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Motivational and Methodological Factors Involved in the Helping Behavior Test in Laboratory Rodents

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Abstract

Empathy allows humans and other animals to share the emotional state of another, adopting that individual's perspective on a given situation. This ability is fundamental for species living in groups. Helping behavior in laboratory animals has been used to study empathy. In this test, subjects are exposed to a conspecific that is trapped and learn to open the cage to release the other animal. However, the interpretation of helping behavior as an emphatically motivated action is still controversial. Here we review the studies that use the helping behavioral test proposed by Ben-Ami Bartal and colleagues in 2011 to better understand motivational factors for this behavior. In addition, we compare methodological aspects of these studies. In conclusion, helping behavior can be driven by empathy, but other factors such as the desire for social contact and learning components cannot be ruled out as motivators. In addition, studies focused on evaluating neurobiological mechanisms underlying helping behavior in laboratory rodents can help elucidate the factors involved in releasing the trapped co-specific.

Keywords

Animal Models, Empathy, Prosocial Behavior, Rats

1. Introduction

Prosocial behaviors encompass a range of actions characterized as beneficial to an individual or a social group, such as cooperation, charity, emotional support, among others [1] [2]. The motivators underlying prosocial behaviors are still a

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topic of debate, but some researchers suggest that empathic ability is a crucial motivator of prosocial behaviors [3].

Classically, empathy is defined as the ability to share another individual's emotional state, understand the reasons for that emotional state, and adopt that individual's perspective on a particular situation [1]. Thus, empathy is a skill that allows an individual to relate to others, being essential for species with social structures, providing parental care and exercising cooperative behaviors to achieve a common goal. Other authors define empathy as a broader term that would encompass the individual's emotional and cognitive reactions when observing another's experience [4].

There has been solid evidence that empathy is not exclusive to humans, being present in other species that live under social structures, mainly, but not exclusively, other mammals [5].

From a neurobiological point of view, the mechanisms underlying empathy are not completely understood. Most of the evidence for brain regions involved in empathy in humans comes from correlational studies using neuroimaging techniques. One of these studies shows that empathy in humans is correlated with simultaneous activation of the prefrontal cortex and amygdala [6]. In addition, Lamm, Nusbaum, Meltzoff *et al.* [3] showed that perceiving pain in others activates somatosensory and affective-motivational circuitries related to physical and emotional processing of pain. The investigation of such mechanisms is relevant because some neuropsychiatric disorders such as autism, schizophrenia and psychopathy are described as conditions in which the individual presents empathic impairments and socialization deficits [7]-[9].

In Neuroscience animal studies, laboratory animals such as rats, mice, birds, and fish are the main experimental subjects [10] [11]. Laboratory rodents have an extensive experimental history [12] [13], low-cost breeding, and easy maintenance. Thus, these animals would provide an interesting approach to the investigation of physiological and pathological aspects of empathy and prosocial behavior. However, most studies of empathy in rodent only address a single aspect of this phenomenon: emotional contagion (*i.e.*, the sharing of emotional reactions to a situation experienced by only one of the subjects) [14]-[16]. Rarer and more complex are studies that evaluate the effectiveness of empathically motivated helping or cooperative behaviors in rodents. Nevertheless, recently, some protocols have been proposed for this purpose.

In a pioneer study, Ben-Ami Bartal, Decety and Mason [17], using Sprague Dawley rats, proposed a model in which a rat should release a conspecific from a potentially stressful situation. For this purpose, the animals are placed in an open field with a central trapping box that can only be opened from the outside. One of the rats is trapped in this box, with restricted movements, which sets up a potentially stressful situation. The other rat remains free in the open field, and can open the box, thereby helping the trapped animal to escape. The opening rate (proportion of animals that open the door) and the latency to perform the behavior (elapsed time between the start of the session and the opening of the box) are the main parameters used to analyze helping behavior in this task.

The results of this first study showed that rats perform helping behavior (open the box) when they perceive a conspecific experiencing an uncomfortable situation [17]. This outcome suggests an altruistic behavior of these animals to minimize the stress experienced by the conspecific. Subsequently, other studies (reviewed here) have proposed using this task, or adaptations of it, to study empathically motivated prosocial behavior. However, the main motivator for this behavior is still controversial. For example, Silberberg, Allouch, Sandfort *et al.* [18] suggest that this behavior would be the consequence of a desire for social contact and not motivated by empathy [18].

Thus, the aim of this review is to identify and analyze studies that used the helping behavioral test originally proposed by Ben-Ami Bartal, Decety and Mason [17] for the study of prosocial behavior possibly driven by empathy. With this survey, we intend to compare the methodological changes that emerged in relation to the original protocol, evaluate the main results found regarding the opening behavior and discuss the conclusions and interpretations of the authors in relation to the motivations to perform it.

2. Methods

We performed a literature search using Medline, PsycInfo and SciELO databases. The following keywords were used: prosocial behavior, helping behavior and empathic behavior. A filter for year of publication (2011-2024) was used to include only studies published after the original of Ben-Ami Bartal, Decety and Mason [17]. Within the works found using these criteria, only those articles that used the helping behavioral protocol in laboratory rodents were included in the review. In addition, we only included articles written in English. The data extracted from the selected studies were year of publication, authors, changes made in the behavioral test compared to the original protocol, results found regarding the behavior of opening the door and main conclusions raised.

Initially, 38 articles were found and fourteen of them were excluded for not meeting the inclusion criteria. The remaining 24 papers were selected for the study and data extracted from these papers are shown in **Table 1**.

The evaluation of the studies showed that helping behavior expressed by opening a door to release a trapped conspecific is highly consistent across species (rats, mice, and hamsters), rat strains (Sprague Dawley, Long Evans and Wistar), sexes and age ranges. In addition, variations in protocols and adaptations of the apparatus did not prevent the execution of the release behavior. However, as observed in **Table 1**, the studies were not homogeneous regarding the interpretation of the helping behavior as being emphatically motivated.

Table 1. Summary of the studies using the helping behavior test in rodents.

Year	First author	Animals	Protocol	Main results	Interpretation	Ref
2011	Ben-Ami Bartal, Inbal	Male and female Sprague Dawley rats	Original. Groups: toy rat, trapped rat, empty box. Variations: modified box (rat could not interact after release); a box containing chocolate or box containing trapped mouse.	Rats open the chamber after the 6th session; higher opening rate for situations with trapped animals; similar opening rate between box with chocolate and box with trapped animal; possibility of social interaction does not interfere with opening behavior.	Emotional factors related to empathy are the motivation to open the door.	[17]
2014	Silberberg, Alan	Female Sprague Dawley rats, 3 - 6 months old	Modified apparatus To control social interaction after release (central restrictor tube and two separate chambers, one containing free animal and the other empty chamber; restrained tube opened by the touch by the free animal; trapped rat, when released, could remain either in the separate chamber or in the chamber with the free animal).	The door opening increases when there is the possibility of social contact.	A possible motivation for the release of the trapped rat is the desire for social contact.	[18]
2014	Ben-Ami Bartal, Inbal	Male Sprague Dawley and Long-Evans rats, 2 - 3 months old	Original. Different experiments to compare the opening rate between animals of their own x other strain; familiar and unfamiliar animals.	Previous social contact increases the opening of the door. Animals can open the door for their own strain or for familiar animals without having had contact before, but for animals from another strain and unfamiliar animals previous social contact is needed.	Familiar animals present a better helping behavior than animals that are strangers to each other.	[46]
2015	Sato, Nobuya	Male and female Sprague Dawley rats, 2 months old	Different apparatus that simulates another stressful condition for the animal An apparatus with different chambers, one containing water, other containing a safe platform, other containing a safe platform with chocolate. A transparent wall and a door that could be opened separated all the chambers. Animal trapped placed in the area with water and free animal in the safe central chamber.	The animals are highly motivated to open the door when the conspecific is in a potentially stressful situation. The opening behavior is attenuated when there is no stress.	Empathic motivation to open the door, trying to alleviate the stress of the other animal.	[21]
2016	Ben-Ami Bartal, Inbal	Male Sprague Dawley rats, 2 months old	Original. Experimental group was administered with Midazolam (MDZ—1.25 mg/kg or 2.0 mg/kg—a benzodiazepine anxiolytic).	The animals that received anxiolytics did not have the expected increase in opening behavior across sessions. MDZ interfered specifically with social affective processing that appears necessary to motivate a free rat to help a trapped rat.	Opening the box depends on an emotional motivator	[22]
2018	Hachiga, Yosuke	Male Sprague Dawley rats, 2 weeks old	Different apparatus. An E maze was used instead of the open field. One end arm contained the restricted box with the trapped animal and at the other end an empty box or a free animal.	The animals explored more the arm containing a trapped animal than the empty arm but did not show preference when exposed to a trapped x a free animal.	Animals were motivated by desire for social contact or exploratory drive rather than empathy.	[19]
2018	Kandis, Sevim	Male Sprague Dawley rats	Modified protocol. Arena with two chambers, one with water and one safe. Free animal could open the door, releasing the other animal to enter the safe arena. Experimental group received paracetamol.	The animals that received high doses of paracetamol presented impaired performance of opening behavior. The behavior is still observed in controls.	Paracetamol reduces oxytocin and vasopressin levels in brain regions linked to empathic behavior. Thus, the animals that received high doses of paracetamol did not show the behavior motivated by empathy.	[23]
2019	Tomek, Seven	Male Sprague Dawley rats	Original. Investigated the effect of opioid dependence on social behavior.	The animals that received opioids demonstrated deficient helping behavior, choosing heroin self-over helping the conspecific.	The use of opioids interferes with prosocial behavior. However, social interaction and prosocial behavior worked as positive reinforcements. Thus, the releasing of the companion is still observed.	[26]
2019	Ueno, Hiroshi	Male mouse strain C57BL/6N	Original. Apparatus dimensions adapted for mice.	The animals open the box for familiar or unfamiliar conspecifics, even if the "trapped" animal is anesthetized.	Animals that have had previous contact exhibit better helping behavior than animals that are strangers to each other. Mice present the release behavior of another animal, probably motivated by empathy.	[47]

Continued

2019	Blystad, Magnus	Female Sprague Dawley rats, 3 months old	Original.	The animals prefer to open the box containing suffering animals than the box containing food, even if they were undergoing food restriction. The study shows that the latency of door opening by females is lower at the beginning of the test when compared with males.	The experiments performed with females corroborate the findings with males and show better performance for the former sex.	[44]
2019	Fontes-Dutra, Mellanie.	Male Wistar rats, 69 - 81 days old	Original/Different animal model. This study used the animal model for autism. Pregnant rats were injected with valproic acid (VPA), resveratrol (VRS), valproic acid plus resveratrol (VPA + VSR) and vehicle. Male offspring aged 61 - 81 days postnatal were used.	The animals in the VPA and VPA + RSV groups exhibited a delayed onset in the manifestation of pro-social helping behavior, with the initial occurence of opening the restrainer at three days later than in the Control group, on average.	The results suggest that VPA may not directly impact neural circuits associated with empathy. Instead, it likely influences processes such as decision-making or anxiety. The VSR was not able to prevent social impairments in the VPA model of autism.	[43]
2020	Cox, Stewart	Male Sprague Dawley rats	Modified apparatus. Arena with two or three chambers (one with water, one dry, and one that hampered social contact between animals).	Animals open the door consistently even when they are prevented from interacting.	The emotional contagion is essential for the observer to carry out the releasing task; the observer maintains the releasing behavior by perceiving the relief of the trapped conspecific.	[24]
2020	Silva, Phietica	Male Wistar rats, 3 - 4 months old	Original. Changing the number of sessions. In the original 12 days, this protocol the tests were repeated for 12 days, but changing the order of sessions with empty box or trapped rat in box.	The door opening behavior is executed even with the empty box, provided that the animals learned to open it in the presence of a trapped conspecific. No correlation was found between latency to open the door and amount of social interaction.	Although social interaction does not seem to be an important motivator, there are multiple factors involved in the drive to open the door, including empathic aspects and the process of learning the task.	[13]
2020	Yamagishi Atsuhito	Male Sprague Dawley rats, 7 - 11 weeks old	Different apparatus that simulates another stressful condition. An apparatus with different chambers, one containing water, another containing a safe platform. The animals were treated with oxytocin or saline before the sessions. The study has four groups: pairs with oxytocin (pair_oxy); pairs with saline (pair_sal); solo with oxytocin (solo_oxy) and solo with saline (solo_sal).	The animals in the solo_oxy group acquired helping behavior more quickly than the pair_oxy group.	The study showed that oxytocin can reduce social stress and favor the performance of tasks, especially for unfamiliar animals.	[34]
2021	Ben-Ami Bartal, Inbal	Male Sprague Dawley and Long-Evans rats (60 - 90 days old)	Original. The experimental protocol was divided into two groups: the ingroup, consisting of animals of the same strain (Sprague Dawley), and the outgroup, consisting of animals of different strains (Sprague Dawley and Long-Evans). The opening of the restraining box was evaluated between these different groups.	Rats belonging to the same strain (ingroup) showed better performance in opening the containment box and releasing the conspecific compared to rats from different strains (outgroup).	Ingroup animals performed the conspecific release task better than outgroup animals, demonstrating an intergroup bias. This finding aligns with the literature, which shows a reduction in the release of unfamiliar animals.	[36]
2021	Heslin, Kelsey	Male Sprague-Dawley rats (9-months)	Different apparatus. The test apparatus was a square arena with choice chambers distributed on four sides with olfactory and auditory cues. The test protocol was divided into two blocks of 12 sessions.	Choice latency increased the longer the exposure to the task; there was a change in the pattern the greater the familiarity with the task	Animals preferred to choose locations that have socialization opportunities, particularly with no restrained animals.	[45]

Continued

2022	Breton, Jocelyn	Male Sprague Dawley and Long-Evans rats (60 - 90 days old) Male Sprague Dawley and Long-Evans rats (32 days old)	Original. The protocol lasted 12 days, tissue was removed for c-Fos quantification. The experimental subjects were divided into four groups: adult outgroup (different strains), adult ingroup (same strain), adolescent outgroup (different strains), and adolescent ingroup (same strain).	Adolescent rats performed similarly to adult rats in conspecific release for the ingroup condition. However, for the outgroup condition, adolescent rats performed better than adult rats, exhibiting a shorter latency in door opening.	Adolescent rats did not exhibit a bias in social behavior when faced with a trapped rat from an outgroup. Their behavior differs when interacting with ingroup versus outgroup rats, demonstrating a distinction between the groups. However, adolescents were still capable of opening the restraining box and freeing the outgroup rat.	[37]
2022	Cox, Stewart	Male Sprague Dawley rats (weighing 250 - 275 g)	Different apparatus. The animals were divided into pairs. Treatment consisted of a local anterior insula (AI) injection of baclofen/muscimol. The social contact-independent targeted helping was measured by placing a target on a wet platform and an observer on a dry platform with access to a chain that opened an automated door.	A longer latency time was observed for the free animal to release the restricted animal for the groups treated with AI injection of baclofen/muscimol in relation to the control group.	AI inhibition can reduce the process of helping a conspecific or social contact.	[48]
2022	Kitano, Kota	Male and Female voles (10 - 62 weeks old)	Modified apparatus and protocol. The protocol consisted of sessions lasting 7 days. The testing apparatus featured an arena with two chambers: one containing water and the other designated as a safe area. These chambers were separated by a transparent wall and connected by a circular door that could be opened by the subjects. The animals were divided into four experimental groups based on sex and the helper-recipient dyad: 1) male helping male, 2) male helping female, 3) female helping female, and 4) female helping male. Additionally, the wildtype and vole Oxtr KO groups were subdivided into two groups: 1) wildtype helping wildtype, and 2) Oxtr KO helping wildtype.	The results suggest that animals learned to open the door and free the trapped animal, especially when it was soaked in water. Vole Oxtr KO animals showed lower performance in helping behavior. No difference observed between the male and female animals.	The oxtr KO animals showed worse help compartment compared to the wild type. Suggesting that oxytocin is important for the development of helping behavior.	[35]
2022	Subhadeep, Duttagupta	Male Adults Wistar Rats	Modified protocol. The animals were divided into two groups: Vehicle Control (VC) and Ventral Subicularis injured (VSL). The pairs for the VC group consisted of "VCfree" and "VCtrapped" rats and the other group "VSLfree" and "VSLtrapped" rats.	"VSLfree" group did not show a pattern in door-opening behavior	The apathetic behavior caused deficit in pro-social behavior, stimulated by ventral subicular lesion	[29]
2022	Sugano, Vieira	Sprague Dawley (2 months old)	Original. The animals were divided into four groups (midazolam MDZ 2.0 mg/kg; MDZ 1.12 mg/kg; saline; not injected; all i.p.) and drugs were administered to the trapped rats. In a second experiment, the animals were anesthetized of sodium pentobarbital at a dose of 40 mg/kg. In the third experiment, two other groups, Metyrapone s.c. were administered 25mg/kg and saline. The animals were placed in an open field and remained there for 30 minutes.	Pre-treatment with MDZ, even at high doses, in trapped animals did not demonstrate significantly different results compared to controls, an asymmetry in the effects of trapped and free rats.	Decreased helping behavior may occur with reduced emotional signals by the trapped rat	[27]
2023	Wu, Wen-Yi	Female Long-Evan Rats (9 - 20 weeks),	Same apparatus as the original test. The animals were grouped in conspecific trapped, toy rat and empty box. They implanted microwire array electrodes on Cingulate Cortex Area (ACC) and Insular Cortex (InC) to quantify and compare neuronal activities of this areas during the helping test and an emotional contagion test (observational distress x self-nociception distress)	Both ACC and InC neurons were more activated on groups with conspecific trapped when compared with control groups (toy rat or empty box). Also, the same neural circuit was activated on the Emotional Contagion test (observational distress induced by laser heat pulse). The authors suggests that ACC had more neurons with mirror properties than InC.	The neural circuit that explains empathy-related helping behavior could be related to the same neural circuit that explains emotional contagion in Rats.	[41]

Continued

2024	Cox, Stewart	Male and Female Sprague Dawley Rats	Different protocol and apparatus. The behavioral tests were divided into Exp1: Social contact-dependent release task and Exp2: Social contact-independent targeted helping task. Immunohistochemical analysis for c-Fos were performed at two different times: Early Acquisition (EA) and Late Acquisition (LA).	No differences were found between male and female for the release of a conspecific with social contact, but there was a difference over the course of the day. In the second experiment, males demonstrated faster chain pulls when compared to females, but there was no time x sex difference	Similar behavioral results, may have different neural mechanisms	[30]
2024	Nugroho, Dwi	Male Campbell's Dwarf hamsters, 3 to 4 weeks old	Different protocol and apparatus. A subject was placed in the center of the experimental chamber for his daily session. Photographs of their movements in the chamber and in the open tube were taken every 10 s during a 20-min session (120 photographs/session). This regimen continued until all subjects had completed their session. Five daily 20-minute sessions defined the experience.	The animals spent more time in the tube quadrant than would be predicted, but outside of it	The results showed that the animals prefer to stay in the tube, in contrast to other studies that suggest staying in the tube is unpleasant for rodents.	[39]

3. Empathy or Desire for Social Contact?

The original study that proposed the behavioral task showed that laboratory rats exhibit helping behavior, releasing their trapped co-specifics from the aversive situation [17]. However, among the studies that attempted to address prosocial behavior using this task there is controversy in the interpretation of the results. Specifically, the major motivator to perform helping behavior is questioned: is it empathy or simply desire for social contact? In order to address this issue, different authors proposed changes to the original protocol, which prevented or limited social interaction between animals after release.

Silberberg, Allouch, Sandfort *et al.* [18] used a modified open field where the restraint box was connected to two chambers, one where the animal remained free and another that remained empty and prevented social interaction between the animals. Two different paradigms were conducted: 1) the trapped animal, when released by the free animal, could remain in the same chamber as the conspecific that released it; 2) after release, the animals remained in separate chambers. The results showed that the opening latency (time until the rat opens the door releasing the trapped companion) decreased across sessions only when the animals could remain together after release [18].

Hachiga, Schwartz, Silberberg *et al.* [19] used an E maze instead of an open field, and the free rat placed in the maze was submitted to three different paradigms: 1) choosing between an empty restraint box and a restraint box containing a trapped conspecific (released upon entrance of the free rat in the arm); 2) choosing between the arm with the trapped rat and an arm with a free animal and 3) choosing between an empty restrain box and an arm with a free animal. The results showed that rats 1) preferred the animal trapped in the restraint box over the empty box; 2) showed no preference between trapped or free rat and 3) preferred the free animal to the empty box [19].

Exploratory behavior is natural for rodents and is expressed when faced with novelty, such as a new object in the environment [20]. The results found by Hachiga, Schwartz, Silberberg *et al*. [19] reflect this exploratory drive, indicating a greater interest in the exploration of the box containing the trapped companion than the empty box. However, differences were found in the comparison of the exploratory behavior between the situation with the free animal and the trapped animal. Thus, overall, that study supports the hypothesis that the animals are motivated by desire of social contact or exploratory drive, rather than empathy, when performing the alleged helping behavior.

For other authors, however, the main motivator for the free animal to perform the opening of the door is the empathic ability [17] [21]-[24]. This discussion is supported mainly by the observation of an emotional feature involved in performance of the behavior. Bartal, in another work published by the group, investigated whether the previous administration of a benzodiazepine anxiolytic would interfere with the release of the conspecific. The results showed that the animals that received anxiolytic treatment did not show a decrease in the latency to open the door across sessions, different from the control group that did not receive the drug [22]. Benzodiazepines potentiate GABAergic neurotransmission, which is involved in information processing in the amygdala, one of the brain regions responsible for emotional control in mammals [25]. Thus, the administration of benzodiazepines may have influenced helping behavior by impairing emotional processing.

In the study by Tomek, Stegmann and Olive [26] the animals were exposed to a similar protocol to that used by Ben-Ami Bartal, Decety and Mason [17]. However, in this case, the free animal could choose between self-administering heroin or freeing the companion from the restriction box. The results demonstrated that opioid use interfered with helping behavior, *i.e.*, the animals preferred to self-administer the opioid instead of releasing the conspecific. However, when the animals that had previously received heroin were without access to the drug, they released the companion, suggesting that helping behavior has a positive reinforcement for the free animal.

Finally, Cox and Reichel [24] showed that animals open the door consistently even when they are prevented from interacting, and Silva, Silva, Lima *et al*. [13] showed that efficiency in performing opening behavior does not correlate with amount of social interaction after release. Thus, although the releasing of the conspecific may be stimulated by the desire for social contact, it is not the only motivator for the helping behavior, and there is evidence for the involvement of at least emotional aspects of empathy.

4. Adapted Protocol: Variation in the Aversive Stimulus

The presence of an aversive situation is necessary for the manifestation of helping or cooperative behavior motivated by empathy. Thus, some authors proposed changes to the original protocol to vary the aversiveness of the situation to which the confined animal is subjected. One of these variations was the addition of water to the chamber the animal in distress would be kept [21] [24].

Using this adaptation, Sato, Tan, Tate *et al*. [21] observed that rats opened the door, releasing the conspecific from the stressful situation (chamber with water) and the latency to open the door decreased throughout the sessions. The same was not observed when animals were free in a safe chamber (without a stressful condition). This outcome confirms that a stressful situation is necessary to stimulate opening behavior, suggesting an empathic motivation to open the door to relieve the other animal's stress [21].

Kandis, Ates, Kizildag *et al*. [23] also used this adaptation and showed that the administration of paracetamol (an anti-inflammatory drug) in high doses (100, 200 or 400 mg/kg) caused a decrease in opening behavior, as well as the administration of midazolam (MDZ) in trapped rats can also change these behaviors [27] [28]. In addition, pharmacological inactivation of some brain structures, such as the ventral subiculum [29] or the anterior insula [30], decreases social interaction and increases the latency for releasing of the conspecific.

Accordingly, paracetamol administration has been correlated with decreased empathic response to pain and decreased emotional processing [31] [32]. In addition, human neuroimaging studies have shown a difference in the pattern of brain activity in regions such as the prefrontal cortex and amygdala between healthy subjects and individuals with autism (condition in which the individual presents empathic impairments and socialization deficits) [9].

In addition, the levels of oxytocin and vasopressin, important modulators of prosocial and empathic behavior in mammals [33], were reduced in the prefrontal cortex and amygdala of the animals that received the drug. Indeed, animals that received oxytocin prior to exposure to the apparatus demonstrated better performance in prosocial behavior compared to the control group [34]. Accordingly, oxytocin knockout (Oxtr KO) animals exhibited a decrease in performance when compared to wildtype animals [35].

Emotional contagion plays a pivotal role in eliciting compassionate and altruistic responses to the perception of others. Recent studies have demonstrated that animals are more inclined to helping cage mates (family members) compared to unfamiliar subjects [36]. However, age-related biases in helping behavior may not be consistently observed across all age groups of helpers. Breton, Eisner, Gandhi *et al*. [37] showed that adolescent animals can help distressed conspecifics regardless of group identity, differing from adult animals in this respect. This indicates that adolescent animals have not yet developed biases in social behavior.

These results reaffirm the need of an aversive situation to evoke helping behavior in laboratory rodents. Furthermore, with this new adaptation in the protocol, we can infer that variation in the intensity of the aversive stimulus can modulate the efficiency of prosocial behavior (releasing the conspecific). However, no studies have specifically addressed this issue by comparing the response to different levels of aversive situations.

5. Emotional Feature of Empathic Behavior

To support the idea that empathic ability is the main motivator for helping behavior, some authors point out that there is an important emotional component in performing the task. Indeed, it has been shown that the administration of a benzodiazepine causes an increase in the latency to open the door, suggesting that the helping behavior depends on emotional arousal [22]. Also, Carvalheiro, Seara-Cardoso, Mesquita *et al*. [38] observed that when a non-pharmacological option to reduce stress is offered to the free rat (escape to a dark area of the arena), the helping behavior was reduced (the latency for opening was higher). In addition, recent work [39] discusses that trapped animals may have a preference to remain in the box, in contrast to the statement that it would be an aversive situation. Furthermore, it was also observed that animals open the door consistently when they observe their cage mate in a very aversive situation (chamber with water), suggesting the existence of emotional contagion between animals prior to the releasing [24].

The involvement of emotional processing in prosocial behaviors motivated by empathy is already well defined. Some authors prefer to differentiate the term empathy in "affective empathy" and "cognitive empathy". The first would be related to sharing an emotional state and, consequently, to neural circuits linked to emotional contagion. Those circuits would be activated when someone observes (and identifies with) another person's emotional state. On the other hand, the second would better answer the question "how can we infer about someone else's thoughts?". This ability would involve brain regions such as prefrontal cortex and mental states like perspective-taking [40]. Thus, the manifestation of empathic behavior can occur in different ways, involving basic and primitive processes, such as emotional contagion, and complex processes, such as perspective-taking [5]. While the former has been shown unequivocally for laboratory rodents, the latter is still a matter of debate. Importantly, if even these animals present those cognitive aspects of empathy, it is not clear if this would be a direct motivator for helping behavior.

Still supporting the idea of an important emotional feature that sustains behavior, the experiments conducted by Carvalheiro, Seara-Cardoso, Mesquita *et al*. [38] elucidated that a positive emotional state and proactive behavior of the free rat were more predictive of door opening than negative emotional state of the trapped rat. In their protocol they used an adapted arena, with a light area containing the trapped rat and a dark area that the free rat could escape to. The proactive behavior was measured by the number of transitions between the light area and dark area, and negative emotional state was measured by struggling behavior of the trapped rat [38]. Following this reasoning, Wu, Yiu, Ophir *et al*. [41] suggest that the neural circuit underlying empathy-related helping behavior can be the same neural circuit that underlies emotional contagion in ats. They showed that cingulate cortex and insular cortex neurons were both activated on observational distress test and on helping behavior test.

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by behavioral alterations, including impairments in social communication and social interaction. In this context, the use of animal models can be an effective tool to investigate the empathic behavior characteristics associated with ASD [42]. Animal models of autism were tested using the protocol established by Ben-Ami Bartal, Decety and Mason [17], demonstrating reduced performance in the development of helping behavior compared to the control group. These animals exhibited delays in starting the task of opening the containment box and releasing the cage mate [43].

6. The Role of Task Learning

As discussed above, the evidence to date suggests that different motivators can may drive restraining box opening behavior, and not exclusively an empathic ability of animals [13] [44]. Among the premises of this discussion is the observation that the decrease in latency to open the door across sessions depends on the content of the box, or an opportunity for social interaction [45]. This decrease in latency overtime is consistent across studies and resembles classical learning curves. Thus, the hypothesis arises that learning the task as an important determinant for the expression of this behavior.

Blystad, Andersen and Johansen [44] attempted to modulate the learning of door opening behavior. For this purpose, they use a conditioning protocol with food as a positive reinforcement, until they observe that the behavior becomes part of the behavioral repertoire of the rats. When the animals were tested in the paradigm containing a conspecific trapped in the restrictor box, they opened the door more consistently and earlier when compared to what was initially observed by Ben-Ami Bartal, Decety and Mason [17]. Thus, they emphasize the importance of learning to the effectiveness of opening behavior and suggest that the content inside the box will determine the success of the task, being the presence of a conspecific or food, both reinforcing factors for learning [44].

Silva, Silva, Lima *et al.* [13] reaffirmed this discussion. Those authors observed that rats continued to open the restriction box even when it was empty, if they had been previously exposed to the situation with the trapped conspecific. Those results corroborated the hypothesis that task learning is a determining factor for the execution of opening behavior. However, the presence of a conspecific animal was necessary for the first occurrence of the behavior, since the animals exposed first to the empty box did not perform it [13]. Thus, the idea of multiple motivators for the helping behavior test is reinforced.

7. Conclusions

The behavioral task originally proposed by Ben-Ami Bartal, Decety and Mason [17] assesses helping behavior among rodents. This original study, as well as others that followed it, show a consistent pattern of this behavior, that is, the opening rate increases and the latency to open decreases over repetitions of the situation. These results reflect the effectiveness of helping behavior in these animals, but the motivation behind the behavior is still not well defined.

The possible motivators for helping behavior identificed so far were empathy (at least its emotional component), the desire for social contact and task learning as strong determinants for the execution of the task. To clarify this question, more studies are needed. For example, protocols focused on evaluating neurobiological mechanisms underlying helping behavior in laboratory rodents can help elucidate the factors involved in the release of the trapped conspecific. Nevertheless, the helping behavior test proposed by Ben-Ami Bartal, Decety and Mason [17] is a consistent and promising approach for the study of empathy and prosocial behavior in laboratory rodents, and for modeling human conditions that involve deficiencies in these phenomena.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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