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Review article

Behavioral and mechanistic insight into rodent empathy

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ABSTRACT

Empathy is a psychological construct that allows individuals to understand and share the emotions of others. The ability to share emotional states relies on basic social mechanisms, such as mimicry and emotional contagion, which are considered building blocks for empathy. Mimicking another's emotional or physical state is essential for successful social interactions and is found in a number of animal species. For the current review we focus on emotional state sharing in rodents, a core feature of empathy that is often measured using pain and fear as proxies; we also discuss prosociality in rodents. The evidence for empathy in rodents shows that rats and mice consistently imitate arousal states and behaviors of conspecifics and will even sacrifice personal gain to relieve the distress of a conspecific. These behaviors support basic processes that are crucial for the survival of individual animals and give us insight into the neural mechanisms that govern empathy-related behaviors.

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1. Introduction

Human societies are based on the formation and maintenance of social relationships. Successful relationships are necessary for acquiring resources, achieving social status within a group, and reproduction, which makes our capacity to understand the emotions of others vital for individual survival. In this regard, empathy-like behaviors are evolutionarily conserved among species and have been described as having ultimate and proximate causes (Preston and de Waal, 2002). *The ultimate causes* of empathy explain the evolutionary advantages of emotional state sharing, such as a greater capacity for learning from social situations (e.g., social cooperation, mentoring, alerting to threats). While the *proximate causes* correspond to the immediate environmental contexts that trigger empathy, such as psychological, neural, and physiological triggers. Here, we delve into the proximate causes of empathy with a primary focus on rodent models. Accordingly, it must be kept in mind that proximate mechanisms possibly originated because they are/were in some way beneficial for individual survival.

Empathy is a catchall term used to capture the understanding and sharing of emotional states. Conceptually, it ranges from simple emotional behaviors that include mimicry and emotional contagion to evolutionarily more complex constructs, such as perspective taking and targeted helping (see Preston and de Waal, 2002 for a review). In this review, we refer to "empathy" as the ability

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to share or relate to the affective state of another by considering aspects of emotional contagion, social modulation of learning and helping behaviors, all essential components of empathy that are within the capability of rodents (Panksepp and Panksepp, 2013; Panksepp and Lahvis, 2011). Since empathy behaviors have been observed in species ranging from mice to elephants, the debate should no longer be about whether animals have empathy; instead, what are the mechanisms that engage it.

Empathy has been described in terms of levels with the lowest tier describing primitive social behaviors (e.g., emotional contagion and mimicry) and subsequent levels adding to the intricacy of these behaviors (Singer and Lamm, 2009). Some species are capable of multiple empathic processes (i.e. emotional contagion and prosociality), but the higher tiers (i.e. cognitive empathy) are reserved for more advanced species, including humans and non-human primates. As shown in Fig. 1., we represent the multiple components of empathy as a staircase with different species expressing different degrees of empathy-related behaviors. A similar scheme proposed by Frans de Waal (2008) used the analogy of a Russian nesting doll. In this model the inner core of the doll consists of contagion effects, with the doll's outer layers adding more complexity until behaviors such as sympathy, perspective taking, and prosocial behavior are included (de Waal, 2008). The Russian nesting doll model is elegant in its simplicity and presumes that even though more complex empathy-related behaviors are found in the outer layers of the doll, empathy still requires the inner core to be complete.

Mimicry and emotional contagion occupy the lowest tier of our empathy staircase (Fig. 1). They are described as matching behaviors, expressions, or emotional states with that of another (Preston and de Waal, 2002). For example, when someone smiles we often unconsciously smile in return, showing that the emotions expressed by one individual can be contagious to another. This does not imply that the observer perceives or understands the emotional state of another, just that the emotion is imitated when observed. There has been skepticism in the empathy field on whether mimicry and emotional contagion significantly contribute to the expression of empathy, however it can be argued that one cannot truly experience empathy for another without mimicking the other's experience (Singer and Lamm, 2009). Interestingly, neuronal activation patterns suggest that emotional contagion stimulates brain areas important for pain empathy. Participants who observed a "loved one" receiving a pain stimulus showed increased brain activation in areas that are typically associated with the affective but not the sensory component of pain (e.g. anterior insula and rostral anterior cingulate cortex (ACC)) indicating that participants were mimicking their loved one's emotional pain. In fact, the self-reported empathy levels were associated with the amount of activation in pain-associated areas, which suggests that more mimicry equates with a stronger empathy experience (Singer et al., 2004).

A higher form of empathy known as 'theory of mind' is the ability to distinguish that the perspective of another individual is different from your own. This is also known as "empathic perspective-taking" (Decety and Svetlova, 2012). Unfortunately, since perspective taking in animal species—the exception being nonhuman primates—is difficult to assess, there is virtually no convincing evidence demonstrating that rodents have this ability. As discussed below, we infer that prosocial and helping behavior studies require some form of perspective taking, but we cannot be certain. Scientists that believe rodents cannot experience empathy argue that higher cognitive processes are needed for such abilities. However, there is research to suggest that rats have higher-level processes such as metacognition, which is the ability to control one's own cognitive processes, especially when learning (i.e., thinking about thinking). Metacognition has been explored using a rat model of operant conditioning that examined decision-making in rats based on the value of information they received (Kirk et al., 2014). Rats were trained to press a lever that delivered an immediate food reward, but pressing the lever would also tell them where more food was hidden. In a maze with 2 arms (a light would turn on in the arm of the maze containing more food), lever pressing was reduced if the lever stopped delivering an immediate food reward, even though it still provided information about where other food was located. However, if the maze contained 8 possible paths instead of just 2, the rats would continue to press the lever at high rates even after the immediate reward was stopped. Presumably this was because in the 2-arm maze they had a 50% chance of choosing the right arm by simply guessing, but in 8-arm maze pressing the lever provided more valuable information and therefore it was more advantageous to continue pressing. Of course these are inferences but demonstrate that rats show information-seeking behavior (i.e., they are assessing the benefits of performing certain behaviors), which is considered a higher-level process; an ability required for higher-order forms of empathy.

2. Seminal studies

Studies conducted in the mid 1900s set the foundation for empathy-related research. One of the first rodent empathy experiments required rats to press a lever in order to obtain a food reward (Church, 1959). Then, the experiment changed so that every time the rat would push the lever, a rat in an adjacent cage received an electric shock. Subjects that previously received an electric shock showed the greatest decline in lever pressing in response to other rats being shocked (maintained for 10 days post-experiment). A control group that had never experienced shocks did not decline in lever pressing. The interpretation of this is that rats that had previously experienced shocks were empathetic to the rat in the other cage (i.e., they did not want to shock them). This was the first study that looked at the shared experience of pain in rodents and implied that a rat could recognize its own negative experience as being experienced by a conspecific. By sharing the negative experience, the effect of punishment on the helping behavior was stronger. This is indicative of the fact that rats were able to distinguish another's affective state as different from their own, an interpretation consistent with perspective-taking in rats, however, it is debatable whether rats truly have the capacity for perspective-taking.

Shortly thereafter, Rice and Gainer (1962) conducted one of the first studies that assessed prosocial behavior in rodents. They trained rats to alleviate the distress of a social partner by pressing a lever to lower a squealing conspecific that was suspended in the air. It was found that rats reliably pressed the lever to lower the squealing rat but would not press the lever to lower a Styrofoam block. This was instrumental in demonstrating that rats are capable of helping and prosocial behaviors—often considered higher order forms of cognitive empathy. However, Lavery and Foley (1963) argued that the squealing of the suspended rat may simply have been annoying, creating arousal that the lever-pressing rat chose to stop. Hence, the same results could be interpreted as mere arousal of the lever-pressing rat; in which case, lever pressing would simply be self-serving.

3. Rodent models for the study of empathy

Even though tests to assess whether rodents experience higher forms of empathy have yet to be developed, empathy-related behaviors including emotional contagion and prosociality can be tested (Ben-Ami Bartal et al., 2011; Langford et al., 2006). Behavioral assays that measure emotional contagion and prosocial behavior in rodents are well suited for understanding the neurobiological mechanisms of empathy and associated processes. To study empa-

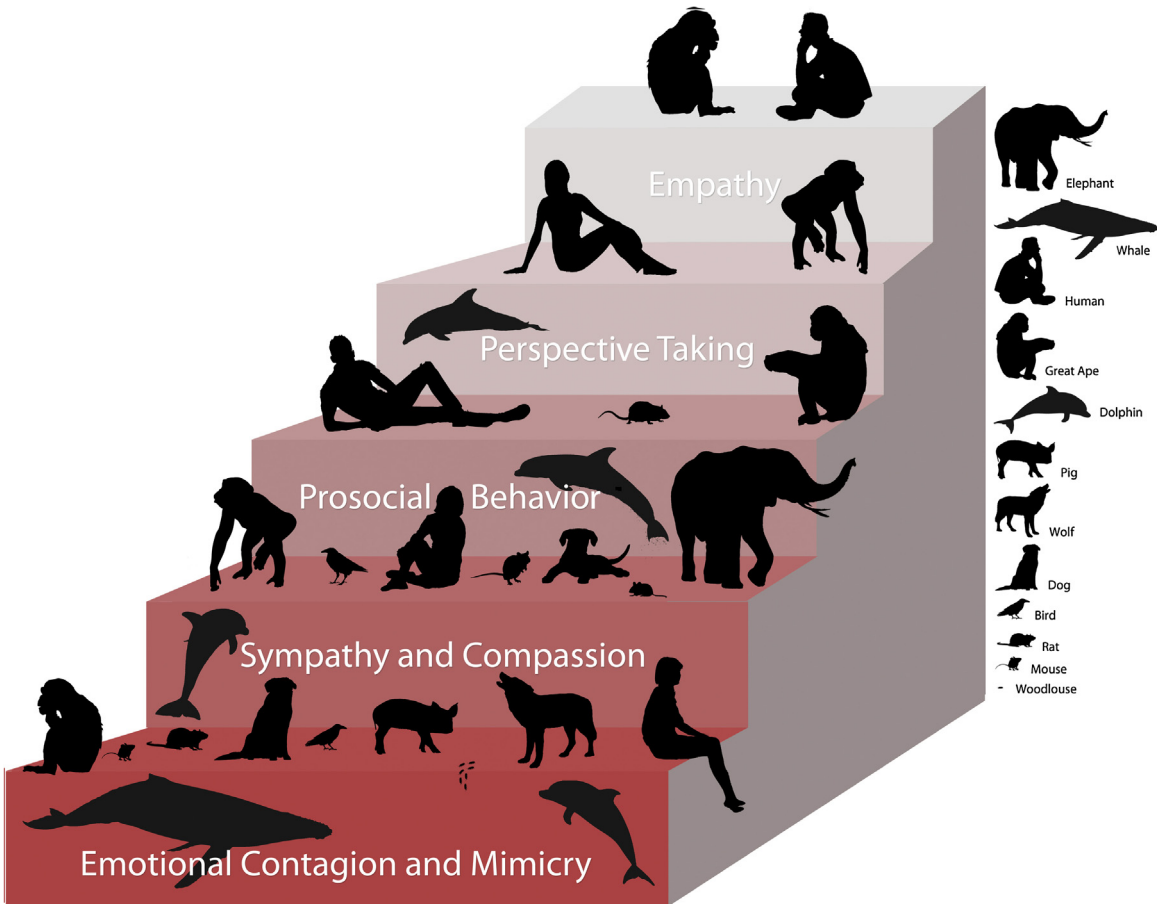

Fig. 1. The different species that display empathy-related behaviors are represented. Many instances of empathy have been recorded throughout many species, including (but not limited to) woodlice, mice, rats, canines, dolphins, elephants, non-human primates, and humans. The largest land mammal known to demonstrate empathy-like behaviors is the Asian elephant, which has been observed to recognize an upset herd mate and offer gentle caresses and chirps of sympathy. In animals, empathy-related responding could have an ulterior motive such as survival, the sharing of food, companionship and pack-oriented mentality. It is difficult to assess an animal's motivation behind an empathically driven response and for some, applying the term empathy to animal behavior is an act of anthropomorphism. This figure only offers a glimpse into the diversity in which empathy-like behaviors have been documented. For instance, we have included the woodlouse as an example of emotional contagion/mimicry in an insect, but honeybees and drosophila are also known to display signs of emotional transference. It should be noted that the animal's used for some of our examples occupy more than one level of the empathy staircase. For example, groups of dolphins will form a body raft to help support an injured or dying dolphin. This one act demonstrates sympathy, prosocial behavior and possibly perspective taking.

thy in rodents, researchers have adopted two basic approaches. One involves testing emotional contagion through experiments that invoke fear or pain and measuring how these states affect another rodent's fear or pain. The second approach measures prosocial behavior by inducing distress in a rodent and assessing an observer's helping behaviors. These two approaches will be explored in further detail below.

4. Emotional contagion: social modulation of fear and pain

There are two major subcomponents of empathy: cognitive empathy and emotional empathy (de Waal, 2008; Hoffman, 2001). Cognitive empathy is the more complex form; it includes perspective-taking and understanding the feelings of others. Since it is debatable whether rodents truly possess this ability, we do not label cognitive empathy as such, and instead focus on experiments that support perspective-taking by measuring prosocial and helping behaviors. As explained above, emotional empathy (or emotional contagion) is experiencing another's feelings or states without conscious awareness and since a core feature of empathy is the ability to share emotional experiences, a number of empathy-related studies have investigated the social transference of fear in rodents (Chen et al., 2009; Jeon et al., 2010; Kavaliers et al., 2001b). This requires the distress of another to be recognized in order to engage empathy-related circuitry. One of the first demonstrations of social fear learning involved placing a demonstrator mouse in a cage with biting flies while an observer mouse watched the demonstrator in distress (Kavaliers et al., 2001a). The next day, the observer mouse was exposed to flies whose biting appendages were removed. The observer mice that did not have experience with biting flies showed distress-like behaviors (i.e., self-burying avoidance) and enhanced analgesic responses, all behaviors previously displayed by the demonstrator mice. This indicates that mice have the ability to recognize distress in conspecifics possibly through a combination of visual and social odor exchanges (Fanselow, 1985). Intriguingly, follow-up studies have demonstrated that kinship, familiarity, and social status (dominance-subordinate relationships) are integral for mediating the behavioral responses to pain in biting flies (Kavaliers et al., 2005). This parallels vicarious fear- and pain-based paradigms, where emotional contagion is stronger among familiars and siblings (Jeon et al., 2010; Jeon and Shin, 2011; Langford et al., 2006; Martin et al., 2015).

Rodents express fear by freezing—usually in response to a foot shock, measured using classical conditioning (Martin et al., 2011). However, shock-naïve mice learn to freeze by simply observing a conspecific receive repetitive foot shocks, a mechanism promoted by social learning (Jeon et al., 2010; Jeon and Shin, 2011). In fact, observer mice have higher fear responses when observing mating

partners or siblings in distress compared to stranger mice (Jeon et al., 2010), indicating that social transfer of fear in rodents is stronger among familiars. The neural mechanisms underlying the social transfer of fear remain largely unknown, however the anterior cingulate cortex (ACC), an area of the brain involved with pain and affect, has emerged as a likely source. The lateral pain system and medial pain system are the two primary pain-processing pathways that relay signals via the thalamic nuclei of the central nervous system (Price, 2000). Social fear learning requires the ACC and the parafascicular and mediodorsal thalamic nuclei, components of the medial pain system. Inactivation of these structures with lidocaine significantly disrupted social fear learning. In contrast, inactivation of the ventral posteromedial (VPM) and ventral posterolateral (VPL) thalamic nuclei, which belong to the lateral sensory pain system, did not affect observational fear learning. This indicates that observational fear learning is dependent on the affective but not the sensory component of pain transmission. Furthermore, an ACC-restricted deletion of Cav1.2 Ca^{2+} channels in mice impaired social fear learning and reduced behavioral pain responses, demonstrating the functional involvement of the affective pain system and Cav1.2 channels of the ACC in observational social fear (Jeon et al., 2010). Observational fear learning has also been shown to activate the dorsomedial prefrontal cortex–basolateral amygdala pathway by increasing the amplitude and slowing the decay of NMDA receptor-mediated currents as well as generating silent synapses (Ito et al., 2015).

The social transference of fear has also been demonstrated to occur between rats in absence of observation. Following fear-conditioning, a brief social interaction between a shocked demonstrator and a naïve cagemate observer increased freezing behavior in the latter. In the absence of observation, the demonstrator's fear was socially transferred to the observer, resulting in a rapid increase in freezing behavior of the observer and neuronal activation throughout the observer's amygdala—including the central, medial, lateral, basal and basomedial nuclei—that parallels neural activation in the amygdala of shocked demonstrators (Knapska et al., 2006). The extent of neural activation (as measured with cFOS labeling) was not driven by acute stress or changes in pain sensitivity and was observed the next day in naïve rats that were familiar with demonstrator rats (Knapska et al., 2010). In addition, when naïve rats socially interact with a fear conditioned cagemate they showed avoidance behaviors (Knapska et al., 2010). Both freezing and avoidance behaviors extinguish over time but are reinstated in the presence of a fearful conspecific (Nowak et al., 2013). These experiments indicate that rodents are able to share emotional and behavioral fear states with other rodents through direct observation and/or through social interaction. It should be noted that, when considering fear as expressed by freezing, these studies have been restricted to males. Recent studies indicate that females employ active avoidance responses and males shift to these when the size of the chamber is increased (Gruene et al., 2015).

While social fear learning has been at the forefront of empathy-related rodent research, the direct transfer of emotional information encoded by painful stimuli is a much more salient approach for studying the neuronal correlates of empathy. This approach does not involve learning and instead examines the pain responses of rodents when they are engaged in various social and observational groupings. For example, mice tested for pain sensitivity in the presence of a cagemate, also in pain, display significantly higher levels of pain behavior than mice tested in isolation or when paired with a stranger mouse in pain (Langford et al., 2006; Martin et al., 2015). In fact, pain behaviors are not only amplified but they can be reduced when mice observe another mouse in pain, but not as much pain (Langford et al., 2006). In a subsequent study, it was demonstrated that the observed difference in pain modulation seen between familiar and unfamiliar mice was mediated through a testosterone-based mechanism that initiated a form of social stress-induced analgesia (Langford et al., 2011). There was no change in pain sensitivity when male mice were paired with unfamiliar female or castrated male conspecifics. It was concluded that the severity of social threat modulates pain sensitivity such that hyperalgesia is produced in mild social conditions while analgesia emerges with more severe stress.

Aside from its modulation of pain responses, stress is emotionally contagious (Burkett et al., 2016) and has been shown to block the emotional contagion of pain (Martin et al., 2015). We have recently shown that pharmacological blockade of the glucocorticoid stress response enabled the expression of pain empathy in mouse and human strangers (Martin et al., 2015). Specifically, we showed that mice and undergraduates tested in familiar, but not stranger pairs, displayed enhanced pain behaviors or ratings, a finding commensurate with Langford et al. (2006). Pharmacological blockade of glucocorticoid synthesis or glucocorticoid and mineralocorticoid receptors enabled the expression of emotional contagion of pain in mouse and human stranger dyads. Conversely, 15 min of restraint stress was sufficient to block the expression of pain empathy in familiar mice. While we believe that increased stress blocks empathy-related behaviors in mice, others have found no such relationship (Li et al., 2014). Conversely, an increased stress response has been found to underlie distress in social observational learning. The repeated witnessing of a conspecific in pain triggers freezing responses in rats, a behavior that eventually decreases and is replaced by spontaneous yawning (Carrillo et al., 2015). In this context, yawning is hypothesized to reflect increased distress and a coping strategy. Blockade of the HPA axis blocks yawning in rats that have previously witnessed a cagemate receive repeated foot shocks. This indicates that the observation of a conspecific in distress may stimulate the stress response and enhance emotional responsiveness. In another rodent species, the prairie vole, the distress of one vole elicited spontaneous social grooming behavior in a cagemate, when the two were reunited (Burkett et al., 2016). Exposure to the stressed cagemate increased activity in the ACC, and an oxytocin receptor antagonist infused into this region abolished the partner-directed response. In this context, social grooming was viewed as consoling and these types of behaviors may be more common than assumed in rodents.

Even the most basic pain-relevant social interaction, simple observation of pain by another, yields a complex pattern of results. In humans, pain experience can be reduced through social interaction, also known as social buffering (Brown et al., 2003). This is in contrast to the Langford et al. (2006) study that showed no measurable reduction in pain behavior when one mouse is in pain and its cagemate is not. Only when both mice are in pain, are pain behaviors enhanced, indicating that the social buffering of pain in mice is inconclusive. However, some research suggests that social buffering of fear and stress is attainable in rats. One study showed that having a naïve cagemate present blocked freezing behavior in a fear-conditioning paradigm. At the cellular level, it was shown that the presence of a social partner inhibited activity in the lateral amygdala, a brain structure important for emotional reactivity (Kiyokawa et al., 2004). In addition, a companion rat was sufficient to lower plasma corticosterone levels and stress-related behaviors when placed in a novel environment. In fact, stress was mitigated proportionally based on physical closeness of the two rats. (Leshem and Sherman, 2006).

The transmission of emotional states can be evoked by both fear and pain stimuli, but only a few studies exist where pain responses in a "demonstrator" modulate pain thresholds in a naïve "observer". A brief social interaction with a rat in pain (injected with bee venom) evokes bilateral mechanical hypersensitivity and an enhanced flinch reflex in naïve observer rats (Li et al., 2014). In line with the preponderance of empathy-related studies in rodents,

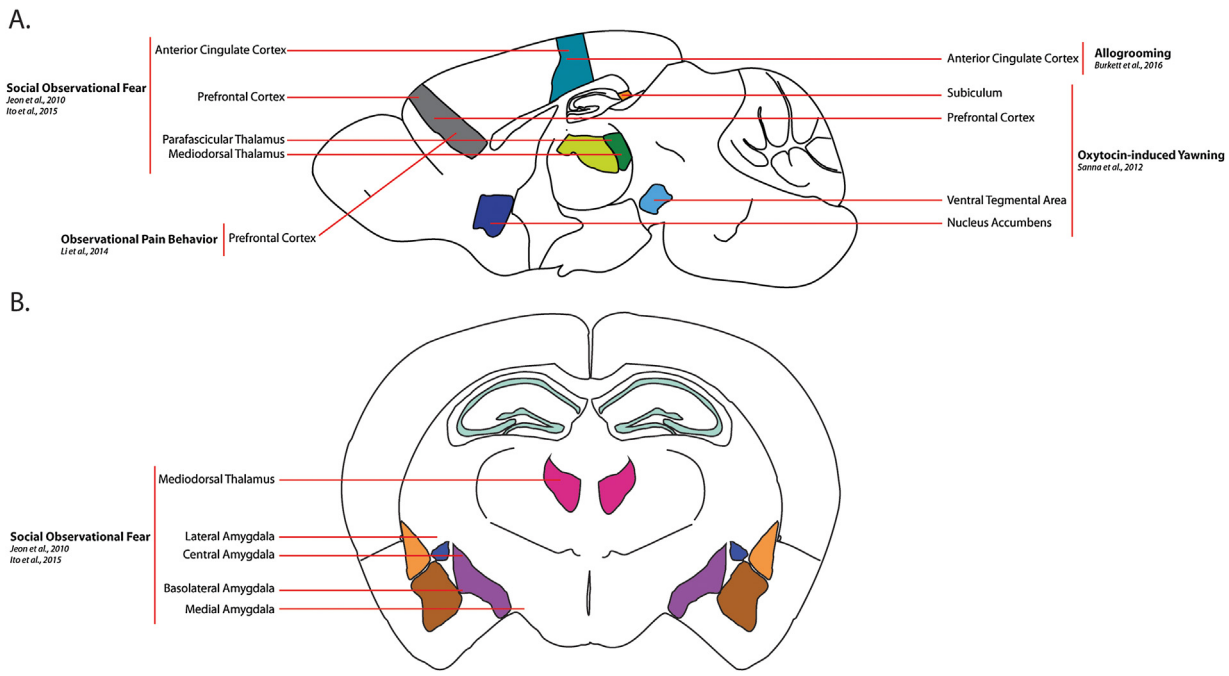

Fig. 2. In this schematic, major brain regions associated with rodent-empathy are illustrated. The specific paradigms that were used to link these brain regions to empathy are indicated. For simplicity, we have linked multiple brain regions with their respective empathy paradigms and in some instances this included more than one study, which is also indicated. Panel A shows a mid-sagittal section of the brain, so that the rostral-caudal aspect can be easily visualized. Panel B shows a coronal section of the brain so lateral brain structures are included.

these results are only true for familiars, as non-cagemates do not demonstrate social enhancement of pain. Interestingly, observing a cagemate in pain increases neuronal activity in the dorsal horn of the spinal cord suggesting that the increased pain behaviors are '*real*' and not just mimicry. In addition, the social enhancement of pain responses were ablated with bilateral lesions of the medial prefrontal cortex but not the amygdala or entorhinal cortex. This suggests that empathy for pain in rodents reflects a top-down biobehavioral process that is driven by the activation of the medial prefrontal cortex through socially interacting with familiar social partners. As a consequence, spinal nociception and vicarious pain behaviors in rats are facilitated by activating the medial prefrontal cortex but not the amygdala or entorhinal cortex. Fig. 2 summarizes the central brain areas that we have reviewed as critical components for empathy in rodents, with most of these regions also important for empathy in people (Singer and Lamm, 2009; Singer et al., 2004).

Another example of emotional state sharing is yawning. Yawning is contagious and represents a form of emotional contagion (Demuru and Palagi, 2012; Platek, 2010). Yawn contagion has been reported to occur in a variety of species including humans, bonobos, chimpanzees, parrots, and dogs (Demuru and Palagi, 2012; Massen et al., 2012; Miller et al., 2012; Romero et al., 2013) and susceptibility to contagious yawning is correlated with social familiarity (Demuru and Palagi, 2012; Romero et al., 2013). Interestingly, rats are known to yawn and in some situations yawning is considered a sign of distress (Carrillo et al., 2015). The neuropeptide oxytocin, known for its role in social communication and bonding (Bartz et al., 2010; Rodrigues et al., 2009) has been shown to induce yawning in male rats (Sanna et al., 2012). Injections of oxytocin into the caudal part of the ventral tegmental area, the hippocampal ventral subiculum and the posteromedial nucleus of the amygdala induced yawning, while oxytocinergic neurons in the paraventricular nucleus of the hypothalamus stimulate yawning by elevating extracellular dopamine in the shell of the nucleus accumbens and the medial prefrontal cortex (Sanna et al., 2012). This suggests that coordinated activity of oxytocin and dopamine in mesolimbic neurons contributes to primitive, unconscious forms of empathy such as yawning and regulates emotional and social reward.

Oxytocin is also known to modulate pain responses through social communication in people (Hurlemann et al., 2010). Through a mechanism that remains unclear, the cagemate of an oxytocin-injected rat displays significantly longer thermal latencies on the hotplate—even though it was never injected with oxytocin—than rats taken from a cage where all rats received saline injections. This effect disappeared in cagemates that were injected with an oxytocin antagonist in lieu of saline (Agren et al., 1997b). The contagion mediated by oxytocin is most likely driven by olfactory detection and in a parallel study, an olfactory-induced tail skin temperature reduction was observed in rats exposed to an oxytocin-injected cagemate, an effect abolished by olfactory impairment (Agren et al., 1997a). Strikingly, exposure of a naïve rat to the odor of a stressed rat that underwent a series of electric shocks promotes analgesia—an effect abolished by the administration of an oxytocin antagonist (Fanselow, 1985). Aside from the brain, oxytocinergic neurons are known to project to the spinal cord (Teclemariam-Mesbah et al., 1997) and stress-induced analgesia is impaired in mice lacking the oxytocin receptor—an effect that is mediated by oxytocinergic projections to the spinal cord (Robinson et al., 2002).

Oxytocin promotes social bonding and communication, however, it is important to consider the roles of social salience, prior familiarity and sex in any discussion pertaining to the neural mechanisms of oxytocin. For instance, acute intranasal oxytocin treatment increased social behaviors towards opposite-sex novel-stimulus female mice, while on the other hand it decreased social exploration of same-sex novel stimulus male mice, without affecting social behaviors towards familiar mice. Conversely, chronic intranasal oxytocin treatment produced a selective reduction of social behaviors concomitant with a reduction of oxytocin receptors throughout the brain (Huang et al., 2014). In addition, social context is another factor known to alter the behavioral responses of oxytocin, where social play is dependent on oxytocin in novel

but not familiar environments (Bredewold et al., 2014). Interestingly, in male rats the intranasal administration of oxytocin limited proactive aggression and increased social exploration of a male resident towards an unfamiliar male intruder (Calcagnoli et al., 2015), which is not in perfect agreement with the promotion of out-group aggression in people (De Dreu et al., 2010). All of these social behaviors are important for a number of reasons including intraspecies communication, survival and adaptability to rapidly changing environments. This is precisely why these types of behaviors are evolutionarily preserved from mice to chimpanzees to humans. By using rodent models to study empathy and socially modulated behavioral responses we are able gain a better understanding of the neural mechanisms that govern higher order and more socially complex behaviors.

5. Prosociality: helping others in distress

In 1962, Rice and Gainer set the stage for exploring rodent models of prosocial behavior and altruism. In the context of this review, we refer to prosocial behaviors as direct actions intended to benefit another at a temporary cost to the self (Eisenberg and Miller, 1987). This is best exemplified by the development of a behavioral task designed to assess prosocial helping behaviors in rats—at a cost of overcoming their innate fear of open spaces (Ben-Ami Bartal et al., 2011). In this paradigm, rats were trained to intentionally open a restrainer containing a distressed cagemate. Once rats learned to open the door, the latency to door opening greatly decreased over time. Rats only opened the door if the restrainer contained a distressed cagemate, not if it was empty or contained an inanimate object. Interestingly, female rats were more likely to free their cagemates than male rats. Additionally, this door-opening behavior does not seem to be driven by the reward of social contact. When rats were given a choice between freeing a trapped conspecific or opening the restrainer for a chocolate treat, rats typically opened both restrainers and shared the chocolate. In a subsequent set of experiments, prosocial helping was found to be modulated by social experience and rats helped release cagemates and strangers that were trapped, but only if the stranger was the same strain of rat (Ben-Ami Bartal et al., 2014). Rats did not help strangers from a different strain, unless they were previously housed with rats from that strain. This provides strong evidence that social relationships are not only important to rats but that they depend on them for social decisions rather than instinctual stereotyped behaviors.

The main criticism of prosocial rodent paradigms is that the underlying motivation for these behaviors is the desire for social contact and not necessarily empathy (Silberberg et al., 2014). Silberberg et al. (2014), have reported that the rescue behavior of rats was present even when rescue was not needed or failed. However, the possibility that contact between rats provided a means of support to their conspecific (which is a form of empathic behavior) cannot be ruled out. In the same study, a new experimental test was designed in which the free rat could not open the restraining door. If the responding of the free rat was empathically motivated then the rat's door opening behavior should disappear, however their results demonstrated the opposite as rats still attempted to open the door even though it did not work. This was further attributed to social contact but again it poses the possibility that social contact was empathic in nature. Assuming that the social contact explanation was true, rats would be motivated to open restrainers and trap doors for distressed and non-distress cagemates indiscriminately, but this is not the case (Ben-Ami Bartal et al., 2014; Sato et al., 2015). It should be realized that a key difference among these various studies is the degree of distress. Empathy cannot be expected from one without the distress or need from another. Also it must be noted that these helping actions were clear and goal-oriented. In Ben-Ami Bartal et al. (2011) opening the door involved physically displacing the door from the restraining tube to release the trapped rat, while in Silberberg et al. (2014) opening the door just required making contact to a metal strip attached to the door creating the possibility that these responses were accidental. In another study, a trapped rat was not restrained but rather soaked in a pool of water (Sato et al., 2015). Observer rats did not engage in helping behavior if their soaked cagemate was not distressed suggesting that door opening is motivated specifically by distress. In a secondary experiment, the rats were split into two groups where one set was conditioned to open the door for a soaked cagemate while the other group was conditioned to open the door for a food reward. Similar to Ben-Ami Bartal et al. (2011) after training was complete, rats had to choose a door that either freed the cagemate from the pool or one that opened to an area with a food reward. Ultimately, both groups regardless of how they were conditioned (soaked cagemate or food) opened the door for the cagemate first. Additionally, door-opening is a non-instinctual behavior that is difficult to learn which makes a strong argument over any sort of neophobic explanations (Mogil, 2012).

Empathy is thought to be the main motivator for prosocial behaviors in rodents. The helping test paradigm of Ben-Ami Bartal et al. (2011) equates the lack of door-opening with a reduced empathic response. This paradigm demonstrates that prosociality is extremely motivating for rats because in order to engage in door-opening they are required to venture into open areas, which they strongly dislike. Although the most common reaction to a rat's own distress is freezing, this behavior was not present in helper rats when freeing their cagemate (Atsak et al., 2011). Affective relations develop in rats through social interaction, which motivates helping. It is surprising to many that rodents are capable of such complex behaviors and that they respond in an appropriate manner to the distress of a conspecific providing strong evidence for the biological roots of prosociality.

6. Genes and empathy

Distinct biological factors contribute to empathy and its related behaviors. Arguably, the most fundamental of all biological factors that regulate behavior is our genes, yet there have only been a handful of genes linked to empathy-related behaviors. The only research into the genetic determinants of empathy and prosocial behaviors has been performed using "candidate gene" association studies in humans. Published associations for only six different genes—*AVPR1A*, *COMT*, *DRD4*, *MAOA*, *NOS1*, and *OXTR*—are relevant for trait empathy, prosocial temperament, altruism, partner bonding, and cooperation (Avinun et al., 2011; Bachner-Melman et al., 2005; Israel et al., 2009; Knafo et al., 2008; Mertins et al., 2011; Retz et al., 2010; Reuter et al., 2011; Rodrigues et al., 2009; Tost et al., 2010; Walum et al., 2008; Wu et al., 2012). In mice, it has been documented that the strain of mouse used is critical for testing empathy-related behaviors. In particular, the BALB/cJ mouse strain displays less social motivation and reduced social fear learning when compared with the more sociable C57BL/6J mouse strain (Chen et al., 2009). However, the genes related to different presentations of empathy-related behaviors between mouse strains have not yet been determined. Albeit, a number of immune genes—*C1qb*, *Cx3cl1*, *H2-d1*, *H2-k1*, *Polr3b*, and *Tnfsf13b*—have been identified to correlate in a seemingly direct manner with sociability (Ma et al., 2015). Studying the genetic determinants of empathy and prosocial behavior provides a stepping-stone towards understanding deficiencies of sociality including autism spectrum disorder, of which a lack of empathy is a defining feature. Future findings will have a great impact on the mechanisms underlying psychosocial suscep-

tibilities such as conduct and antisocial personality disorder, which also have lack of empathy as a central feature.

7. Conclusions and the power of rodent research

It has been traditionally assumed that there is a discontinuity between human social interactions and those displayed by lower order animals including rodents. Even with smaller prefrontal areas, this is evolutionarily implausible since rodents are perfectly capable of showing empathy-related behaviors such as emotional contagion and prosociality; two key features of empathy. Social neuroscience in laboratory animals is in its infancy, and the emotional communication of pain and fear in laboratory animals has not yet been studied systematically. The understanding of the self and others as being distinct is essential for the production of altruistic behaviors (e.g., prosocial and helping behavior). The existence of such social abilities has been considered an unlikely phenomenon in non-primate animals. We have provided strong evidence that rodents show empathy-related behaviors, but the detailed underlying neural mechanisms remain unclear. More studies are simply required before a greater understanding of rodent empathy is achieved. However, rodent models of empathy have the potential to provide a strong framework for understanding these highly important, complex, and interesting behaviors. These models have the power to provide unprecedented insight into the central mechanisms that engage empathy-related neural circuits at a detailed cellular and molecular level. This is a transformative step towards bridging the gap between social behavior and its underlying mechanisms, which will allow us to embark on a new stage of integrative studies in the domains of social behavior and rodent empathy.

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