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REVIEW

Observational fear behavior in rodents as a model for empathy

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Empathy enables social mammals to recognize and share emotion with others and is well-documented in non-human primates. During the past few years, systematic observations have showed that a primal form of empathy also exists in rodents, indicating that empathy has an evolutionary continuity. Now, using rodents exhibiting emotional empathy, the molecular and cellular study of empathy in animals has begun in earnest. In this article, we will review recent reports that indicate that rodents can share states of fear with others, and will try to highlight new understandings of the neural circuitry, biochemistry and genetics of empathic fear. We hope that the use of rodent models will enhance understanding of the mechanisms of human empathy and provide insights into how to treat social deficits in neuropsychiatric disorders characterized by empathy impairment.

KEYWORDS

affective empathy, anterior cingulate cortex, Cacna1c, Chd5, neurexin, observational fear, oxytocin, rodent, serotonin, social transmission of emotion, somatostatin interneuron, vicarious freezing

1 | INTRODUCTION

Empathy is the ability to feel, understand and share the mental state of others. It can range over diverse behaviors, including mimicry, emotional contagion, perspective taking and targeted helping.[1] As many mental disorders are characterized by atypical regulation of empathy that can be either too low or too high, there is both clinical and scientific value in understanding how empathy works biologically. Empathy is traditionally categorized into two different types: emotional and cognitive.[2,3] *Emotional empathy* is thought to involve several underlying processes, such as emotional contagion, in which one is affected by another's emotional or arousal state. In contrast, *cognitive empathy* is described as a cognitive role-taking ability, or the capacity to adopt another's psychological point of view.[4] The cognitive empathic perspective-taking system is considered more advanced than emotional empathy, and involves higher cognitive functions such as mental state attribution.[5]

Evidence suggests that empathy is evolutionarily conserved from rodents to humans, and rodents such as the mouse and the rat have showed affective sensitivity to their social partners.[6,7] Like humans, mice and rats exhibit observational fear,[8–10] social modulations of pain,[11] consolation,[12] and prosocial helping behavior.[13] Observational fear is a rodent behavioral model for assessing empathic fear.[2,8,14–17] In observational fear, a mouse is vicariously fear conditioned by observing a conspecific receive aversive foot shocks.[8] Human performance in a similar observational fear process was correlated with trait measures of empathy,[18,19] suggesting that observational fear contains a fundamental feature of empathy that is conserved across species.[20] These findings have led to a surge in new research exploring methods and variables of indirect fear learning where one animal observes another animal experiencing pain and expresses fear. In the past decade, paradigms designed to investigate indirect fear conditioning to previously neutral cues in laboratory rodents have rapidly emerged in the literature, but variations in the design and execution of these paradigms allow for notably different interpretations of the results (Table 1).

In this review, we discuss recent developments in the field of rodent empathy research with a special emphasis on the neural basis for observational fear. We begin by reviewing observational fear paradigm, discussing how the observational fear is distinct from fear conditioning and is representative of affective empathy. Then, we discuss behavioral and biological factors affecting the degree to which observers respond to another's distress in the observational fear paradigm. Considered lastly is the role of genes and neuromodulators in

TABLE 1 Rodent observational fear studies since 2009 that investigated behavioral or molecular mechanisms

Study	Subject species (strain)	Observer's sex	Housing condition after weaning	Observer's age	US to demonstrator and CS	Prior experience	Familiarity	Vicarious freezing during conditioning	24 h retrieval	Region, circuit, genes, or other mechanisms involved
Chen et al[17]	Mouse (C57BL/6 J, BALB/cJ)	Male and female	5 per cage (but isolated for 24 h before testing)	Adolescent (4 weeks vs 7-8 weeks)	0.5 mA; 2 s; tone cued (paired or unpaired) or context only	Only naïve tested	Only strangers tested	Yes	No	Not tested; BALB/cJ and C57BL/6 J differed in freezing, USV, and heart rate change.
Jeon et al[8]	Mouse (C57BL/6)	Male	2-3 per cage	12-15 weeks	1 mA; 2 s; context only	Only naïve tested	Siblings or mating partners as demonstrator elicited higher freezing	Yes	Yes	ACC; parafascicular; mediodorsal thalamus; lateral amygdala (LA)
Atsak et al[9]	Rat (long Evans)	Female	2-4 per cage	Adult	0.8 mA; 5 s; context only	Experienced observer (0.8 mA) expressed higher freezing	Only cagemates tested	Yes	Yes	Not tested
Yusufishaq et al[35]	Rat (Sprague-Dawley)	Male	2 per cage or isolated	Not specified	0.5 or 0.8 mA; 1 s; tone cued	Only naïve tested	Only strangers tested	Yes	Yes	Not tested
Sanders et al[33]	Mouse (C57BL/6 J)	Male and female	2-4 per cage	2-6 months	0.7 mA; 1 s; context only	Experienced observer expressed higher freezing	Only strangers tested	Yes	No	Not tested
Kim et al[99]	Mouse (C57BL/6)	Male	Not specified	8-11 weeks	1.3 mA; 2 s; context only	Only naïve tested	Only strangers tested	Yes	Yes	ACC; serotonin; dopamine; oscillations in ACC
Jones et al[104]	Rat (Sprague-Dawley)	Female	3 per cage	Average 130 days	0.7 mA; 0.5 s; tone cued	Only naïve tested	Sisters vs non-sisters (shared cage for 1 week prior to test)	Fear conditioning by proxy	Yes	Not tested
Gonzalez-Liencres et al[29]	Mouse (C57BL/6 J)	Male	6 per cage	22-24 weeks	0.5 mA; 1 s; context only	Only naïve tested	Familiar demonstrator elicited higher freezing	Yes	No	Not tested; fecal droppings of demonstrators and observers were correlated.
Ito et al[55]	Mouse (129SvEv/C57BL/6 N F1 hybrid)	Male	2 per cage	9-11 weeks	1 mA; 2 s; context only	Only naïve tested	Only cagemates tested	Yes	Passive avoidance training and testing	dmPFC − > BLA glutamatergic transmission and silent synapses; ketamine
Keum et al[37]	Mouse (C57BL/6 J, C57BL/6NTac,	Male and female	4-5 per cage		1 mA; 2 s; context only	Only naïve tested	Only strangers tested	Yes	Yes	Not tested

TABLE 1 (Continued)

Study	Subject species (strain)	Observer's sex	Housing condition after weaning	Observer's age	US to demonstrator and CS	Prior experience	Familiarity	Vicarious freezing during conditioning	24 h retrieval	Region, circuit, genes, or other mechanisms involved
	129S1/SvImJ, 129S4/SvJae, BTBR T(+) Itpr3(tf) /J, AKR/J, BALB/cByJ, C3H/HeJ, DBA/2 J, FVB/NJ and NOD/ShiLtJ)			4, 8, 12, or 16 weeks; 13 weeks						
Pisansky et al[30] (OXT)	Mouse (C57/B6)	Male and female	2-6 vs 7-12 per cage (smaller litter size resulted in familiarity difference)	8-12 weeks	0.8 mA, 1.5 s; context only	Only naïve tested	Familiar demonstrator elicited higher freezing in males	Yes	Yes	ACC; intranasal oxytocin; Oxtr; PVN oxytocinergic neurons
Pisansky et al [78] (Chd5)	Mouse (C57BL/6 J)	Male	2 per cage	10-15 weeks	0.8 mA, 1.5 s; tone	Only naïve tested	Familiar demonstrator elicited higher freezing	Yes	Yes	Chd5
Lidhar et al[105]	Degu	Male and female	Not specified	5-18 months	1 mA; 2 s; context only	Only naïve tested	Familiar demonstrator elicited higher freezing in males	Yes	Yes	Not tested
Twining et al[36]	Rat (Sprague-Dawley)	Male	2-3 per cage	9-12 weeks	0.5-0.6 mA; 1 s; cued (tone)	Only naïve tested	Only strangers tested	Yes	48 hours context, then tone	LA – > MeA; Nrxn1
Allsop et al[34]	Mouse (C57BL/6 J)	Male	Not specified	8-12 weeks	1.5 mA (>30 g body weight) or 1 mA (<30 g); 2 s; cued (light and tone)	Experienced (1 trial) mice expressed higher freezing	Only strangers tested	Yes	Yes	ACC – > BLA projection neurons
Keum et al[38]	Mouse (C57BL/6 J)	Male	2-5 per cage	10-14 weeks	1 mA; 2 s; context only	Only naïve tested	Only strangers tested	Yes	Yes	ACC; inhibitory neurons; Nrxn3

the regulation of the observational fear and in neuropsychiatric disorders characterized by impairments in empathy.

2 | MAIN BODY

2.1 | Observational fear as a model of affective empathy

Fear is a subjective state expressed as behavioral and physiological responses to threatening environmental stimuli. Fear responses are triggered by direct exposure to harmful stimuli, such as an electric shock. In typical fear conditioning paradigms, an emotionally neutral conditioned stimulus (CS), such as a tone or a context, is paired with an innately aversive unconditioned stimulus (US) during the acquisition phase. Following the experience of the aversive US coupled with a CS, exposure to the previously neutral CS can elicit a fearful response.[21]

In addition to the direct acquisition of fear toward the CS through Pavlovian fear conditioning, fear responses can be indirectly acquired through social transmission. Even a brief social exposure to a distressed demonstrator modifies the behavioral performance of an observer in associative fear learning in rodents, implying that social interaction with a distressed partner facilitates the new association and alters the emotional response of the observer.[22–24] These findings show that (1) the emotional state of one rodent can influence the behavior of another, and (2) rodents can learn a fear association in the absence of direct experience of the aversive stimulus.

Fear behaviors also develop vicariously by observational fear conditioning. In the observational fear behavioral paradigm, a mouse learns CS-US contingency by pairing a CS (context or tone) with a conspecific's distressed response which serves as the US.[8,17] Without receiving direct aversive stimuli, mice are conditioned for fear vicariously by witnessing conspecifics experience repetitive foot shocks, and the contextual fear memory can be measured the next day in the absence of the demonstrator or the foot shocks.[8] The expressed distress of the demonstrator alone can elicit an association between the affective experience of the observer and the specific environmental context. Similarly, in a tone-based observational fear conditioning paradigm, mice that witnessed a demonstrator mouse being presented with a tone and paired foot shocks subsequently showed increased freezing to the tone alone.[17] This subsequent effect is distinct from emotional contagion or mimicry because this freezing behavior takes place in the absence of the demonstrator long after its exposure to it.

There are many similarities between observational fear and classical fear conditioning; both result in fear expression by making new associations between CS and US, and involve overlapping brain circuitries.[19,25] However, fear conditioning is elicited by direct experience of the aversive stimuli. By contrast, vicarious freezing in observational fear conditioning is evoked by social transmission of the demonstrator animal's affective state and should therefore be dependent on social perception and the integrated social cognitive processes.[8,15,26] And the process by which recognition of the demonstrator's distress triggers fear in the observer is, by definition, a form of affective empathy, a critical factor involved in social fear transmission and the ensuing observational learning.[2,6,14,16] Observational fear learning studies in primates and humans, where subjects recognize fear by observing a conspecific suffering, showed that trait empathy was positively associated with stronger vicarious fear response.[18,27,28]

2.2 | What factors affect the degree of vicarious freezing in observer mice?

Multiple studies show that factors affecting sensory modalities or social cues have modulatory effects on observational fear response. Here, we discuss familiarity, prior experience, rearing condition, sensory modalities and the sex and age of the animal (Table 1).

2.2.1 | Familiarity

The vicarious freezing response of the observer mouse is positively influenced by the animal's familiarity or kinship with the demonstrator. Vicarious freezing during conditioning and contextual 24 hours retrieval were significantly enhanced when the demonstrators were siblings of the observer mice. Additionally, female mating partners that had been housed together with the male observer for more than 10 weeks elicited significantly higher freezing response as demonstrators compared to other females, non-cage mates or mates less than 10 weeks together.[8] Thus, the demonstrator being a sibling or a long-time mating partner tended to trigger a higher fear response in the observer. Similarly, observer mice showed higher freezing and increased number of fecal droppings, both signs of distress, when a familiar cage mate was receiving the electric foot shocks, compared to an unfamiliar stranger mouse.[29] Such dependence on familiarity appears more prominently in male observer mice.[30] These results indicate that especially for males, familiarity of the demonstrator enhances the level of vicarious freezing in the observer.

2.2.2 | Prior shocks

Human studies showed that aversive experiences facilitate the ability to recognize and share similar distressing emotions with others.[31,32] Likewise, previous experience of an aversive stimulus similar to what the demonstrator receives can enhance the observational fear response of the observing rodents. Indeed, observer mice froze more when they had a similar shock experience 24 hours earlier.[33] A recent study also showed that observer mice with prior shock experience showed more robust acquisition of tone-dependent fear response than naïve mice during observational conditioning.[34] Rat study shows consistent results, in that a prior shock experience potentiates fear response to witnessing the shock of another animal.[9] In fact, the ability of naïve rats to exhibit any vicarious freezing is still not entirely clear. A study that compared naïve and experienced observers reported no vicarious freezing in naïve female Long Evans rats,[9] while two other studies that tested only naïve observers reported vicarious freezing by naïve male Sprague-Dawley rats.[35,36] This difference might be analogous to the strain difference in the degree of observational fear among inbred strains of naïve mice, where 9 of 18 inbred strains did not exhibit detectable observational fear.[37,38] Because the minute differences in the environment, handling, and protocols

between different laboratories can affect the behaviors of laboratory rodents even when the behavioral assays are standardized,[39] we cannot be sure until multiple strains of naïve and experienced rats are tested for observational fear learning.

It has been suggested that an observer's vicarious freezing after a prior shock is not directly related to a non-specific effect of heightened anxiety that results from having had a prior stressful experience, because having a stressful experience qualitatively different from the observed stressor to the demonstrator subdued the vicarious freezing.[33] This is consistent with the priming effect of prior experience in direct, not vicarious, fear conditioning, where different kinds of traumatic experience was not generalized, but only the similar kind of experience had the priming effect.[40] In addition, when the experienced observer enters the chamber where it has been lightly shocked, it does not show more freezing than naïve observers until the demonstrator starts to receive foot shocks coupled with the tone.[34] In this study, because naïve observer mice did not learn to avoid the shock floor after watching demonstrator mice being shocked there, the authors further interpreted that the experienced mice learned the predictive value of the cue independent of contextual conditioning. This behavior thus is a form of cued fear conditioning where the animal learned the association of the CS (tone) and the US (foot-shock). The US here, however, is dependent on the memory of its own foot-shock experience which has been activated/augmented by observing the demonstrator receiving the tone and foot shocks. Therefore, this observational fear response should consist of two components, the fear induced by the socially evoked recall of its own shock experience and the empathy-related fear. It appears that the first component is robust while the second component is very weak in this behavioral paradigm.

2.2.3 | Social conditions

Socially transmitted fear differs between familiar and unfamiliar male mice, depending on the size of the observer's litter. For observer mice reared within a small litter of 2-6 pups, the average freezing count was significantly higher in response to familiar demonstrators than to unfamiliar demonstrators.[30] However, for observer mice reared within a larger litter of 7-12 pups, a more socially stressful condition, the observational fear responses were similarly low for familiar and unfamiliar demonstrators. Therefore, crowded rearing conditions can influence the development of the observer's ability to respond to social stimuli, including the affective response to social partners. Furthermore, isolation diminishes vicarious freezing, as much as congestion does. Using mice raised either socially or in solitude during their adolescence that then underwent cue-conditioned observational fear learning, researchers found that socially reared mice had stronger "long-term" (24-hour postconditioning) vicarious fear memories than "short-term" (15-minute postconditioning) memories, whereas the opposite was true for mice reared in isolation. The social and solitude rearing groups had a greater difference in vicarious fear responses than for directly acquired fear.[41] In addition, rats reared in social isolation for 3 weeks displayed impaired vicarious freezing and tone memory at postnatal weeks 4 and 5, compared to pair-housed rats. In both cases, the demonstrators were unfamiliar strangers.[35] These results show that both congested housing and social isolation can impair normal development of sociality, and observational fear learning. Further investigation is needed to determine whether different housing conditions specifically impair different processes—recognition of the demonstrator's distress signals and subsequent trigger of empathic fear and observational learning, for example.

2.2.4 | Sensory modalities: Sound/vocalization/vision

The degree of the vicarious fear response showed by the observer depends on the sensory modalities that carry the information of fear expressed by the demonstrator to the observer. These modalities include vision and sound. Sound information includes demonstrator's fear-signaling vocalization, or silence due to cessation of any movement by the demonstrator.[10]

Watching the demonstrator's response to the shocks and subsequent freezing may not explain the entirety of the vicarious freezing, but a significant portion of it requires visual information: replacing the transparent partition with an opaque one attenuated the observer's vicarious freezing significantly.[8]

In addition to the passive, conditioned fear response of immobility, vocal communication has been shown to affect social transfer of fear in mice in a tone-based observational fear conditioning.[17] The number of ultrasonic vocalizations (USV) emitted by a fearful rat demonstrator is positively associated with the level of fear expressed by an observer rat.[42,43] However, USVs recorded from observer-demonstrator pairs during the training alone did not produce significant vicarious freezing either in naïve nor shock-experienced rats.[9] Furthermore, the duration of vocalization was significantly longer for female demonstrators, which suggested enhanced nociceptive sensitivity or heightened communication of distress.[30] In case of the males, only the familiar and not the unfamiliar pairs showed both high vicarious freezing responses and a correlation between the observer freezing counts and the demonstrator vocalization durations. The authors did not see any qualitative difference between the fear expression by the demonstrators through vocalization or freezing, which supports the notion that the dependence on familiarity results from differential processing of fear vocalization by the observer between familiar and unfamiliar demonstrators.

We have investigated whether various behavioral reactions of different out-group demonstrator strains to foot shocks (ie, jumping, freezing, running or vocalization) can trigger the differential level of vicarious freezing in observer mice. Despite potential difference in demonstrator's vocalization or social cues between different inbred strains,[44,45] we found that C57BL/6 J (B6J) male observer mice exhibited similar levels of observational fear toward different out-group FVB/NJ and 129S1/SvImJ demonstrator strains.[37] If demonstrator's vocalization or social cues differed between the mice of these three inbred strains, these differences did not significantly determine the degree of vicarious fear expressed by the B6J observer mice.

In summary, the role of vocalization in the social transmission of fear between mice is still not clear. Rodents seem to perceive more than just the vocal or visual signals and may be additionally aware of various nuanced environmental details that influence their interpretation of and response to the social cues. For example, a recent study

showed that rats use the cessation of movement-evoked sound to detect freezing by another rat, suggesting that silence could constitute an environmental cue that can affect social fear transmission.[10]

A source of debate is that the observer could be freezing because the vocalization or jumping by the demonstrator was aversive, rather than it being socially meaningful. However, then it is hard to explain the increase in freezing for familiar demonstrators. Also, the correlation between the duration of vocalizations and the length of freezing bouts was observed to be different between familiar and unfamiliar demonstrators,[30] suggesting that the vicarious freezing is at least somewhat socially incited, rather than simply another conspecific's distressed vocalization being inherently aversive.

2.2.5 | Sex and age

In one of our previous studies, difference in age or sex did not significantly affect the level of observational fear in B6J mice. Although 4 weeks old adolescent male mice showed higher vicarious fear than older mice, their level of freezing in fear conditioning was also higher than that of adult mice.[37] Consistent with our findings, a previous study also showed that mice at the early adolescent stage acquired and expressed conditioned fear response to a greater degree as compared to adults.[46] Thus, it is likely that the increased level of vicarious freezing in 4 weeks old mice might be due to enhanced acquisition of conditioned fear.

Likewise, we did not see a difference between male and female B6J mice in observational fear learning. Others also reported no sex difference in auditory cue-based observational fear,[17] and in mice reared in isolation.[41] Socially reared females, however, had higher vicarious freezing than socially reared males.

However, gender difference in human empathy is well-documented,[47] and Pisansky et al[30] showed that female observers exhibited similarly high vicarious freezing responses to familiar and unfamiliar demonstrators, while males showed high freezing only in response to familiar demonstrators. The authors pointed out difference in the duration of vocalization as a possible clue to explain this sex difference. The discrepancy among these studies could also be due to the different behavioral procedures including handling for acclimation and shock protocols.

2.3 | Brain regions and cell types involved in observational fear learning

The Pavlovian fear conditioning is believed to take place by the convergence of the CS and US. The innate ability to learn to recognize and respond to direct cues that predict danger is mediated by the amygdala, as well-understood through numerous studies.[21,48] How does the brain then associate an aversive US with a neutral CS simply through observation? A key difference is the unique involvement of the anterior cingulate cortex (ACC) that projects to the amygdala during acquisition of observational fear.[8,34]

The ACC has been implicated in fundamental cognitive processes including executive processing, attention, affective emotion, and social cognition.[49–51] Importantly, the prefrontal cortical areas, the ACC and the insular cortex have been shown activated when humans detect distress in others.[19,50,52,53] Likewise, the activity of the ACC is augmented in mice engaged in observational fear,[30,34] and its role in the acquisition of vicarious freezing has been showed using neuroanatomical lesions, electrical stimulations and optogenetic manipulations.[8,34,54]

Along with the ACC, cortical-amygdalar circuits are required for generating observational fear in mice.[8,25] Jeon et al found that during observational fear conditioning, the ACC and the lateral amygdala (LA) neuronal activities were highly synchronized at the 4-7 Hz theta rhythm frequency, suggesting that an interactive communication through this cortico-amygdalar connection is critical for observational fear. A subsequent lidocaine lesion study showed that ACC activity was required for learning, but not for recall, of the vicarious fear, whereas the LA activity was required for freezing during both the vicarious fear conditioning and the contextual recall,[8] suggesting that the ACC encodes affective and cognitive information required to induce social fear but not for its expression, which is probably controlled by the BLA. How the ACC interacts with and drives the BLA to express fear response needs to be studied further.

In a recent report using tone-based observational fear conditioning in mice which were primed with a prior foot shock,[34] the authors performed in vivo single unit recording and showed that BLA-projecting ACC neurons in the observer mice responded to cues that predict shocks to the demonstrator. In further detailed analysis of neural dynamics, ACC neurons showed baseline firing changes in the context of the demonstrator's distress and some BLA neurons were dependent on ACC input during the cue presentations. They interpreted that these data suggest that BLA-projecting ACC neurons encode aversive information derived by socially activated memory of its own experience of the same aversive stimuli.[34] However, the function of those neuronal firing in their observational fear conditioning is not clear, because photo-inhibition of the ACC-to-BLA projecting neurons did not affect the observational fear conditioning on Day 1, suggesting that firing of these neurons is not necessary for the fear response in this behavioral paradigm. In addition, in the Day 2 memory test, the photo-inhibited group showed a much enhanced baseline freezing compared to the control group. The basal level of freezing notwithstanding, the level of cue-induced freezing was not different between the photo-inhibited group and the control. This raises a significant obstacle in making a valid interpretation of the data. Therefore, it is not clear to decide whether the firing of the BLA-projecting ACC neurons are the driver for the fear response in the observational fear conditioning of mice with a prior foot shock experience.

In mice that underwent observation fear conditioning the neurons in the ACC (the 1.3 mm anterior, 0.4 lateral, 1.3 ventral target region was named dmPFC, but it is very similar to our 1.2 mm, 0.3, 1.2 target for ACC, and the histology image also shows what many rodent studies discussed here identify as ACC, so we will call it ACC for the ease of discussion) and the basolateral amygdala (BLA) were activated, and the ACC-to-BLA NMDAR-mediated currents displayed increased amplitude and slowed decay.[55] The physiological function of these changes, plasticity, is not clear. All we can tell for now is that they are not required for the fear memory expression next day.

A surprising observation was the cortical lateralization of observational fear to the right hemisphere of the ACC.[54] Experiments utilizing intracranial electrical stimulation or lidocaine lesion showed that only

the right side ACC is involved in control of observational fear learning. Thus electrical stimulation in the right ACC but not in the left ACC enhances, while lidocaine inactivation of the right but not the left ACC suppresses observational fear learning. This was the first case where hemispheric lateralization of a cognitive function had been clearly defined in the mouse.

However, we still have limited knowledge on how the ACC circuit is integrating social cognitive information during observational fear. A specific network of neurons within the ACC that includes a particular subgroup of the GABAergic inhibitory neurons appears critical in controlling the level of observational fear. We recently learned that the somatostatin-expressing (SST+) neurons in the ACC bi-directionally control the degree of socially transmitted fear in mice.[38] Observational fear was enhanced by optogenetic inhibition of SST+ neurons, and impaired by activation of SST+ neurons. Inhibition of the SST+ neurons would have decreased the inhibition of ACC pyramidal neurons at the dendrites. By contrast, decreasing inhibition of the pyramidal neurons via optogenetic suppression of soma-targeting parvalbumin-expressing (PV+) neurons did not change the behavior. This suggests that SST+ neuron-specific mechanisms, not just any inhibition, control empathic fear responses.

Pain signals are relayed through the thalamus via the lateral and medial pain systems.[56] Human neuroimaging data and rodent models of empathy suggest that the medial affective pain system involving the ACC, insula, amygdala, and MD, is active during both self-experienced pain and observation of pain. Such overlapping activities are believed to enable an inference of another's pain, facilitating empathy.[57–62] Inactivation of the medial affective pain system, including the ACC, the parafascicular, or the mediodorsal thalamic nuclei (MD) by lidocaine injection significantly impaired observational fear learning, whereas inactivation of the lateral sensory pain system did not affect observational fear learning.[8,50] This indicates that observational fear learning is dependent on the affective but not the sensory component of pain transmission.[8,50]

2.4 | Genes and neuromodulation of observational fear

Although there is a considerable genetic contribution to individual variability in human empathy,[63–66] identification of specific genes has been largely limited, primarily because it is difficult to control the social context in humans. While psychological research has shown the importance of empathy in sociality and substantial amount of information has accumulated about executive neural circuitry controlling observational fear, far less is known about distinct genetic factors influencing empathy.

2.4.1 | Neurexins (*Nrxn1 and Nrxn3*)

It has been well known that different inbred mouse strains show different emotional responses to social stress, and such differences have been attributed to genetic differences of the strains.[67,68] We previously surveyed multiple inbred mouse strains and found that the vicarious freezing response was highly variable among different strains. Importantly, the variability in observational fear was not significantly associated with the strain-specific differences in other behaviors such as conditioned fear, locomotor activity, anxiety, or 3-chamber sociability, suggesting that observational fear-specific genetic variations exist in inbred strains of mice.[37] Intriguingly, we found that a mouse strain, 129S1/SvImJ (129S1), exhibited a vicarious freezing response substantially higher than any of 18 other inbred mouse strains. By comparing a panel of genetically nearly identical 129 steel-lineage (129S) substrains, we identified that a gene variant present only in the 129S1 strain was a candidate for their enhanced empathy fear. Using forward genetics combined with whole-genome sequencing and the CRISPR/Cas9 genome editing, a missense variant (Arg498Trp, or R498W) in the *Nrxn3* gene was confirmed as a causative variant for the selective enhancement in observational fear without altering classical fear conditioning.[38]

Moreover, we showed that *Nrxn3* was selectively required for inhibitory synaptic transmission in SST+ interneurons in the ACC. Dysfunctional inhibitory circuits in the ACC of SST+ neuron-specific *Nrxn3* knockout mice caused hyperactivity of excitatory pyramidal neurons, resulting in elevated observational fear, which is similar to the behavior of the 129S1 mice. Thus, this study has identified a novel role of Nrxn3-dependent SST+ interneurons in the ACC in controlling the affective capacity in empathy fear behavior in mice.[38]

Nrxn3 R498W mutant and SST-specific conditional KO B6J mice showed similar levels of fear conditioning as wild-type controls, suggesting that neither the R498W allele nor the deletion of *Nrxn3* affects classical fear conditioning. Other behaviors such as 3-chamber social behavior or Elevated Plus Maze (EPM) were not performed on these *Nrxn3* Knockout mice, so whether the gene plays a critical role in those behaviors is yet unclear. Nonetheless, these findings suggest that the *Nrxn3* gene may play a crucial role in neural circuits specific to observational fear, independent of direct conditioned fear.

Neurexins have been known to have important roles in neurotransmission and synaptic connectivity in the brain,[69] leading to the expectation of abnormal social behaviors in rodents with neurexin mutations. In the tone-based observational behavioral paradigm, a recent study showed that *Nrxn1* knockout rats showed impaired observational fear and 48 hours social fear memory due to reduced synaptic transmission in an intra-amygdala circuit from the LA to the MeA. The authors further showed that the *Nrxn1*-dependent LA-MeA circuit is required for associating the affective content of social cues (distress of conspecific) with the predictive cues of the external environment and LA-MeA strength was correlated with the level of freezing in social fear conditioning.[36] Consistently, a recent study supports that the distinct MeA neuronal populations are implicated in encoding of social and defensive information in mice.[70]

Nrxn1 KO rats showed enhanced freezing during classical fear conditioning, although their fear memory retrieval and forepaw withdrawal threshold were normal. Considering their observational fear is decreased, it is unlikely that they exhibit impaired observational fear because of an enhanced associative fear learning.

Similar to the rats, Nrxn1 mutant mice exhibit many of the phenotypes seen in patients with ASD or schizophrenia. For example, Nrxn1α—an isoform harboring a longer extracellular domain -KO mice displayed increased aggression and anxiety in males, altered social approach, reduced social investigation, reduced nest building, and reduced locomotor activity in novel environments.[71–74]

These results suggest that the role of Nrxn1 in observational fear is specific to social behaviors, and does not affect most sensory abilities, novel object preference, and anxiety, as tested.

2.4.2 | L-type Cav1.2 calcium channel (*Cacna1c*)

Previously, the L-type calcium channel gene, CaV1.2 (*Cacna1c*), in the ACC was shown to be required for the observational fear behavior.[8] Mice with an ACC-limited deletion of the Cav1.2 gene exhibited impaired observational fear. Notably, the ACC-specific KO mice displayed reduced pain responses to formalin and acetic acid, which are known to have affective pain components, but there was no difference in acute pain behavior, either mechanical or thermal. These suggest that functional Cav1.2 channels in the ACC are required for social fear learning and are critical for pain response modulation by the brain. These mice, however, showed normal responses in other behavioral assays, including EPM, light/dark transition, open field, novel object recognition, predator exposure, and classical fear conditioning behaviors.[8] These results further support the involvement of the ACC in affective or emotional dimension of noxious or aversive stimuli. Nonetheless, it is still not clear how the Cav1.2 channel in the ACC is involved in modulation of observational fear. The impairment in observational fear by Cav1.2 deletion could be simply due to a general decrease of excitability in the ACC. However, it has become evident that the Cav1.2 gene is a critical mediator of transcription-dependent neural plasticity. Signaling via the influx of calcium ion[75] and dysregulated Ca2+ as a consequence of altered Cav1.2 channel function can affect social behavior.[76] A recent study clearly showed that abnormality of nonionic conformational signaling of Cav1.2 channel is associated with neurological dysfunction in Timothy syndrome, a highly penetrant autism spectrum disorder (ASD). Mice expressing a constitutively active G406R mutation in the *Cacna1c* gene, causative of Timothy syndrome in humans, manifested a number of behavioral changes reminiscent of autism including repetitive behaviors, altered social interaction and ultrasonic vocalizations, and enhanced fear memory.[76] Similarly, mice with excitatory neuron-specific deletion of Cav1.2 in the forebrain exhibited anxiety-like behaviors and deficits in sociability in the 3-chamber test.[77] Further electrophysiological or pharmacological experiments will show the role of Cav1.2 in observational fear.

2.4.3 | Chd5

A recent study showed that the chromodomain helicase DNA-binding 5 (*Chd5*) gene, a chromatin remodeling protein known to regulate neuronal differentiation, was involved in observational fear.[78] In this study, the level of observational fear in *Chd5* KO mice was similar to those seen in wild-type control mice. However, despite no behavioral deficit in conditioned fear, the *Chd5* KO observers failed to show enhanced observational fear response to familiar cagemate demonstrators.[78] Because the Chd5 gene was implicated in ASDs and the KO mice also exhibited altered vocalization and reduced sociability, the authors concluded that failure to respond to familiar conspecifics in *Chd5*-deficient observer mice could be due to impaired social recognition.

It is interesting to note that all these genes involved in observational fear we discussed above have been also implicated in ASD. Deletions or copy number variations of the neurexin genes (*NRXN1* and *NRXN3*) were directly implicated as genetic risk factors for ASD.[79,80] Similarly, the *Chd* gene family and dysregulated neuronal Ca^{2+} signaling via L-type Cav1.2 are also known to be associated with autism.[76,78,81,82] Disturbance of empathy is a salient feature of ASD. Many patients with ASDs show impaired emotional processing with deficit of social recognition and empathy.[83–85] However, other studies show that emotional empathy response can be intact, or even heightened, in ASD patients.[84,86,87] Intriguingly, a recent work showed that participants high in autistic traits showed an elevated observational fear response.[18,88] This heterogeneity suggests a more general affective imbalance in neurocognitive capacity in ASDs, resulting from a complicated matrix of genes, brain regions and behavioral correlates.[84,89] Dysfunction of these genes (*Nrxn1*, *Nrxn3*, *Chd5* and *Cacna1c*) may likely perturb the neural circuitry underlying sociability and repetitive behaviors at different nodes in ASD patients.[90–92] Similarly, a locus in 3p26.1 was significantly associated with cognitive empathy in women[66] and deletion of this locus has been implicated in ASDs.[93,94] Although certain features of affective empathy can be modeled in rodents, finding a common underlying molecular mechanism that are functionally relevant to sociability and empathy will require additional studies.

Brain-specific Chd5 knockout mice showed abnormal sociocommunicative behaviors (fewer pup separation-induced ultrasonic vocalizations, adult three-chamber social approach test) and lack of preference for a novel mouse. Chd5 knockout in the brain did not affect Pavlovian fear conditioning, however, as these mice were no different from the wildtype control in Pavlovian fear conditioning.[78]

2.5 | Neuromodulation of observational fear

2.5.1 | Oxytocin

Recent research has examined the role of the neuropeptide oxytocin in empathy due to its involvement in a wide range of socioemotional processes.[95] Although the administration of exogenous oxytocin often produces no effect, more recent work has shown that oxytocin administration increases empathy traits.[96,97] Moreover, genetic variations in oxytocin receptor (OXTR) are associated with empathy traits.[64,98] A recent study examined the involvement of oxytocin in observational fear in mice.[30] Both acute and chronic intranasal oxytocin administration enhanced vicarious freezing in response to unfamiliar demonstrators. Similarly, chemogenetic stimulation of hypothalamic oxytocinergic neurons rendered male mice sensitive to the distress of unfamiliar demonstrators. Moreover, systemic injection of an oxytocin receptor antagonist impaired acquisition of observational fear in familiar conspecifics. This study clearly implicated oxytocin in observational fear in association with increased cellular activities in the ACC. Further cell-type-specific electrophysiological or circuit-based investigations will be important to show the underlying neural mechanism of oxytocin signaling in observational fear.

2.5.2 | Serotonin

Serotonin (5-HT) modulates a variety of brain functions, and altered levels of this neurotransmitter has been implicated in the pathophysiology of affective disorders. Kim et al injected 5-HT into the ACC, which reduced the observational fear in mice.[99] And extracellular application of 5-HT to ACC slices reduced the excitability of ACC neurons, but the molecular mechanisms underlying the 5-HT-mediated decrease in the observational fear response is still not clear. On a related note, 5-HT1A serotonin receptor is thought to be involved in emotion-related behavioral phenotypes such as aggression and sociability.[100,101] Previous studies showed that the activation of this receptor within the prefrontal cortex caused neuronal inhibition.[102] Thus, it is possible that heightened 5-HT action results in a hyperactivation of the 5-HT1A receptor, which may lead to a dysregulation of affective and emotional behaviors such as empathy. Intriguingly, a functional polymorphism in the regulatory region (5-HTTLPR) of the 5-HT transporter (5-HTT) gene was shown to be associated with enhanced observational fear response in humans.[103]

Similar to circuit studies made possible by animal models, neurochemical studies of observational fear in rodents can shed light on the biological mechanisms of mammalian emotional empathy that may be also shared by humans. Such knowledge can help better understand facets of human empathy and treat symptoms of social, emotional disorders involving dysfunctions of emotional empathy.

3 | CONCLUSION

Over the years, a wealth of data has accumulated to show that rodents can share the emotional states of their conspecifics, as well as exhibit prosocial behavior. Rodent observational fear has surprising anatomical, neurochemical, and behavioral similarities with human emotional empathy. Both are critically controlled by the following: familiarity of the demonstrator, the activation of anterior cingulate, medial thalamus, and amygdala, and oxytocin signaling. This strongly suggests that mice experience what is homologous to our pain empathy. On the other hand, it is still in debate whether rodent observational fear indicates empathy, because "higher cognitive functions" such as self-recognition are considered crucial for empathy and rodents do not pass tests for self-recognition such as the mirror test. Still, rodent observational fear involves the key cognitive function of fear learning, followed by the retrieval of the fear memory in the absence of the demonstrator to incite fear. This places observational fear apart from simultaneous emotional contagion which requires the presence of the demonstrator.

Rodent behavior models have major advantages, because they allow for the precise manipulation of neural circuits and single-cell resolution mapping of neuronal activity in vivo. Future investigation of the molecular, cellular and circuitry mechanisms underlying observational fear in rodents will offer insights into the foundations of human empathy. Identification of causal genes may uncover novel genetic pathways and underlying neural mechanisms that modulate empathy and may ultimately provide new targets for therapeutic intervention in human mental disorders causing dysregulations in empathy.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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