Galea LAM, Sohrabji F, Frick KM. Sex differences in the brain: Implications for behavioral and biomedical research. Neurosci Biobehav Rev. 2018 Feb;85:126–45. Doi: 10.1016/j.neubiorev.2017.07.005.

110. Burke AR, McCormick CM, Pellis SM, Lukkes JL. Impact of adolescent social experiences on behavior and neural circuits implicated in mental illnesses. Neuroscience & Biobehavioral Reviews. 2017 May 1;76:280–300. Doi: 10.1016/j.neubiorev.2017.01.018.

111. Panksepp JB, Jochman KA, Kim JU, Koy JJ, Wilson ED, Chen Q, et al. Affiliative Behavior, Ultrasonic Communication and Social Reward Are Influenced by Genetic Variation in Adolescent Mice. PLOS ONE. 2007 Apr 4;2(4):e351.Doi: 10.1371/journal.pone.0000351.

112. Schneider P, Bindila L, Schmahl C, Bohus M, Meyer-Lindenberg A, Lutz B, et al. Adverse Social Experiences in Adolescent Rats Result in Enduring Effects on Social Competence, Pain Sensitivity and Endocannabinoid Signaling. Front Behav Neurosci. 2016 Oct 20;10:203. Doi: 10.3389/fnbeh.2016.00203.

113. Shoji H, Mizoguchi K. Aging-related changes in the effects of social isolation on social behavior in rats. Physiology & Behavior. 2011 Jan 10;102(1):58–62. Doi: 10.1016/j.physbeh.2010.10.001.

114. Kumar G, Talpos J, Steckler T. Strain-dependent effects on acquisition and reversal of visual and spatial tasks in a rat touchscreen battery of cognition. Physiology & Behavior. 2015 May 15;144:26–36. Doi: 10.1016/j.physbeh.2015.03.001.

115. Ku KM, Weir RK, Silverman JL, Berman RF, Bauman MD. Behavioral Phenotyping of Juvenile Long-Evans and Sprague-Dawley Rats: Implications for Preclinical Models of Autism Spectrum Disorders. PLOS ONE. 2016 Jun 28;11(6):e0158150. Doi: 10.1371/journal.pone.0158150.

116. Moy SS, Nadler JJ, Perez A, Barbaro RP, Johns JM, Magnuson TR, et al. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav. 2004 Oct;3(5):287–302. Doi: 10.1111/j.1601-1848.2004.00076.x.

117. Manduca A, Servadio M, Campolongo P, Palmery M, Trabace L, Vanderschuren LJMJ, et al. Strain- and context-dependent effects of the anandamide hydrolysis inhibitor URB597 on social behavior in rats. European Neuropsychopharmacology. 2014 Aug 1;24(8):1337–48. Doi: 10.1016/j.euroneuro.2014.05.009.

118. Decety J, Svetlova M. Putting together phylogenetic and ontogenetic perspectives on empathy. Developmental Cognitive Neuroscience. 2012 Jan 1;2(1):1–24. Doi: 10.1016/j.dcn.2011.05.003.

119. Ben-Ami Bartal I, Rodgers DA, Bernardez Sarria MS, Decety J, Mason P. Pro-social behavior in rats is modulated by social experience. Fernald R, editor. eLife. 2014 Jan 14;3:e01385. Doi: 10.7554/eLife.01385.

120. Gachomba MJM, Esteve-Agraz J, Caref K, Maroto AS, Bortolozzo-Gleich MH, Laplagne DA, et al. Multimodal cues displayed by submissive rats promote prosocial choices by dominants. Current Biology. 2022 Aug 8;32(15):3288-3301.e8. Doi: 10.1016/j.cub.2022.06.026.

121. Morgan KN, Tromborg CT. Sources of stress in captivity. Applied Animal Behaviour Science. 2007 Feb 1;102(3):262–302. Doi: 10.1016/j.applanim.2006.05.032.

122. Balcombe JP, Barnard ND, Sandusky C. Laboratory Routines Cause Animal Stress. Journal of the American Association for Laboratory Animal Science. 2004 Nov 15;43(6):42–51.

123. Novak J, Jaric I, Rosso M, Rufener R, Touma C, Würbel H. Handling method affects measures of anxiety, but not chronic stress in mice. Sci Rep. 2022 Dec 3;12(1):20938. Doi: 10.1038/s41598-022-25090-9.

124. Cloutier S, LaFollette MR, Gaskill BN, Panksepp J, Newberry RC. Tickling, a Technique for Inducing Positive Affect When Handling Rats. J Vis Exp. 2018 May 8;(135):57190. Doi: 10.3791/57190.

125. Tsai PP, Schlichtig A, Ziegler E, Ernst H, Haberstroh J, Stelzer HD, et al. Effects of different blood collection methods on indicators of welfare in mice. Lab Animal. 2015 Aug 1;44(8):301–11. Doi: 10.1038/laban.738.

126. Kumar M, Dandapat S, Sinha MP, Kumar A, Raipat BS. Different blood collection methods from rats: A review. Doi: 10.12680/balneo.2017.141.

127. Hoggatt AF, Hoggatt J, Honerlaw M, Pelus LM. A Spoonful of Sugar Helps the Medicine Go Down: A Novel Technique to Improve Oral Gavage in Mice. Journal of the American Association for Laboratory Animal Science. 2010 May 15;49(3):329–34.

128. Stuart SA, Robinson ESJ. Reducing the stress of drug administration: implications for the 3Rs. Sci Rep. 2015 Sep 23;5:14288. Doi: 10.1038/srep14288.

129. Foley PL, Kendall LV, Turner PV. Clinical Management of Pain in Rodents. Comparative Medicine. 2019 Dec 1;69(6):468–89. Doi: 10.30802/AALAS-CM-19-000048.

130. Cora MC, Kooistra L, Travlos G. Vaginal Cytology of the Laboratory Rat and Mouse: Review and Criteria for the Staging of the Estrous Cycle Using Stained Vaginal Smears. Toxicol Pathol. 2015 Aug;43(6):776–93. Doi: 10.1177/0192623315570339.

131. Gouveia A, dos Santos UD, Felisbino FE, de Afonseca TL, Antunes G, Morato S. Influence of the estrous cycle on the behavior of rats in the elevated T-maze. Behavioural Processes. 2004 Sep 30;67(2):167–71. Doi: 10.1016/j.beproc.2004.03.018.

132. Lovick TA, Zangrossi H. Effect of Estrous Cycle on Behavior of Females in Rodent Tests of Anxiety. Frontiers in Psychiatry. 2021, 12. Doi: 10.3389/fpsyt.2021.711065.

133. Sharp J, Zammit T, Azar T, Lawson D. Stress-like Responses to Common Procedures in Individually and Group-Housed Female Rats. Journal of the American Association for Laboratory Animal Science. 2003 Jan 15;42(1):9–18.

134. Becegato M, Meurer YSR, Paiva-Santos MA, Lima AC, Marinho GF, Bioni VS, et al. Impaired discriminative avoidance and increased plasma corticosterone levels induced by vaginal lavage procedure in rats. Physiology & Behavior. 2021 Apr 1;232:113343. Doi: 10.1016/j.physbeh.2021.113343.

135. Ekambaram G, Sampath Kumar SK, Joseph LD. Comparative Study on the Estimation of Estrous Cycle in Mice by Visual and Vaginal Lavage Method. J Clin Diagn Res. 2017 Jan;11(1):AC05–7. Doi: 10.7860/JCDR/2017/23977.9148.

136. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertility Research and Practice. 2020 Mar 14;6(1):5. Doi: 10.1186/s40738-020-00074-3.

137. Byers SL, Wiles MV, Dunn SL, Taft RA. Mouse Estrous Cycle Identification Tool and Images. PLOS ONE. 2012 Apr 13;7(4):e35538. Doi: 10.1371/journal.pone.0035538.

138. Jirkof P, Rudeck J, Lewejohann L. Assessing Affective State in Laboratory Rodents to Promote Animal Welfare—What Is the Progress in Applied Refinement Research? Animals. 2019 Dec;9(12):1026. Doi: 10.3390/ani9121026.

139. Ben-Ami Bartal I, Shan H, Molasky NMR, Murray TM, Williams JZ, Decety J, et al. Anxiolytic Treatment Impairs Helping Behavior in Rats. Front Psychol. 2016 Jun 8;7, Doi: 10.3389/fpsyg.2016.00850.

140. Muroy SE, Long KLP, Kaufer D, Kirby ED. Moderate Stress-Induced Social Bonding and Oxytocin Signaling are Disrupted by Predator Odor in Male Rats. Neuropsychopharmacol. 2016 Jul;41(8):2160–70. Doi: 10.1038/npp.2016.16.

141. Mateo JM. Kin Recognition in Ground Squirrels and Other Rodents. Journal of Mammalogy. 2003 Nov 21;84(4):1163–81. Doi: 10.1644/BLe-011.

142. Schweinfurth MK, Taborsky M. Relatedness decreases and reciprocity increases cooperation in Norway rats. Proceedings of the Royal Society B: Biological Sciences. 2018 Mar 7;285(1874):20180035. Doi: 10.1098/rspb.2018.0035.

143. Taborsky M, Frommen JG, Riehl C. Correlated pay-offs are key to cooperation. Philosophical Transactions of the Royal Society B: Biological Sciences. 2016 Feb 5;371(1687):20150084. Doi: 10.1098/rstb.2015.0084.

144. Mogil JS. Social modulation of and by pain in humans and rodents. PAIN. 2015 Apr;156:S35. Doi: 10.1097/01.j.pain.0000460341.62094.77.

145. Martin LJ, Tuttle AH, Mogil JS. The interaction between pain and social behavior in humans and rodents. Curr Top Behav Neurosci. 2014;20:233–50. Doi: 10.1007/7854_2014_287.

146. Bartal IBA. What’s familiarity got to do with it? Neural mechanisms of observational pain in siblings and strangers. Neuron. 2022 Jun 15;110(12):1887–8. Doi: 10.1016/j.neuron.2022.05.013.

147. Szenczi P, Bánszegi O, Groó Z, Altbäcker V. Development of the Social Behavior of Two Mice Species With Contrasting Social Systems. Aggressive Behavior. 2012;38(4):288–97. Doi: 10.1002/ab.21431.

148. Weber EM, Dallaire JA, Gaskill BN, Pritchett-Corning KR, Garner JP. Aggression in group-housed laboratory mice: why can't we solve the problem? Lab Anim (NY). 2017 Mar 22;46(4):157–61. Doi: 10.1038/laban.1219.

149. Beery AK, Shambaugh KL. Comparative Assessment of Familiarity/Novelty Preferences in Rodents. Front Behav Neurosci. 2021 Apr 13;15:648830. Doi: 10.3389/fnbeh.2021.648830.

150. Ennaceur A. Omission of the habituation procedure in the acquisition of a working memory task – evidence from Balb/c, C57/BL6J, and CD-1 mice. Behavioural Brain Research. 2011 Sep 30;223(1):203–10. Doi: 10.1016/j.bbr.2011.04.035.

151. Sih A, Bell A, Johnson JC. Behavioral syndromes: an ecological and evolutionary overview. Trends in Ecology & Evolution. 2004 Jul 1;19(7):372–8. Doi: 10.1016/j.tree.2004.04.009.

152. Kozma K, Kassai F, Ernyey AJ, Gyertyán I. Establishment of a rodent cooperation assay as a model of social cognition. Journal of Pharmacological and Toxicological Methods. 2019 May 1;97:44–51. Doi: 10.1016/j.vascn.2019.03.003.

153. Noldus LPJJ, Spink AJ, Tegelenbosch RAJ. EthoVision: A versatile video tracking system for automation of behavioral experiments. Behavior Research Methods, Instruments, & Computers. 2001 Aug 1;33(3):398–414. Doi: 10.3758/BF03195394.

154. Kafkafi N, Agassi J, Chesler EJ, Crabbe JC, Crusio WE, Eilam D, et al. Reproducibility and replicability of rodent phenotyping in preclinical studies. Neuroscience & Biobehavioral Reviews. 2018 Apr 1;87:218–32. Doi: 10.1016/j.neubiorev.2018.01.003.

155. Friard O, Gamba M. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution. 2016;7(11):1325–30. Doi: 10.1111/2041-210X.12584.

156. Isik S, Unal G. Open-source software for automated rodent behavioral analysis. Frontiers in Neuroscience. 2023;17. Doi: 10.3389/fnins.2023.1149027.

157. O'Connor BP. Simple and flexible SAS and SPSS programs for analyzing lag-sequential categorical data. Behavior Research Methods, Instruments, & Computers. 1999 Dec 1;31(4):718–26. Doi: 10.3758/BF03200753.

158. Casarrubea M, Leca JB, Gunst N, Jonsson GK, Portell M, Di Giovanni G, et al. Structural analyses in the study of behavior: From rodents to non-human primates. Frontiers in Psychology 2022;13. Doi: 10.3389/fpsyg.2022.1033561.

159. Arakawa H. Ethological approach to social isolation effects in behavioral studies of laboratory rodents. Behavioural Brain Research. 2018 Apr 2;341:98–108. Doi: 10.1016/j.bbr.2017.12.022.

160. Mellen J, Sevenich MacPhee M. Philosophy of environmental enrichment: Past, present, and future. Zoo Biology. 2001;20(3):211–26. Doi: 10.1002/zoo.1021.

161. Bayne K, Würbel H. The impact of environmental enrichment on the outcome variability and scientific validity of laboratory animal studies. Rev Sci Tech. 2014 Apr;33(1):273–80. Doi: 10.20506/rst.33.1.2282.

162. Sparling JE, Barbeau K, Boileau K, Konkle ATM. Environmental enrichment and its influence on rodent offspring and maternal behaviours, a scoping style review of indices of depression and anxiety. Pharmacology Biochemistry and Behavior. 2020 Oct 1;197:172997. Doi: 10.1016/j.pbb.2020.172997.

163. Nithianantharajah J, Hannan AJ. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat Rev Neurosci. 2006 Sep;7(9):697–709. Doi: 10.1038/nrn1970.

164. Amorim L, Dá Mesquita S, Jacinto L, Castelhano-Carlos MJ, Santos NC, Leite-Almeida H, et al. Shaping social behavior in an enriched environment. Frontiers in Behavioral Neuroscience. 2022;16. Doi: 10.3389/fnbeh.2022.999325.

165. Parra-Cruz JC, Martin-Neira V, Martínez-Muñoz ND, Jacobo-Suarez SC, Nieto-Capador D, Cortés-Patiño DM, et al. Environmental Enrichment and Prosocial Behavior in Wistar Rats: an Exploratory Study. Revista Brasileira de Terapia Comportamental e Cognitiva. 2022;24:1–17. Doi: 10.31505/rbtcc.v24i1.1752.

166. Joushi S, Taherizadeh Z, Esmaeilpour K, Sheibani V. Environmental enrichment and intranasal oxytocin administration reverse maternal separation-induced impairments of prosocial choice behavior. Pharmacology Biochemistry and Behavior. 2022 Feb 1;213:173318. Doi: 10.1016/j.pbb.2021.173318.

167. Barkus C, Bergmann C, Branco T, Carandini M, Chadderton PT, Galiñanes GL, et al. Refinements to rodent head fixation and fluid/food control for neuroscience. Journal of Neuroscience Methods. 2022 Nov 1;381:109705. Doi: 10.1016/j.jneumeth.2022.109705.

168. Dolivo V, Taborsky M. Norway rats reciprocate help according to the quality of help they received. Biology Letters. 2015 Feb;11(2):20140959. Doi: 10.1098/rsbl.2014.0959.

169. Miller AL, Leach MC. Determining the preferred liquid reward in adult C57BL/6 mice. Lab Anim. 2023 Jun 1;57(3):332–5. Doi: 10.1177/00236772221138628.

170. Rowland NE. Food or Fluid Restriction in Common Laboratory Animals: Balancing Welfare Considerations with Scientific Inquiry. Comparative Medicine. 2007 Apr 1;57(2):149–60.

171. Viana DS, Gordo I, Sucena É, Moita MAP. Cognitive and Motivational Requirements for the Emergence of Cooperation in a Rat Social Game. PLOS ONE. 2010 Jan 13;5(1):e8483. Doi: 10.1371/journal.pone.0008483.

172. Silberberg A, Allouch C, Sandfort S, Kearns D, Karpel H, Slotnick B. Desire for social contact, not empathy, may explain "rescue" behavior in rats. Anim Cogn. 2014 May;17(3):609–18. Doi: 10.1007/s10071-013-0692-1.

173. Lahvis GP. Social Reward and Empathy as Proximal Contributions to Altruism: The Camaraderie Effect. Curr Top Behav Neurosci. 2017;30:127–57. Doi: 10.1007/7854_2016_449.

174. Ben-Ami Bartal I, Breton JM, Sheng H, Long KL, Chen S, Halliday A, et al. Neural correlates of ingroup bias for prosociality in rats. Flagel SB, Wassum KM, editors. eLife. 2021 Jul 13;10:e65582. Doi: 10.7554/eLife.65582.

175. Cox SS, Reichel CM. Rats display empathic behavior independent of the opportunity for social interaction. Neuropsychopharmacol. 2020 Jun;45(7):1097–104. Doi: 10.1038/s41386-019-0572-8.

176. Han S, Chen YQ, Zheng B, Wang YX, Yin B. An Empirical Study on the Motivation of Helping Behavior in Rat.2023. Doi: 10.1101/2023.02.01.526568.

177. Heslin KA, Brown MF. No preference for prosocial helping behavior in rats with concurrent social interaction opportunities. Learn Behav. 2021 Dec;49(4):397–404. Doi: 10.3758/s13420-021-00471-8.

CHAPTER FIVE

General discussion

1. Brief overview of all chapters

Empathy in humans facilitates survival and successful interpersonal relationships. However, various physical and mental health disorders can impact empathy, significantly decreasing the quality of life for those affected. While empathy represents a subjective internal state, prosocial behaviors can provide insight into actions that benefit others, allowing researchers to objectively test this concept in animals. Rodent models can yield valuable information about the underlying mechanisms regulating prosociality and related impairments. The primary goal of this thesis was to advance the concept of prosociality and study its expression in rodent models, thereby addressing significant gaps in methodological reporting within the current literature. The main findings of the three studies presented in this thesis are as follows: (1) Three main categories of prosocial tasks (helping, cooperation, and sharing), each investigating a different domain of prosociality, were identified in Study Two and reframed within a conceptual framework in Study Three using top-down and bottom-up theoretical approaches; (2) Behavioral analyses were found to be a core element for a deeper understanding of prosociality in rodents, as demonstrated in Studies One and Two and illustrated in Study Three; and (3) Studies One and Two identified critical factors mediating prosocial responses, while Study Three offered a conceptual framework of the identified key elements (i.e., individual, environmental, and contextual factors) in the regulation of prosociality in rodents.

1.1 Summary of findings

The goal of Study One was to investigate prosocial behaviors in a sharing paradigm amongst two strains of adolescent rats. Moreover, pretraining sessions were used as a potentially mediating variable in the expression of prosociality in rodents. The paradigm enabled the assessment of whether an actor rat would work harder to share a reward with a congener. Results revealed that pretraining facilitated the expression of prosocial behaviors in adolescent rats. Strain analyses indicated that Long-Evans rats were more active than Sprague-Dawley rats. Analyses of transitional probabilities found complex behavioral sequences and increased visual communication between pretrained dyads, while decreased visual communication was observed in the naive group, highlighting the importance of behavioral analysis and pretraining sessions in the study of prosociality in rodents. Finally, observer rats failed to learn the task vicariously, further supporting the significance of pretraining sessions in shaping prosociality.

The objective of the second study was to extensively examine and consolidate existing research in the field of rodent prosociality, enabling the identification of gaps in current knowledge. In total, 80 studies from 2000 to 2023 met the inclusion criteria. The analysis of the surveyed data allowed for the definition and examination of prosociality in rats through three main behavioral paradigms: helping, sharing, and cooperation. This in-depth examination revealed significant gaps in the reporting of important details related to methodologies and behavioral analyses. Finally, important mediating factors were highlighted as determinants in the assessment of prosociality in rodents, including sex, age, strain, housing, familiarity, food restriction, aggression, and dominance.

The last study aimed to consolidate findings from Studies One and Two into a conceptual framework designed to facilitate the selection and design of study paradigms in the field of rodent prosociality, while also considering current theories on the topic. Initially conceptualized through affective and cognitive theories, rodent prosociality was subsequently characterized as a behavioral response observed in situations involving helping, cooperation, and sharing tasks.

Furthermore, key elements related to experimental conditions—specifically, individual, environmental, and contextual features that influence the promotion or prevention of prosocial responses—were identified. Additionally, this research resulted in an applied version of the developed conceptual framework, introduced through a decision tree, which can provide informed guidance to future researchers. The decision tree is a robust tool offering a foundation for the ongoing utilization of current models and the creation of novel paradigms.

2. Redefining prosociality from top-down to bottom-up: helping, cooperation, and sharing tasks

While prosociality has been tested in rodents for decades, no research had previously been conducted to organize, classify, and assess the efficacy of existing experimental models. As such, this thesis defined three important categories of prosocial tasks (i.e., helping, cooperation, sharing). While some reviews have been done on social behavior tests (File & Seth, 2003; Arakwa, 2023; Cox & Reichel, 2021; Sivaselvachandran et al., 2018; Keysers et al., 2022), none have documented the characteristics and findings of prosocial (i.e., action-based) models. Therefore, this thesis represents a unique contribution by providing the first classification of experimental paradigms used to study prosocial responses in rodents. Three distinctive categories of protocols were defined, each targeting specific characteristics of prosociality. This classification will promote study replication and enhance the ecological validity of prosocial behaviors observed in rats and mice.

2.1 Helping tasks

Helping tasks, as outlined in Study Two, entail actions that provide benefit or assistance to another. Tasks in this category involve a conspecific experiencing distress, fear, or discomfort, such as being restrained, exposed to uncomfortable conditions (e.g., a soaked area), or subjected to electric foot shocks (e.g., affective prosociality). As suggested in Study Three, helping tasks likely involve bottom-up processing of perceptual information. The animal must initially perceive signs of distress using external cues such as sight, smell, or sound (referred to as bottom-up interpretation). Subsequently, the animal interprets this information as indicative of a conspecific in need of assistance or relief from distress, involving a higher level of appreciation, consistent with the bottom-up approach described in Decety's model (Decety & Jackson, 2006; Decety & Meyer, 2008). Furthermore, in such situations, research suggests that visual, olfactory, and auditory cues play essential roles in prompting helping behavior and emotional contagion amongst mice and rats, whereas physical contact with a congener may not be as pivotal in initiating a response (Geng et al., 2020; Ueno et al., 2018; Yu et al., 2024; Cox & Reichel, 2020). This suggests that external cues function as critical stimuli in fostering context-specific helping behaviors, contrasting with earlier assumptions that social contact primarily drives helping behaviors (Silberberg et al., 2014). Collectively, these findings indicate that the decision of a rodent to help a conspecific is primarily influenced by the actor's interpretation of external cues.

2.2 Cooperation tasks

Cooperation tasks are distinctive in that they involve the combined or coordinated behavior of two or more individuals for mutual benefit or to benefit the recipient, with or without a disadvantage for the donor. Cooperation, as assessed in rodents, requires a learned behavioral response (e.g., pressing a lever, coordinated behavior) in which the understanding of the impact of one’s actions on another motivates the actor’s response. The top-down approach necessitates that the animal draw upon prior knowledge (e.g., through pretraining and habituation) to execute an action (i.e., top), followed by perceiving the consequences of that action on others (i.e., down). Similar to helping behaviors, sensory cues play a crucial role in the manifestation of cooperative behaviors amongst rodents. For example, Łopuch and Popik (2011) reported reduced cooperation in rats deprived of all sensory modalities (e.g., visual, auditory, olfactory, and physical cues), while such responses remained unaffected when restrictions were limited to physical cues. Similarly, Schweinfurth and Taborsky (2019) demonstrated that rats utilized auditory signals to express their willingness to cooperate in a prisoner's dilemma scenario, potentially using this behavior to capture the attention of their conspecifics and thereby enhance cooperation. Furthermore, Conde-Moro and colleagues (2019) highlighted the necessity of visual cues for the coordination of platform climbing in rats. They demonstrated that a leader rat would initiate platform climbing and then pause, awaiting the follower rat's replication of the action in its compartment, resulting in both rats receiving a mutual reward upon synchronized behavior. Interestingly, rodents appear to draw upon past knowledge and experiences when deciding whether to cooperate with a previously generous or selfish congener (Schneeberger et al., 2012). Taken together, these findings suggest that rodents may lean towards employing top-down information processing mechanisms when engaging in cooperation with conspecifics, utilizing feedback from their counterparts to guide subsequent actions.

2.3 Sharing tasks

Sharing tasks are tasks in which two or more individuals take turns helping each other. Results from Study One are consistent with findings from Study Two: rodents can learn to donate food to a conspecific. In sharing tasks, rodents must grasp the contingencies of levers or compartments (indicative of a top-down process) and observe the consequences of their choices on their peers (such as receiving a reward or not), incorporating this information into future decisions (indicative of a bottom-up process). Multiple trials of a sharing task allow for the investigation of a feedback loop between top-down and bottom-up processing. Notably, this feedback loop was also demonstrated in the behavioral sequences identified through transitional probabilities established in Study One (see section below). Consequently, the conceptual framework proposed in Study Three suggests that sharing tasks encompass elements of both affective and cognitive aspects of prosocial behavior. Affective ‘bottom-related’ aspects are found in the state of the conspecific (e.g., food or water restricted) and the apparatus that allows for communication between rodents (i.e., conveying information about the state of the conspecific). In contrast, the cognitive aspect lies within the task itself: rodents undergo multiple trials to select between selfish and mutual options (drawing on prior knowledge; top) and observe the repercussions of their actions on the recipient (down).

2.3.1 Exploring the dynamics of sharing: sensory influences on rodent behavior

The findings from Study One align with those presented in the existing literature, supporting the significance of sensory cues in the emergence of sharing behaviors (Márquez et al., 2015; Gerber et al., 2020). Specifically, Study One reported heightened visual communication amongst rats following food-sharing actions, indicating their sensitivity to conspecific behaviors. Similarly, Márquez and colleagues (2015) trained recipient rats (i.e., observers) in a food-sharing task to exhibit "food-seeking behaviors," wherein rats were taught to nose-poke a port. This behavior allowed the recipient rat to signal its preference for the prosocial choice to the actor before decision-making using visual cues. The findings indicated that displaying food-seeking behavior influenced the actor rats’ decisions, increasing their preference for the prosocial option over the selfish one (Márquez et al., 2015). This suggests that rodents employ bottom-up processing when evaluating the state of their peers before engaging in sharing behaviors. Similarly, Gerber and colleagues (2020) found that rodents distinguished between generous and defective partners using olfactory cues and exhibited increased sharing with generous partners based on this information. This suggests that rodents employ top-down information processing by drawing on past knowledge and experiences to guide their decision-making. In summary, current literature suggests that rodents are attuned to their peers' exhibited behaviors both before and after sharing actions, potentially supporting the involvement of a feedback loop between top-down and bottom-up approaches, which facilitates the expression of prosocial behaviors.

3. From top-down to bottom-up: cerebral activation during prosocial behaviors

Characterizing a broad set of prosocial responses, Study 3 supported the activation of similar brain circuits in rodents and humans. Four main brain regions have been highlighted: the anterior insula (AI), the anterior cingulate cortex (ACC), the basolateral amygdala (BLA), and the prefrontal cortex (PFC), especially the infralimbic cortex (IL).

The AI, activated during helping tasks in rodents (Cox et al., 2022; Ben-Ami Bartal et al., 2021), is similarly recruited in humans when observing another individual in pain (Singer et al., 2004). Notably, individuals diagnosed with autism spectrum disorder do not show AI activation in similar tasks (Gu et al., 2015), supporting the critical role of this brain region in regulating the affective aspects of prosociality, such as recognizing a conspecific in distress and engaging in targeted helping behaviors. Furthermore, the insular cortex is suggested to play a role in social decision-making by processing inputs from various brain regions associated with sensory, social, and reward-related stimuli (Rogers-Carter & Christianson, 2019). In humans, AI activation has been linked to cooperative behaviors during the decision phase of the Prisoner’s Dilemma game (Thompson et al., 2021). In Sprague-Dawley rats, AI lesions impair decision-making, resulting in reduced performance during a gambling task compared to their pre-lesion performance and that of sham-operated rats (Daniel et al., 2017). Together, these findings highlight the insular cortex's importance in both the affective (e.g., perceiving others' emotional states) and cognitive (e.g., decision-making) aspects of prosociality.

Another important brain locus is the ACC, recently shown to modulate helping behaviors in mice (Song et al., 2023). Activation of both the ACC and AI has been observed in mice housed with conspecifics in pain (Benassi-Cezar et al., 2021). Notably, inhibiting oxytocin receptors in the ACC resulted in delayed acquisition of helping behavior in rats (Yamagishi et al., 2020). In humans, the ACC plays a crucial role in affect regulation, with key connections to the limbic system and prefrontal cortex, supporting the interplay between cognitive and affective aspects of prosociality (Davis et al., 2005; Stevens et al., 2011). Interestingly, a recent study using a fear conditioning paradigm demonstrated that enhanced BLA activation is associated with emotional contagion, observed in both human-to-human and mouse-to-mouse interactions, as well as cross-species responses (Kaźmierowska et al., 2023). Yamagishi and colleagues (2020) noted increased c-Fos expression in oxytocin receptors in the ACC and BLA during helping behaviors, further supporting the mediating role of these regions in affective tasks. While often linked to emotional responses, the BLA also regulates cognitive aspects of prosociality. Conde-Moro and colleagues (2024) showed that BLA electrical activity significantly increases prior to cooperative responses, suggesting its role in anticipating task outcomes. This notion is supported by findings indicating that BLA lesions impair learning and performance in trust games (Rosenberger et al., 2019). Additionally, the BLA is crucial for promoting sharing decisions in both mice (Scheggia et al., 2023) and rats (Hernandez-Lallement et al., 2016; Schönfeld et al., 2020). It also contributes to the learning and decision-making phases of prosocial tasks (Hernandez-Lallement et al., 2016). In humans, BLA activation is linked to social affiliation and learning (Rosenberger et al., 2019), underscoring its role in regulating cognitive aspects of prosociality.

Finally, the infralimbic cortex, including the dorsomedial and ventromedial prefrontal regions, is of interest as a regulator of the cognitive aspects of prosociality. The mPFC is associated with top-down cognitive information processing in both humans and rodents (Ko, 2017; Kietzman & Gourley, 2023). In humans, the dmPFC is involved in cognitive perspective-taking (i.e., inferring what another person thinks), while the vmPFC is involved in affective perspective-taking (i.e., inferring what another person feels; Kietzman & Gourley, 2023; Lombardo et al., 2010). In both humans and rodents, the dmPFC and vmPFC are involved in decision-making and social cognition (Lockwood et al., 2022; Howland et al., 2022). In rodents, the prefrontal cortex, including the infralimbic cortex, is also crucial for the expression of cognitive prosociality (Bicks et al., 2015; Jiang et al., 2021; Conde-Moro et al., 2019).

Based on current literature, this thesis is the first to suggest and apply such an approach to prosociality in rodents. By categorizing sharing, helping, and cooperation tasks according to a framework based on their reliance on a differential use of top-down and bottom-up information processing, this thesis offers a novel and unique conceptualization that advances our understanding of prosociality in rodents. Moreover, the similarities in cerebral activation observed between rodents and humans support the proposed three task categories to delineate distinctive aspects of rodent prosociality and highlight potential translation to behavioral responses observed in humans.

3. Behavioral analyses at the core of understanding rodent prosociality

While human studies often rely on self-reported measures to investigate emotions and thoughts (Neumann et al., 2015), research with rodents primarily involves the observation and recording of actions and behaviors. Interestingly, results from the scoping review conducted in Study Two revealed significant gaps in behavioral analyses amongst the reviewed articles. Behavioral neuroscience has much to gain in terms of the translatability of findings by relating behaviors to brain network activation and mechanisms; however, behavioral analyses are often omitted. As illustrated in Study One, rodents exhibit complex sequences of behaviors that can provide additional insights into the mechanisms of prosociality. To maximize data from these recordings, transitional probability analyses were performed using the work of O'Connor (1999). These statistics generate probabilities for sequences of behaviors to occur, which is especially relevant in the study of animal behavior (O'Connor, 1999). Despite the availability of this method for over 25 years, its utilization in assessing animal behavior has been limited. This could be attributed to the general gap in the analysis of animal behavior found in the field of behavioral neuroscience, as indicated by the findings of Study Two. Consequently, Study One selected this method to promote and maximize behavioral analyses.

The behavioral sequences found in Study One demonstrated that: (1) rodents understood the contingency of the levers, (2) the actor rat recognized the impact of pressing the hard lever by its tendency to look at its congener after pressing, (3) visual communication persisted even in the absence of direct physical contact, and (4) the behavior of the observer rat (i.e., looking at the actor rat, staying near the acrylic wall, looking into its feeder after a lever press, and following this behavior with visual communication) facilitated the sharing behavior of the actor rat. These findings align with those from Study Two, which revealed that rodents are sensitive to the behavior of their congeners. For instance, Kozma and colleagues (2019) found that actor rats tended to stay close to the cooperation apparatus and to their congener, likely to monitor cooperative actions. This discovery is significant and corroborates the results of Study One, indicating that the behavior of the observer rat significantly influences the prosocial decisions made by the actor.

Videorecording of rodents and coding of predefined behaviors are not the sole methods for monitoring their behavioral patterns. As discussed in Study Two, other behavioral analyses include recording ultrasonic vocalizations (USVs), observing social behaviors such as allogrooming and play-fighting, and monitoring biological rhythm behaviors such as sleeping and feeding. Rodents emit USVs to convey both positive and negative affective states, with 22 kHz calls typically associated with negative states and 50 kHz calls with positive states (Simola & Granon, 2019). Studies have shown that rodents trapped in a helping task often emit distress calls, such as 22 kHz calls (Subhadeep et al., 2022), while positive 50 kHz calls are emitted during cooperation tasks (Łopuch & Popik, 2011). Increased allogrooming has been noted between rodents when one conspecific is experiencing pain and is thus considered a consolation behavior (Lu et al., 2018; Li et al., 2018). Play fighting is predominantly displayed by juvenile rodents and is considered critical to this developmental phase, necessary for the acquisition of social skills, communication, and prosociality (Lahvis, 2017; Pellis et al., 2022; Pellis et al., 2023). These social interactions are important and can offer further insights into the behavioral processes that regulate the expression of prosocial behavior in rodents when analyzed in conjunction with task performance.

Other behaviors influenced by biological rhythms, such as sleep and feeding, can also impact prosociality in rodents. For example, studies have shown that sleep deprivation can impair emotional contagion in mice (Xue et al., 2023), while the feeding behavior of mice is influenced by social factors such as the presence or absence of satiated or restricted conspecifics (Ueno et al., 2019b). Exploring social behaviors and biological rhythms can yield insights potentially valuable for explaining prosociality—or the lack thereof—and promote a deeper understanding of such complex behaviors in rodents. With current technological advancements, behavior coding software is becoming more accessible. In Study One, an open-source coding software, BORIS (Friard & Gamba, 2016), was employed for the manual coding of predefined behaviors. Many resources are now available, from paid software (e.g., Ethovision, Spink et al., 2001) to open-source tools (Isik & Unal, 2023). Access to these tools enables more researchers to conduct behavioral analyses in rodents, thereby enhancing our understanding of prosociality.

Study Three used findings from Studies One and Two to reframe the concept of prosociality as a set of complex behaviors emerging in response to environmental determinants that cannot be reduced to a single set of data. As demonstrated in Study Two, while performance indices such as the number of lever presses or door openings are valuable for analysis and interpretation, they alone do not provide a comprehensive understanding of the underlying processes in prosocial tasks, including helping, cooperation, and sharing tasks. Furthermore, additional research into behavioral sequences and patterns amongst rodents has the potential to uncover crucial mediating factors influencing the manifestation of prosocial behavior.

4. Methodological considerations: individual, environmental, and contextual factors mediating prosocial behaviors

As Study Three exemplified, prosociality represents a set of complex behaviors that emerge in response to environmental determinants. This includes various mediating factors illustrated throughout this research program. Study Two revealed significant gaps in the reported methodologies of the included articles, highlighting the need to further promote the important role of commonly omitted mediating factors that can influence the expression of prosociality in rodents.

4.1 Age and strain mediate the expression of rodent prosociality

Study One demonstrated that the strain of rat plays a role in the expression of prosocial behaviors, with Long-Evans rats showing more activity in this regard compared to Sprague-Dawley rats. This aligns with existing literature indicating that albino rats, such as Sprague-Dawley, typically exhibit lower activity levels during daylight hours compared to pigmented strains like Long-Evans (Andrews et al., 1995). These findings are corroborated by similar results reported in other studies (Turner & Burne, 2014; Schwarting et al., 2018).

Moreover, Study One explored prosocial behavior in adolescent rats, a developmental stage known for increased activity, including play fighting and exploration (Pellis et al., 2023). Adolescence is characterized by significant cerebral changes and heightened impulsivity, which may influence the expression of prosocial behaviors (Doremus-Fitzwater et al., 2012). This aligns with the results of a recent study examining helping behaviors in adolescent versus adult rats (Breton et al., 2022). The study revealed that while both age groups released a conspecific, adolescent rats were more inclined to release both ingroup and outgroup members, whereas adults predominantly released ingroup members. This significant finding implies that ingroup and outgroup bias may emerge during adulthood (Breton et al., 2022). Similar patterns have been observed in mice, where strain and age differences affect the display of helping behaviors (Panksepp & Lahvis, 2023). Notably, rescuing behavior in helping tasks appears to decrease in older rats compared to adults (Hosgorler et al., 2023), highlighting the importance of age as a factor influencing prosocial behavior.

4.2 Mediating effect of pretraining on performance and behavior

Habituation refers to the opportunity for rodents to explore a novel environment before an experiment, aiming to reduce the novelty and stress associated with the unfamiliar setting (Leussis & Bolivar, 2006). Conversely, pretraining involves an experimental session designed to teach specific actions, such as lever pressing or door opening (Brady & Floresco, 2015).

In Study One, analysis of transitional probabilities indicated that rats subjected to pretraining (e.g., trained to understand lever contingencies) demonstrated sequences of behaviors that were more varied and intricate compared to naïve rats (e.g., those without pretraining). Pretrained rats also opted for the sharing option more frequently than non-pretrained rats of the same strain. This finding suggests that pretraining sessions are a significant mediating factor in the expression of prosocial behaviors in rodents.

Study Two revealed that pretraining periods were more prevalent in non-aversive tasks, such as sharing and cooperation paradigms. Amongst the screened articles, pretraining was commonly utilized in sharing tasks, while cooperation tasks required longer pretraining periods (averaging 18 to 20 days), likely due to the need for enhanced cognitive resources to coordinate behaviors with a conspecific (Rutte & Taborsky, 2008). In contrast, pretraining was least emphasized in helping tasks, possibly due to their aversive nature, which may rely on instinctive responses (e.g., survival instinct; Panksepp & Panksepp, 2013). Notably, many articles did not employ pretraining to teach rodents how to open a restrainer, yet still reported significant results (Ben-Ami Bartal et al., 2011; Cox & Reichel, 2020; Carvalheiro et al., 2019).

Several studies using sharing or cooperation tasks proceeded without pretraining or habituation. For example, sharing behavior was observed by the 4th or 5th session in a prosocial choice task, despite the absence of a pretraining (Schönfeld et al., 2020). Similarly, in a cooperation task, learning occurred after four days without pretraining (one session per day; Han et al., 2020b). Conversely, a cooperation task without pretraining did not yield significant cooperative behaviors in mice (Raz, 2013), suggesting that the brief duration of four days may not have allowed for adequate learning.

Although pretraining is less common with aversive tasks, some studies reported increased rescuing behavior between the third and sixth days of training (Cox & Reichel, 2020; Ben-Ami Bartal et al., 2011; Carvalheiro et al., 2019). Additionally, some studies included only the last few sessions of training in their statistical analyses (e.g., the last 3 to 5 sessions) (Schwartz et al., 2017; Tomek et al., 2019). Overall, it appears to take 4 to 6 days of experimental sessions for prosocial behaviors to fully establish, indicating that the development of these behaviors necessitates a learning component, consistent with findings from other studies using prosocial paradigms (Heyes, 2018; Meyza et al., 2017; Keysers et al., 2022). This highlights that pretraining influences the emergence of prosocial behaviors, and in its absence, the frequency and duration of sessions also play a critical role.

The factors explored in this thesis—such as age, strain, and pretraining—demonstrated significant mediating effects on the development of prosocial behaviors in rodents. Additionally, Studies Two and Three highlighted the importance of environmental factors, including stress variables (procedures and manipulation), familiarity, choice of paradigm and reward, housing conditions, and food/water restrictions. This further highlights the complexity of the processes underlying prosocial behaviors in rodents, indicating a need for a more comprehensive and integrated assessment of its different aspects. This approach could improve understanding of the neurophysiological and behavioral signatures of prosociality and may link these findings to empathic responses seen in humans.

5. Strengths and specific contributions

Collectively, the in vivo assessment of prosociality in rodents, the scoping review, and the conceptual framework developed in this thesis collectively introduce a new tool for analyzing behavioral interactions in rodents and offer a comprehensive understanding of research on prosociality in these animals. Additionally, the affective and cognitive processes that regulate prosocial expression are central to the conceptual framework presented in this thesis, which aims to refine current scientific knowledge about prosocial behaviors in rodents.

Study One established a sharing model capable of assessing prosocial behaviors in two strains of adolescent male rats while also examining the potential mediating effect of pretraining sessions on the expression of prosocial behavior. Although similar sharing models have been previously published, this paradigm was the first to investigate the impact of a heavier lever on rats' willingness to exert more effort for mutual benefit. Moreover, this study was the first to explore the impact of pretraining on the emergence of prosocial behaviors while also using extensive transitional probabilities of behavioral sequences in a rat dyad. Results demonstrated that pretrained rats were willing to work harder to share a mutual reward, showing more complex behavioral sequences than their naive counterparts. This important finding significantly contributes to the field by highlighting crucial methodological considerations, including pretraining and behavioral analyses for the emergence of prosociality in rodents.

Study Two conducted a scoping review aimed at consolidating current knowledge and gaps in the field of rodent prosociality. This study was the first to categorize prosocial models into three important categories: helping, sharing, and cooperation tasks. Moreover, it identified and highlighted important mediating factors potentially impacting the expression of prosociality in rodents, as well as significant gaps in reported methodologies and the lack of behavioral analyses. This opened the door for Study Three to further redefine rodent prosociality.

Study Three introduced a conceptual framework aimed at integrating findings from the preceding studies and existing theories on rodent prosocial behavior. This framework represents the first endeavor of its kind within this field and makes a substantial contribution by providing a classification of prosociality and its associated tasks in rodents. Additionally, mediating factors were highlighted and discussed to address the gaps in methodological reporting found in Study Two. By redefining an entire domain of research, this conceptual framework will promote the standardization and replicability of prosocial tasks in rodents.

Collectively, this research program significantly advances the field of rodent prosocial behavior by redefining the criteria for conducting prosocial tests in both rats and mice. Whether aimed at gaining deeper insights into the cerebral mechanisms underlying prosocial behavior or investigating its deficits observed in mental, neurodevelopmental, and neurological disorders, this thesis addresses a crucial gap and will improve the standardization and replication of future studies.

6. Limitations and future directions

While the three studies in this thesis distinctly contribute to expanding knowledge in the field of rodent prosocial behavior, there are certain limitations worth noting. Firstly, Study One did not explore prosocial behaviors in female rodents or in age groups other than adolescence. Unfortunately, the COVID-19 pandemic, which occurred during this thesis work, severely affected access to facilities and resources. Consequently, to mitigate this limitation, sex differences were further examined in the articles included in Study Two. Additionally, while Study One conducted an extensive analysis of behaviors using transitional probabilities, incorporating the recording and analysis of ultrasonic vocalizations (USVs) could have provided valuable insights into the behaviors of rat dyads. Moreover, like many studies involving rodents, the findings of this research are constrained by a small sample size. Regarding Study Two, it is important to acknowledge the possibility of missing pertinent studies during the process of collecting and including relevant articles, as is common in reviews. Furthermore, the focus of the scoping review was limited to prosocial tasks, thereby excluding other studies exploring social behaviors in rodents, such as those investigating emotional contagion or imitation experiments. Lastly, while Study Three provides a concise conceptual framework, it is important to note that prosociality is a complex behavior that is challenging to operationalize precisely. Although the framework is thorough, some aspects may not be fully captured due to gaps in current knowledge. Additionally, this conceptual framework specifically applies to rodent models and may not be directly applicable to other animal models or humans.

Future research may benefit from utilizing findings from this thesis to advance our understanding of behavioral processes, cerebral mechanisms, and mediating factors involved in the expression of prosocial behavior in rodents. This work sets the stage for enhanced standardization and replicability of existing paradigms while also encouraging the development of new models.

7. Conclusion

Engaging in prosocial behaviors is inherent to both human and animal existence, facilitating connection, communication, meaningful relationships, resource sharing, and positive interactions amongst individuals. In humans, deficits in prosocial behavior have been associated with a significant decline in quality of life, as evidenced by various mental, neurodevelopmental, and neurological disorders. Consequently, animal models provide valuable insights into the cerebral and behavioral mechanisms underlying prosocial processes. Studies within this research program have highlighted significant findings that deepen our understanding of prosocial behavior in rodents, including its behavioral manifestations, cerebral mechanisms, and associated mediating factors. Furthermore, numerous gaps in reporting have been addressed to improve the standardization and replicability of future studies. As the study of prosocial behavior in rodents remains a relatively new and rapidly expanding field, this thesis lays the groundwork for a more comprehensive understanding of prosocial processes in these animals. It aims to advance knowledge of the cerebral and behavioral mechanisms underlying prosociality and its associated impairments

References

(General introduction, General discussion)

Adams, A. G., Schweitzer, D., Molenberghs, P., & Henry, J. D. (2019). A meta-analytic review of social cognitive function following stroke. *Neuroscience & Biobehavioral Reviews*, *102*, 400–416. https://doi.org/10.1016/j.neubiorev.2019.03.011

Aival-Naveh, E., Rothschild-Yakar, L., & Kurman, J. (2019). Keeping culture in mind: A systematic review and initial conceptualization of mentalizing from a cross-cultural perspective. *Clinical Psychology: Science and Practice*, *26*(4), 25–25. https://doi.org/10.1037/h0101757

Alves, R. L., Portugal, C. C., Summavielle, T., Barbosa, F., & Magalhães, A. (2020). Maternal separation effects on mother rodents' behaviour: A systematic review. *Neuroscience and Biobehavioral Reviews*, *117*, 98–109. https://doi.org/10.1016/j.neubiorev.2019.09.008

American Psychiatric Association. (2013). Diagnostic and statistical manual of mental disorders (5th ed.). https://doi.org/10.1176/appi.books.9780890425596

Andrews, J. S., Jansen, J. H., Linders, S., Princen, A., & Broekkamp, C. L. (1995). Performance of four different rat strains in the autoshaping, two-object discrimination, and swim maze tests of learning and memory. *Physiology & Behavior*, *57*(4), 785–790. https://doi.org/10.1016/0031-9384(94)00336-x

Arakawa, H. (2023). Revisiting sociability: Factors facilitating approach and avoidance during the three-chamber test. *Physiology & Behavior*, *272*, 114373. https://doi.org/10.1016/j.physbeh.2023.114373

Atrooz, F., Alkadhi, K. A., & Salim, S. (2021). Understanding stress: Insights from rodent models. *Current Research in Neurobiology*, *2*, 100013. https://doi.org/10.1016/j.crneur.2021.100013

Avital, A., Aga-Mizrachi, S., & Zubedat, S. (2016). Evidence for social cooperation in rodents by automated maze. *Scientific Reports*, *6*(1), 29517. https://doi.org/10.1038/srep29517

Bahader, G. A., Naghavi, F., Alotaibi, A., Dehghan, A., Swain, C. C., Burkett, J. P., & Shah, Z. A. (2024). Neurobehavioral and inflammatory responses following traumatic brain injury in male and female mice. *Behavioural Brain Research*, *456*, 114711. https://doi.org/10.1016/j.bbr.2023.114711

Bartochowski, Z., Gatla, S., Khoury, R., Al-Dahhak, R., & Grossberg, G. T. (2018). Empathy changes in neurocognitive disorders: A review. *Annals of Clinical Psychiatry: Official Journal of the American Academy of Clinical Psychiatrists*, *30*(3), 220–232.

Baskin-Sommers, A., Krusemark, E., & Ronningstam, E. (2014). Empathy in narcissistic personality disorder: From clinical and empirical perspectives. *Personality Disorders: Theory, Research, and Treatment*, *5*(3), 323–333. https://doi.org/10.1037/per0000061

Beesdo-Baum, K., & Knappe, S. (2014). Epidemiology and Natural Course. In *The Wiley Handbook of Anxiety Disorders* (pp. 26–46). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118775349.ch3

Ben-Ami Bartal, I. B.-A., Decety, J., & Mason, P. (2011a). Empathy and Pro-Social Behavior in Rats. *Science*, *334*(6061), 1427–1430. https://doi.org/10.1126/science.1210789

Ben-Ami Bartal, I., Shan, H., Molasky, N. M. R., Murray, T. M., Williams, J. Z., Decety, J., & Mason, P. (2016). Anxiolytic Treatment Impairs Helping Behavior in Rats. *Frontiers in Psychology*, *7*. https://doi.org/10.3389/fpsyg.2016.00850

Ben-Ami Bartal, I., Breton, J. M., Sheng, H., Long, K. L., Chen, S., Halliday, A., Kenney, J. W., Wheeler, A. L., Frankland, P., Shilyansky, C., Deisseroth, K., Keltner, D., & Kaufer, D. (2021). Neural correlates of ingroup bias for prosociality in rats. eLife, 10, e65582. https://doi.org/10.7554/eLife.65582

Benassi-Cezar, G., Carmona, I. M., Baptista-de-Souza, D., Nunes-de-Souza, R. L., & Canto-de-Souza, A. (2021). Differential modulation of the anterior cingulate and insular cortices on anxiogenic-like responses induced by empathy for pain. *Neuropharmacology*, *192*, 108413. https://doi.org/10.1016/j.neuropharm.2020.108413

Bicks, L. K., Koike, H., Akbarian, S., & Morishita, H. (2015). Prefrontal Cortex and Social Cognition in Mouse and Man. Frontiers in Psychology, 6. https://doi.org/10.3389/fpsyg.2015.01805

Blakemore, S.-J., & Decety, J. (2001). From the perception of action to the understanding of intention. *Nature Reviews Neuroscience*, *2*(8), 561–567. https://doi.org/10.1038/35086023

Blystad, M. H. (2019). A critical review of the rodent social release paradigm: Empathy or social reinforcement. *Revista Mexicana de Análisis de La Conducta*, *45*(2), 199–222.

Bodnar, C. N., Roberts, K. N., Higgins, E. K., & Bachstetter, A. D. (2019). A Systematic Review of Closed Head Injury Models of Mild Traumatic Brain Injury in Mice and Rats. *Journal of Neurotrauma*, *36*(11), 1683–1706. https://doi.org/10.1089/neu.2018.6127

Bonfils, K. A., Lysaker, P. H., Minor, K. S., & Salyers, M. P. (2016). Affective empathy in schizophrenia: A meta-analysis. *Schizophrenia Research*, *175*(1), 109–117. https://doi.org/10.1016/j.schres.2016.03.037

Brady, A. M., & Floresco, S. B. (2015). Operant Procedures for Assessing Behavioral Flexibility in Rats. *Journal of Visualized Experiments : JoVE*, *96*, 52387. https://doi.org/10.3791/52387

Breton, J. M., Eisner, J. S., Gandhi, V. S., Musick, N., Zhang, A., Long, K. L. P., Perloff, O. S., Hu, K. Y., Pham, C. M., Lalchandani, P., Barraza, M. K., Kantor, B., Kaufer, D., & Ben-Ami Bartal, I. (2022). Neural activation associated with outgroup helping in adolescent rats. *iScience*, *25*(6), 104412. https://doi.org/10.1016/j.isci.2022.104412

Bruchey, A. K., Jones, C. E., & Monfils, M.-H. (2010). Fear conditioning by-proxy: Social transmission of fear during memory retrieval. *Behavioural Brain Research*, *214*(1), 80–84. https://doi.org/10.1016/j.bbr.2010.04.047

Byom, L. J., & Mutlu, B. (2013). Theory of mind: Mechanisms, methods, and new directions. *Frontiers in Human Neuroscience*, *7*. https://doi.org/10.3389/fnhum.2013.00413

Campbell, B. C. V., De Silva, D. A., Macleod, M. R., Coutts, S. B., Schwamm, L. H., Davis, S. M., & Donnan, G. A. (2019). Ischaemic stroke. *Nature Reviews Disease Primers*, *5*(1), 1–22. https://doi.org/10.1038/s41572-019-0118-8

Campos, C., Pasion, R., Azeredo, A., Ramião, E., Mazer, P., Macedo, I., & Barbosa, F. (2022). Refining the link between psychopathy, antisocial behavior, and empathy: A meta-analytical approach across different conceptual frameworks. *Clinical Psychology Review*, *94*, 102145. https://doi.org/10.1016/j.cpr.2022.102145

Cao, Q., Tan, C.-C., Xu, W., Hu, H., Cao, X.-P., Dong, Q., Tan, L., & Yu, J.-T. (2020). The Prevalence of Dementia: A Systematic Review and Meta-Analysis. *Journal of Alzheimer's Disease*, *73*(3), 1157–1166. https://doi.org/10.3233/JAD-191092

Carlson, S. M., Koenig, M. A., & Harms, M. B. (2013). Theory of mind. *WIREs Cognitive Science*, *4*(4), 391–402. https://doi.org/10.1002/wcs.1232

Carvalheiro, J., Seara-Cardoso, A., Mesquita, A. R., de Sousa, L., Oliveira, P., Summavielle, T., & Magalhães, A. (2019). Helping behavior in rats (Rattus norvegicus) when an escape alternative is present. *Journal of Comparative Psychology*, *133*(4), 452–462. https://doi.org/10.1037/com0000178

Cavigelli, S. A., Michael, K. C., West, S. G., & Klein, L. C. (2011). Behavioral responses to physical vs. Social novelty in male and female laboratory rats. *Behavioural Processes*, *88*(1), 56–59. https://doi.org/10.1016/j.beproc.2011.06.006

Cheluvappa, R., Scowen, P., & Eri, R. (2017). Ethics of animal research in human disease remediation, its institutional teaching; and alternatives to animal experimentation. *Pharmacology Research & Perspectives*, *5*(4), e00332. https://doi.org/10.1002/prp2.332

Chen, J. (2018). Empathy for Distress in Humans and Rodents. *Neuroscience Bulletin*, *34*(1), 216–236. https://doi.org/10.1007/s12264-017-0135-0

Choi, J., & Jeong, Y. (2017). Elevated emotional contagion in a mouse model of Alzheimer's disease is associated with increased synchronization in the insula and amygdala. *Scientific Reports*, *7*(1), 46262. https://doi.org/10.1038/srep46262

Church, R. M. (1959). Emotional reactions of rats to the pain of others. *Journal of Comparative and Physiological Psychology*, *52*(2), 132–134. https://doi.org/10.1037/h0043531

Coan, J. A., & Sbarra, D. A. (2015). *Social Baseline Theory*: The social regulation of risk and effort. *Current Opinion in Psychology*, *1*, 87–91. https://doi.org/10.1016/j.copsyc.2014.12.021

Conde-Moro, A. R., Rocha-Almeida, F., Gebara, E., Delgado-García, J. M., Sandi, C., & Gruart, A. (2024). Involvement of prelimbic cortex neurons and related circuits in the acquisition of a cooperative learning by pairs of rats. Cognitive Neurodynamics. https://doi.org/10.1007/s11571-024-10107-y

Conde-Moro, A. R., Rocha-Almeida, F., Sánchez-Campusano, R., Delgado-García, J. M., & Gruart, A. (2019). The activity of the prelimbic cortex in rats is enhanced during the cooperative acquisition of an instrumental learning task. *Progress in Neurobiology*, *183*, 101692. https://doi.org/10.1016/j.pneurobio.2019.101692

Corniquel, M. B., Koenigsberg, H. W., & Likhtik, E. (2019). Toward an animal model of borderline personality disorder. *Psychopharmacology*, *236*(8), 2485–2500. https://doi.org/10.1007/s00213-019-05289-x

Corradini, A., & Antonietti, A. (2013). Mirror neurons and their function in cognitively understood empathy. *Consciousness and Cognition*, *22*(3), 1152–1161. https://doi.org/10.1016/j.concog.2013.03.003

Coundouris, S. P., Adams, A. G., & Henry, J. D. (2020). Empathy and theory of mind in Parkinson's disease: A meta-analysis. *Neuroscience & Biobehavioral Reviews*, *109*, 92–102. https://doi.org/10.1016/j.neubiorev.2019.12.030

Cox, S. S., & Reichel, C. M. (2020). Rats display empathic behavior independent of the opportunity for social interaction. *Neuropsychopharmacology*, *45*(7), 1097–1104. https://doi.org/10.1038/s41386-019-0572-8

Cox, S. S., & Reichel, C. M. (2021). Current Rodent Models for the Study of Empathic Processes. *Behavioural Pharmacology*, *32*(2-#x000263), 96–111. https://doi.org/10.1097/FBP.0000000000000590

Cox, S. S., Kearns, A. M., Woods, S. K., Brown, B. J., Brown, S. J., & Reichel, C. M. (2022). The role of the anterior insula during targeted helping behavior in male rats. Scientific Reports, 12, 3315. https://doi.org/10.1038/s41598-022-07365-3

Cruz, A., Heinemans, M., Márquez, C., & Moita, M. A. (2020). Freezing Displayed by Others Is a Learned Cue of Danger Resulting from Co-experiencing Own Freezing and Shock. *Current Biology*, *30*(6), 1128-1135.e6. https://doi.org/10.1016/j.cub.2020.01.025

Curzer, H. J., Perry, G., Wallace, M. C., & Perry, D. (2016). The Three Rs of Animal Research: What they Mean for the Institutional Animal Care and Use Committee and Why. *Science and Engineering Ethics*, *22*(2), 549–565. https://doi.org/10.1007/s11948-015-9659-8

Daniel, M. L., Cocker, P. J., Lacoste, J., Mar, A. C., Houeto, J. L., Belin-Rauscent, A., & Belin, D. (2017). The anterior insula bidirectionally modulates cost-benefit decision-making on a rodent gambling task. European Journal of Neuroscience, 46(10), 2620–2628. https://doi.org/10.1111/ejn.13689

Darwin, C., & Prodger, P. (1998). *The Expression of the Emotions in Man and Animals*. Oxford University Press.

Davis, K. D., Taylor, K. S., Hutchison, W. D., Dostrovsky, J. O., McAndrews, M. P., Richter, E. O., & Lozano, A. M. (2005). Human Anterior Cingulate Cortex Neurons Encode Cognitive and Emotional Demands. The Journal of Neuroscience, 25(37), 8402–8406. https://doi.org/10.1523/JNEUROSCI.2315-05.2005 de Carvalho, L. C., dos Santos, L., Regaço, A., Barbosa, T. B., da Silva, R. F., de Souza, D. das G., & Sandaker, I. (2018). Cooperative responding in rats maintained by fixed- and variable-ratio schedules. *Journal of the Experimental Analysis of Behavior*, *110*(1), 105–126. https://doi.org/10.1002/jeab.457

de la Zerda, S. H., Netser, S., Magalnik, H., Briller, M., Marzan, D., Glatt, S., Abergel, Y., & Wagner, S. (2022). Social recognition in laboratory mice requires integration of behaviorally-induced somatosensory, auditory and olfactory cues. *Psychoneuroendocrinology*, *143*, 105859. https://doi.org/10.1016/j.psyneuen.2022.105859

de Sousa, A., McDonald, S., Rushby, J., Li, S., Dimoska, A., & James, C. (2010). Why don’t you feel how I feel? Insight into the absence of empathy after severe Traumatic Brain Injury. *Neuropsychologia*, *48*(12), 3585–3595. https://doi.org/10.1016/j.neuropsychologia.2010.08.008

de Waal, F. B. M. (2007). The “Russian doll” model of empathy and imitation. In *On being moved: From mirror neurons to empathy* (pp. 49–69). John Benjamins Publishing Company. https://doi.org/10.1075/aicr.68.06waa

Decety, J., Bartal, I. B.-A., Uzefovsky, F., & Knafo-Noam, A. (2016). Empathy as a driver of prosocial behaviour: Highly conserved neurobehavioural mechanisms across species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *371*(1686), 20150077. https://doi.org/10.1098/rstb.2015.0077

Decety, J., & Holvoet, C. (2021). The emergence of empathy: A developmental neuroscience perspective. *Developmental Review*, *62*, 100999. https://doi.org/10.1016/j.dr.2021.100999

Decety, J., & Jackson, P. L. (2006). A Social-Neuroscience Perspective on Empathy. *Current Directions in Psychological Science*, *15*(2), 54–58. https://doi.org/10.1111/j.0963-7214.2006.00406.x

Decety, J., & Meyer, M. (2008). From emotion resonance to empathic understanding: A social developmental neuroscience account. *Development and Psychopathology*, *20*(4), 1053–1080. https://doi.org/10.1017/S0954579408000503

Decety, J., Norman, G. J., Berntson, G. G., & Cacioppo, J. T. (2012). A neurobehavioral evolutionary perspective on the mechanisms underlying empathy. *Progress in Neurobiology*, *98*(1), 38–48. https://doi.org/10.1016/j.pneurobio.2012.05.001

Deci, E. L., & Ryan, R. M. (2000). The "What" and "Why" of Goal Pursuits: Human Needs and the Self-Determination of Behavior. *Psychological Inquiry*, *11*(4), 227–268. https://doi.org/10.1207/S15327965PLI1104_01

Delmas, G. E., Lew, S. E., & Zanutto, B. S. (2019). High mutual cooperation rates in rats learning reciprocal altruism: The role of payoff matrix. *PLOS ONE*, *14*(1), e0204837. https://doi.org/10.1371/journal.pone.0204837

Deschênes, M., Moore, J., & Kleinfeld, D. (2012). Sniffing and whisking in rodents. *Current Opinion in Neurobiology*, *22*(2), 243–250. https://doi.org/10.1016/j.conb.2011.11.013

Díaz, V. (2022). Minds in action: Evidence that linguistic diversity helps children build a theory of mind. *Bilingualism: Language and Cognition*, *25*(1), 70–80. https://doi.org/10.1017/S1366728921000109

Dinsdale, N., & Crespi, B. J. (2013). The borderline empathy paradox: Evidence and conceptual models for empathic enhancements in borderline personality disorder. *Journal of Personality Disorders*, *27*(2), 172–195. https://doi.org/10.1521/pedi.2013.27.2.172

Donovan, A., Ryan, E., & Wood, R. I. (2020). Cooperative responses in rats playing a 2 × 2 game: Effects of opponent strategy, payoff, and oxytocin. Psychoneuroendocrinology, 121, 104803. https://doi.org/10.1016/j.psyneuen.2020.104803

Doremus-Fitzwater, T. L., Barreto, M., & Spear, L. P. (2012). Age-related differences in impulsivity among adolescent and adult Sprague-Dawley rats. *Behavioral Neuroscience*, *126*(5), 735–741. https://doi.org/10.1037/a0029697

Ebbesen, C. L., & Froemke, R. C. (2021). Body language signals for rodent social communication. *Current Opinion in Neurobiology*, *68*, 91–106. https://doi.org/10.1016/j.conb.2021.01.008

Engelhardt, K.-A., Schwarting, R. K. W., & Wöhr, M. (2018). Mapping trait-like socio-affective phenotypes in rats through 50-kHz ultrasonic vocalizations. *Psychopharmacology*, *235*(1), 83–98. https://doi.org/10.1007/s00213-017-4746-y

Engelhardt, S. C., & Taborsky, M. (2024). Reciprocal altruism in Norway rats. Ethology, 130(4), e13418. https://doi.org/10.1111/eth.13418

Eslinger, P. J., Moore, P., Anderson, C., & Grossman, M. (2011). Social cognition, executive functioning, and neuroimaging correlates of empathic deficits in frontotemporal dementia. *The Journal of Neuropsychiatry and Clinical Neurosciences*, *23*(1), 74–82. https://doi.org/10.1176/jnp.23.1.jnp74

Fendt, M., Gonzalez-Guerrero, C. P., & Kahl, E. (2021). Observational Fear Learning in Rats: Role of Trait Anxiety and Ultrasonic Vocalization. *Brain Sciences*, *11*(4), Article 4. https://doi.org/10.3390/brainsci11040423

Fenwick, N., Griffin, G., & Gauthier, C. (2009). The welfare of animals used in science: How the “Three Rs” ethic guides improvements. *The Canadian Veterinary Journal*, *50*(5), 523–530.

Festucci, F., Buccheri, C., Cerniglia, L., Paciello, M., Cimino, S., Curcio, G., & Adriani, W. (2020). A new paradigm for Prosocial Behavior and Reciprocity, assessed in WT and HET rats for the DAT gene. *Behavioural Brain Research*, *393*, 112746. https://doi.org/10.1016/j.bbr.2020.112746

File, S. E., & Seth, P. (2003). A review of 25 years of the social interaction test. *European Journal of Pharmacology*, *463*(1–3), 35–53. https://doi.org/10.1016/S0014-2999(03)01273-1

Finlayson, K., Lampe, J. F., Hintze, S., Würbel, H., & Melotti, L. (2016). Facial Indicators of Positive Emotions in Rats. *PLOS ONE*, *11*(11), e0166446. https://doi.org/10.1371/journal.pone.0166446

Fischer, A., Landeira-Fernandez, J., Sollero de Campos, F., & Mograbi, D. C. (2019). Empathy in Alzheimer’s Disease: Review of Findings and Proposed Model. *Journal of Alzheimer’s Disease*, *69*(4), 921–933. https://doi.org/10.3233/JAD-180730

Fluri, F., Schuhmann, M. K., & Kleinschnitz, C. (2015). Animal models of ischemic stroke and their application in clinical research. *Drug Design, Development and Therapy*, *9*, 3445–3454. https://doi.org/10.2147/DDDT.S56071

Freidin, E., Carballo, F., & Bentosela, M. (2017). Direct reciprocity in animals: The roles of bonding and affective processes. *International Journal of Psychology*, *52*(2), 163–170. https://doi.org/10.1002/ijop.12215

Friard, O., & Gamba, M. (2016). BORIS: A free, versatile open-source event-logging software for video/audio coding and live observations. Methods in Ecology and Evolution, 7(11), 1325–1330. https://doi.org/10.1111/2041-210X.12584

Fulenwider, H. D., Caruso, M. A., & Ryabinin, A. E. (2022). Manifestations of domination: Assessments of social dominance in rodents. *Genes, Brain, and Behavior*, *21*(3), e12731. https://doi.org/10.1111/gbb.12731

Geng, K.-W., Du, R., Wei, N., Li, C.-L., Wang, Y., Sun, W., Chen, T., Wei, D.-Y., Yu, Y., He, T., Luo, W.-J., Wang, R.-R., Chen, Z.-F., & Chen, J. (2020). Image-Forming Visual Basis of Empathy for Pain in Mice. Neuroscience Bulletin, 36(12), 1563–1569. https://doi.org/10.1007/s12264-020-00528-0

Gerber, N., Schweinfurth, M. K., & Taborsky, M. (2020). The smell of cooperation: Rats increase helpful behaviour when receiving odour cues of a conspecific performing a cooperative task. *Proceedings of the Royal Society B: Biological Sciences*, *287*(1939), 20202327. https://doi.org/10.1098/rspb.2020.2327

Gilman, S. L. (1984). Charles Bell, The anatomy of expression (1806): Die Ausdruckstheorie des Anatomen und Chirurgen Sir Charles Bell (1774–1842) und ihre Beziehung zur Ästhetik des 19. Jahrhunderts. *Medical History*, *28*(1), 85–86.

Greene, J. T. (1969). Altruistic behavior in the albino rat. *Psychonomic Science*, *14*(1), 47–48. https://doi.org/10.3758/BF03336420

Gu, X., Eilam-Stock, T., Zhou, T., Anagnostou, E., Kolevzon, A., Soorya, L., Hof, P. R., Friston, K. J., & Fan, J. (2015). Autonomic and brain responses associated with empathy deficits in autism spectrum disorder. Human Brain Mapping, 36(9), 3323–3338. https://doi.org/10.1002/hbm.22840

Guariglia, P., Palmiero, M., Giannini, A. M., & Piccardi, L. (2023). The Key Role of Empathy in the Relationship between Age and Social Support. *Healthcare*, *11*(17), Article 17. https://doi.org/10.3390/healthcare11172464

Hachiga, Y., Schwartz, L. P., Silberberg, A., Kearns, D. N., Gomez, M., & Slotnick, B. (2018). Does a rat free a trapped rat due to empathy or for sociality? *Journal of the Experimental Analysis of Behavior*, *110*(2), 267–274. https://doi.org/10.1002/jeab.464

Hakulinen, C., Pulkki-Råback, L., Jokela, M., Ferrie, J. E., Aalto, A.-M., Virtanen, M., Kivimäki, M., Vahtera, J., & Elovainio, M. (2016). Structural and functional aspects of social support as predictors of mental and physical health trajectories: Whitehall II cohort study. *J Epidemiol Community Health*, *70*(7), 710–715. https://doi.org/10.1136/jech-2015-206165

Han, Y., Sichterman, B., Carrillo, M., Gazzola, V., & Keysers, C. (2020a). Similar levels of emotional contagion in male and female rats. *Scientific Reports*, *10*(1), 2763. https://doi.org/10.1038/s41598-020-59680-2

Han, K. A., Yoon, T. H., Shin, J., Um, J. W., & Ko, J. (2020b). Differentially altered social dominance- and cooperative-like behaviors in Shank2- and Shank3-mutant mice. *Molecular Autism*, *11*, 87. https://doi.org/10.1186/s13229-020-00392-9

Hatfield, E., Bensman, L., Thornton, P. D., & Rapson, R. L. (2014). New Perspectives on Emotional Contagion: A Review of Classic and Recent Research on Facial Mimicry and Contagion. *Interpersona: An International Journal on Personal Relationships*, *8*(2), Article 2. https://doi.org/10.5964/ijpr.v8i2.162

Helt, M. S., Fein, D. A., & Vargas, J. E. (2020). Emotional contagion in children with autism spectrum disorder varies with stimulus familiarity and task instructions. Development and Psychopathology, 32(1), 383–393. https://doi.org/10.1017/S0954579419000154

Hernandez-Lallement, J., Attah, A. T., Soyman, E., Pinhal, C. M., Gazzola, V., & Keysers, C. (2020). Harm to Others Acts as a Negative Reinforcer in Rats. Current Biology, 30(6), 949-961.e7. https://doi.org/10.1016/j.cub.2020.01.017

Hernandez-Lallement, J., Gómez-Sotres, P., & Carrillo, M. (2022). Towards a unified theory of emotional contagion in rodents—A meta-analysis. *Neuroscience & Biobehavioral Reviews*, *132*, 1229–1248. https://doi.org/10.1016/j.neubiorev.2020.09.010

Hernandez-Lallement, J., van Wingerden, M., & Kalenscher, T. (2018). Towards an animal model of callousness. *Neuroscience & Biobehavioral Reviews*, *91*, 121–129. https://doi.org/10.1016/j.neubiorev.2016.12.029

Hernandez-Lallement, J., van Wingerden, M., Marx, C., Srejic, M., & Kalenscher, T. (2015). Rats prefer mutual rewards in a prosocial choice task. *Frontiers in Neuroscience*, *8*. https://doi.org/10.3389/fnins.2014.00443

Hernandez-Lallement, J., van Wingerden, M., Schäble, S., & Kalenscher, T. (2016). Basolateral amygdala lesions abolish mutual reward preferences in rats. Neurobiology of Learning and Memory, 127, 1–9. https://doi.org/10.1016/j.nlm.2015.11.004

Hess, E. M., Venniro, M., & Gould, T. D. (2023). Relative to females, male rats are more willing to forego obtaining sucrose reward in order to prevent harm to their cage mate. Psychopharmacology. https://doi.org/10.1007/s00213-023-06435-2

Heyes, C. (2018). Empathy is not in our genes. *Neuroscience & Biobehavioral Reviews, 95*, 499–507. https://doi.org/10.1016/j.neubiorev.2018.11.001

Holt-Lunstad, J. (2021). The Major Health Implications of Social Connection. *Current Directions in Psychological Science, 30*(3), 251–259. https://doi.org/10.1177/0963721421999630

Holy, T. E., & Guo, Z. (2005). Ultrasonic Songs of Male Mice. *PLOS Biology, 3*(12), e386. https://doi.org/10.1371/journal.pbio.0030386

Hosgorler, F., Akkaya, E. C., Ilgin, R., Koc, B., Kizildag, S., Gumus, H., & Uysal, N. (2023). The ameliorative effect of midazolam on empathy-like behavior in old rats. *Naunyn-Schmiedeberg's Archives of Pharmacology, 396*(11), 3183–3193. https://doi.org/10.1007/s00210-023-02526-1

Hosgorler, F., Koc, B., Kizildag, S., Canpolat, S., Argon, A., Karakilic, A., Kandis, S., Guvendi, G., Ates, M., Arda, N. M., & Uysal, N. (2020). Magnesium Acetyl Taurate Prevents Tissue Damage and Deterioration of Prosocial Behavior Related with Vasopressin Levels in Traumatic Brain Injured Rats. *Turkish Neurosurgery*, *30*(5). https://doi.org/10.5137/1019-5149.JTN.29272-20.1

Hostinar, C. E., Sullivan, R. M., & Gunnar, M. R. (2014). Psychobiological mechanisms underlying the social buffering of the hypothalamic–pituitary–adrenocortical axis: A review of animal models and human studies across development. *Psychological Bulletin*, *140*(1), 256–282. https://doi.org/10.1037/a0032671

Howland, J. G., Ito, R., Lapish, C. C., & Villaruel, F. R. (2022). The rodent medial prefrontal cortex and associated circuits in orchestrating adaptive behavior under variable demands. Neuroscience and Biobehavioral Reviews, 135, 104569. https://doi.org/10.1016/j.neubiorev.2022.104569

Hua, A. Y., Sible, I. J., Perry, D. C., Rankin, K. P., Kramer, J. H., Miller, B. L., Rosen, H. J., & Sturm, V. E. (2018). Enhanced Positive Emotional Reactivity Undermines Empathy in Behavioral Variant Frontotemporal Dementia. *Frontiers in Neurology*, *9*. https://doi.org/10.3389/fneur.2018.00402

Inagaki, H., Kuwahara, M., & Tsubone, H. (2004). Effects of psychological stress on autonomic control of heart in rats. Experimental Animals, 53(4), 373–378. https://doi.org/10.1538/expanim.53.373

Ishii, A., Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2016). Social buffering ameliorates conditioned fear responses in female rats. *Hormones and Behavior*, *81*, 53–58. https://doi.org/10.1016/j.yhbeh.2016.03.003

Ishiyama, S., & Brecht, M. (2016). Neural correlates of ticklishness in the rat somatosensory cortex. *Science*, *354*(6313), 757–760. https://doi.org/10.1126/science.aah5114

Isik, S., & Unal, G. (2023). Open-source software for automated rodent behavioral analysis. Frontiers in Neuroscience, 17. https://doi.org/10.3389/fnins.2023.1149027

Jabarin, R., Netser, S., & Wagner, S. (2022). Beyond the three-chamber test: Toward a multimodal and objective assessment of social behavior in rodents. *Molecular Autism*, *13*(1), 41. https://doi.org/10.1186/s13229-022-00521-6

Jahoda, G. (2005). Theodor Lipps and the Shift from “Sympathy” to “Empathy.” *Journal of the History of the Behavioral Sciences*, *41*(2), 151–163. https://doi.org/10.1002/jhbs.20080

Jiang, M., Wang, M., Shi, Q., Wei, L., Lin, Y., Wu, D., Liu, B., Nie, X., Qiao, H., Xu, L., Yang, T., & Wang, Z. (2021). Evolution and neural representation of mammalian cooperative behavior. Cell Reports, 37(7), 110029. https://doi.org/10.1016/j.celrep.2021.110029

Jones, C. E., & Monfils, M.-H. (2018). Chapter 8—The Social Transmission of Associative Fear in Rodents—Individual Differences in Fear Conditioning by Proxy. In K. Z. Meyza & E. Knapska (Eds.), *Neuronal Correlates of Empathy* (pp. 93–109). Academic Press. https://doi.org/10.1016/B978-0-12-805397-3.00008-5

Karakilic, A., Kizildag, S., Kandis, S., Guvendi, G., Koc, B., Camsari, G. B., Camsari, U. M., Ates, M., Arda, S. G., & Uysal, N. (2018). The effects of acute foot shock stress on empathy levels in rats. *Behavioural Brain Research*, *349*, 31–36. https://doi.org/10.1016/j.bbr.2018.04.043

Kas, M. J., Glennon, J. C., Buitelaar, J., Ey, E., Biemans, B., Crawley, J., Ring, R. H., Lajonchere, C., Esclassan, F., Talpos, J., Noldus, L. P. J. J., Burbach, J. P. H., & Steckler, T. (2014). Assessing behavioural and cognitive domains of autism spectrum disorders in rodents: Current status and future perspectives. *Psychopharmacology*, *231*(6), 1125–1146. https://doi.org/10.1007/s00213-013-3268-5

Kaźmierowska, A. M., Kostecki, M., Szczepanik, M., Nikolaev, T., Hamed, A., Michałowski, J. M., Wypych, M., Marchewka, A., & Knapska, E. (2023). Rats respond to aversive emotional arousal of human handlers with the activation of the basolateral and central amygdala. Proceedings of the National Academy of Sciences, 120(46), e2302655120. https://doi.org/10.1073/pnas.2302655120

Keller, D., Láng, T., Cservenák, M., Puska, G., Barna, J., Csillag, V., Farkas, I., Zelena, D., Dóra, F., Küppers, S., Barteczko, L., Usdin, T. B., Palkovits, M., Hasan, M. T., Grinevich, V., & Dobolyi,

A. (2022). A thalamo-preoptic pathway promotes social grooming in rodents. *Current Biology*, *32*(21), 4593-4606.e8. https://doi.org/10.1016/j.cub.2022.08.062

Kettler, N., Schweinfurth, M. K., & Taborsky, M. (2021). Rats show direct reciprocity when interacting with multiple partners. *Scientific Reports*, *11*(1), 3228. https://doi.org/10.1038/s41598-021-82526-4

Keum, S., & Shin, H.-S. (2019). Neural Basis of Observational Fear Learning: A Potential Model of Affective Empathy. *Neuron*, *104*(1), 78–86. https://doi.org/10.1016/j.neuron.2019.09.013

Keysers, C., Knapska, E., Moita, M. A., & Gazzola, V. (2022). Emotional contagion and prosocial behavior in rodents. *Trends in Cognitive Sciences*, *26*(8), 688–706. https://doi.org/10.1016/j.tics.2022.05.005

Kietzman, H. W., & Gourley, S. L. (2023). How social information impacts action in rodents and humans: The role of the prefrontal cortex and its connections. Neuroscience and Biobehavioral Reviews, 147, 105075. https://doi.org/10.1016/j.neubiorev.2023.105075

Kilner, J. M., & Lemon, R. N. (2013). What We Know Currently about Mirror Neurons. *Current Biology*, *23*(23), R1057–R1062. https://doi.org/10.1016/j.cub.2013.10.051

Kim, A., Keum, S., & Shin, H.-S. (2019). Observational fear behavior in rodents as a model for empathy. *Genes, Brain and Behavior*, *18*(1), e12521. https://doi.org/10.1111/gbb.12521

Kiyokawa, Y., Kawai, K., & Takeuchi, Y. (2018). The benefits of social buffering are maintained regardless of the stress level of the subject rat and enhanced by more conspecifics. *Physiology & Behavior*, *194*, 177–183. https://doi.org/10.1016/j.physbeh.2018.05.027

Knapska, E., Mikosz, M., Werka, T., & Maren, S. (2010). Social modulation of learning in rats. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *17*(1), 35–42. https://doi.org/10.1101/lm.1670910

Knop, J., Joëls, M., & van der Veen, R. (2017). The added value of rodent models in studying parental influence on offspring development: Opportunities, limitations and future perspectives. *Current Opinion in Psychology*, *15*, 174–181. https://doi.org/10.1016/j.copsyc.2017.02.030

Ko, J. (2017). Neuroanatomical Substrates of Rodent Social Behavior: The Medial Prefrontal Cortex and Its Projection Patterns. Frontiers in Neural Circuits, 11. https://doi.org/10.3389/fncir.2017.00041

Kondrakiewicz, K., Kostecki, M., Szadzińska, W., & Knapska, E. (2019). Ecological validity of social interaction tests in rats and mice. *Genes, Brain and Behavior*, *18*(1), e12525. https://doi.org/10.1111/gbb.12525

Kozma, K., Kassai, F., Ernyey, A. J., & Gyertyán, I. (2019). Establishment of a rodent cooperation assay as a model of social cognition. Journal of Pharmacological and Toxicological Methods, 97, 44–51. https://doi.org/10.1016/j.vascn.2019.03.003

Krakauer, J. W., Ghazanfar, A. A., Gomez-Marin, A., MacIver, M. A., & Poeppel, D. (2017). Neuroscience Needs Behavior: Correcting a Reductionist Bias. *Neuron*, *93*(3), 480–490. https://doi.org/10.1016/j.neuron.2016.12.041

Krupenye, C., & Call, J. (2019). Theory of mind in animals: Current and future directions. *Wiley Interdisciplinary Reviews. Cognitive Science*, *10*(6), e1503. https://doi.org/10.1002/wcs.1503

Lahvis, G. P. (2017). Social Reward and Empathy as Proximal Contributions to Altruism: The Camaraderie Effect. Current Topics in Behavioral Neurosciences, 30, 127–157. https://doi.org/10.1007/7854_2016_449

Langford, D. J., Crager, S. E., Shehzad, Z., Smith, S. B., Sotocinal, S. G., Levenstadt, J. S., Chanda, M. L., Levitin, D. J., & Mogil, J. S. (2006). Social modulation of pain as evidence for empathy in mice. *Science (New York, N.Y.)*, *312*(5782), 1967–1970. https://doi.org/10.1126/science.1128322

Langford, D. J., Tuttle, A. H., Brown, K., Deschenes, S., Fischer, D. B., Mutso, A., Root, K. C., Sotocinal, S. G., Stern, M. A., Mogil, J. S., & Sternberg, W. F. (2010). Social approach to pain in laboratory mice. *Social Neuroscience*, *5*(2), 163–170. https://doi.org/10.1080/17470910903216609

Leigh, R., Oishi, K., Hsu, J., Lindquist, M., Gottesman, R. F., Jarso, S., Crainiceanu, C., Mori, S., & Hillis, A. E. (2013). Acute lesions that impair affective empathy. *Brain*, *136*(8), 2539–2549. https://doi.org/10.1093/brain/awt177

Leussis, M. P., & Bolivar, V. J. (2006). Habituation in rodents: A review of behavior, neurobiology, and genetics. *Neuroscience & Biobehavioral Reviews*, *30*(7), 1045–1064. https://doi.org/10.1016/j.neubiorev.2006.03.006

Li, C.-L., Yu, Y., He, T., Wang, R.-R., Geng, K.-W., Du, R., Luo, W.-J., Wei, N., Wang, X.-L., Wang, Y., Yang, Y., Yu, Y.-Q., & Chen, J. (2018). Validating Rat Model of Empathy for Pain: Effects of Pain Expressions in Social Partners. Frontiers in Behavioral Neuroscience, 12. https://doi.org/10.3389/fnbeh.2018.00242

Lindgren, H. S., & Dunnett, S. B. (2012). Cognitive dysfunction and depression in Parkinson's disease: What can be learned from rodent models? *European Journal of Neuroscience, 35*(12), 1894–1907. https://doi.org/10.1111/j.1460-9568.2012.08162.x

Lockwood, P. L., Wittmann, M. K., Nili, H., Matsumoto-Ryan, M., Abdurahman, A., Cutler, J., Husain, M., & Apps, M. A. J. (2022). Distinct neural representations for prosocial and self-benefiting effort. Current Biology, 32(19), 4172-4185.e7. https://doi.org/10.1016/j.cub.2022.08.010

Lombardo, M. V., Chakrabarti, B., Bullmore, E. T., Wheelwright, S. J., Sadek, S. A., Suckling, J., MRC AIMS Consortium, & Baron-Cohen, S. (2010). Shared neural circuits for mentalizing about the self and others. Journal of Cognitive Neuroscience, 22(7), 1623–1635. https://doi.org/10.1162/jocn.2009.21287

Łopuch, S., & Popik, P. (2011). Cooperative behavior of laboratory rats (Rattus norvegicus) in an instrumental task. *Journal of Comparative Psychology (Washington, D.C.: 1983), 125*(2), 250–253. https://doi.org/10.1037/a0021532

Lu, Y.-F., Ren, B., Ling, B.-F., Zhang, J., Xu, C., & Li, Z. (2018). Social interaction with a cagemate in pain increases allogrooming and induces pain hypersensitivity in the observer rats. Neuroscience Letters, 662, 385–388. https://doi.org/10.1016/j.neulet.2017.10.063

Márquez, C., Rennie, S. M., Costa, D. F., & Moita, M. A. (2015). Prosocial Choice in Rats Depends on Food-Seeking Behavior Displayed by Recipients. *Current Biology*, *25*(13), 1736–1745. https://doi.org/10.1016/j.cub.2015.05.018

Martin, L. J., Poulson, S. J., Mannan, E., Sivaselvachandran, S., Cho, M., Setak, F., & Chan, C. (2022). Altered nociceptive behavior and emotional contagion of pain in mouse models of autism. Genes, Brain and Behavior, 21(1), e12778. https://doi.org/10.1111/gbb.12778

Meyza, K. Z., Bartal, I. B.-A., Monfils, M. H., Panksepp, J. B., & Knapska, E. (2017). The roots of empathy: Through the lens of rodent models. *Neuroscience and Biobehavioral Reviews*, *76*(Pt B), 216–234. https://doi.org/10.1016/j.neubiorev.2016.10.028

Michalec, B., & Hafferty, F. W. (2021). Challenging the clinically-situated emotion-deficient version of empathy within medicine and medical education research. *Social Theory & Health*, *20*(3), 306–324. https://doi.org/10.1057/s41285-021-00174-0

Morris, R. G. M. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation*, *12*(2), 239–260. https://doi.org/10.1016/0023-9690(81)90020-5

Moyaho, A., Rivas-Zamudio, X., Ugarte, A., Eguibar, J. R., & Valencia, J. (2015). Smell facilitates auditory contagious yawning in stranger rats. *Animal Cognition*, *18*(1), 279–290. https://doi.org/10.1007/s10071-014-0798-0

Murlanova, K., Kirby, M., Libergod, L., Pletnikov, M., & Pinhasov, A. (2022). Multidimensional nature of dominant behavior: Insights from behavioral neuroscience. *Neuroscience and Biobehavioral Reviews*, *132*, 603–620. https://doi.org/10.1016/j.neubiorev.2021.12.015

Nair, T. K., Waslin, S. M., Rodrigues, G. A., Datta, S., Moore, M. T., & Brumariu, L. E. (2024). A meta-analytic review of the relations between anxiety and empathy. *Journal of Anxiety Disorders*, *101*, 102795. https://doi.org/10.1016/j.janxdis.2023.102795

Nakamura, K., Ishii, A., Kiyokawa, Y., Takeuchi, Y., & Mori, Y. (2016). The strain of an accompanying conspecific affects the efficacy of social buffering in male rats. *Hormones and Behavior*, *82*, 72–77. https://doi.org/10.1016/j.yhbeh.2016.05.003

Neumann, D., Zupan, B., Babbage, D. R., Radnovich, A. J., Tomita, M., Hammond, F., & Willer, B. (2012). Affect Recognition, Empathy, and Dysosmia After Traumatic Brain Injury. *Archives of Physical Medicine and Rehabilitation*, *93*(8), 1414–1420. https://doi.org/10.1016/j.apmr.2012.03.009

Neumann, David. L., Chan, R. C. K., Boyle, Gregory. J., Wang, Y., & Rae Westbury, H. (2015). Measures of Empathy. In *Measures of Personality and Social Psychological Constructs* (pp. 257–289). Elsevier. https://doi.org/10.1016/B978-0-12-386915-9.00010-3

Nijsse, B., Spikman, J. M., Visser-Meily, J. M., Kort, P. L. de, & Heugten, C. M. van. (2019). Social Cognition Impairments in the Long Term Post Stroke. *Archives of Physical Medicine and Rehabilitation*, *100*(7), 1300–1307. https://doi.org/10.1016/j.apmr.2019.01.023

O'Connell, L. A., & Hofmann, H. A. (2011). Genes, hormones, and circuits: An integrative approach to study the evolution of social behavior. *Frontiers in Neuroendocrinology*, *32*(3), 320–335. https://doi.org/10.1016/j.yfrne.2010.12.004

O'Connor, B. P. (1999). Simple and flexible SAS and SPSS programs for analyzing lag-sequential categorical data. *Behavior Research Methods, Instruments, & Computers*, *31*(4), 718–726. https://doi.org/10.3758/BF03200753

O'Keefe, L. M., Doran, S. J., Mwilambwe-Tshilobo, L., Conti, L. H., Venna, V. R., & McCullough, L. D. (2014). Social isolation after stroke leads to depressive-like behavior and decreased BDNF levels in mice. *Behavioural Brain Research*, *260*, 162–170. https://doi.org/10.1016/j.bbr.2013.10.047

Panksepp, J. B., & Lahvis, G. P. (2023). Sociability versus empathy in adolescent mice: Different or distinctive? *Learning and Motivation, 83*, 101892. https://doi.org/10.1016/j.lmot.2023.101892

Panksepp, J., & Panksepp, J. B. (2013). Toward a cross-species understanding of empathy. *Trends in Neurosciences*, *36*(8), 10.1016/j.tins.2013.04.009. https://doi.org/10.1016/j.tins.2013.04.009

Pasquini, L., Nana, A. L., Toller, G., Brown, J. A., Deng, J., Staffaroni, A., Kim, E.-J., Hwang, J.-H. L., Li, L., Park, Y., Gaus, S. E., Allen, I., Sturm, V. E., Spina, S., Grinberg, L. T., Rankin, K. P., Kramer, J. H., Rosen, H. J., Miller, B. L., & Seeley, W. W. (2020). Salience Network Atrophy Links Neuron Type-Specific Pathobiology to Loss of Empathy in Frontotemporal Dementia. *Cerebral Cortex*, *30*(10), 5387–5399. https://doi.org/10.1093/cercor/bhaa119

Patrick, H., Knee, C. R., Canevello, A., & Lonsbary, C. (2007). The role of need fulfillment in relationship functioning and well-being: A self-determination theory perspective. *Journal of Personality and Social Psychology*, *92*(3), 434–457. https://doi.org/10.1037/0022-3514.92.3.434

Peen, N. F., Duque-Wilckens, N., & Trainor, B. C. (2021). Convergent neuroendocrine mechanisms of social buffering and stress contagion. *Hormones and Behavior*, *129*, 104933. https://doi.org/10.1016/j.yhbeh.2021.104933

Pellis, S. M., Pellis, V. C., Ham, J. R., & Achterberg, E. J. M. (2022). The rough-and-tumble play of rats as a natural behavior suitable for studying the social brain. *Frontiers in Behavioral Neuroscience*, *16*. https://doi.org/10.3389/fnbeh.2022.1033999

Pellis, S. M., Pellis, V. C., Ham, J. R., & Stark, R. A. (2023). Play fighting and the development of the social brain: The rat's tale. *Neuroscience & Biobehavioral Reviews*, *145*, 105037. https://doi.org/10.1016/j.neubiorev.2023.105037

Pérez-Manrique, A., & Gomila, A. (2018). The comparative study of empathy: Sympathetic concern and empathic perspective-taking in non-human animals. *Biological Reviews*, *93*(1), 248–269. https://doi.org/10.1111/brv.12342

Pérez-Manrique, A., & Gomila, A. (2022). Emotional contagion in nonhuman animals: A review. *WIREs Cognitive Science*, *13*(1), e1560. https://doi.org/10.1002/wcs.1560

Phillips, H. L., Dai, H., Choi, S. Y., Jansen-West, K., Zajicek, A. S., Daly, L., Petrucelli, L., Gao, F.-B., & Yao, W.-D. (2023). Dorsomedial prefrontal hypoexcitability underlies lost empathy in frontotemporal dementia. *Neuron*, *111*(6), 797-806.e6. https://doi.org/10.1016/j.neuron.2022.12.027

Piguet, O., Hornberger, M., Mioshi, E., & Hodges, J. R. (2011). Behavioural-variant frontotemporal dementia: Diagnosis, clinical staging, and management. *The Lancet Neurology*, *10*(2), 162–172. https://doi.org/10.1016/S1474-4422(10)70299-4

Pijnenborg, G. H. M., Spikman, J. M., Jeronimus, B. F., & Aleman, A. (2013). Insight in schizophrenia: Associations with empathy. *European Archives of Psychiatry and Clinical Neuroscience*, *263*(4), 299–307. https://doi.org/10.1007/s00406-012-0373-0

Politis, M., Wu, K., Molloy, S., G. Bain, P., Chaudhuri, K. R., & Piccini, P. (2010). Parkinson's disease symptoms: The patient's perspective. *Movement Disorders*, *25*(11), 1646–1651. https://doi.org/10.1002/mds.23135

Powell, S. B., & Swerdlow, N. R. (2023). The Relevance of Animal Models of Social Isolation and Social Motivation for Understanding Schizophrenia: Review and Future Directions. *Schizophrenia Bulletin*, *49*(5), 1112–1126. https://doi.org/10.1093/schbul/sbad098

Premoli, M., Pietropaolo, S., Wöhr, M., Simola, N., & Bonini, S. A. (2023). Mouse and rat ultrasonic vocalizations in neuroscience and neuropharmacology: State of the art and future applications. *European Journal of Neuroscience*, *57*(12), 2062–2096. https://doi.org/10.1111/ejn.15957

Preston, S. D., & de Waal, F. B. M. (2002a). Empathy: Its ultimate and proximate bases. *The Behavioral and Brain Sciences*, *25*(1), 1–20; discussion 20-71. https://doi.org/10.1017/s0140525x02000018

Preston, S. D., & de Waal, F. B. M. (2002b). The communication of emotions and the possibility of empathy in animals. In *Altruism & altruistic love: Science, philosophy, & religion in dialogue* (pp. 284–308). Oxford University Press. https://doi.org/10.1093/acprof:oso/9780195143584.003.0025

Rana, A.-N., Gonzales-Rojas, R., & Lee, H. Y. (2022). Imitative and contagious behaviors in animals and their potential roles in the study of neurodevelopmental disorders. *Neuroscience & Biobehavioral Reviews*, *143*, 104876. https://doi.org/10.1016/j.neubiorev.2022.104876

Raz, S. (2013). Ameliorative effects of brief daily periods of social interaction on isolation-induced behavioral and hormonal alterations. *Physiology & Behavior*, *116–117*, 13–22. https://doi.org/10.1016/j.physbeh.2013.03.009

Rex, A., Voigt, J.-P., Gustedt, C., Beckett, S., & Fink, H. (2004). Anxiolytic-like profile in Wistar, but not Sprague–Dawley rats in the social interaction test. *Psychopharmacology*, *177*(1), 23–34. https://doi.org/10.1007/s00213-004-1914-7

Ripoll, L. H., Snyder, R., Steele, H., & Siever, L. J. (2013). The Neurobiology of Empathy in Borderline Personality Disorder. *Current Psychiatry Reports*, *15*(3), 344. https://doi.org/10.1007/s11920-012-0344-1

Rogers-Carter, M. M., & Christianson, J. P. (2019). An Insular View of the Social Decision-Making Network. Neuroscience and Biobehavioral Reviews, 103, 119–132. https://doi.org/10.1016/j.neubiorev.2019.06.005

Rosenberger, L. A., Eisenegger, C., Naef, M., Terburg, D., Fourie, J., Stein, D. J., & van Honk, J. (2019). The Human Basolateral Amygdala Is Indispensable for Social Experiential Learning. Current Biology, 29(20), 3532-3537.e3. https://doi.org/10.1016/j.cub.2019.08.078

Roth, G., Vansteenkiste, M., & Ryan, R. M. (2019). Integrative emotion regulation: Process and development from a self-determination theory perspective. *Development and Psychopathology*, *31*(3), 945–956. https://doi.org/10.1017/S0954579419000403

Runyan, A., Lengel, D., Huh, J. W., Barson, J. R., & Raghupathi, R. (2021). Intranasal Administration of Oxytocin Attenuates Social Recognition Deficits and Increases Prefrontal Cortex Inhibitory Postsynaptic Currents following Traumatic Brain Injury. *eNeuro*, *8*(3). https://doi.org/10.1523/ENEURO.0061-21.2021

Rutte, C., & Taborsky, M. (2007). Generalized Reciprocity in Rats. *PLOS Biology*, *5*(7), e196. https://doi.org/10.1371/journal.pbio.0050196

Rutte, C., & Taborsky, M. (2008). The influence of social experience on cooperative behaviour of rats (Rattus norvegicus): Direct vs generalised reciprocity. *Behavioral Ecology and Sociobiology*, *62*(4), 499–505. https://doi.org/10.1007/s00265-007-0474-3

Saltzman, W., Harris, B. N., De Jong, T. R., Perea-Rodriguez, J. P., Horrell, N. D., Zhao, M., & Andrew, J. R. (2017). Paternal Care in Biparental Rodents: Intra- and Inter-individual Variation. *Integrative and Comparative Biology*, *57*(3), 589–602. https://doi.org/10.1093/icb/icx047

Sato, N., Tan, L., Tate, K., & Okada, M. (2015). Rats demonstrate helping behavior toward a soaked conspecific. *Animal Cognition*, *18*(5), 1039–1047. https://doi.org/10.1007/s10071-015-0872-2

Scheggia, D., La Greca, F., Maltese, F., Chiacchierini, G., Italia, M., Molent, C., Bernardi, F., Coccia, G., Carrano, N., Zianni, E., Gardoni, F., Diluca, M., & Papaleo, F. (2022). Reciprocal cortico-amygdala connections regulate prosocial and selfish choices in mice. Nature Neuroscience, 25(11), 1505–1518. https://doi.org/10.1038/s41593-022-01179-2

Schneeberger, K., Dietz, M., & Taborsky, M. (2012). Reciprocal cooperation between unrelated rats depends on cost to donor and benefit to recipient. BMC Evolutionary Biology, 12(1), 41. https://doi.org/10.1186/1471-2148-12-41

Schönfeld, L.-M., Schäble, S., Zech, M.-P., & Kalenscher, T. (2020). 5-HT1A receptor agonism in the basolateral amygdala increases mutual-reward choices in rats. *Scientific Reports*, *10*, 16622. https://doi.org/10.1038/s41598-020-73829-z

Schwarting, R. K. W. (2018). Ultrasonic vocalization in juvenile and adult male rats: A comparison among stocks. *Physiology & Behavior*, *191*, 1–11. https://doi.org/10.1016/j.physbeh.2018.03.023

Schwartz, L. P., Silberberg, A., Casey, A. H., Kearns, D. N., & Slotnick, B. (2017). Does a Rat Release a Soaked Conspecific Due to Empathy? *Animal Cognition*, *20*(2), 299–308. https://doi.org/10.1007/s10071-016-1052-8

Schweinfurth, M. K. (2020). The social life of Norway rats (Rattus norvegicus). *eLife*, *9*, e54020. https://doi.org/10.7554/eLife.54020

Schweinfurth, M. K., Aeschbacher, J., Santi, M., & Taborsky, M. (2019). Male Norway rats cooperate according to direct but not generalized reciprocity rules. *Animal Behaviour*, *152*, 93–101. https://doi.org/10.1016/j.anbehav.2019.03.015

Shinozuka, K., Tajiri, N., Ishikawa, H., Tuazon, J. P., Lee, J.-Y., Sanberg, P. R., Zarriello, S., Corey, S., Kaneko, Y., & Borlongan, C. V. (2020). Empathy in stroke rats is modulated by social settings. *Journal of Cerebral Blood Flow & Metabolism*, *40*(6), 1182–1192. https://doi.org/10.1177/0271678X19867908

Shirenova, S. D., Khlebnikova, N. N., & Krupina, N. A. (2023). Changes in Sociability and Preference for Social Novelty in Female Rats in Prolonged Social Isolation. *Neuroscience and Behavioral Physiology*, *53*(1), 103–118. https://doi.org/10.1007/s11055-023-01395-8

Silberberg, A., Allouch, C., Sandfort, S., Kearns, D., Karpel, H., & Slotnick, B. (2014). Desire for social contact, not empathy, may explain “rescue” behavior in rats. *Animal Cognition*, *17*(3), 609–618. https://doi.org/10.1007/s10071-013-0692-1

Silveira, K. M., & Joca, S. (2023). Learned Helplessness in Rodents. In J. Harro (Ed.), *Psychiatric Vulnerability, Mood, and Anxiety Disorders: Tests and Models in Mice and Rats* (pp. 161–184). Springer US. https://doi.org/10.1007/978-1-0716-2748-8_9

Simola, N., & Granon, S. (2019). Ultrasonic vocalizations as a tool in studying emotional states in rodent models of social behavior and brain disease. *Neuropharmacology*, *159*, 107420. https://doi.org/10.1016/j.neuropharm.2018.11.008

Singer, T., Seymour, B., O'Doherty, J., Kaube, H., Dolan, R. J., & Frith, C. D. (2004). Empathy for pain involves the affective but not sensory components of pain. Science (New York, N.Y.), 303(5661), 1157–1162. https://doi.org/10.1126/science.1093535

Singewald, N., & Holmes, A. (2019). Rodent models of impaired fear extinction. *Psychopharmacology*, *236*(1), 21–32. https://doi.org/10.1007/s00213-018-5054-x

Sivaselvachandran, S., Acland, E. L., Abdallah, S., & Martin, L. J. (2018). Behavioral and mechanistic insight into rodent empathy. *Neuroscience and Biobehavioral Reviews*, *91*, 130–137. https://doi.org/10.1016/j.neubiorev.2016.06.007

Skversky-Blocq, Y., Haaker, J., & Shechner, T. (2021). Watch and Learn: Vicarious Threat Learning across Human Development. *Brain Sciences, 11*(10), Article 10. https://doi.org/10.3390/brainsci11101345

Slaughter, V. (2015). Theory of Mind in Infants and Young Children: A Review. *Australian Psychologist*. https://doi.org/10.1111/ap.12080

Sommer, C. J. (2017). Ischemic stroke: Experimental models and reality. *Acta Neuropathologica*, *133*(2), 245–261. https://doi.org/10.1007/s00401-017-1667-0

Song, D., Wang, C., Jin, Y., Deng, Y., Yan, Y., Wang, D., Zhu, Z., Ke, Z., Wang, Z., Wu, Y., Ni, J., Qing, H., & Quan, Z. (2023). Mediodorsal thalamus-projecting anterior cingulate cortex neurons modulate helping behavior in mice. Current Biology, 33(20), 4330-4342.e5. https://doi.org/10.1016/j.cub.2023.08.070

Spink, A. J., Tegelenbosch, R. A. J., Buma, M. O. S., & Noldus, L. P. J. J. (2001). The EthoVision video tracking system—A tool for behavioral phenotyping of transgenic mice. Physiology & Behavior, 73(5), 731–744. https://doi.org/10.1016/S0031-9384(01)00530-3

Stein, D. J., Scott, K. M., de Jonge, P., & Kessler, R. C. (2017). Epidemiology of anxiety disorders: From surveys to nosology and back. *Dialogues in Clinical Neuroscience*, *19*(2), 127–136.

Stevens, F. L., Hurley, R. A., Taber, K. H., Hurley, R. A., Hayman, L. A., & Taber, K. H. (2011). Anterior Cingulate Cortex: Unique Role in Cognition and Emotion. The Journal of Neuropsychiatry and Clinical Neurosciences, 23(2), 121–125. https://doi.org/10.1176/jnp.23.2.jnp121

Sturm, V. E., Yokoyama, J. S., Seeley, W. W., Kramer, J. H., Miller, B. L., & Rankin, K. P. (2013). Heightened emotional contagion in mild cognitive impairment and Alzheimer's disease is associated with temporal lobe degeneration. *Proceedings of the National Academy of Sciences*, *110*(24), 9944–9949. https://doi.org/10.1073/pnas.1301119110

Subhadeep, D., Srikumar, B. N., Shankaranarayana Rao, B. S., & Kutty, B. M. (2022). Ventral subicular lesion impairs pro-social empathy-like behavior in adult Wistar rats. Neuroscience Letters, 776, 136535. https://doi.org/10.1016/j.neulet.2022.136535

Sullivan, R. M., Wilson, D. A., Ravel, N., & Mouly, A.-M. (2015). Olfactory memory networks: From emotional learning to social behaviors. *Frontiers in Behavioral Neuroscience*, *9*. https://doi.org/10.3389/fnbeh.2015.00036

Sveinbjornsdottir, S. (2016). The clinical symptoms of Parkinson's disease. *Journal of Neurochemistry*, *139*(S1), 318–324. https://doi.org/10.1111/jnc.13691

Thoma, P., Zalewski, I., von Reventlow, H. G., Norra, C., Juckel, G., & Daum, I. (2011). Cognitive and affective empathy in depression linked to executive control. *Psychiatry Research*, *189*(3), 373–378. https://doi.org/10.1016/j.psychres.2011.07.030

Thompson, K., Nahmias, E., Fani, N., Kvaran, T., Turner, J., & Tone, E. (2021). The Prisoner's Dilemma paradigm provides a neurobiological framework for the social decision cascade. PLOS ONE, 16(3), e0248006. https://doi.org/10.1371/journal.pone.0248006

Titchener, E. B. (2014). Introspection and Empathy. *Dialogues in Philosophy, Mental and Neuro Sciences*, *7*(1), 25–30.

Tomek, S. E., Stegmann, G. M., & Olive, M. F. (2019). Effects of Heroin on Rat Prosocial Behavior. *Addiction Biology*, *24*(4), 676–684. https://doi.org/10.1111/adb.12633

Turner, K. M., & Burne, T. H. J. (2014). Comprehensive Behavioural Analysis of Long Evans and Sprague-Dawley Rats Reveals Differential Effects of Housing Conditions on Tests Relevant to Neuropsychiatric Disorders. *PLoS ONE*, *9*(3), e93411. https://doi.org/10.1371/journal.pone.0093411

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Okamoto, M., Matsumoto, Y., Aoki, S., & Ishihara, T. (2018). Empathic behavior according to the state of others in mice. Brain and Behavior, 8(7), e00986. https://doi.org/10.1002/brb3.986

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Takahashi, Y., Matsumoto, Y., Okamoto, M., & Ishihara, T. (2019a). Rescue-like Behaviour in Mice is Mediated by Their Interest in the Restraint Tool. *Scientific Reports*, *9*(1), 10648. https://doi.org/10.1038/s41598-019-46128-5

Ueno, H., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Takahashi, Y., Matsumoto, Y., Okamoto, M., & Ishihara, T. (2019b). Feeding Behavior of Mice under Different Food Allocation Regimens. Behavioural Neurology, 2019, e1581304. https://doi.org/10.1155/2019/1581304

Van Bogaert, M. J. V., Groenink, L., Oosting, R. S., Westphal, K. G. C., Van Der Gugten, J., & Olivier, B. (2006). Mouse strain differences in autonomic responses to stress. Genes, Brain and Behavior, 5(2), 139–149. https://doi.org/10.1111/j.1601-183X.2005.00143.x

Vanderschuren, L. J. M. J., Achterberg, E. J. M., & Trezza, V. (2016). The neurobiology of social play and its rewarding value in rats. *Neuroscience and Biobehavioral Reviews*, *70*, 86–105. https://doi.org/10.1016/j.neubiorev.2016.07.025

Venna, V. R., Xu, Y., Doran, S. J., Patrizz, A., & McCullough, L. D. (2014). Social interaction plays a critical role in neurogenesis and recovery after stroke. *Translational Psychiatry*, *4*(1), e351–e351. https://doi.org/10.1038/tp.2013.128

Vernay, A., Sellal, F., & René, F. (2015). Evaluating Behavior in Mouse Models of the Behavioral Variant of Frontotemporal Dementia: Which Test for Which Symptom? *Neurodegenerative Diseases*, *16*(3–4), 127–139. https://doi.org/10.1159/000439253

Vingill, S., Connor-Robson, N., & Wade-Martins, R. (2018). Are rodent models of Parkinson's disease behaving as they should? *Behavioural Brain Research*, *352*, 133–141. https://doi.org/10.1016/j.bbr.2017.10.021

Wang, J., Mann, F., Lloyd-Evans, B., Ma, R., & Johnson, S. (2018). Associations between loneliness and perceived social support and outcomes of mental health problems: A systematic review. *BMC Psychiatry*, *18*(1), 156. https://doi.org/10.1186/s12888-018-1736-5

Wearne, T. A., Osborne-Crowley, K., Logan, J. A., Wilson, E., Rushby, J., & McDonald, S. (2020). Understanding how others feel: Evaluating the relationship between empathy and various aspects of emotion recognition following severe traumatic brain injury. *Neuropsychology*, *34*(3), 288–297. https://doi.org/10.1037/neu0000609

Wilson, C. A., & Koenig, J. I. (2014). Social interaction and social withdrawal in rodents as readouts for investigating the negative symptoms of schizophrenia. *European Neuropsychopharmacology*, *24*(5), 759–773. https://doi.org/10.1016/j.euroneuro.2013.11.008

Wilson, S. P. (2017). Modelling the emergence of rodent filial huddling from physiological huddling. *Royal Society Open Science*, *4*(11), 170885. https://doi.org/10.1098/rsos.170885

Wood, R. I., Kim, J. Y., & Li, G. R. (2016). Cooperation in rats playing the iterated Prisoner's Dilemma game. *Animal Behaviour*, *114*, 27–35. https://doi.org/10.1016/j.anbehav.2016.01.010

Wu, Y. E., Dang, J., Kingsbury, L., Zhang, M., Sun, F., Hu, R. K., & Hong, W. (2021). Neural control of affiliative touch in prosocial interaction. *Nature*, *599*(7884), 262–267. https://doi.org/10.1038/s41586-021-03962-w

Xue, J., Li, B., Huang, B., Feng, H., Li, X., Liang, S., Yuan, F., Wang, S., Shi, H., Shao, J., & Shi, Y. (2023). Sex-dependent and long-lasting effects of adolescent sleep deprivation on social behaviors in adult mice. Pharmacology Biochemistry and Behavior, 232, 173657. https://doi.org/10.1016/j.pbb.2023.173657

Yamagishi, A., Lee, J., & Sato, N. (2020). Oxytocin in the anterior cingulate cortex is involved in helping behaviour. Behavioural Brain Research, 393, 112790. https://doi.org/10.1016/j.bbr.2020.112790

Yan, Z., Zeng, X., Su, J., & Zhang, X. (2021). The dark side of empathy: Meta-analysis evidence of the relationship between empathy and depression. *PsyCh Journal*, *10*(5), 794–804. https://doi.org/10.1002/pchj.482

Yang, X., Fang, Y., Chen, H., Zhang, T., Yin, X., Man, J., Yang, L., & Lu, M. (2021). Global, regional and national burden of anxiety disorders from 1990 to 2019: Results from the Global Burden of Disease Study 2019. *Epidemiology and Psychiatric Sciences, 30*, e36. https://doi.org/10.1017/S2045796021000275

Yeh, Z.-T., & Tsai, C.-F. (2014). Impairment on theory of mind and empathy in patients with stroke. *Psychiatry and Clinical Neurosciences*, *68*(8), 612–620. https://doi.org/10.1111/pcn.12173

Yu, D., Bao, L., & Yin, B. (2024). Emotional contagion in rodents: A comprehensive exploration of mechanisms and multimodal perspectives. Behavioural Processes, 216, 105008. https://doi.org/10.1016/j.beproc.2024.105008

Yu, Y.-Q., Barry, D. M., Hao, Y., Liu, X.-T., & Chen, Z.-F. (2017). Molecular and neural basis of contagious itch behavior in mice. *Science, 355*(6329), 1072–1076. https://doi.org/10.1126/science.aak9748

Yüksel, O., Ateş, M., Kızıldağ, S., Yüce, Z., Koç, B., Kandiş, S., Güvendi, G., Karakılıç, A., Gümüş, H., & Uysal, N. (2019). Regular Aerobic Voluntary Exercise Increased Oxytocin in Female Mice: The Cause of Decreased Anxiety and Increased Empathy-Like Behaviors. *Balkan Medical Journal*, *36*(5), 257–262. https://doi.org/10.4274/balkanmedj.galenos.2019.2018.12.87

Zamora-González, E. O., Santerre, A., Palomera-Avalos, V., & Morales-Villagrán, A. (2013). A chronic combinatory stress model that activates the HPA axis and avoids habituation in BALB/C mice. Journal of Neuroscience Methods, 213(1), 70–75. https://doi.org/10.1016/j.jneumeth.2012.10.015

Zhang, T. D., Kolbe, S. C., Beauchamp, L. C., Woodbridge, E. K., Finkelstein, D. I., & Burrows, E. L. (2022). How Well Do Rodent Models of Parkinson’s Disease Recapitulate Early Non-Motor Phenotypes? A Systematic Review. *Biomedicines*, *10*(12), Article 12. https://doi.org/10.3390/biomedicines10123026

Appendix A: Study Two - Complete search strategy

The following databases were initially searched on January 26, 2021:

- APA PsycInfo (Ovid) – 3920 results
- Embase (Ovid) – 6759 results
- MEDLINE (Ovid) – 5745 results
- Scopus – 3948 results
- Web of Science (Clarivate) – 4113 results

The following strategies were used for each database.

APA PsycInfo (Ovid)

1. rodents/
2. mice/
3. exp rats/
4. rodent*.ti,ab
5. (mouse or mice or "mus musculus").ti,ab
6. (rat or rats or rattus).ti,ab
7. or/1-6
8. empathy/
9. sympathy/
10. kindness/
11. altruism/
12. "sharing (social behavior)"/
13. exp animal emotions/
14. animal social behavior/
15. animal cooperation/
16. empathy.ti,ab
17. sympathy.ti,ab
18. (prosocial* or "pro social*").ti,ab
19. (compassion* or altru* or kindness).ti,ab
20. ((share* or sharing or help* or assist* or care* or caring or emotion* or empath* or sympath*) adj3 behav*).ti,ab
21. or/8-20
22. 7 and 21
23. limit 22 to yr="2000 -Current"

Embase (Ovid)

1. rodent/
2. exp mouse/
3. exp rat/
4. rodent*.ti,ab
5. (mouse or mice or "mus musculus").ti,ab

6. (rat or rats or rattus).ti,ab
7. or/1-6
8. empathy/
9. altruism/
10. social behavior/
11. care behavior/
12. cooperation/
13. empathy.ti,ab
14. sympathy.ti,ab
15. (prosocial* or "pro social*").ti,ab
16. (compassion* or altru* or kindness).ti,ab
17. ((share* or sharing or help* or assist* or care* or caring or emotion* or empath* or sympath*) adj3 behav*).ti,ab
18. or/8-17
19. 7 and 18
20. limit 19 to yr="2000 -Current"
21. 20 not human/

MEDLINE (Ovid)

1. rodentia/
2. exp mice/
3. exp rats/
4. rodent*.ti,ab
5. (mouse or mice or "mus musculus").ti,ab
6. (rat or rats or rattus).ti,ab
7. or/1-6
8. empathy/
9. altruism/
10. social behavior/
11. cooperative behavior/
12. helping behavior/
13. empathy.ti,ab
14. sympathy.ti,ab
15. (prosocial* or "pro social*").ti,ab
16. (compassion* or altru* or kindness).ti,ab
17. ((share* or sharing or help* or assist* or care* or caring or emotion* or empath* or sympath*) adj3 behav*).ti,ab
18. or/8-17
19. 7 and 18
20. limit 19 to yr="2000 -Current"
21. 20 not humans/

Scopus

1. TITLE-ABS-KEY(rodent*)

2. TITLE-ABS-KEY(mouse or mice or "mus musculus")
3. TITLE-ABS-KEY(rat or rats or rattus)
4. #1 OR #2 OR #3
5. TITLE-ABS-KEY(empathy)
6. TITLE-ABS-KEY(sympathy)
7. TITLE-ABS-KEY(prosocial* or "pro social*")
8. TITLE-ABS-KEY(compassion* or altru* or kindness)
9. TITLE-ABS-KEY((share* or sharing or help* or assist* or care* or caring or emotion* or empath* or sympath*) w/3 behav*)
10. #5 OR #6 OR #7 OR #8 OR #9
11. #4 AND #10
12. PUBYEAR > 1999
13. #11 AND #12

Web of Science (Clarivate)

1. TS=(rodent*)
2. TS=(mouse or mice or "mus musculus")
3. TS=(rat or rats or rattus)
4. #1 OR #2 OR #3
5. TS=(empathy)
6. TS=(sympathy)
7. TS=(prosocial* or "pro social*")
8. TS=(compassion* or altru* or kindness)
9. TS=((share* or sharing or help* or assist* or care* or caring or emotion* or empath* or sympath*) near/3 behav*)
10. #5 OR #6 OR #7 OR #8 OR #9
11. #4 AND #10
12. PY=(2000-2021)
13. #11 AND #12

Appendix B: Study Two - Characteristics of included articles

Original search – Characteristics of the included studies (2000-2020)

Table 1. Results of all Included Studies – Animals.

Reference	Type of rodent	#rodents	#males	#females	Age	Strain	Weight (g)	Water re-striction	Food restriction
Avital et al., 2016	Rats	40	20	20	NA	Wistar	240-260	Yes	No
Bartal et al., 2011	Rats	97	85	12	3-6 months	Sprague-Dawley	270g-380	No	No
Bartal et al., 2014	Rats	59	59	NA	8-11 weeks	Sprague-Dawley + Long-Evans	NA	No	No
Bartal et al., 2016	Rats	154	154	NA	2 months	Sprague-Dawley	NA	No	No
Blystad et al., 2019	Rats	30	NA	30	100pnd	Sprague-Dawley	150-200	No	Yes
Carvalheiro et al., 2019	Rats	18	18	NA	41 to 53pnd	Wistar	NA	No	No
Conde-Moro et al., 2019	Rats	12	12	NA	3 months old	Lister Hooded	250-300	No	Yes
Cox et al., 2020	Rats	102	102	NA	NA	Sprague-Dawley	250-275	No	Yes
Daghestani et al., 2017	Rats	60	NA	NA	PND7	Sprague-Dawley	NA	No	No
de Carvalho et al., 2018	Rats	20	20	NA	exp1: 3 to 6 months, exp2: 3 months	Wistar	NA	Yes	No
Delmas et al., 2019	Rats	30	30	NA	2 months	Long-Evans	300-330	No	Yes
Dolivo & Taborsky 2015 (A)	Rats	43	NA	43	NA	Wild-type Norway	NA	No	No
Dolivo & Taborsky 2015 (B)	Rats	20	NA	20	22 months	Wild-type Norway	NA	No	No
Donovan et al., 2020	Rats	80	80	NA	6 weeks	Long-Evans	200	No	No
Festucci et al., 2020	Rats	8	8	NA	at least 4 months	Wistar-Han DAT knockout	300-400	NA	NA
Fontes-Dutra et al., 2019	Rats	50	50	NA	69-81 days experi-mental sessions	Wistar	NA	NA	NA
Gerber et al., 2020	Rats	115	NA	115	NA	Wild-type Norway	300	No	No

Hachiga et al,. 2018	Rats	18	18	NA	2 weeks	Sprague-Dawley	NA	NA	NA
Han et al., 2020	Mice	NA	unknown	NA	8-12 week	KO and WT littermates	NA	Yes	NA
Havlik et al., 2020	Rats	56	56	NA	8-11 weeks	Sprague-Dawley and Long-Evans	NA	No	No
Hernandez-Lallement et al., 2015	Rats	68	68	NA	NA	Long-Evans	NA	No	Yes
Hernandez-Lallement et al., 2016	Rats	34	36	NA	adult	Long-Evans	250-450	No	Yes
Hernandez-Lallement et al., 2020	Rats	314	302	12	30 days	Sprague-Dawley	M:302.4;F:240.8	No	Yes
Hosgorler et al., 2020	Rats	55	NA	55	3-6 months	Sprague-Dawley	200-250	No	No
Kandis et al., 2018	Rats	32	32	NA	Adult	Sprague-Dawley	NA	No	No
Karakilic et al., 2018	Rats	30	30	NA	Adult	Sprague-Dawley	NA	No	No
Kentrop et al., 2020	Rats	160	124	36	8–10 weeks	Wistar	NA	No	Yes
Kozma et al., 2019	Rats	44	44	NA	1.5 y/o (LE) and 1 y/o (LH)	Long-Evans and Lister Hooded	LE:401-514; LH:377-556	NA	Yes
Li & Wood, 2017	Rats	26	26	NA	Adult	Long-Evans	200	No	Yes
Lopuch & Popik, 2011	Rats	20	20	NA	NA	Sprague-Dawley	225-250	No	No
Marquez et al., 2015	Rats	74	74	NA	NA	Sprague-Dawley	375-425	No	No
Oberliessen et al., 2016	Rats	23	23	NA	4-5 months	Long-Evans	400-533	No	Yes
Raz, 2013	Rats	81	81	NA	pnd 49	Wistar	190	No	No
Rutte & Taborsky, 2007	Rats	36	NA	36	NA	Wild-type Norway	NA	No	No
Rutte & Taborsky, 2008	Rats	23	NA	23	NA	Wild-type	NA	No	No
Sato et al., 2015	Rats	56	46	10	10 weeks	Sprague-Dawley	EXP1 (214-f, 362-m), EXP2: 350, EXP3: 291	No	No
Schmid et al., 2017	Rats	36	NA	36	NA	Wild-type Norway	NA	NA	NA
Schneeberger et al., 2012	Rats	14	NA	14	NA	Wild-type Norway	NA	No	Yes
Schonfeld et al., 2020	Rats	48	NA	NA	10 weeks	NA	NA	NA	Yes
Schwartz et al., 2017	Rats	29	20	9	15 weeks. Exp 6: 10 months	Sprague-Dawley	NA	No	No

Schweinfurth & Taborsky, 2016	Rats	48	NA	48	Adult	Wild-type Norway	NA	NA	NA
Schweinfurth & Taborsky, 2017	Rats	40	NA	40	1 y/o	Wild-type Norway	300	No	No
Schweinfurth & Taborsky, 2018 (A)	Rats	50	NA	50	2 y/o	Norway rats	300g to 400	No	Yes
Schweinfurth & Taborsky, 2018 (B)	Rats	74	NA	74	adult	Wild-type Norway	avg 300	No	No
Schweinfurth & Taborsky, 2018 (C)	Rats	21	21	NA	19 months	Wild-type Norway	607	NA	NA
Schweinfurth & al., 2019	Rats	41	23	NA	adult	Wild-type Norway	Median of 627	No	No
Schweinfurth & Taborsky, 2020	Rats	54	NA	54	adult	Wild-type Norway	350	NA	NA
Silberberg et al., 2014	Rats	12	NA	12	3-6 months	Sprague-Dawley	NA	No	No
Silva et al., 2020	Rats	52	52	NA	3-4 months	Wistar	NA	No	No
Tomek et al., 2019	Rats	64	64	NA	NA	Sprague-Dawley	250	Yes	Yes
Tomek et al., 2020	Rats	99	99	NA	NA	Sprague-Dawley	250	No	No
Tsoory et al., 2012	Rats	27	27	NA	7-8 weeks	Wistar	NA	Yes	No
Ueno et al., 2019 (A)	Mice	NA	NA	NA	10 weeks	C57BL/6N	NA	No	No
Ueno et al., 2019 (B)	Mice	40	40	NA	10 weeks	C57BL/6N	NA	No	No
Viana et al., 2010	Rats	12	12	NA	NA	Sprague-Dawley	NA	No	Yes
Wood et al., 2016	Rats	48	32	16	6 weeks	Long-Evans	200	No	Yes
Yamagishi et al., 2020 (A)	Rats	72	72	NA	7 or 11 weeks	Sprague-Dawley	314.62g	No	No
Yamagishi et al., 2020 (B)	Rats	82	70	12	EXP1: 11-weeks, EXP2: 12 weeks	Sprague-Dawley	Exp 1: M: 370.64 (335-401); F: 244.92 (227-269g range); Exp 2 (M only): 398.35 (range 351-512g)	No	No
Yuksel et al., 2019	Mice	32	16	16	Adult	Balb-c	NA	No	No

Table 2. Results of all Included Studies – Housing Conditions and Apparatus.

Reference	**Housing (per cage)**	**Relationship**	**Light-Dark Cycle**	**Enrichment**	**Operant Paradigm**	**Video/vocalization recording**	**Can animals see/hear/smell each other?**
Avital et al., 2016	3-4	NA	12:12 dl lights on @ 7am	No	Cooperation learning task	yes (video, ethovision)	Yes
Bartal et al., 2011	2	NA	12:12 dl	No	Freeing task (tube)	Yes (video, ultrasonic voc)	Yes
Bartal et al., 2014	2	NA	12:12 dl	No	Freeing task (tube)	Yes (video and voc)	Yes
Bartal et al., 2016	2	NA	12:12 dl	No	Freeing task (tube)	No	Yes (+ touch)
Blystad et al., 2019	2	NA	12:12 dl lights on @ 7	No	Freeing task (tube)	Yes (video, ethovision)	Yes
Carvalheiro et al., 2019	2	Littermates	12:12 dl	No	Freeing task (tube)	Yes (video)	Yes
Conde-Moro et al., 2019	2	NA	12:12 dl	No	Cooperation learning task	Yes (video)	Yes
Cox et al., 2020	2	NA	R12:12 dl lights on @ 6pm	No	Freeing task (soaked area)	No	Yes (smell)
Daghestani et al., 2017	10	Split litter design	12:12 dl	No	Freeing task (tube)	No	Yes
de Carvalho et al., 2018	2	NA	12:12 dl	No	Cooperation learning task	No	Yes (see)
Delmas et al., 2019	1-2	NA	12:12 ld lights @ 9am	No	Prisoner's dilemma	No	Yes (see/smell -white noise machine preventing hearing)
Dolivo & Taborsky 2015 (A)	5	Related (housing); unrelated for task	R12:12 dl lights on @ 20h00	Yes	Repeated donation game	No	See/hear/smell in visual contact but not see specifically for the blind test
Dolivo & Taborsky 2015 (B)	5	Related (housing); unrelated for task	R 12:12 ld lights on @ 20:00	No	Repeated donation game	No	Yes
Donovan et al., 2020	2	NA	R14L:10D photoperiod	No	Prisoner's dilemma	No	Yes
Festucci et al., 2020	2	NA	NA	No	Repeated donation game - Box ('open field')	No	Yes
Fontes-Dutra et al., 2019	NA	Littermates	12:12 dl	No	Freeing task (tube)	No	Yes
Gerber et al., 2020	5	Littermates	12:12dl	No	Repeated donation game	No	Yes
Hachiga et al,. 2018	3	NA	12:12 dl lights @ 8am	No	Freeing task (tube)	No	Yes
Han et al., 2020	2-4	Littermates	NA	No	Cooperation learning task	Yes (video, ethovision)	Yes
Havlik et al., 2020	2	NA	12:12ld lights on 6am	No	Freeing task (tube)	Yes (video)	Yes
Hernandez-Lallement et al., 2015	4	NA	NA	No	Prosocial choice task - Double T Maze	No	Yes

Hernandez-Lallement et al., 2016	3	NA	R12:12 dl	No	Prosocial choice task - Double T Maze	No	Yes (hear/smell)
Hernandez-Lallement et al., 2020	4	NA	R12:12 lights OFF @ 7am	No	Not harming - Operant box	Yes (video and sound)	Yes
Hosgorler et al., 2020	2	NA	NA	No	Freeing task (soaked area)	Yes (video, ethovision)	NA
Kandis et al., 2018	2	NA	12:12 dl	No	Freeing task (soaked area)	No	Yes
Karakilic et al., 2018	2	NA	12:12 dl	No	Freeing task (soaked area)	No	NA
Kentrop et al., 2020	6-10	NA	12:12 ld lights on @ 8am	Yes	Sharing task (lever presses)	No	Yes
Kozma et al., 2019	3	NA	R12:12 ld lights on @ 17h	No	Cooperation learning task (nose poke)	No	Yes (+ touch)
Li & Wood, 2017	2	NA	R14L:10D photoperiod	No	Repeated donation game	No	Yes
Lopuch & Popik, 2011	4	NA	12:12 dl lights on @ 7am	No	Cooperation learning task (nose poke)	Yes (video and ultrasound voc)	Yes + touch (except in phase 2)
Marquez et al., 2015	2	NA	R12:12 ld lights OFF @ 10 am	No	Prosocial choice task - Double T Maze	Yes (video)	Yes
Oberliessen et al., 2016	2-3	Non-related	R12:12 ld lights off @ 7am	No	Prosocial choice task - Double T Maze	No	Yes
Raz, 2013	5-6	NA	12:12 dl	No	Cooperation learning task	Yes (video, ethovision)	Yes
Rutte & Taborsky, 2007	3-7	Littermates	R12:12 lights on @ 20h	No	Lever presses - Box ('open field')	No	Yes
Rutte & Taborsky, 2008	3-7	Littermates	R12:12 ld lights on @ 8pm	No	Repeated donation game	Yes (video)	Yes
Sato et al., 2015	2	Non-related	16:8 ld lights on @ 8 am	No	Freeing task (soaked area)	No	Yes
Schmid et al., 2017	3-6	NA	R12:12 dl	No		Yes (video)	Yes
Schneeberger et al., 2012	3-5	Related (housing); unrelated for task	R12:12 ld lights on @ 8 pm	No	Repeated donation game	No	Yes
Schonfeld et al., 2020	3	NA	R12:12ld	No	Prosocial choice task - Double T Maze	No	Yes
Schwartz et al., 2017	3	NA	12:12 ld lights @ 8am	No	Freeing task (soaked area)	No	NA
Schweinfurth & Taborsky, 2016	3-5	Related (housing); unrelated for task	Reversed 12:12 ld lights on @8pm	No	Repeated donation game	No	Yes
Schweinfurth & Taborsky, 2017	5	Littermates	R12:12 dl lights on @ 8pm	Yes	Repeated donation game	No	Yes
Schweinfurth & Taborsky, 2018 (A)	3-5	Littermates	R12:12 ld lights on @ 8pm	Yes	Repeated donation game	Yes (video, ultrasonic voc)	Yes

Schweinfurth & Taborsky, 2018 (B)	3-5	Littermates	R12:12 ld lights on @ 8pm	Yes	Repeated donation game	No	Yes
Schweinfurth & Taborsky, 2018 (C)	4	Non-related	R12:12 ld lights on @ 8pm	No	Sharing task (stick pulling)	No	Yes
Schweinfurth & al., 2019	4	NA	R12:12 lights on @ 20h	Yes	Repeated donation game	No	Yes
Schweinfurth & Taborsky, 2020	1-5	Related (housing); unrelated for task	R12:12 ld lights on @ 8pm	Yes	Prisoner's dilemma	No	Yes
Silberberg et al., 2014	2	NA	12:12 dl	No	Freeing task (soaked area)	Yes (video)	Yes
Silva et al., 2020	2	NA	12:12 dl	No	Freeing task (tube)	Yes (video)	Yes
Tomek et al., 2019	2	NA	R12:12 ld lights @ 7pm light off at 7am; 22= reversed 12:12dl,	No	Freeing task (tube)	No	Yes
Tomek et al., 2020	2	NA	R12:12 ld light on @ 7am	No	Freeing task (tube)	Yes (video)	Yes
Tsoory et al., 2012	2-5	NA	12:12 ld lights on @ 7am	No	Cooperation learning task	No	NA
Ueno et al., 2019 (A)	5	NA	12:12 ld lights on @ 8am	No	Freeing task (tube)	Yes (video)	Yes (see)
Ueno et al., 2019 (B)	5	NA	12:12 ld	No	Freeing task (tube)	No	Yes
Viana et al., 2010	2	Non-related	12:12 dl	No	Prisoner's dilemma in a double T-Maze	No	Yes (see/smell)
Wood et al., 2016	2	NA	R14:10 dl	No	Prisoner's dilemma in operant box	No	Yes
Yamagishi et al., 2020 (A)	1-2	NA	16:8 dl	No	Freeing task (soaked area)	Yes (video)	Yes (see)
Yamagishi et al., 2020 (B)	2	NA	12:12 dl	No	Freeing task (soaked area)	Yes (video)	Yes (see)
Yuksel et al., 2019	2	NA	12:12 dl	No	Freeing task (soaked area)	No	NA

Table 3. Results of all Included Studies – Methodology.

Reference	Duration (in min)	# of Testing Days	Habituation	Pretraining	Reward/Punishment	Control Group	Other Behavioral Tests	Drugs or Surgical Interventions
Avital et al., 2016	15	24	Yes	No	70ul of sucrose water solution (20%)	Yes	NA	NA
Bartal et al., 2011	40-60	128	Yes	No	Social Reward/chocolate chips	Yes	NA	NA
Bartal et al., 2014	40-60	12	Yes	No	Social reward	No	Open field	NA
Bartal et al., 2016	40-60	12	Yes	No	Social reward/chocolate chips	No	Open field	MDZ (2mg & 1.25mg/kg, i.p.), nadolol (10mg/kg) or saline
Blystad et al., 2019	5-10	15	Yes	Yes	Food Pellets/social reward	Yes	Light vs dark envi.	NA
Carvalheiro et al., 2019	60	12	Yes	No	dark box/social reward	Yes	NA	NA
Conde-Moro et al., 2019	40presses	until reached criterion	Yes	Yes	Pellets	Yes	NA	Rats implanted with 2 sets of recording electrodes at the right prelimbic cortex
Cox et al., 2020	5	5	No	No	social contact/ no social contact	Yes	NA	NA
Daghestani et al., 2017	30	3	No	No	Social reward	Yes	Hole-board test, social contact test, open field, self-grooming test	BV (subcutaneous 0.5 mg/kg/ day for 31 days) PPA (oral, 250-mg/kg/day body weight for 3 days)
de Carvalho et al., 2018	45-75 water deliveries	10 sessions	No	Yes	Water	Yes	NA	NA
Delmas et al., 2019	45sec	until reached criterion	No	Yes	Sugar pellets	NA	NA	NA
Dolivo & Taborsky 2015 (A)	NA	18 sessions	No	Yes	food pellet	Yes	NA	NA
Dolivo & Taborsky 2015 (B)	7	4	No	Yes	Piece of banana or carrot, cereal flake	NA	NA	NA
Donovan et al., 2020	24 trials	10 sessions	No	Yes	45mg sucrose pellet	Yes	NA	i.p. of saline or Oxytocin (0.1 mg/kg)
Festucci et al., 2020	NA	12	No	Yes	2g of pellets	Yes	NA	NA
Fontes-Dutra et al., 2019	40	12	Yes	No	social reward	Yes	NA	injections of RSV, VPA, RSV+VPA or saline
Gerber et al., 2020	14	NA	No	Yes	1 oat flake	Yes	NA	NA
Hachiga et al,. 2018	3	24 sessions	No	Yes	social reward	NA	NA	NA

Han et al., 2020	15	12	Yes	No	70 ul 20% sucrose	NA	Dominance test	NA
Havlik et al., 2020	40	12	Yes	No	Social reward	Yes	Open field, elevated plus maze	benzodiazepine (2 mg/kg i.p. increased at 4)
Hernandez-Lallement et al., 2015	8/10forced+15free choice trials	40	Yes	Yes	3 pellets	Yes	NA	NA
Hernandez-Lallement et al., 2016	6forced + 25free choice trials	12	Yes	Yes	3 sucrose pellets	Yes	Magnitude discrimination task	22 actors had surgery in BLA
Hernandez-Lallement et al., 2020	4forced+20free choice trials	NA	Yes	No	Pellets + foot shocks	Yes	NA	Muscimol or saline, cannulas in ACC
Hosgorler et al., 2020	5	12	No	No	NA	Yes	Rotarod test, plus maze, open field, forced swim test	Magnesium sulphate (30 mg/kg intramuscularly); Magnesium citrate (27 mg/kg perorally by gavage); Magnesium acetyl laurate (50 mg/kg perorally by gavage)+Head trauma
Kandis et al., 2018	5	12	Yes	No	NA	Yes	Open field, Elevated plus maze, Rotarod performance test	Acetaminophen once a day orally for 11 days (100, 200, 400mg)
Karakilic et al., 2018	1	12	Yes	No	Social reward	Yes	Open field, elevated plus maze	NA
Kentrop et al., 2020	30	19 (f) 30 (m)	Yes	No	Sucrose pellets	No	Boldness test	NA
Kozma et al., 2019	20	until reached criterion	No	Yes	Sucrose pellet	Yes	NA	NA
Li & Wood, 2017	72trials	10 days per condition	No	Yes	Sucrose pellet	Yes	NA	NA
Lopuch & Popik, 2011	NA	44	No	Yes	liquid sucrose (10ul; sucrose solution 20%)	No	NA	NA
Marquez et al., 2015	40	8 days on average	Yes	Yes	Sucrose pellet	Yes	NA	NA
Oberliessen et al., 2016	NA	24	Yes	Yes	sucrose pellet	Yes	Hierarchy assessment	NA
Raz, 2013	30	4	Yes	No	0.5ml sucrose solution (10%)	No	Open field, sucrose preference test, acoustic startle response, two-way active shuttle avoidance	NA
Rutte & Taborsky, 2007	7	17	No	Yes	oat flake	Yes	NA	NA

Rutte & Taborsky, 2008	7	20	No	Yes	1 oat flake	No	NA	NA
Sato et al., 2015	5	66	No	Yes	6 pieces of chocolate cereal (Kellogg's Japan Chocowa)	Yes	Preference test	NA
Schmid et al., 2017	7	6	No	Yes	Raisins halves	Yes	NA	NA
Schneeberger et al., 2012	14	NA	No	Yes	1 oat flake	Yes	NA	NA
Schonfeld et al., 2020	6forced+15free choice trials	NA	Yes	No	3 sucrose pellets	Yes	Open field, Magnitude discrimination task	Daily injections of 50 ng of the 5-HT1A receptor agonist 8-OH-DPAT or Ringer solution;cannulas targeting BLA
Schwartz et al., 2017	3	40 sessions	No	No	Social contact	Yes	NA	NA
Schweinfurth & Taborsky, 2016	14	NA	No	Yes	Oat flake	Yes	NA	NA
Schweinfurth & Taborsky, 2017	14	NA	No	Yes	1 oat flake	Yes	NA	NA
Schweinfurth & Taborsky, 2018 (A)	14	NA	No	Yes	Piece of banana	No	NA	NA
Schweinfurth & Taborsky, 2018 (B)	20+7	NA	No	Yes	1 oat flake	Yes	NA	NA
Schweinfurth & Taborsky, 2018 (C)	14	NA	No	Yes	1 oat flake	Yes	Kin discrimination test	NA
Schweinfurth & al., 2019	7	20	No	Yes	Oat flake	No	NA	NA
Schweinfurth & Taborsky, 2020	7	10	No	Yes	Oat flake	Yes	Test of memory capacity	NA
Silberberg et al., 2014	30	57 daily sessions	Yes	No	Freeing/social contact	Yes	NA	NA
Silva et al., 2020	30	12 days per condition	Yes	No	Social reward	No	NA	NA
Tomek et al., 2019	60-30	Phase1: 14 days, Phase 2:10-14 days, Phase 3: 3 days	Yes	No	social reward, heroin, and sucrose	No	NA	Heroin (0.06mg/kg), intravenous catheters in jugular vein
Tomek et al., 2020	60-30	24	Yes	No	Social reward	Yes	NA	buprenorphine (0.05 mg/kg), control virus (AAV8-CaMKIIα-EGFP), DREADD virus (AAV8-CaMKIIα-hM3D(Gq), inhibitory DREADD virus (AAV8-CaMKIIα-hM4D(Gi), +

								Clozapine-N-Oxide (CNO) 1.5mg/kg i.p. injection); intravenous catheters in jugular vein
Tsoory et al., 2012	20	10	Yes	Yes	0.04 ml of a sweet saccharine solution [0.06%]	Yes	NA	NA
Ueno et al., 2019 (A)	90	7	No	Yes	Social reward or food reward	Yes	Social interaction test	NA
Ueno et al., 2019 (B)	90	10	No	Yes	Social reward	Yes	NA	Oxytocin (100 µg/ kg)
Viana et al., 2010	20 trials	10	No	Yes	Food pellets + tail pinches	Yes	NA	NA
Wood et al., 2016	25 trials	4	No	Yes	Sucrose pellets	Yes	Dominance test	Female rats were ovariectomized
Yamagishi et al., 2020 (A)	10-15	4-20	Yes	No	Social reward	No	NA	intraperitoneal injections oxytocin (1.0 mg/kg)
Yamagishi et al., 2020 (B)	10	10	Yes	No	Social reward	Yes	NA	0.2 µl oxytocin receptor antagonist + cannula into ACC
Yuksel et al., 2019	5	1	Yes	Yes	Escape water	Yes	Elevated plus maze, open field	NA

Table 4. Result of all Included Studies - Performance and Behavioral Analyses.

Reference	**Performance index**	**Behavioral Analyses**
Avital et al., 2016	# of coordinated behavior (learning and latent social coop. learning can be achieved; divider that enabled perception of sensory modalities ↑ coop. Enabling tactile perception led to poor performance while visual availability ↑ performance.)	NA
Bartal et al., 2011	Frequency and latency of door openings (Rats were motivated to move and act specifically in presence of trapped rat. In trapped condition, the % of rats that opened the door ↑, and latency to door-opening ↓. Rats in chocolate condition shared in half the trials.)	Sig more alarm calls were recorded during trapped condition (13%) than during empty and object conditions (3 to 5%).
Bartal et al., 2014	Frequency and latency of door openings (Most rats in both SD cagemate (6/8, 75%) and SD stranger (10/12, 83%) conditions became openers. Rats were as motivated to help strangers as they were to help cagemates.)	Openers spent more time around closed restrainer than non-openers. After freeing rats, sig less fights were observed for openers vs non-openers. Rats were less active before door-opening vs after.
Bartal et al., 2016	Door opening latency (↓ over days in all groups indicating learning, except MDZ injected rats)	NA
Blystad et al., 2019	# and latency of door openings (door opening was largely the same as when the restrainer was baited with food. Latency was shortest when restrainer contained food, intermediate when it contained a cagemate and longest when it was empty. Latencies for dark (89.9s) and light (82.7s.))	NA
Carvalheiro et al., 2019	# of door openings (prosocial behavior still occurred when given escape alternative. Rats that could escape showed sig fewer door openings (first opening within 8 days) and took more time to open the door than rats that could not escape (first opening within 2 days). All rats, except one, opened the door after 12 sessions.)	Trapped rat's struggling behavior (associated with restraint stress) did not affect door-opening latency. ↑ exploratory behavior was predictive of faster door opening.
Conde-Moro et al., 2019	# of coordinated press (rats synchronized their platform climbs sig more when paired than when individual.)	One rat (leader) was the first to climb onto the platform most of the times and seemed to wait on the platform for the partner (follower) to climb for each coop. trial.
Cox et al., 2020	Chain pull latency (when removing social interaction rats still learned to release a distressed cage mate and retained the task for an extended period. When the distressed Target or Rtarget was either replaced or removed in 3-chamber task, chain pull latency of the Observers and R-observers sig ↑.)	NA
Daghestani et al., 2017	# of door openings (Sig lower # of trials of PPA-treated rat pups to open the restrainers and help the encaged rat pups vs control. Other treated groups, particularly the BV-treated group, displayed a comparatively high pro-social behavior vs PPA-treated group.)	NA
de Carvalho et al., 2018	# of coop. responses (Coop. rates systematically ↑ as a function of ratio value for some of the dyads; for other dyads, responding remained constant or ↓ between.)	NA
Delmas et al., 2019	# of coop. choices (High levels of coop. (86,11%) and mutual coop. (76,32%))	NA
Dolivo & Taborsky 2015 (A)	# and latency of pulls (with no visual information exchange, test rats rewarded cooperators earlier than defectors.)	Rats ↓ help propensity by showing aggression, non-cooperators ↑ help propensity of partners by attacking them.
Dolivo & Taborsky 2015 (B)	Frequency of providing partner with reward (Rats adjust their help levels to the quality of help previously obtained. Rats pulled after a shorter delay for a cooperator who had provided them with preferred bananas than for one who had provided them with same amount of nonpreferred carrots.)	NA
Donovan et al., 2020	# of coordinated lever presses (Tendency for Subjects to be nicer when their Stooge partner was also nice.)	NA
Festucci et al., 2020	# of pushes (WT rats pushed regardless of reward. WT rats facing a WT partner pushed sig more than HET ones. When food pellet was used as reward, data for same genotype did not show sig differences.)	NA

Fontes-Dutra et al., 2019	# of door openings (VPA animals are able to open restrainer, and continue to do so at the same frequency as controls. Rats of the VPA and VPA+RSV groups showed a delay in the expression of this helping behavior, opening the restrainer for the first time on average 3 days later than controls.)	NA
Gerber et al., 2020	# of food sharing (Rats donated food more often and earlier when olfactory information from their partner was available, irrespective of partner's previous helpfulness.)	NA
Hachiga et al,. 2018	# of goal box choices (Except for S4, all subjects preferred to go to rat locked in restraint tube more frequently when other box was empty (Cond.1) than when choice was between 2 rats, one in a tube and one free (Cond.2). All rats preferred to go to a rat outside restraint tube (Cond.3) than going to a rat locked in tube.)	NA
Han et al., 2020	# of coordinated running (Shank2Δ6−7 and Shank3Δ9 mice displayed opposite behaviors in social dominance and coop. tests. Shank2Δ6−7 exhibited a trend (not sig) towards improved performance, in association with higher activity and elevated efficacy suggesting similar levels of motivated behavior between WT and Shank2Δ6−7. Shank3Δ9 exhibited ↓ social coop. behavior, a ↓ in the # of mutual rewards and reduced activity.)	Most male Shank2Δ6−7 exhibited frequent aggressive behaviors and ↑ anxiety level, likely accounting for the ↑ dominance in tube test.
Havlik et al., 2020	Door opening latencies (Rats tested with incompetent helpers had fewer consecutive door-openings. SD rats familiar with LE strain tested with a LE incompetent helper resembled rats tested with one or two incompetent SD helpers in that they opened less frequently than controls. SD rats unfamiliar with LE behaved similarly to control.)	NA
Hernandez-Lallement et al., 2015	# of BR choice (Actors have preference for BR compartment when paired with partner. Sig higher % of BR choices in partner vs toy condition. Negative correlation between normalized partner weight and SB scores.)	NA
Hernandez-Lallement et al., 2016	NA	NA
Hernandez-Lallement et al., 2020	# of lever presses (Many individual differences across rats, with only a subset showing strong switching. Actors that switched more delayed and shortened reward consumption following shocks to victim and oriented more toward the victim in shock trials. An actor's prior experience with shocks ↑ switching. No effect of familiarity on harm aversion.)	USV of victim: pain squeaks + freezing was not associated with switching. Victims that ↓ time spent close to divider (due to shock-induced behavioral activity) were paired with actors with higher switching scores.
Hosgorler et al., 2020	# of door openings (No sig difference between groups. On 3rd post-traumatic day, door opening time was sig longer in both trauma and Mg sulphate group than in control.)	NA
Kandis et al., 2018	# of door openings (Reduced latency of freeing in all acetaminophen administrations (with 400mg being longer than 100 and 200mg.))	NA
Karakilic et al., 2018	# of door openings (the mean opening door latency ↓ in all animals. Low intensity stress group was quicker in opening door vs control. Low intensity acute stress improved empathic behavior. Higher intensity acute stress associated with less anxiety indicators.)	NA
Kentrop et al., 2020	# of BR lever presses (Males had a preference for the option that yields a reward for both rats. Females did not show preference for prosocial option, regardless of estrous cycle. Rats in complex. housing did not show preference for pro-social lever pressing. Costly prosocial behavior ↓ pro-social choice.)	Complex housed rats were found to be either prosocial (52 %) or not prosocial (48 %), while the standard housed rats were prosocial (64 %), indifferent (18 %), or not prosocial (18 %)
Kozma et al., 2019	# of nose poke (Analysis of the nose-poke latency data, sample video-recordings and the sig ↓ performance of rats in control experiment suggests real coop.)	Trained rats of both strains performed coop. though alternative ways. Both regular place exchanges of LE rats and close body contact of LH rats may have served to control action/presence of partner.
Li & Wood, 2017	# of rewards (when partnered with cagemate in FV block, responses were consistent with reciprocal altruism. When paired with another partner (a stranger or good stooge), Responder response rates ↓. Ehen paired with good stooge, rats failed to adjust their Donor responses to obtain more pellets.)	NA

Lopuch & Popik, 2011	# of nose poke (Coop. developed gradually as # of coop. responses ↑ during training. Coop. was ↓ by a partition restricting visual, acoustic, and physical communication but not by partition restricting only physical contact.)	Coop. was related to the # of 50 kHz USV “happy” calls and to intensity of social interactions. No difference in high frequency communication.
Marquez et al., 2015	# of BR choice (Rats acquired preference for prosocial option, providing recipient with access to food-baited arm. Rats rapidly, but gradually, acquired preference for the prosocial side, possibly through learning the contingency between their choice and the outcome to the recipient.)	The # of social investigation bouts was similar across protocols, except for the ↑ # of social investigations in rats of ‘‘no display of preference’’ condition during time between focals’ decision and recipients’ retrieval of food.
Oberliessen et al., 2016	# of BR rewards (Rats preferred equal outcomes more in social than in toy condition, although the effect was relatively small.)	NA
Raz, 2013	# of reward obtained (Rats in social isolation received less rewards than those in partial isolation.)	NA
Rutte & Taborsky, 2007	# of pulling (Rats that recently experienced help pulled more often than when they had not. Pulling frequency was on average 21% higher in helper treatment than in non helper treatment. Rats with previous experience of help pulled on average four times earlier.)	NA
Rutte & Taborsky, 2008	# of stick pulling (Exp1: rats pulled more often for partner that had pulled for them in previous interactions than for a partner that had not. Exp 2: pulling frequency was higher for partner that had helped before than for a new partner after having received help from others.)	NA
Sato et al., 2015	# of door openings (Exp1: 9/10 helpers showed door-opening behavior. In role-reversal sessions, all helper rats (soaked rats in previous sessions) exhibited door-opening behavior more rapidly than helper in door-opening sessions. Exp2: 1/8 helper showed opening behavior. Exp3: the # of sessions were not sig different between the two groups.)	NA
Schmid et al., 2017	Pulling frequencies (Rats pulled sig more often for partners that had pulled for them before than for partners that had not.)	NA
Schneeberger et al., 2012	# of stick pulling (Rats provided more help to cooperative than defective partners. The amount of help provided ↓ more strongly with ↑ costs when experimental partner was a defector. Hungry rats received more help for food if they were light, whereas if receiver was satiated, rats provided more help for heavy partners.)	NA
Schonfeld et al., 2020	# of BR choices (There was neither a sig interaction effect between condition (partner vs. toy) and group (50 ng 8-OHDPAT, 25 ng 8-OH-DPAT or vehicle), nor sig main effects of condition or group on % BR-choices in learning phase. A sig interaction effect between condition and group on % BR-choices in the expression phase was found once BR-side assignment was fully learned.)	NA
Schwartz et al., 2017	# of compartment entering with wet vs dry rat (Exp 1: Free rats preferred mingling with wet rat over dry rat in both conditions. Exp2: free rats chose goal box with wet rat on 0.66 of the trials during last 5 sessions. Exp5: Rats chose wet box on 0.72 of free-choice trials over last 5 sessions. Exp6: Free rat sig preferred trapped rat over empty goal box in initial and reversal conditions.)	
Schweinfurth & Taborsky, 2016	Frequency and latency of pulling (Rats distinguished between pulling for cooperator vs defector; they pulled sig less often for the latter. Rats pulled more often and earlier for cooperative partners than for an empty cage.)	NA
Schweinfurth & Taborsky, 2017	# of stick pulling/pushing (Rats helped previously cooperative partners more often than previously defecting partners. Rats responded to the coop. of social partners and transferred experienced to different coop. task.)	NA
Schweinfurth & Taborsky, 2018 (A)	Frequency and latency of pulling (Hungry rats pulled more often for social partner than satiated ones and started earlier to do so. Regardless of whether focal rats were hungry or satiated, pulling rates were always lower for empty cage than for social partners.)	Rats showed food reaching behaviors: stretching paws or sniffing towards reward, vocalized in 50-kHz, 13/25 rats showed ‘attention-grabbing ‘ (noisy behaviors directed towards potential donor.)

Schweinfurth & Taborsky, 2018 (B)	# of stick pulling (Rats provided more help for previously cooperating than non-cooperating partners. Rats groomed previously cooperative food providers more often than non-cooperative ones and provided more food to previously cooperating high groomers than low.)	In response to ↑ allogrooming, 20 rats ↑ whereas 11 ↓ food provisioning. In response to receiving food, 21 ↑ whereas 11 ↓ allogrooming rate.
Schweinfurth & Taborsky, 2018 ©	# of stick pulling (Rats helped unrelated partners more often than related ones. Rats provided less food to previously defecting partners than cooperating ones.)	NA
Schweinfurth & al., 2019	# of food donations (Rats provided more food to previously experienced cooperators than defectors. Experiencing cooperating or defecting partners prior to providing food to an unknown partner did not alter rats' donation rate.)	NA
Schweinfurth & Taborsky, 2020	# of stick pulling (Rats donated food less often to partners that were defecting. Pulling rates were lower for partners that they had experienced for 4 days with conflicting coop. experiences than for partners that they only met cooperating/defecting once. Rats provided food more often to partners that had been coop. during last encounter prior to test than to partners that had been defecting during last encounter.)	NA
Silberberg et al., 2014	Latencies and frequencies of door openings (Exp 1: Latencies ↑ and response rate ↓ for all free rats. Exp 2: latencies ↓ for all free rats over sessions. Exp 3: touch-contact frequencies were higher for all rats than in Condition 1 even though between-condition contingencies were identical in the 2 conditions.)	3/6 free rats spent most of their session time in contact with tube; 4/6 trapped rats spent most of their session time inside the tube after the rear door was opened. Most previously restrained rats returned for substantial periods of time to the tube, presumably to be next to free rats, which also spent much of their time in contact with the tube, presumably to be near the trapped rat.
Silva et al., 2020	# of door openings (Exp 1: no sig results from phase 1 to 3, sig result between phase 1 and 4 being lower rates of opening in phase 1 than 4. Exp2: Phase 4 was sig higher than phase 1,2,3 Exp3: Phase 1 was sig higher than toy or empty box. Latency Opening: exp1 no sig, exp2 no sig in phase 1,2,3 but lower latency in phase 4. exp3 no sig diff between phases.)	Reduced social interaction in phase 3 (Random rat) than phase 1 and 2.
Tomek et al., 2019	# of rescue rates (Rats with history of sucrose self-administration continue to rescue cagemate, while rats with history of heroin self-administration choose to continue heroin intake and not rescue cagemate.)	No observed evidence of sig heroin-induced stupor or other opioid-induced behaviors during test sessions.
Tomek et al., 2020	# of door openings (History of heroin self-administration ↓ prosocial behaviors. Chemogenetic activation of AIC restored prosocial behaviors following heroin intake. No sig difference in heroin intake between animals receiving active or control virus, chemogenetic inhibition of the insula had no effect on prosocial behaviors or heroin intake.)	NA
Tsoory et al., 2012	# of coordinated behavior (COOP rats learned to cooperate by coordinating their shuttles. COOP and IND rats did not differ in terms of individual shuttles and obtained reinforcements.)	NA
Ueno et al., 2019 (A)	# of door openings (Mice engaged in tube-opening behavior to free conspecifics. Mice did not open paper lid of empty tubes. Mice showed tube-opening behavior both to free cagemates and strangers. Hungry mice tended to open tube containing food before the conspecific. Mice freed conspecifics even at the cost of personal discomfort (wet floor).	Mice freed conspecifics and then entered the tube. Even in the absence of expression of distress or vocalisation by the conspecific, the mouse exhibited tube-opening behavior.
Ueno et al., 2019 (B)	# of door openings (Mice did not open tube containing ball of yarn. No sig difference between latency to lid-opening with 1 and 2 cagemates. No sig difference in the latency to lid-opening between tubes placed close to and far from the constrained cagemate.)	NA
Viana et al., 2010	# of cooperative choices (Rats cooperated more often than they defected. Food deprived rats defected more often. When playing against a reciprocating opponent, rats displayed behavior composed of both coop. (reward trials) and alternating reciprocity (alternating temptation and sucker trials.))	NA
Wood et al., 2016	# of cooperative choices (Coop. was reduced under food restriction, rats made sig more operant responses but received fewer pellets.)	No effect of dominance status on responses made or pellets received.

Yamagishi et al., 2020 (A)	Latencies of door openings (Latency of door-opening in all groups across sessions. Rats in Solo Oxtgroup learned door-opening faster than PairOxt group.)	NA
Yamagishi et al., 2020 (B)	Latency of door openings (Exp 1: latency of door-opening in OTA group was longer than SLN group, latency in both OTA and SLN groups ↓ across sessions. Exp. 2: door opening latencies were no different in Early and Late groups in first session, but those in Early group were longer than Late group in last session.)	
Yuksel et al., 2019	# of door openings (Mean door-opening duration progressively ↓ with time in all groups. Exercised groups opened the door quicker than controls in empathy-learning period. Voluntary physical activity ↓ anxiety and ↑ empathy-like behavior in both males and females.)	

Table 5. Results of all Included Studies - Other Analyses.

Reference	**Sex Differences**	**Strain Differences**
Avital et al., 2016	Females better in social coop. ↑ in mutual rewards rate observed in females is positively correlated with activity level.	NA
Bartal et al., 2011	More females (6/6) than males (17/24) became door-openers. Females opened restrainer at shorter latency than males on days 7-12. Females were more active in trapped but not in empty condition.	NA
Festucci et al., 2020	NA	No differences for the 2 genotypes
Kentrop et al., 2020	Females did not show preference for BR side.	NA
Kozma et al., 2019	NA	LE rats required sig more days to complete training than LH. LE in experienced- experienced pairs learnt faster than the other two. Naive-experienced pairs did not perform better than naive-naïve.
Wood et al., 2016	For direct reciprocity, females were more likely than males to deliver food pellets to their partner, but both showed no sig preference to give pellets to their cagemate over unfamiliar same-sex partner. Responses of both sexes were reduced when tested with an unresponsive partner (bad stooge.)	NA
Yamagishi et al., 2020 (B)	No sex differences were found.	NA

Articles that had no reported information regarding results in this table were excluded from it

Table 6. Results of all Included Studies – Reported definition.

Reference	Task	Reported definition
Avital et al., 2016	Cooperation learning task	Social cooperation is defined as a joint action for mutual benefit1 that depends not only on the individual behavior but also on the behaviors of others.
Bartal et al., 2011	Freeing task (tube)	Pro-social behavior refers to actions that are intended to benefit another.
Bartal et al., 2014	Freeing task (tube)	Pro-social behavior comprises actions that improve the well-being of others
Bartal et al., 2016	Freeing task (tube)	Helping refers to actions that intentionally benefit others. In humans, helping is often motivated by an empathic response to the distress and pain of others.
Blystad et al., 2019	Freeing task (tube)	The term empathy originates from a description of feeling at one with aesthetic experience and was proposed to denote the feeling/understanding of the thoughts and behavior of others
Carvalheiro et al., 2019	Freeing task (tube)	Prosocial behavior refers to "voluntary actions that are intended to help or benefit another individual" such as helping an individual in need, sharing resources, and cooperating with others to achieve common goals.
Conde-Moro et al., 2019	Cooperation learning task	According to game theory, cooperation is considered when two or more individuals work together toward a common goal
Cox et al., 2020	Freeing task (soaked area)	Empathy can be defined as the capacity for shared emotional valence, which generates shared affective states and therefore drives behaviors most appropriate to the emotional condition of others
Daghestani et al., 2017	Freeing task (tube)	NA
de Carvalho et al., 2018	Cooperation learning task	The experimental analysis of cooperative behavior investigates the effects of consequences contingent on the combined or coordinated behavior of two or more individuals
Delmas et al., 2019	Prisoner's dilemma	Altruism is a behavior by an individual that may be to his disadvantage but benefits others individuals.
Dolivo & Taborsky 2015 (A)	Repeated donation game	Altruistic behavior, in the sense that an individual performs a costly act that temporarily reduces its Darwinian fitness to the benefit of a social partner, is usually explained by kin selection
Dolivo & Taborsky 2015 (B)	Repeated donation game	Direct reciprocity, according to the decision rule 'help someone who has helped you before', reflects cooperation based on the principle of postponed benefits.
Donovan et al., 2020	Prisoner's dilemma	When participants incur costs from social interaction (loss of resources or risk of harm), they must decide whether the costs are worth the potential benefits. In circumstances where participants interact repeatedly, mutual cooperation can offer long-term benefits to overcome short-term costs
Festucci et al., 2020	Repeated donation game - Box ('open field')	The definition of prosocial behavior refers to actions aimed to provide help to individuals or groups, with or without expecting external rewards.
Fontes-Dutra et al., 2019	Freeing task (tube)	Empathy is a complex phenomenon that could be understood in humans as the ability to understand and share the internal states of others, while generating an emotional response more appropriate to someone else's situation than to one's own. Therefore, empathy is frequently demonstrated through caring and helping behavior toward others
Gerber et al., 2020	Repeated donation game	"Here, we define cooperation in a descriptive, general sense as simultaneous or consecutive acting together of two or more individuals, without implying fitness costs and benefits to either partner. Reciprocity is defined as a helpful act apparently benefitting a receiver at immediate costs to the actor, which increases the probability to receive a helpful act in return
Hachiga et al,. 2018	Freeing task (tube)	empathic action—that is, behavior in a rescuer intended solely to relieve distress in another.
Han et al., 2020	Cooperation learning task	NA

Havlik et al., 2020	Freeing task (tube)	NA
Hernandez-Lallement et al., 2015	Prosocial choice task - Double T Maze	Pro-sociality,i.e.,the preference for outcomes that produce benefits for other individuals
Hernandez-Lallement et al., 2016	Prosocial choice task - Double T Maze	NA
Hernandez-Lallement et al., 2020	Not harming - Operant box	Empathy, the ability to share another individual's emotional state and/or experience
Hosgorler et al., 2020	Freeing task (soaked area)	NA
Kandis et al., 2018	Freeing task (soaked area)	Empathy is the ability to recognize, process and respond to another's emotional state
Karakilic et al., 2018	Freeing task (soaked area)	"Empathy is defined as recognizing and internalizing the motivation of someone else's feelings, situation or behavior. Two fundamental types of empathy have been defined; emotional empathy and cognitive empathy. Emotional empathy ('I feel what you feel') is considered as a primitive behavior. Emotional contagion and imitation mimicry are considered within this context. Cognitive empathy ("I understand what you feel") is considered as a higher-level process, involving cognitive processes such as glance acquisition and altruistic behaviors"
Kentrop et al., 2020	Sharing task (lever presses)	Pro-social behavior, defined as behavior that is aimed to benefit others, is a key element in many aspects of everyday life. It is proposed to be driven by the motivation to maintain social relations and is hypothesized to emerge from different forms of empathy, from emotional contagion (i.e. the ability to experience and share emotions) to more cognitive forms of empathy like adopting the other's point of view
Kozma et al., 2019	Cooperation learning task (nose poke)	A crucial component of social cognition is the theory of mind, that is, the ability to make inferences on someone else's mental state (thoughts, emotions, or intentions) and predict his/her future behavior based on social signals and the context of the situation. For example, even if children with ASD are capable to recognize another person's goal and help him/her in attaining it, notwithstanding they are incapable to cooperate, suggesting that controlling the behavior of two individuals to reach a common goal is a far more complex task
Li & Wood, 2017	Repeated donation game	NA
Lopuch & Popik, 2011	Cooperation learning task (nose poke)	Cooperation can be defined as the voluntary joint action of two or more individuals that benefits the recipient(s) (Brosnan & de Waal, 2002; Hamilton, 1964). An act of altruism benefits the recipient at the cost to the actor (Hamilton, 1964), while mutual benefits follow a joint action resulting in the simultaneous benefit for all individuals involved (Dugatkin, 2002; Krebs & Davis, 1993).
Marquez et al., 2015	Prosocial choice task - Double T Maze	Animals often are prosocial, displaying behaviors that result in a benefit to one another
Oberliessen et al., 2016	Prosocial choice task - Double T Maze	Beyond maximizing one's own material gains, fairness plays an important role in human behavior and economic decision making. The tendency to base decisions not solely on selfish motives but considering others' outcomes as well has often been studied with economic games. Disadvantageous inequity aversion (IA) is a behavioral response to an inequitable outcome distribution yielding a smaller reward to oneself than to a conspecific, given comparable efforts to obtain the reward.
Raz, 2013	Cooperation learning task	NA
Rutte & Taborsky, 2007	Lever presses - Box ('open field')	Cooperation among unrelated individuals may be achieved by reciprocal altruism in which two or more individuals help each other in turn. The decision to cooperate is based on expected future help, which may be judged from past interactions.
Rutte & Taborsky, 2008	Repeated donation game	The logic of reciprocal altruism is that the decision to pay some cost for the benefit of another individual is based on expected future help, which may be judged from past interactions. Cooperation among non-kin has been attributed sometimes to reciprocal altruism: Two or more individuals exchange behavior that benefits the respective partner.
Sato et al., 2015	Freeing task (soaked area)	Helping behavior is a prosocial behavior whereby an individual helps another irrespective of disadvantages to him or herself. (definition from conclusion: Empathy is thought to be divided into two major subcomponents: cognitive empathy and affective (emotional) empathy (de Waal

		2008; Hoffman 2000). Cognitive empathy is the ability to understand the thoughts, feelings, and desires of other individuals, and emotional empathy is the ability to share the emotional states of other individuals). Sensitivity to the emotions of conspecifics, through empathy or emotional contagion, is important to facilitate smooth communication with others and is necessary for an adaptive social life. As opposed to antisocial behavior, prosocial behavior is socially desirable behavior that benefits other individuals
Schmid et al., 2017		In an iterated prisoner's dilemma game, individuals base their decision to provide help to a partner or not on the latter's previous help provided to them (direct reciprocity; Axelrod & Hamilton, 1981) or to others (indirect reciprocity; Nowak & Sigmund, 1998). Alternatively, an individual may help someone if it had previously received help from somebody else (generalised reciprocity
Schneeberger et al., 2012	Repeated donation game	NA
Schonfeld et al., 2020	Prosocial choice task - Double T Maze	The choice for a mutual reward does not lead to any direct benefit for the rat that makes the decision, it is assumed that a preference for mutual rewards reflects rodent social behavior, specifically prosocial decision making
Schwartz et al., 2017	Freeing task (soaked area)	Empathic action—aiding a recipient despite cost to a donor—
Schweinfurth & Taborsky, 2016	Repeated donation game	Three forms of reciprocal cooperation among animals have been described: direct reciprocity where individuals help those that have helped them before; generalized reciprocity, where the decision to help a social partner is based on help received from someone else; and indirect reciprocity where the decision to help a partner is dependent on the helpfulness of this partner towards others.
Schweinfurth & Taborsky, 2017	Repeated donation game	Individuals showing direct reciprocity help those that have previously helped them (the concept of reciprocity is applied only to situations where the same social service or commodity is returned to the same social partner in a similar context)
Schweinfurth & Taborsky, 2018 (A)	Repeated donation game	Negotiation about mutual help might be an important mechanism responsible for the evolution of cooperation because it can generate greater fitness rewards and lead to higher levels of cooperation than kin selection. Cooperation among conspecifics, such as one individual helping another, is common in animals
Schweinfurth & Taborsky, 2018 (B)	Repeated donation game	The evolution and maintenance of cooperative interactions between unrelated individuals can be explained by the reciprocal trading of given and received help
Schweinfurth & Taborsky, 2018 (C)	Sharing task (stick pulling)	altruistic behaviors, entailing immediate costs without compensation by immediate benefits, are widespread in nature
Schweinfurth & al., 2019	Repeated donation game	Evolutionary theory predicts individuals will behave in their own interest. Nevertheless, in many species individuals cooperate by providing costly help to others. The evolution of such cooperation depends on its costs and benefits.
Schweinfurth & Taborsky, 2020	Prisoner's dilemma	The reciprocal exchange of help between social partners can lead to stable cooperation by taking turns
Silberberg et al., 2014	Freeing task (soaked area)	empathically motivated behavior (altruism) consists of actions in one animal (the donor) to redress the perceived needs of another (the recipient).
Silva et al., 2020	Freeing task (tube)	Empathy is the ability to (a) be affected by and share the emotional state of another; (b) assess the reasons for the other's state; and (c) identify with the other, adopting their perspective.
Tomek et al., 2019	Freeing task (tube)	NA
Tomek et al., 2020	Freeing task (tube)	Here, we define prosocial behavior as those that occur with the intent to interact with others. One important aspect of prosocial behavior is empathy, the ability to perceive and understand the emotions or situations of others.
Tsoory et al., 2012	Cooperation learning task	Cooperation is broadly defined as a situation in which an individual's outcomes depend not only on its own behaviors but also on the behaviors of others
Ueno et al., 2019 (A)	Freeing task (tube)	"Prosocial behavior comprises actions that benefit others and is said to include informing, comforting, sharing, and helping. Helping behavior, a form of prosocial behavior, involves acting for the benefit of others (e.g. rescuing others from difficult situations) in the absence of reward"
Ueno et al., 2019 (B)	Freeing task (tube)	Expressing socially desirable behaviors for conspecifics without external compensation is called prosocial behavior

Viana et al., 2010	Prisoner's dilemma in a double T-Maze	a cooperative act constitutes a truly altruistic behavior emerging from a reward value attributed to the perception of benefit to others. Alternatively, from a strictly economic perspective, it is proposed that animals cooperate whenever it entails a benefit, either immediate or in the future, regardless of the consequence of its action to the other interacting individual
Wood et al., 2016	Prisoner's dilemma in operant box	direct reciprocity is a dyadic interaction, representing the repeated reciprocal exchange of equivalent benefits between two parties. When delivering a benefit to their partner, each participant experiences a temporary net cost, which is exceeded by the benefit they subsequently receive from a partner working on their behalf
Yamagishi et al., 2020 (A)	Freeing task (soaked area)	prosocial behavior (e.g., helping, consolation, and food sharing), voluntary behavior through which they provide benefits to other individuals. Prosocial behavior is considered to be motivated by empathy, or the ability to share and understand others’ emotions
Yamagishi et al., 2020 (B)	Freeing task (soaked area)	Observing others’ emotional responses elicits emotional reactions from observers. Such an ability to share others’ emotions is called empathy
Yuksel et al., 2019	Freeing task (soaked area)	Empathy is the recognition and internalization of someone else’s feelings, condition, or behavior.

Updated search – Characteristics of the included studies (2021-2023)

Table 7. Results of all Included Studies – Animals (updated search).

Reference	Type of rodent	#rodents	#males	#females	Age	Strain	Weight(g)	Water restriction	Food restriction
Asadi et al., 2021	Rats	30	30	NA	Adult	Wistar	NA	No	No
Ben-Ami Bartal & al., 2021	Rats	83	83	NA	Adult	Long-Evans + Sprague-Dawley	NA	No	No
Breton et al., 2022	Rats	90	90	NA	Ado: PND32 Adult: PND 60-90	Long-Evans + Sprague-Dawley	NA	No	No
Conde-Moro et al., 2022	Rats	38	38	NA	3 months	Lister Hooded	250-300g	No	Yes
Cox et al., 2022 [A]	Rats	18	18	NA	NA	Sprague-Dawley	250-275g	No	Yes
Cox et al., 2022 [B]	Rats	16	8	8	NA	Sprague-Dawley	250-275g	No	Yes
de Carvalho et al., 2020	Rats	10	10	NA	3 months	Wistar	NA	Yes	No
Gachomba et al., 2022	Rats	86	74	12	3-3.5 months	Sprague-Dawley	226-250g	No	No
Heslin et al., 2021	Rats	43	43	NA	9 months	Sprague-Dawley	NA	NA	NA
Joushi et al., 2022	Rats	NA	NA	NA	PND34	Wistar	NA	No	No
Kalamari et al., 2021	Rats	NA	NA	NA	adults	Wistar	NA	No	No
Misiolek et al., 2023	Mice	89	45	44	10-12 weeks	C57BL/6	17-29 g	No	Yes
Paulsson & Taborsky, 2021	Rats	25	NA	25	NA	Norway	NA	No	No
Scheggia et al., 2022	Mice	68	NA	NA	2-6 months	C57BL/6J	NA	No	Yes
Schweinfurth, 2021	Rats	52	NA	52	Adult	Norway	256-408g	No	No
Segura et al., 2019	Rats	8	8	NA	Adult	Wistar	228-264g	No	Yes
Sen et al., 2021	Rats	16	16	NA	Adult	Sprague-Dawley	NA	No	No
Shima et al., 2022	Mice	16	16	NA	8 weeks	C57BL/6	NA	No	No
Subhadeep et al., 2022	Rats	32	32	NA	Adult	Wistar	200-240g	No	No
Wan et al., 2021	Rats	6	NA	6	NA	Sprague-Dawley	NA	No	Yes
Wu et al., 2023	Rats	24	NA	24	9-20weeks	Long-Evans	200-300g	No	Yes

Table 8. Results of all Included Studies – Housing Conditions and Apparatus (updated search).

Reference	Housing (per cage)	Relationship	Light-Dark Cycle	Enrichment	Operant Paradigm	Video/vocalization recording	Can animals see/hear/smell each other?
Asadi et al., 2021	paired	littermates	12:12 dl lights on @ 7am	No	Freeing task (soaked area)	Videorecording	NA
Ben-Ami Bartal & al., 2021	paired	NA	12:12 dl	No	Freeing task (tube)	Videorecording	Yes
Breton et al., 2022	paired	Littermates (ado)	12:12 dl lights on @ 7am	No	Freeing task (tube)	Videorecording	Yes
Conde-Moro et al., 2022	paired	NA	12:12 dl	No	Cooperation	No	Yes
Cox et al., 2022 [A]	paired	NA	reversed 12:12dl lights on @ 18:00	No	Freeing task (soaked area)	Ultrasonic voc	NA
Cox et al., 2022 [B]	paired	NA	Reversed 12:12dl	No	Freeing task (soaked area)	Ultrasonic voc	NA
de Carvalho et al., 2020	paired	NA	12:12 dl	No	Cooperation	Videorecording	Yes
Gachomba et al., 2022	paired	NA	Reversed 12:12 dl lights off @ 8:30am	No	Prosocial choice task	Videorecording + Ultrasonic voc	Yes
Heslin et al., 2021	paired	NA	Reversed 12:12 dl	No	Freeing task (tube)	No	Yes (smell and hear)
Joushi et al., 2022	6-9 per cage	littermates	12:12 dl	Yes	Prosocial choice task	Videorecording	Yes
Kalamari et al., 2021	10 males per cage	littermates	Reversed 12:12dl lights on @ 20:00	Yes	Freeing task (tube)	Ultrasonic voc	Yes
Misiolek et al., 2023	paired	littermates	12:12 dl lights on @ 7am	No	Prosocial choice task	Videorecording	Yes
Paulsson & Taborsky, 2021	5 sisters	littermates	Reversed 12:12 lights off @ 8am	No	Repeated donation game	Videorecording	Yes
Scheggia et al., 2022	2-4 per cage	littermates	12:12 dl lights on @ 7am	No	Sharing task	Videorecording	Yes
Schweinfurth, 2021	5 per cage	NA	Reversed 12:12dl lights on @ 20:00	Yes	Repeated donation game	No	Yes
Segura et al., 2019	single	NA	12:12 dl lights on @ 7am	No	Cooperation	Videorecording	Yes (except in opaque condition)
Sen et al., 2021	paired	NA	12:12 dl	No	Freeing task (soaked area)	NA	NA
Shima et al., 2022	NA	NA	12:12 dl lights on @ 8am	No	Freeing task (soaked area)	NA	NA
Subhadeep et al., 2022	paired	NA	12:12 dl lights on @ 6am	No	Freeing task (tube)	Ultrasonic voc + Videorecording	Yes
Wan et al., 2021	paired	NA	12:12 dl	No	Freeing task (tube)	NA	Yes
Wu et al., 2023	single	NA	12:12 dl lights off @ 18:00	No	Freeing task (tube)		Yes

Table 9. Results of all Included Studies – Methodology (updated search).

Reference	**Duration (in min)**	**# of Testing Days**	**Habituation**	**Pretraining**	**Reward**	**Control Group**	**Other Behavioral Tests**	**Drugs or Surgical Intervention**
Asadi et al., 2021	5	12 days	No	No	NA	No	NA	NA
Ben-Ami Bartal & al., 2021	40-60	12 days	Yes	No	social contact	Yes	Boldness, open field	NA
Breton et al., 2022	40-60	12 days	Yes	No	social contact	No	Open field	NA
Conde-Moro et al., 2022	Until reached criterion	At least 2 consecutive days	Yes	Yes	pellet	Yes	Open field, elevated plus maze, water and food competition tests	Animals were chronically implanted with 2 sets of recording electrodes+ implantation of cannulas for micro injection
Cox et al., 2022 [A]	5	2 sessions per day for 8 days	No	No	social contact	No	Social reward place conditioning	AAV8-CaMKIIα-enhanced green fluorescent protein or inhibitory DREADD virus, AAV8-CaMKIIα-hM4D(Gi)- mCherry
Cox et al., 2022 [B]	5	2 sessions per day for 8 days	No	No	social contact	No	Social reward place conditioning	NA
de Carvalho et al., 2020	60	10 sessions	No	Yes	water	Yes	NA	NA
Gachomba et al., 2022	40	5 sessions	Yes	Yes	pellet	No	Food competition test	NA
Heslin et al., 2021	8	24 days (2 blocks of 12 sessions)	Yes	Yes	social contact	Yes	NA	NA
Joushi et al., 2022	6 forced+ 25 free choice trials	12 sessions	Yes	Yes	2 sucrose pellets	Yes	NA	Oxytocin
Kalamari et al., 2021	5-10	NA	Yes	Yes	sucrose pellets or social contact	Yes	boldness test	NA
Misiolek et al., 2023	Until reached criterion	4 days	Yes	Yes	2 chocolate chips	No	Social conditioned place preference, affective state discrimination	NA
Paulsson & Taborsky, 2021	7	3 sessions	No	Yes	oat flake	Yes	NA	NA
Scheggia et al., 2022	40-120	Reached criterion for 3 consecutive days.	No	No	14mg of test diet	Yes	Dominance tube test, observational fear conditioning	AAV5-CamKIIa-mCherry Virus injected into BLA.
Schweinfurth, 2021	7	18 sessions	No	Yes	1 oat flake	No	NA	NA
Segura et al., 2019	4 blocks of 4 min	30 sessions	No	Yes	1 pellet	No	NA	NA
Sen et al., 2021	5	12 days	Yes	Yes	social contact	Yes	Open field, elevated plus maze, force swimming test	NA
Shima et al., 2022	3	1 day	No	Yes	NA	Yes	NA	NA

Subhadeep et al., 2022	30	8 days	Yes	Yes	social contact	Yes	Boldness test	Ibotenic acid (1 µg/µl/site)infused into the vSUB bilaterally
Wan et al., 2021	30	4-13 sessions (8.5 avg)	Yes	Yes	food or social contact	Yes	NA	NA
Wu et al., 2023	30 trials	NA	Yes	Yes	social contact	Yes	Observational distress test	Microelectrode implantation

Table 10. Result of all Included Studies - Performance and Behavioral Analyses (updated search).

Reference	**Performance index**	**Behavioral Analyses**
Asadi et al., 2021	# of door openings (# responses in HMC group in last 2 sessions was ↑ than LMC group. Rats that received higher maternal care early in life responded faster to rescue conspecific.)	NA
Ben-Ami Bartal & al., 2021	# of door openings (rats released cagemates of the same strain, but not strangers of an unfamiliar strain, demonstrating an ingroup bias for prosocial behavior.)	Rats in HBT ingroup condition were more active in total. Rats in ingroup condition also spent more time in the area around the restrainer
Breton et al., 2022	# of door openings (adults and ado tested with ingroup members were motivated to release cagemates. Unlike adults, ado released outgroup members as expressed by a sig. ↑ in the % of door-openings and ↓ latency. Nearly all ado (n = 6/8) consistently released trapped outgroup member vs 0/16 in adults.)	Ado in ingroup condition demonstrated movement patterns that reflect ↑ interest in the trapped rat, which may indicate motivation to release the trapped cagemate. Ado in both conditions were more active and spent more time near the trapped rat than adults.
Conde-Moro et al., 2022	# of coordinated climb (all pairs learned to climb onto the platform to mutually obtain a reward and reached criterion between sessions 4-10. For each pair, the rat that climbed onto the platform sig. more times in first place (initiated more cooperation trials) was classified as the leader while the partner was classified as follower.)	Leader rats spent sig. more time in the open arms of the elevated plus-maze showing ↓ levels of anxiety. Follower rats showed a sig. ↑ level of social dominance than leader rats during these tests.
Cox et al., 2022 [A]	# of door openings (Inhibition of the AI sig. blunts release behavior during social contact-independent targeted helping task. The change in Observers' helping behavior due to AI inhibition correlates to an ↑ in distress of the Target as measured by USV.)	Target rats of Observer partners that received B/M infusions had a sig. larger proportion of their total USV calls fall within the distress range compared to Targets on days where their corresponding Observers received PBS control.
Cox et al., 2022 [B]	# of door opening (Rats released a distressed conspecific at similar rates in the 3 timepoints evaluated)	Female Targets had a sig. larger proportion of their total USV calls fall within the distress range, and sig. fewer within the prosocial range compared to other groups.
de Carvalho et al., 2020	# of simultaneous press (↓ in rates and proportion of coordination were observed at larger FR values. Successive ↑ in FR requirements produced systematic ↑ in post reinforcement pauses. Response requirement and reinforcement rates were critical determinants of coordinated responding maintained by FR schedules of mutual reinforcement.)	NA
Gachomba et al., 2022	# of both-choice reward (Rats' prosocial preferences in food-foraging contexts emerged over the testing sessions independently of familiarity or sex. Male rats	Both groups acquired a preference for prosocial option over the days, but social hierarchy drastically modulated the emergence of this choice. Dominant animals acquired faster

	displayed similar levels of prosociality when interacting with their cagemates or unfamiliar conspecifics.)	prosocial tendencies and reached higher prosociality levels than submissive decisionmakers. Submissive recipients are more attentive: they display more direct gazing prior to choice and increase proximity to their focals, specifically when decision-makers are going to be selfish (i.e., following them around the choice area). Dominant decisionmakers might respond to these cues by showing ↑ social attention to their recipients which is reflected in ↑ sniffing time directed to the animal that needs help. All USVs recorded were of the 50-kHz family (no alarm calls were observed)
Heslin et al., 2021	# of door openings (Choices made by the 19 subjects indicated an overall preference for selecting the nonrestrained rat chamber. Subjects initially preferred choosing locations that resulted in socialization opportunities, particularly with nonrestrained animals.)	NA
Joushi et al., 2022	# of both-choice reward (Sig. ↑ proportion of BR choices in partner condition vs toy condition. % of BR choices in partner condition was sig. ↓ in MS group vs CTRL group. Being exposed to EE enhanced BR choices in partner condition in MS+EE group vs MS group. Exposure to EE reversed the impairment in mutual reward preferences caused by MS. % of BR choices in partner condition was sig.↓ in MS.saline group vs CTRL.saline group.)	NA
Kalamari et al., 2021	# of door openings (MD rats seemed less motivated to liberate a trapped cagemate as seen by a lower number of completed ratios, but this effect was (just) not sig., although the effect size was quite large.)	Housing did not affect the emission of alarm calls before door opening. The emission of 22 kHz and appetitive calls were comparable between standard and complex housed rats.
Misiolek et al., 2023	# of both-choice reward (In female mice, the preference for the prosocial compartment sig. ↑ while males appeared to show no change from their initial choices.)	NA
Paulsson & Taborsky, 2021	# of stick pulling (Rats showed more reaching behaviors when a partner capable of providing food was present than when none was present but not in the presence of only a partner without food that it could have fetched for the focal subject.)	NA
Scheggia et al., 2022	# of altruistic choices (Mice intentionally engaged in choices that favor another conspecific or only themselves. Prosocial actions, even if they required more effort or had no direct benefit to the actor mouse, were more generally observed toward familiar, hungry males with the highest hierarchical distance to the actor.)	Dominance test: most mice that displayed a preference for selfish over altruistic choices were subordinate to their recipient and belonged to an intermediate rank.
Schweinfurth, 2021	# of cooperative choices (Rats distinguished between both conditions and provided more food to able food-providing partners vs unable partners that could no provide food as the device was blocked. Subjects helped able and willing partners more often than able and unwilling partners. Unable partners attempted to help by pulling the stick that was connected to the blocked platform, suggesting that they had cooperative intentions. Rats seem to not consider their partner's intention to help but base their decision on outcomes or abilities to help.)	NA
Segura et al., 2019	#of coordinated actions (Rats from Pairs 1 and 2 coordinated their activities only when the reinforcement ratio was larger in the mutual option (4:1) while Pairs 3 and 4 failed to coordinate their actions.)	NA

Sen et al., 2021	# of door openings (Mean opening door latency ↓ progressively in all animals. Door opening times after chronic restraint stress protocol were compared, no sig. difference found between the groups.)	No sig. difference between the groups in the open field test and elevated plus maze test.
Shima et al., 2022	Light-intensity exercise enhances helping behavior with upregulated levels of BDNF mRNA in the insular cortex.	NA
Subhadeep et al., 2022	# of door openings (On the 5th day, 4 out of 8 (50%) VSLfree rats could successfully open the restrainer door, which did not improve further in the subsequent days. On the 8th day, only 2 out of 8 (25%) VSLfree rats successfully opened the door.)	During the task, both 22-kHz and 50-kHz USV calls were emitted by the pair of rats in the arena. Most of the 22-kHz calls were emitted before the restrainer door was opened. 50-kHz calls were emitted after the free rats successfully opened the door. The # of 22-kHz calls emitted by the VSL rats before door-opening on day 1 was the least, which gradually increased in the subsequent sessions. VSL rats continued to emit more 22-kHz calls and fewer 50-kHz calls even after door-opening.
Wan et al., 2021	# of door opening containing the congener vs the food (Rats chose food and social release with similar latencies and rats willingly share food with their social partner, even if it comes at a cost to the individual.)	NA
Wu et al., 2023	# of door opening (8/12 rats opened the restrainer more often when a conspecific was in the restrainer.)	NA

Table 11. Results of all Included Studies - Other Analyses (updated search).

Reference	**Sex Differences**	**Age Differences**
Breton et al., 2022	NA	Adults released trapped ingroup members, but ado rats helped both ingroup and out-group members, suggesting ingroup bias emerges in adulthood.
Cox et al., 2022 [B]	No sex differences	NA
Misiolek et al., 2023	Females, but not males, C57BL/6 mice showed significant preference for prosocial behavior toward a familiar partner.	NA
Scheggia et al., 2022	Males are more prosocial than females.	NA

Articles that had no reported information regarding results in this table were excluded from it

Table 12. Results of all Included Studies – Reported definition (updated search).

Reference	**Task**	**Reported definition**
Asadi et al., 2021	Freeing task (soaked area)	Empathy has been defined as the ability to vicariously experience a shared affective state as another person, coupled with provoked caring and concern for others' good.
Ben-Ami Bartal & al., 2021	Freeing task (tube)	acting with the intention of benefiting others or improving their well-being + helping others in need
Breton et al., 2022	Freeing task (tube)	Prosocial actions are any that occur with the intention of benefiting others or improving their well-being
Conde-Moro et al., 2022	Cooperation	cooperation: a powerful way of improving the access to resources and require a precise synchronization of animal activities
Cox et al., 2022 [A]	Freeing task (soaked area)	Empathy is the capacity to share the feelings of another and generate an appropriate response to those shared feelings
Cox et al., 2022 [B]	Freeing task (soaked area)	Empathy is a complex suite of behaviors that works to convey an understanding of the affective states of others
de Carvalho et al., 2020	Cooperation	NA
Gachomba et al., 2022	Prosocial choice task	performing actions that benefit others
Heslin et al., 2021	Freeking task (tube)	Prosocial behavior is any behavior that provides a benefit to another individual, with little or no cost to the actor
Joushi et al., 2022	Prosocial choice task	Pro-sociality, i.e., the preference for outcomes that produce benefits for other individuals
Kalamari et al., 2021	Freeing task (tube)	behavior that benefits others,
Misiolek et al., 2023	Prosocial choice task	Prosocial behavior, defined as acting to meet the perceived need of another individual, is regarded as the highest form of empathy
Paulsson & Taborsky, 2021	Repeated donation game	Reciprocal altruism or "reciprocity," where a cost is accepted by an individual to provide a service to a social partner for a delayed benefit,
Scheggia et al., 2022	Sharing task	NA
Schweinfurth, 2021	Repeated donation game	Reciprocity is the selective helping of those who were cooperative before
Segura et al., 2019	Cooperation	Cooperative behavior has been defined as "joint action for mutual benefit"
Sen et al., 2021	Freeing task (soaked area)	Empathy, can be defined as understanding and internalizing someone else's emotions, current situation, or behavior
Shima et al., 2022	Freeing task (soaked area)	NA
Subhadeep et al., 2022	Freeing task (tube)	empathy: the ability to understand and share the emotions of others.
Wan et al., 2021	Freeing task (tube)	Pro-social behavior has been defined as behavior that produces benefits for another, sometimes even at a cost to the individual
Wu et al., 2023	Freeing task (tube)	Emotional contagion, the ability to experience the distress of others, is closely associated with prosocial behavior that benefits others