Helping Behavior in Prairie Voles: A Model of Empathy and the Importance of Oxytocin

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26 **Abstract**

27 Accumulated evidence across animals suggests that helping behavior is
 28 triggered by empathy. We examined whether prairie voles, one of the more highly
 29 social animals, help conspecifics who are soaked in water by opening a door to
 30 a safe area. Door-opening latency decreased as task sessions progressed,
 31 suggesting that prairie voles learn helping behavior. In addition, when the
 32 conspecific was not soaked in water, the latency of the door-opening did not
 33 decrease, suggesting that the distress of the conspecific is necessary for the
 34 learning of the door-opening. Thus, the door-opening behavior in prairie voles
 35 can be considered a helping behavior that is motivated by empathy for the
 36 distress of conspecifics. We also examined the helping behavior in prairie voles
 37 in which oxytocin receptors were genetically knocked out. Oxytocin receptor
 38 knockout voles demonstrated impaired learning of the door-opening. This
 39 suggests that oxytocin is important for the emergence of helping behavior.

40

Introduction

Empathy is an innate ability to experience and share the mental state of others (Decety et al., 2016; de Waal & Preston, 2017; Preston & de Waal, 2002; Meyza et al., 2017). In mammals, prosocial behaviors such as helping and consolation are essential for the development of society and are thought to be elicited by empathy (de Waal & Preston, 2017). The existence of empathy has been suggested in a variety of species, including non-human primates (Campbell & de Waal, 2011; Koski & Sterck, 2010; Pruetz, 2011), dogs (Palagi et al., 2015) birds (Gallup et al., 2015), and even rodents (Bartal et al., 2011; Bartal et al., 2016; Sato et al., 2015). Several studies have examined empathy in rodents using behavioral indicators, such as vicarious learning of fear (Atsak et al., 2011; Pisansky et al., 2017) and helping behavior like releasing others from a distressed situation (Bartal et al., 2011; Bartal et al., 2016; Sato et al., 2015; Yamagishi et al., 2019). However, understanding of empathy in rodents is still inadequate. It has been shown that helping behavior, in which an actor pays a cost and gives a benefit to others, is based on empathy. Helping behavior has been observed in animals that are considered to be highly intelligent, such as chimpanzees (Pruetz, 2011; Yamamoto et al., 2012), elephants (Schulte, 2000), and dolphins (Kuczaj et al., 2015). Recent studies suggest that rats also demonstrate helping behavior to conspecifics in distress, such as rats in narrow tubes (Bartal et al., 2011; Bartal et al., 2016) and water (Sato et al., 2015; Yamagishi et al., 2019). Detecting others' distress is a prerequisite for helping behavior (Cronin, 2012; Decety et al., 2016).

This suggests that rats may have an ability of social cognition that allows them to individually recognize conspecifics and an ability of empathy that allows them to perceive their distress.

Prairie voles (*Microtus ochrogaster*) are known to show more of various social behaviors, such as social bonding, nurturing, allogrooming, and huddling, compared to other rodents such as mice and rats (Aragona & Wang, 2004; Carter & Getz, 1993; Getz & Carter, 1996; Getz & Hofmann, 1986). Because of this feature, many studies on social behavior have been carried out using prairie voles (Aragona & Wang, 2004; Young & Wang, 2004). Previous studies suggest that prairie voles show empathy-like behavior, such as freezing caused by emotional contagion of conspecifics' fear and consolation (Burkett et al., 2016; Stetzk et al., 2018; Wardwell et al., 2020). However, helping behavior that rescues conspecifics from distressed situations has not been investigated in prairie voles. The purpose of this study was to investigate whether prairie voles display helping behavior using a door-opening paradigm, which has been used to examine helping behavior in rats (Bartal et al., 2011; Sato et al., 2015).

Several studies have reported that oxytocin and oxytocin receptors in rodents affect sociality, including affiliative behavior, social cognition, and empathic response (Bartz et al., 2010; Marlin & Froemke, 2017; Rogers-Carter et al., 2018; Ross & Young, 2009; Winslow & Insel, 2002; Young & Barrett, 2015). However, the role of oxytocin in helping behavior is largely unexamined except in studies that examined the effect of oxytocin administration and of blocking of oxytocin receptors in rats (Yamagishi et al., 2020; Yamagishi et al., 2019). In this study, we examined the effects of oxytocin on helping behavior using oxytocin receptor knockout (OXTrKO) voles.

Results

Prairie Voles Show Helping Behavior Quickly

Fourteen pairs of same-sex prairie voles (seven pairs of males and seven pairs of females) were tested with a door-opening task. One member of each pair was assigned to be a soaked vole, and the other was assigned to be a helper vole. The experimental apparatus consisted of a pool area and a ground area (Figure 1). The helper vole in the ground area rescued the soaked vole in the pool area by opening a circular door. The helper vole could see the soaked vole through a transparent acrylic plate to which the circular door was attached. The task session started when the helper vole was placed in the ground area after the soaked vole was placed in the pool area. We measured the latency of door-opening. After the helper vole opened the door within 10 minutes, the helper and soaked voles were allowed to interact for two minutes. Before the door-opening task, the helper voles were placed in the ground area for five minutes for two days to habituate to the experimental apparatus. Because the circular door was opened and placed on the floor of the ground area during the habituation, the helper voles could go back and forth between the ground and pool areas. We measured the helper voles' preference for water by counting time they spent in the ground and the pool areas.

Figure 1. Experimental apparatus. (A) Schematic diagram of the experimental apparatus. A helper vole could rescue a soaked vole from the distressing situation of being in water by opening the circular door. (B) A photo image during the door-opening task.

The latency of the door-opening decreased over the seven sessions in both the male and the female pairs (Figures 2A and 2B). All of the helper voles showed door-opening behavior by the fourth session (Figures 2C, S1A and S1B). A two-way analysis of variance (ANOVA) for mixed design with a between-subject factor of group (male pairs, female pairs) and a within-subject factor of session (7) revealed a significant effect of sessions ($F(6, 72) = 19.34, p < .001$). Neither a main effect of sex ($F(1, 12) = 0.09, p = .764$) nor a sex $\times$ session interaction ($F(6, 72) = 1.71, p = .131$) was observed. This suggests that the helper voles learned the door-opening behavior to free the soaked voles from the pool area, and that there is no difference in learning of helping behavior between male and female voles. During the habituation period, the helper voles stayed longer in the ground area (550.50 ± 6.54 s, mean $\pm$ SD) than the pool area. The time spent in the ground area was significantly longer than the expected value (300 s, half of the habituation period, $t(13) = 36.93, p < .001$). This suggests that prairie voles have

an aversion to water, and that the helper voles rescue the soaked voles from distress by opening the circular door.

Figure 2. Behavioral results. (A) The mean latency of door-opening in helper voles of the male and female pairs of wildtype voles. The error bars indicate the standard error of means. (B) The mean latency of door-opening in helper voles of all groups. (C) The percentage of door-opening pairs of all groups. (D) The mean duration of huddling after opening the door in the wildtype and OXTrKO voles. The error bars indicate the standard error of means.

Figure supplement 1. Individual data (Figure 2 – figure supplement 1).

Prairie Voles Demonstrate Less Helping Behavior When Cagemates Are Not Soaked in Water

In the first experiment, the door-opening behavior was potentially learned through factors other than empathy, for instance, social interaction. We tested the door-opening behavior of the helper voles when the cagemate was not soaked in water to examine whether helping behavior is motivated by empathy. The helper voles ($n = 8$), which were different from those used in the previous experiment, did not demonstrate a substantial decrease in the latency of door-opening when their cagemate was in the ground area instead of soaked in water (Figures 2B and S1C). A one-way ANOVA revealed no main effect of sessions ($F(6, 42) = 0.98$, $p = .454$). Next, we compared the latencies for the door-opening behavior when the cagemates were soaked in water (data of the aforementioned experiment) with those when they were on the ground. A two-way ANOVA for mixed design with a between-subject factor of group (cagemate soaked in water, cagemate not soaked in water) and a within-subject factor of session (7) on the latency of door-opening behavior revealed main effects of group ($F(1, 20) = 12.49$, $p = .002$), session ($F(6, 120) = 8.20$, $p < .001$) and group $\times$ session interaction ($F(6, 120) = 2.31$, $p = .038$). Simple main effects analysis showed no significant difference in sessions when cagemates were not soaked in water ($p = .701$). This suggests that the learning of the door-opening behavior is prevented when cagemates are not soaked in water. Thus, the negative emotions of the cagemate in the aversive situation may be necessary for the learning of door-opening behavior in the helpers.

Oxytocin Receptor Knockout Voles Demonstrate Less Helping Behavior

To investigate the impact of the elimination of oxytocin on helping behavior, we compared the learning of door-opening behavior in oxytocin receptor knockout (OXTrKO) voles with that in wildtype voles. The OXTrKO voles were homozygous for the knocked out oxytocin receptor gene. The OXTrKO voles were paired with wildtype littermates. All OXTrKO voles ($n = 8$) were assigned to be helpers.

The OXTrKO voles did not demonstrate a consistent decrease in the latency of door-opening behavior (Figure 2B). In addition, two voles did not show consistent door-opening after the fourth session (Figures 2C and S1D). A one-way ANOVA revealed a significant effect of sessions ($F(6, 42) = 5.84, p < .001$). However, Holm's multiple comparisons revealed no significant difference in the latency of door-opening between all the sessions. Next, the latency of door-opening was compared between the OXTrKO and wildtype voles (data from the first experiment). A two-way ANOVA for mixed design with a between-subject factor of group (OXTrKO, wildtype) and a within-subject factor of session (7) revealed main effects of group ($F(1, 20) = 27.93, p < .001$) and session ($F(6, 120) = 18.84, p < .001$), while the effect of group $\times$ session interaction was close to significant ($F(6, 120) = 2.01, p = .070$). These results suggest that the OXTrKO voles demonstrate less helping behavior than the wildtype voles.

We measured the duration of huddling as an indicator of social attachment in the OXTrKO and wildtype voles (Figure 2D). Huddling was defined as touching part of each other's trunks and was measured for two minutes during

the interaction period after the door-opening. A two-way ANOVA for mixed design with a between-subject factor of group (OXTrKO, wildtype) and a within-subject factor of session (7) on the duration of huddling revealed a main effect of group ($F(1, 20) = 9.49, p = .006$). Neither a main effect of session ($F(6, 120) = 1.51, p = .181$) nor a group $\times$ session interaction ($F(6, 120) = 0.66, p = .685$) was observed. This suggests that oxytocin affects social attachment in prairie voles.

Discussion

In this study, we investigated helping behavior in prairie voles using the door-opening paradigm. The results demonstrated that the helper voles quickly learned the door-opening behavior to free the soaked voles from water. However, the prairie voles did not immediately learn to open the door when the cagemates were not soaked in water as compared to when they were soaked. This suggests that the fact that the cagemate is in a distressed situation is necessary for the learning of door-opening behavior. We also found that the OXTrKO voles demonstrated less learning of door-opening behavior than the wildtype voles. This suggests that oxytocin is important for the emergence of helping behavior.

Helping behavior has been observed in rats when conspecifics are distressing situations, such as being trapped (Bartal et al., 2011) or soaked in water (Sato et al., 2015). It has been explained that helping behavior is learned through emotional contagion, a form of empathy, which involves sharing the distress of others (Bartal et al., 2011; Decety et al., 2016; Sato et al., 2015). In

the present study, the helper vole could see the soaked vole through a transparent acrylic plate. This suggests that prairie voles display helping behavior that is driven by perceiving their conspecifics' distress. When the helper vole observed the soaked vole in distress, the helper vole might have shared the soaked vole's distress through emotional contagion and this might have motivated the helper vole to open the door (Sato et al., 2015).

In the present study, soaking in water was set as an aversive situation. In the preference test, the helper voles stayed much longer in the ground area than the pool area, even when the helper voles could go back and forth between the ground and pool areas. This suggests that prairie voles have an aversion to water and is consistent with previous studies that reported water aversion in rodents (Morris, 1984; Sato et al., 2015).

When cagemates were not soaked in water, prairie voles learned less helping behavior. There is an argument that in rats, social interaction instead of empathy motivates door-opening behavior (Silberberg et al., 2014), although this has been rebutted (Sato et al., 2015). In this study, the helper and soaked voles were able to socially interact after the door was opened. Nevertheless, when the cagemate was not soaked in water, the learning of door-opening in the helper voles was not evident, although the helper and the cagemate could interact with each other after the door-opening, because the situation in which the cagemate was not distressed did not induce empathy in the helper vole. This implies that empathy for distressed others, rather than social interaction, is important for

helping behavior in prairie voles. Previous studies have also shown that learning of helping behavior is suppressed in rats when others are not in distressing situations (Bartal et al., 2011; Sato et al., 2015). Additionally, it was suggested that helping behaviors may occur even when the effects of social interaction are excluded (Cox & Reichel, 2019). These studies support the view that empathy for the distress of others may be more influential than social interaction as a trigger for door-opening behavior.

In this study, all helper and soaked voles were littermates. Various factors of social context are involved in empathy (Bartal et al., 2014), such as familiarity and kinship. A previous study examining the emotional contagion of pain in mice suggests that pain of familiar individuals is more contagious than that of strangers (Langford et al., 2006). Prairie voles exhibit allogrooming that functions as social buffering to relieve the fear of their partner. The allogrooming is observed more with a cagemate or a sibling than with a stranger (Burkett et al., 2016). Since the prairie voles in the current study were littermates, the door-opening behavior might have been learned more easily. It is unclear whether prairie voles exhibit helping behavior with strangers. The effects of familiarity and kinship on helping behavior in prairie voles should be investigated in the future.

Oxytocin works as a hormone and as a neurotransmitter, which is involved in a variety of socialities, such as nurturing, familiarity, empathy, and social attachment (Bosch & Neumann, 2012; Decety et al., 2016; Insel, 2010; King et al., 2016; Rodrigues et al., 2009; Young & Wang, 2004). Many studies have shown that oxytocin and oxytocin receptors are involved in a variety of social

behaviors in humans, non-human primates, and rodents (Amico et al., 2004; Anacker & Beery, 2013; Ferguson et al., 2000; Pobbe et al., 2012; Rich et al., 2014). Especially in rodents, previous studies indicated that oxytocin gene knockout caused a lack of social recognition (Bielsky & Young, 2004; Ferguson et al., 2001), formation of social bonds (Liu & Wang, 2003; Young & Wang, 2004), and social rewards (Dölen et al., 2013; Hung et al., 2017). High sociality in prairie voles may be related to a higher density of oxytocin receptors in the brain as compared to close relative species (Olazábal & Young, 2006; Ross et al., 2009). A previous study reported that oxytocin receptor knockout voles demonstrated autism-like behavior such as a lack of interest in social novelty (Horie et al., 2019). In the present study, the decrease in the latency of the door-opening in the OXTrKO voles was slower than in the wildtype voles. The duration of huddling in the OXTrKO voles was also shorter than that in the wildtype voles. These findings suggest that blocking the function of oxytocin has a significant impact on helping behavior and social attachment. Nurturing and social play behavior in oxytocin knockout or oxytocin receptor knockout rodents as well as wildtype rodents are influenced by social context (Bredewold et al., 2014) and sexuality (Nishimori et al., 1996; Young III et al., 1996; Zimmermann-Peruzatto et al., 2017). Helping behavior in oxytocin knockout voles may also be affected by social context and sexual interaction. Future studies will examine the interaction between the oxytocin receptor knockout and these social elements.

Oxytocin has been gaining attention for its effects on social cognition in

recent years. Specifically, several studies have reported that intranasal administration of oxytocin can alleviate some symptoms of autism (Guastella et al., 2010; Hollander et al., 2003; Preti et al., 2014). The present study implies a relationship between helping behavior and oxytocin in prairie voles. Further studies will be needed to manifest the details, and they will shed light on the mechanisms of social cognition and related psychological disorders. Experiments in prairie voles will be beneficial for examining social cognition functions.

288 Materials and Methods

289 Key Resources Table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
strain, strain background and (<i>Microtus ochrogaster</i>)	Wildtype and OXTrKO prairie voles	Kwansei Gakuin University	N/A	
Gene (<i>Microtus ochrogaster</i>)	OXTr	Horie et al. (2019)	Gene ID: 101979991	
Sequence-based reagent	OXTr_F	FASMAC	N/A	5'-AGA TCA GTG CCC GGG GGG TGC CC-3'

Sequence -based reagent	OxTr_R	FASMAC	N/A	5'-TCG AGC GAC ATA AGC AGC AG-3'
software, algorithm	Excel	Microsoft Office	https://www.mi crosoft.com/	
software, algorithm	R	R foundation	https://www.r- project.org/	
software, algorithm	Rstudio	R Studio	https://www.rst udio.com/	

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291 Subjects

292 We used 44 experimentally naïve wildtype (30 males and 14 females)
 293 and 16 naïve oxytocin receptor knockout (OXTrKO, 8 males and 8 females)
 294 prairie voles (*Microtus ochrogaster*) maintained at a vivarium at Kwansei Gakuin
 295 University. The wildtype voles were captured in Illinois, USA. The OXTrKO voles
 296 were generated in Tohoku University (v4 strain in Horie et al., 2019) and bred in
 297 Kwansei Gakuin University. At the beginning of the experiments, the mean age
 298 of the prairie voles was 22.6 weeks (range: 10–62 weeks) and the mean weight
 299 was 38.3 g (range: 25–63 g). They were housed in plastic home cages (320 ×
 300 212 × 130 mm, or 425 × 265 × 155 mm) with paper-chip bedding. The average
 301 number of prairie voles per cage was 3.8 (range: 2–6). Prairie voles in the same
 302 cage were littermates and were individually marked by ear punches. Helper and

soaked roles were randomly assigned to individuals in the same cage. These pairs, consisting of one helper and one soaked vole, were all same-sex pairs. All animals were allowed free access to food (Labo MR Stock, NOSAN, Japan) and water. The vivarium was maintained at a constant temperature of 22 °C and 60% humidity. The light-dark cycle was set to 12:12 h with lights on at 9:00 am. This experiment was approved by the Animal Experimentation Committee of Kwansei Gakuin University (2018–44, 2019–06).

Genotyping

OXTrKO voles were created in Horie et al. (2019). Genotyping to discriminate between wildtype (OXT+/+) and OXTrKO voles was done using the method of Horie et al. (2019). To summarize, we collected samples from prairie voles' ear tissues and used them for a polymerase chain reaction. In the polymerase chain reaction, the forward polymer was 5'-AGA TCA GTG CCC GGG GGG TGC CC-3' and the reverse polymer was 5'-TCG AGC GAC ATAAGC AGC AG-3'. We used the prairie voles that were homozygous for the knocked out oxytocin receptor gene in the experiment.

Experimental setup

The experimental apparatus was similar to that used in Sato et al. (2015). The size of the experimental box was smaller (240 × 120 × 210 mm) and was made of gray polyvinyl chloride boards (5 mm in thickness). It consisted of ground

and pool areas separated by a transparent acrylic plate (5 mm in thickness). During the experiment, the pool area had water with a depth of 25 mm to create an aversive situation for the prairie voles. The transparent acrylic plate had a circular hole (51 mm in diameter). On the ground area side, the hole was covered with a circular door (60 mm in diameter, 3 mm in thickness). The door completely separated the ground area from the pool area. A helper vole in the ground area opened the door, allowing a soaked vole in the pool area to escape to the ground area. The difficulty of opening the circular door was set to a constant level by holding it between two transparent acrylic fragments (Figure 1A). One pushed up the circular door with springs from below and the other was attached to the transparent acrylic plate. To prevent soaked voles from interfering with the circular door, a thin transparent sheet was applied from the pool area to the transparent acrylic plate. The transparent sheet had three small holes in the area that overlapped the circular door. After the start of the experiment, two thin transparent plates (150 × 150 mm, 5 mm in thickness) were placed on top of the experimental apparatus.

Habituation

Before the experiment, we habituated the helper voles to the experimental apparatus. The habituation was performed for 10 minutes per trial for two days for each helper vole. During the habituation, the door was detached from the dividing plate and was laid on the floor of the ground area. The helper

voles could move freely between the ground and pool areas during the habituation. The pool area was filled with water.

Task procedure

Before the door-opening task, the experimental apparatus was cleaned with a 20% alcohol solution, the circular door was placed on the plate, and water was poured into the pool area to a depth of 25 mm. Immediately after the soaked vole was placed in the pool area, the helper vole was placed in the ground area. We measured the latency of the door-opening from the placement of the helper vole in the ground area. A trial of the task was carried out for a maximum of 10 minutes. If the helper vole opened the door in 10 minutes, we pulled out the sheet attached on the pool side of the plate to allow the soaked vole to escape to the ground area and the two voles to interact with each other. The duration of the interaction was two minutes. If the helper voles did not open the door within 10 minutes, the experimenter slightly opened the door to the right side and continued the trial for five more minutes. When the door-opening behavior was observed during those five minutes, the transparent sheet was removed to allow for interaction. If the helper voles did not open the door in the extra five minutes, the experimenter opened the circular door completely and removed the sheet to allow the interaction. This door-opening task was carried out in one trial per day, for a total of seven days.

Data analysis

We analyzed the latency of door-opening during the first 10 minutes of the task. If the helper voles did not open the door for 10 minutes, the latency of door-opening of the trial was recorded as 600 seconds. We also measured whether the helper and soaked voles displayed huddling in two minutes of the interaction period after the door-opening. Huddling was defined as touching parts of each other's trunks. During the habituation of the helper voles, we measured the time that the helper voles stayed in the ground and pool areas. The experiment was recorded by a video camera (HDR-CX590, Sony) mounted above the experimental apparatus. RStudio (version 1.1.453) was used for all statistical analyses.

Data and code availability

Data and software can be obtained from the corresponding author on request.

Acknowledgments

This work was supported by JSPS KAKENHI Grant Numbers 16H01490 and 18K07357 and the Ministry of Education, Culture, Sports, Science and Technology (MEXT), JAPAN, for the Strategic Research Foundation at Private Universities (2015-2019; Project number S1511032) to the Center for Applied Psychological Science (CAPS), Kwansei Gakuin University.

Competing interests

The authors declare no competing interests.

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Figure 2 – figure supplement 1. Individual data. (A) Individual latency of door-opening in the male pairs of wildtype voles. (B) Individual latency of door-opening in the female pairs of wildtype voles. (C) Individual latency of door-opening in the wildtype voles when the cagemate is not soaked in water. The subjects are different individuals from those in A and B. (D) Individual latency of door-opening in the OXTrKO voles.