

Reviewed Preprint

v1 • February 4, 2026

Not revised

✉ For correspondence:

lingyunli@ccmu.edu.cn

yatangli@cibr.ac.cn

† These authors contributed equally to this work.

Competing interests: No competing interests declared

Funding: See page 16

Reviewing editor: Peng Cao, National Institute of Biological Sciences, Beijing, China

© 2026, Li et al. This article is distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use and redistribution provided that the original author and source are credited.

Rank- and Threat-Dependent Social Modulation of Innate Defensive Behaviors

Ling-yun Li^{1,†}✉, Xinjian Gao^{2,3,†}, Ya-tang Li^{2,3}✉

¹Department of Neurobiology, School of Basic Medical Sciences, Capital Medical University, Beijing, China •

²Beijing Institute for Brain Research, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China •

³Chinese Institute for Brain Research, Beijing, China

eLife Assessment

The authors investigate how dominance hierarchy shapes defensive strategies in mice under two naturalistic threats: a transient visual looming stimulus and a sustained live rat. This study provides **important** insights into how social context and dominance hierarchy modulate innate defensive behaviors across distinct naturalistic threats. The strength of evidence is **convincing**, with detailed classification and analysis of behaviors.

<https://doi.org/10.7554/eLife.109571.1.sa4>

Abstract

Fear and defense are among the most fundamental survival behaviors and are profoundly influenced by the social environment in group-living animals. While previous research has largely focused on learned fear paradigms, these approaches fail to capture the complexity of naturalistic threats. It remains poorly understood how social context—and particularly dominance hierarchy, a defining feature of many social species—modulates defensive strategies under ethologically relevant conditions. To address this question, we investigated the social modulation of innate fear in mice exposed to two ethologically relevant threats: a transient visual looming stimulus and a sustained live rat. Pair-housed mice with established dominance ranks were tested alone or with their social partner, and their behaviors were quantitatively analyzed. We found that social presence alleviates threat-induced stress and modulates defensive behaviors in a rank- and threat-specific manner: during looming exposure, it reduces immediate defensive responses and alleviates post-looming anxiety, with dominants showing greater benefit; during rat exposure, it promotes a shift from passive to active defense, again most prominently in dominants. Furthermore, threat exposure reinforces social roles and enhances group cohesion. Together, these findings demonstrate how dominance hierarchy modulates defensive responses to distinct naturalistic threats and, in turn, how threat experience shapes social behavior, providing a framework for probing the neural basis of socially modulated innate fear.

Introduction

Innate fear of predation has been a powerful driver of the evolution of defensive strategies (Cooper and Blumstein, 2015 [↗](#); Lima and Dill, 1990 [↗](#); Mobbs et al., 2024 [↗](#)). In group-living species, these responses are further shaped by social context (Evans et al., 2019 [↗](#); Krause and Ruxton, 2002 [↗](#)). Collective behaviors—such as the circle defense of muskoxen against wolves, the coordinated retreat of meerkats to burrows, mobbing of raptors by smaller birds, and predator-specific alarm calls in primates—demonstrate how social context enhances survival by coordinating individual responses. In humans and other social animals, individuals adjust their fear perception and defensive behaviors based on conspecific cues through mechanisms such as social buffering (Kikusui et al., 2006 [↗](#); Qi et al., 2021 [↗](#)), emotional contagion (de Gelder et al.,

2004 [Olsson and Phelps, 2007](#)), and social appraisal (Mumenthaler and Sander, 2012). Disruptions in these processes increase vulnerability to fear-related disorders, leading to maladaptive fear responses and impaired social functioning (Grammer and Zelikowsky, 2022 [Ozbay et al., 2007](#) [Top et al., 2016](#)). Understanding the neural mechanisms underlying the social regulation of fear is therefore crucial for treating these neurological disorders.

Most studies on the social regulation of fear have focused on learned fear paradigms (Kikusui et al., 2006 [Morozov and Ito, 2019](#)). In these paradigms, conspecific presence typically reduces fear (Davitz and Mason, 1955), yet the effect depends on partner identity and emotional state: familiar partners provide stronger buffering (Kiyokawa et al., 2014), while frightened partners can diminish or even reverse this effect (Kiyokawa et al., 2004 [Morozov and Ito, 2019](#)). While these studies have advanced our understanding of how social cues modulate fear responses, they rely on conditioning and therefore do not capture ethologically relevant threat scenarios (Cisek and Green, 2024 [Dennis et al., 2021](#)).

In contrast, innate fear paradigms provide complementary insights into naturalistic defensive responses (Adolphs, 2013 [Gross and Canteras, 2012](#) [Rosen, 2004](#)). Because innate fear responses are evolutionarily conserved, their underlying neural mechanisms are likely to generalize across individuals and species (Hein et al., 2018 [Peek and Card, 2016](#)). Recent work using such paradigms has begun to uncover how social cues regulate defensive behaviors across taxa—including flies, fish, rodents, primates, and humans—with modulation depending on the properties of threats and social cues (Blanchard and Blanchard, 1989 [Coan et al., 2006](#) [Faustino et al., 2017](#) [Ferreira et al., 2022](#) [Gutzeit et al., 2020](#) [Testard et al., 2024](#) [Vogt et al., 1981](#)). Despite these advances, how dominance hierarchy—a fundamental organizing principle of many animal societies—regulates defensive behavior, and whether such regulation depends on the nature of the threats, remains poorly understood.

To address these questions, we developed a behavioral paradigm in which pair-housed mice with established dominance hierarchies encountered two distinct naturalistic threats, a transient visual looming stimulus (Yilmaz and Meister, 2013) and a sustained live rat (Kennedy et al., 2020 [Kunwar et al., 2015](#)), both alone and with their partners. Social presence alleviates threat-induced stress and modulates defensive strategies in a rank- and threat-dependent manner, attenuating looming-evoked defense while promoting active coping during rat exposure, with the effects more pronounced in dominants. Furthermore, shared threat experience reinforces social roles and strengthens group cohesion. Together, these findings reveal reciprocal interactions between defensive and social behaviors across distinct threat contexts, providing a behavioral framework for future investigations into the neural mechanisms underlying social modulation of innate fear.

Results

A behavioral paradigm to probe dominance-hierarchy modulation of innate defense across distinct threat contexts

To investigate how dominance hierarchy modulates innate defensive responses, we developed a behavioral paradigm that incorporates two ethologically relevant predatory stimuli (Figure 1A). Adult male sibling mice were pair-housed for two weeks to establish stable dominance hierarchies, assessed using the tube test (Fan et al., 2019). Subsequently, they were divided into two cohorts: one exposed to a transient visual looming stimulus that mimics the sudden approach of an aerial predator (Yilmaz and Meister, 2013), and the other to a live rat that serves as a sustained threat (Kennedy et al., 2020). Before threat exposure, all mice were habituated to the arena. Each mouse was tested both alone and in the presence of their cage mate. Rank stability was reassessed after threat exposure; only one pair showed a rank reversal and was excluded from further analysis.

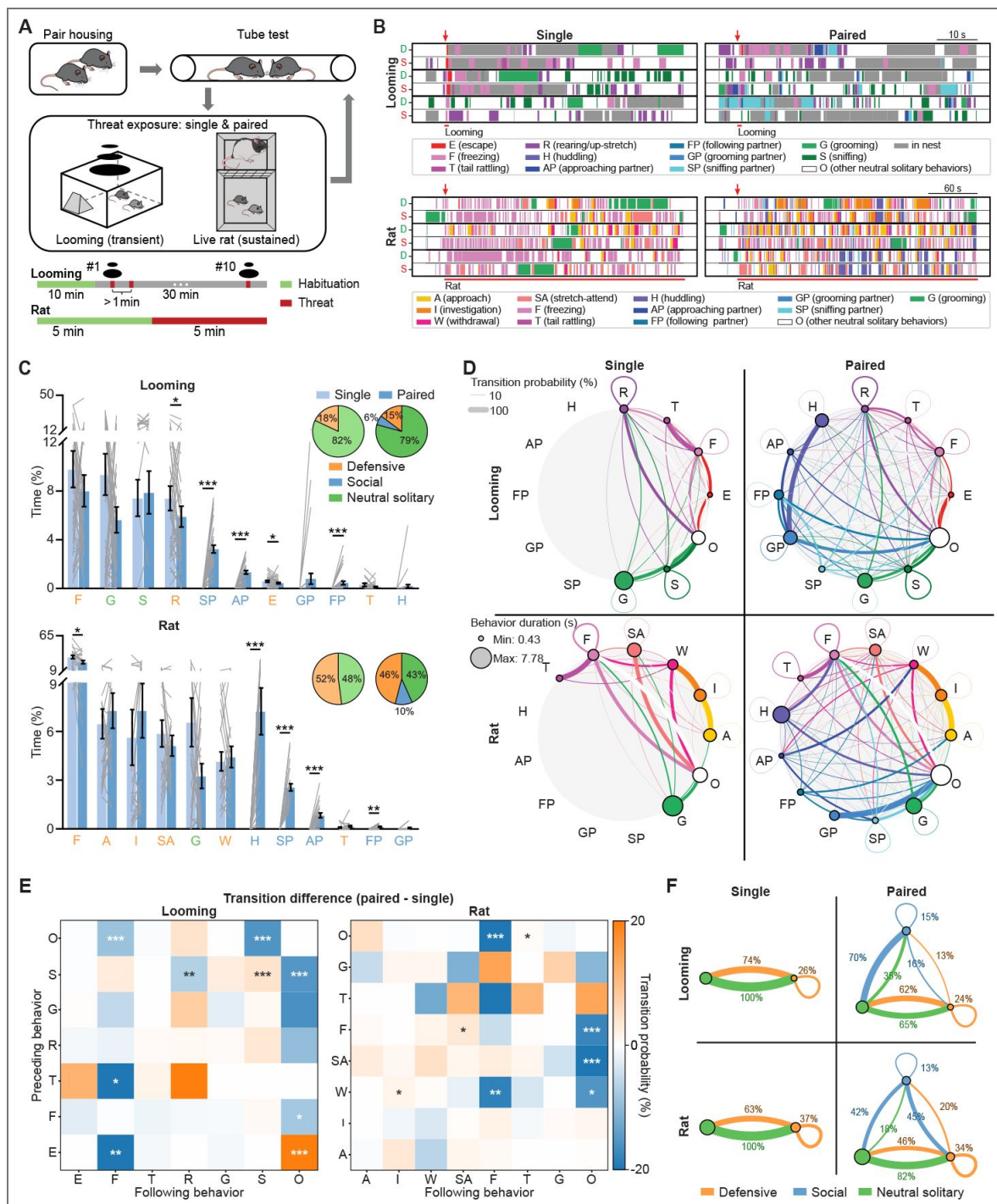


Figure 1. A behavioral paradigm to probe dominance-hierarchy modulation of innate defense across distinct threat contexts.

(A) Schematic of behavioral paradigms. (B) Representative raster plots of behaviors during single and paired exposures. Top: looming exposure; bottom: live rat exposure. D: dominant; S: subordinate. (C) Bar graph of average time allocation for each annotated behavior. Pie charts show the proportion of behavioral categories. Paired *t*-test; *N* = 40 mice for looming exposure, *N* = 22 mice for rat exposure. (D) Behavior transition maps. Node size indicates the average duration of each behavior. Line thickness scales with the transition probability. (E) Matrices of behavior transition differences between single and paired conditions (paired-single). Paired *t*-test. (F) Transition maps of behavioral categories. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

During habituation, mice primarily engaged in neutral solitary behaviors, with occasional spontaneous freezing (Figures S1A [↗](#) and S1B [↗](#)). When exposed to threats alone, both looming and rat stimuli elicited robust defensive responses (Figure 1B [↗](#)). Some behaviors, such as freezing and tail rattling, were common across paradigms, whereas others were threat-specific: escape occurred exclusively during looming exposure, while stretch-attend, approach, investigation, and withdrawal were unique to rat exposure. The presence of a social partner reduced defensive behaviors more than neutral ones (Figures 1B [↗](#) and 1C [↗](#)), suggesting an attenuation of fear under social conditions.

To probe the structure underlying these behaviors, we generated their transition maps (Figures 1D [↗](#)). Three patterns emerged. First, threat-specific defensive structures were evident. During looming exposure, freezing typically followed escape, and about one-third of bouts transitioned into rearing, reflecting heightened vigilance after a transient threat. In contrast, during rat exposure, freezing occurred more frequently after withdrawal, and about one-third entered another freezing bout, consistent with sustained, elevated stress levels. Rat exposure also revealed an approach–investigation–withdrawal sequence, indicating an active threat-evaluation strategy. Second, social context modulated transition dynamics, particularly those leading to freezing (Figures 1E [↗](#), S1C, S1D [↗](#)). During looming exposure, post-escape freezing was partially replaced by neutral behavior in paired conditions; during rat exposure, post-withdrawal freezing was partially replaced by social behaviors. These behavioral shifts are consistent with reduced fear levels. Third, the nature of the threat shaped social modulation of behavior: during transient looming threats, huddling and approaching the partner were often followed by grooming the partner, indicative of stress-buffering interactions, whereas during persistent rat exposure, huddling was more often followed by freezing, reflecting heightened and sustained fear. Such threat-specific social modulation was also evident in transitions among defensive, social, and neutral behaviors. Compared to looming exposure, social behaviors were more often followed by defensive behavior during rat exposure (Figure 1F [↗](#)). Together, these findings reveal that social context modulates defensive strategies in a threat-specific manner.

Social context attenuates looming-evoked defensive behavior in a rank-dependent manner

Although looming stimuli trigger immediate defensive responses, their impact on behavior and emotional state can persist beyond the initial reaction. To examine how social context modulates the expression and progression of defensive responses, we analyzed behavior in two time windows: an early phase (0–5 s after looming onset) and a late phase (20–60 s after onset), each compared to the pre-looming baseline.

During baseline, mice showed a strong preference for the nest and stayed close to the arena walls when exploring outside, consistent with thigmotaxis in the open-field test (Figures 2A, 2B [↗](#), S2A, S2B [↗](#)). Looming exposure altered both spatial occupancy and locomotion speed, with distinct effects across the transient and sustained phases (Figures 2A–2D [↗](#), S2B [↗](#)). In the transient phase, it shifted the distribution of nest occupancy ($p < 0.001$, Kolmogorov–Smirnov (KS) test) and center-zone speed ($p < 0.001$, KS test), reflecting a rapid behavioral change immediately after threat onset. In the sustained phase, looming exposure increased nest occupancy and reduced locomotion speed within the nest (Figures 2A–2D [↗](#), S2B [↗](#)), indicative of heightened anxiety. The presence of a social partner increased the locomotion speed in the nest (Figures S2F–G [↗](#)), suggesting that social interaction attenuates anxiety-related suppression of movement. Notably, nest occupancy remained unchanged (Figures S2C–E [↗](#)), which may reflect opposing social influences: reduced anxiety could promote exploration outside the nest, whereas social opportunities may motivate them to remain inside (Figure S2H [↗](#)).

To directly assess how social context influences defense, we quantified manually annotated behaviors. During the early phase, looming-evoked behaviors were classified into four decision types: escape (E), escape after assessment (F+E), freezing (F), and no response (N) (Figures 2E–F [↗](#)). Social context significantly altered decision patterns in both dominant and subordinate mice (Figure 2F [↗](#)), with rank-specific modulation of escape speed and freezing duration. Escape speed

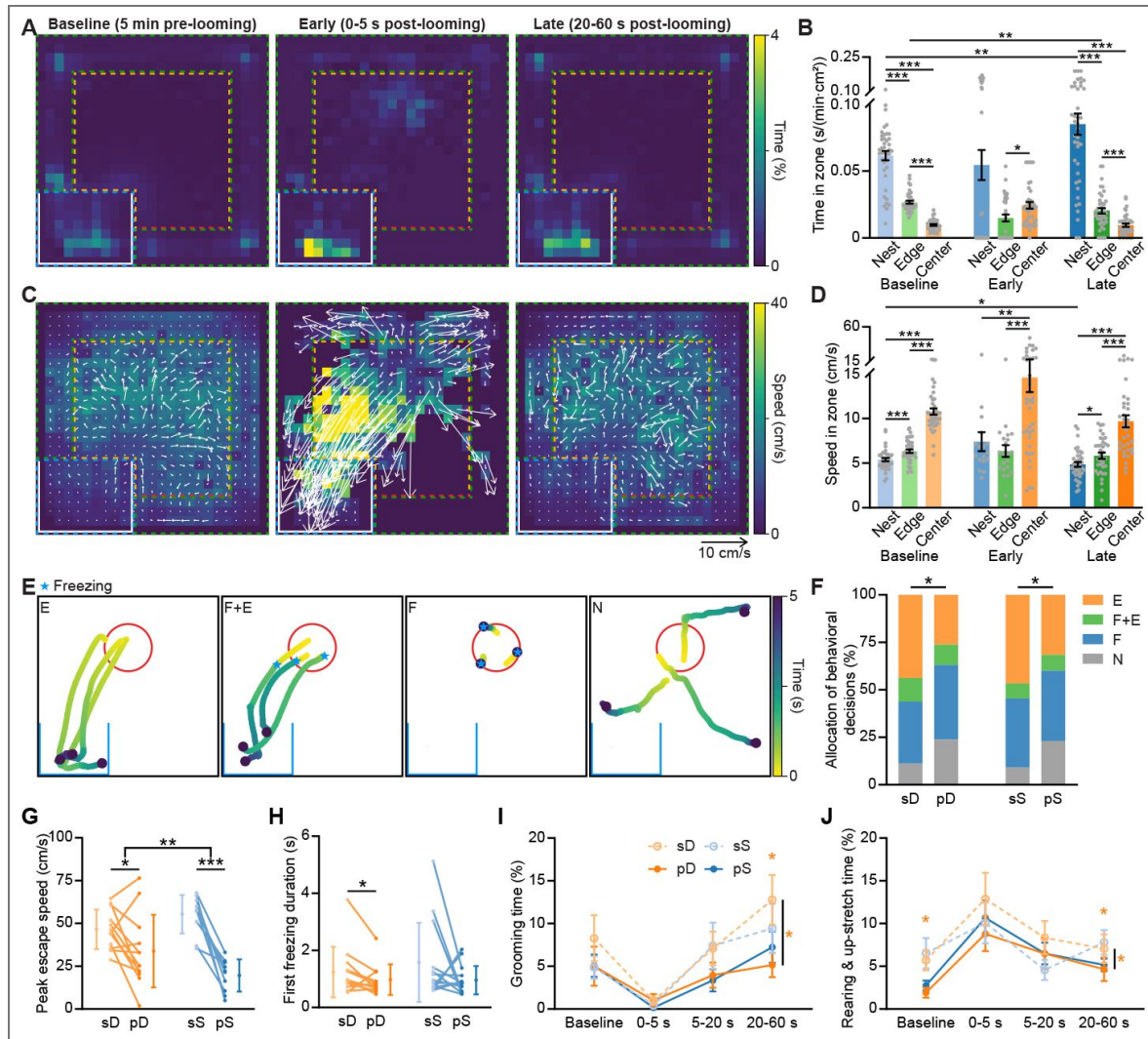


Figure 2. Social context attenuates looming-evoked defensive behavior in a rank-dependent manner.

(A) Heatmaps of average time allocation in nest, edge, and center zones during baseline, early, and late phases under single exposure. (B) Time spent in each zone per minute, normalized by zone area. Paired *t*-test; *N* = 40 mice. (C) Heatmaps of average locomotion speed across zones, with arrows indicating average velocity vectors. (D) Locomotion speed in each zone. (E) Representative moving trajectories of example mice exhibiting different behavior decisions within 5 s after looming onset. (F) Distribution of behavioral decisions following looming exposure. Chi-square test; *N* = 89, 84, 88, 95 trials for sD (single dominant), pD (pair dominant), sS (single subordinate), and pS (pair subordinate) groups, respectively. (G) Peak escape speed. Paired *t*-test; *N* = 12, 12, 10, 10 mice. (H) Duration of the first looming-evoked freezing bout. Paired *t*-test; *N* = 12, 12, 13, 13 mice. (I) Time spent on self-grooming across time windows. Two-way ANOVA with post hoc Tukey's range test; *N* = 20 mice for all groups. (J) Time spent on rearing and up-stretch across time windows. Two-way ANOVA with post hoc Tukey's range test; *N* = 20 mice for all groups. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

was reduced in both groups, with a great reduction in subordinates (Figure 2G [↗](#)), whereas freezing duration was significantly shortened only in dominants (Figure 2H [↗](#)). This reduction in freezing was unlikely due to direct social interruption, as only a small fraction of freezing episodes transitioned to or overlapped with social behaviors (Figures S2I-K [↗](#)).

Rank-specific effects persisted into the late phase. Under social conditions, stress-induced self-grooming was alleviated only in dominants (Figures 2I [↗](#) and S2L [↗](#)). A similar rank-dependent reduction was observed for anxiety-associated rearing and up-stretch behaviors, evident both after looming exposure and during baseline (Figure 2J [↗](#)).

Together, these results demonstrate that social context shapes both early and late defensive responses to transient visual threats in a rank-dependent manner, modulating not only the occurrence but also the expression of specific defensive and anxiety-related behaviors.

Social context alleviates sustained predatory stress and fine-tunes defensive strategies across ranks

In natural environments, prey must cope not only with sudden, unpredictable predatory strikes but also with the sustained presence of predators, which imposes prolonged stress and elicits a broader repertoire of defensive behaviors. To investigate how social context modulates such defenses, we developed a rat-exposure paradigm (Figure 1A [↗](#)). Following a 5-minute habituation, a live rat was placed in an adjacent perforated, transparent chamber, allowing multisensory cues to reach the mouse for 5 minutes.

Mice allocated their time differently across near, middle, and far zones relative to the predator (Figures 3A, 3B [↗](#), S3A-C [↗](#)), and their locomotion speed peaked in the middle zone when moving toward the far zone (Figures 3C [↗](#) and 3D [↗](#)). These patterns suggest a gradient of perceived risk: highest near the predator, moderate in the middle zone, and lowest in the far zone. Social context increased the locomotion speed in the far zone without altering time allocation (Figures S3D [↗](#) and S3E [↗](#)), echoing findings from the looming paradigm and indicating reduced anxiety.

To examine social modulation of specific behaviors, we grouped them into passive defense, active defense, social, and neutral categories (Figures 3E-F [↗](#)). Social context reshaped the distribution across these categories in both ranks and amplified rank differences (Figure 3F [↗](#)). This amplification was driven by a greater shift from passive to active defense in dominants under paired conditions (Figures 3G [↗](#) and 3I [↗](#)). Specifically, freezing decreased in both ranks, with a more pronounced reduction in dominants (Figures 3H [↗](#) and S3F-H [↗](#)). Approach frequency increased in both ranks (Figure 3J [↗](#)), while average investigation time—longer in dominants—remained unaffected by social context (Figure 3K [↗](#)). Together, these results indicate that social context alleviates sustained predator-induced stress while fine-tuning defensive strategies in a rank-dependent manner, paralleling effects observed in the looming assay.

Threat exposure reinforces social roles and promotes cohesive behavior

Having established how social context shapes defensive behaviors toward distinct threats, we next asked the reciprocal effect: how does threat exposure influence social behavior? We quantified both the total time spent on social behaviors and the average duration of social bouts (Figures 4A-D [↗](#)). Looming exposure increased both measures only in subordinates, whereas rat exposure affected both ranks, indicating a threat-specific modulation of social behaviors. In particular, during rat exposure, huddling emerged as the predominant social behavior across ranks (Figure 4E [↗](#)), likely serving to reduce individual risk and enhance collective security under sustained threat.

To further examine how threat exposure alters the dynamics of social interaction, we analyzed proactive and reactive behaviors across ranks (Figure 4F [↗](#)). Looming exposure increased the proportion of proactive bouts in dominants but decreased it in subordinates (Figure 4G [↗](#)), with a similar trend during rat exposure (Figure 4K [↗](#)). Threats also lengthened proactive bouts in

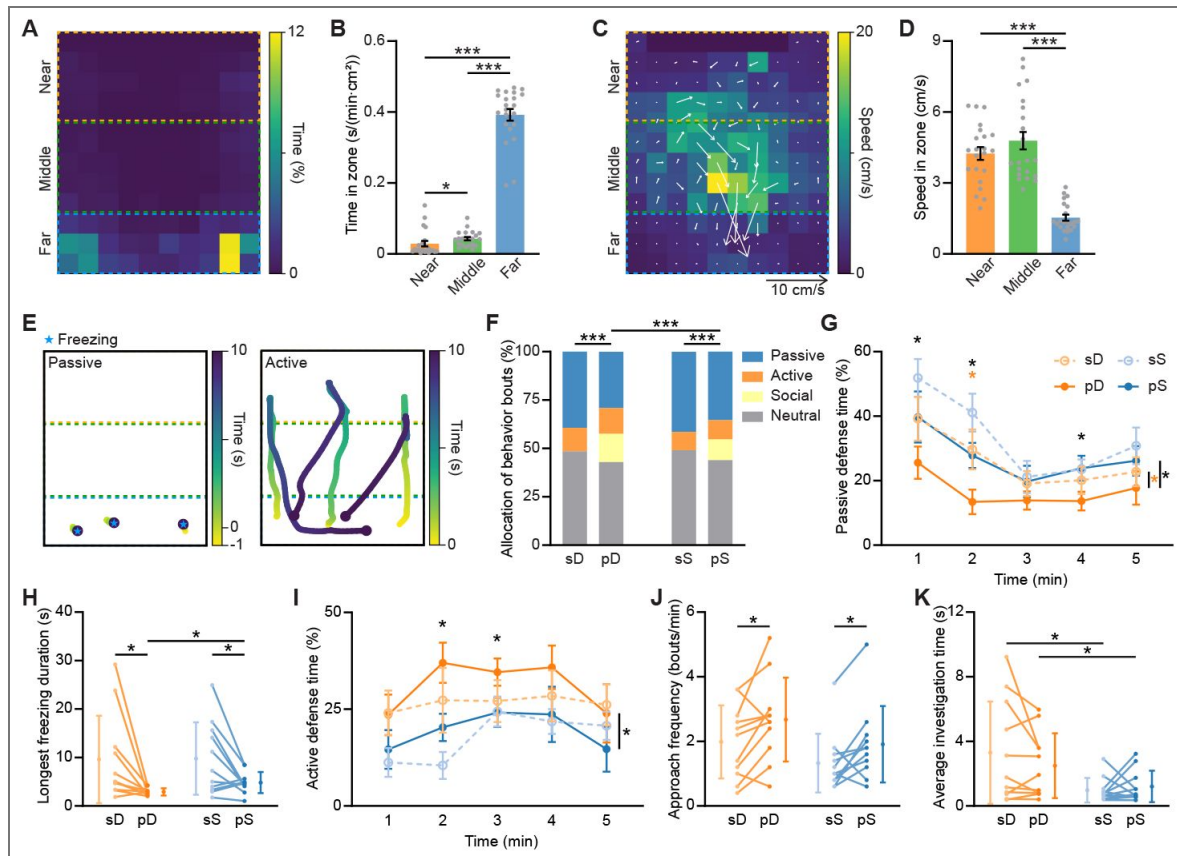


Figure 3. Social context alleviates sustained predatory stress and fine-tunes defensive strategies across ranks.

(A) Heatmaps of average time allocation in near, center, and far zones during rat exposure. (B) Time spent in each zone per minute, normalized by zone area. Paired *t*-test; *N* = 22 mice. (C) Heatmaps of average locomotion speed, with arrows indicating average velocity vectors. (D) Speed in each zone. Paired *t*-test; *N* = 22 mice. (E) Representative trajectories of example mice displaying passive and active defense during rat exposure. (F) Distribution of behavioral categories during rat exposure. Chi-square test; *N* = 11 mice for each group. (G) Time spent on passive defense across time windows. Two-way ANOVA with post hoc Tukey's range test; *N* = 11 mice for each group. (H) Longest freezing duration. Paired *t*-test; *N* = 11 mice for each group. (I) Time spent on active defense across time windows. Two-way ANOVA with post hoc Tukey's range test; *N* = 11 mice for each group. (J) Approach frequency toward the rat. Paired *t*-test; *N* = 11 mice for each group. (K) Average investigation time toward the rat. Paired *t*-test; *N* = 11 mice for each group. **p* < 0.05; *** *p* < 0.001.

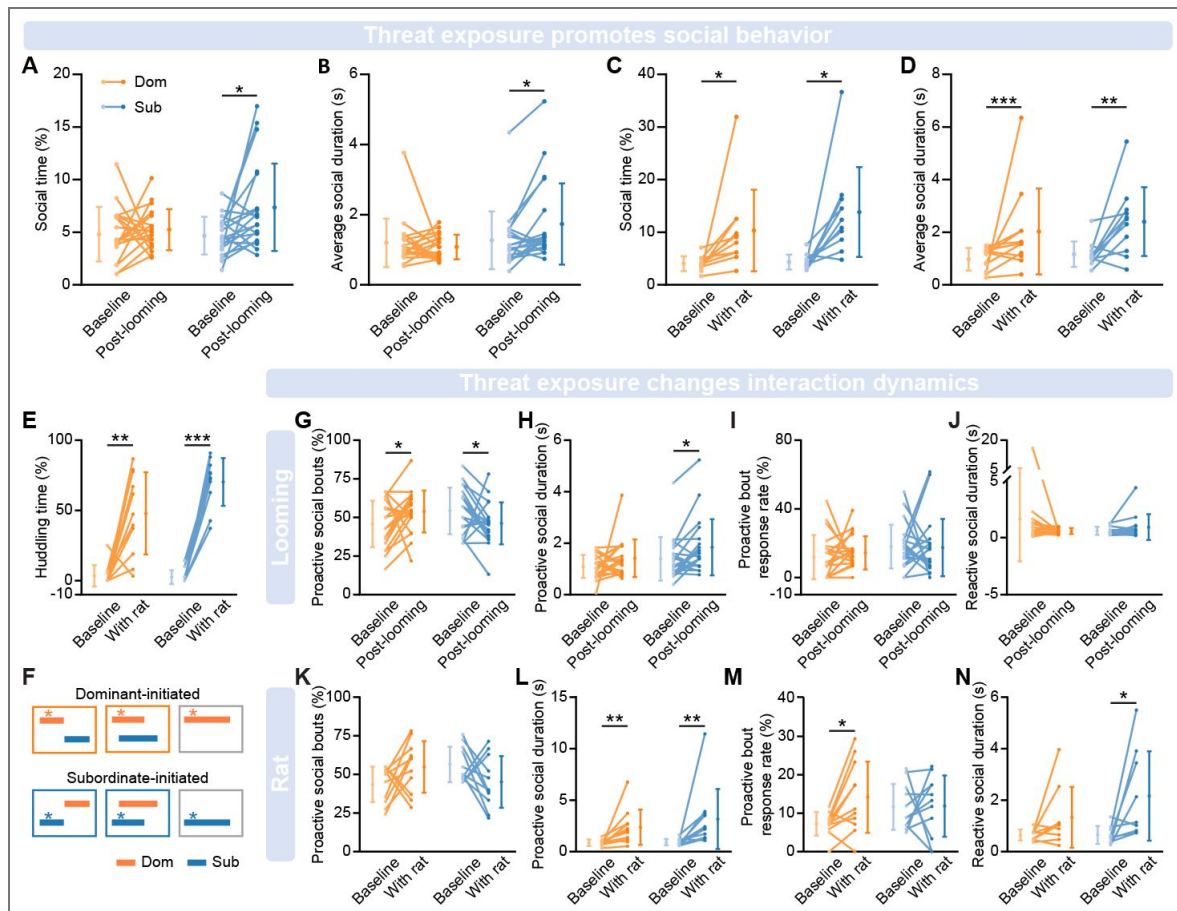


Figure 4. Threat exposure reinforces social roles and promotes cohesive behavior.

(A) Time spent on social behaviors within a 1-minute window before and after looming exposure. Paired *t*-test; *N* = 19, 20 mice for Dom and Sub group. (B) Average bout duration of social behaviors before and after looming exposure. Paired *t*-test; *N* = 19, 20 mice for Dom and Sub group. (C) Time spent on social behaviors during a 5-minute window before and during rat exposure. Paired *t*-test; *N* = 11 mice for both groups. (D) Average bout duration of social behaviors before and during rat exposure. Paired *t*-test; *N* = 11 mice for both groups. (E) Percentage of social time spent on huddling before and during rat exposure. Paired *t*-test; *N* = 11 mice for both groups. (F) Schematic illustrating social interactions. * denotes proactive social behaviors. (G-J) Social interactions in the looming paradigm: (G) Percentage of proactive social bouts across ranks. (H) Average duration of proactive bouts. (I) Response rate to proactive bouts. (J) Average duration of reactive bouts. Paired *t*-test; *N* = 17, 13 mice. (K-N) Same plots for the rat paradigm. *N* = 9, 9 mice. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

subordinates after looming exposure and in both ranks during rat exposure (Figures 4H [↗](#) and 4L [↗](#)). Moreover, only during rat exposure, proactive initiations by dominants elicited higher response rates from subordinates (Figures 4I [↗](#) and 4M [↗](#)), who in turn exhibited longer reactive social durations (Figure 4J [↗](#) and 4N [↗](#)).

These findings indicate that naturalistic threats, particularly sustained predator presence, enhance social engagement in a rank-dependent manner, potentially promoting cohesion and collective coping under threat.

Discussion

Our study introduces a behavioral paradigm to investigate how dominance hierarchy shapes innate defensive behaviors under distinct naturalistic threats (Figure 1 [↗](#)). Using both transient looming stimulus and sustained rat exposure, we found that the presence of a social partner alleviates threat-induced stress in both dominant and subordinate mice, while fine-tuning their defensive strategies in a rank- and threat-dependent manner. Under looming threats, dominants showed stronger social modulation, with reduced freezing and faster recovery (Figure 2 [↗](#)). During sustained rat exposure, social context promoted a shift from passive to active defense, again more prominently in dominants (Figure 3 [↗](#)). Threat exposure also shaped social behaviors in a rank-dependent manner: dominants engaged more proactively, while subordinates became more responsive to dominant initiations, potentially reinforcing their social roles (Figure 4 [↗](#)).

These findings broaden our understanding of how social context regulates fear responses, which has been largely studied in learned fear paradigms (Kikusui et al., 2006 [↗](#); Morozov and Ito, 2019 [↗](#)). Our finding that conspecific presence reduces fear aligns with previous evidence across species, including zebrafish (Faustino et al., 2017 [↗](#)), flies (Ferreira et al., 2022 [↗](#)), rats (Bowen et al., 2013 [↗](#)), monkeys (Testard et al., 2024 [↗](#)), and humans (Coan et al., 2006 [↗](#)). However, the role of social hierarchy in modulating innate fear has remained largely unexplored. Prior work has shown that dominant and subordinate rats behave differently when exposed to a predator (Blanchard and Blanchard, 1989 [↗](#)), possibly reflecting the additional stress imposed on subordinates by their hierarchical status (Blanchard and Blanchard, 1990 [↗](#); Blanchard et al., 1993 [↗](#)). Consistent with this, we found that dominants investigated predators longer than subordinates when tested alone. Yet the earlier study lacked paired versus single comparisons, leaving the role of social context unresolved. Our work addresses this gap, showing that defensive responses are jointly shaped by threat type and social rank, with an emphasis on survival-critical behaviors rather than fear or stress alone.

This rank-specific regulation likely has adaptive value for group survival. For transient threats, stronger fear reduction in dominants may allow leaders to recover rapidly and guide group decision-making. For sustained threats, the shift of dominants toward active defense mirrors natural patterns in which leaders take on greater risk-assessment responsibilities (Blanchard and Blanchard, 1989 [↗](#); Davis et al., 2009 [↗](#)). Such role differentiation could enhance group survival by distributing vigilance and risk-taking across the hierarchy. Furthermore, the increase in social interactions following threat exposure likely reflects an evolutionarily conserved coping mechanism that strengthens group cohesion and collective security—a phenomenon observed across multiple species, including humans (Morris et al., 1976 [↗](#); Preston et al., 2011 [↗](#); Taylor, 1981 [↗](#); von Dawans et al., 2012 [↗](#)).

What neural circuits mediate the social modulation of innate defensive behavior? We propose that the medial prefrontal cortex (mPFC) serves as a central hub in this process. Evidence from learned fear paradigms supports a modulatory role of the mPFC: it is densely interconnected with the amygdala and is essential for both the expression and extinction of conditioned fear (Marek et al., 2013 [↗](#); Sotres-Bayon and Quirk, 2010 [↗](#)). Moreover, the mPFC is involved in social behavior and dominance hierarchy (Kingsbury et al., 2019 [↗](#); Wang et al., 2011 [↗](#)). These findings indicate that the mPFC plays a key role in the social regulation of fear. Indeed, inhibition of the mPFC abolishes social buffering of fear responses (Lungwitz et al., 2014 [↗](#)), whereas its activation can reproduce this buffering effect (Gutzeit et al., 2020 [↗](#)).

By contrast, innate defensive behaviors are mediated by threat-specific subcortical circuits. Defensive responses to visual threats rely on the superior colliculus (SC) (Evans et al., 2018 [↗](#); Shang et al., 2015 [↗](#); Wei et al., 2015 [↗](#)), whereas responses to predator odors or multimodal cues depend on the dorsomedial part of the ventromedial hypothalamus (VMHdm) (Silva et al., 2016 [↗](#)). Importantly, the SC and VMHdm are not isolated but interconnected (Benavidez et al., 2021 [↗](#); Oh et al., 2014 [↗](#)): the SC projects to VMHdm via distributed relay nodes, including the anterior hypothalamic nuclei (AHN), thalamus, and periaqueductal gray, and VMHdm sends direct projections back to the SC. This bidirectional connectivity positions VMHdm as a key integrator of internal fear state triggered by naturalistic threats. Crucially, VMHdm receives top-down modulation from the mPFC, relayed by the amygdala and AHN (Canteras, 2002 [↗](#); Hintiryan et al., 2025 [↗](#); Silva et al., 2016 [↗](#); Swanson, 2000 [↗](#)). These findings raise the possibility that the mPFC regulates innate defensive behaviors by modulating neural activity in VMHdm and related subcortical circuits. Alternatively, the mPFC may function through parallel, modality-specific pathways. For instance, its direct projections to the SC (Benavidez et al., 2021 [↗](#)) could selectively influence visually evoked defensive responses. Disentangling these models—convergent modulation of a unified fear state versus parallel control over distinct threat circuits—remains an important goal for future research.

One limitation of our study is the exclusive use of male mice, as the estrous cycles of female mice introduce additional variability that is difficult to control experimentally. Because female rodents show distinct defensive and social patterns under threat compared to males (Blanchard et al., 1991 [↗](#); Pentkowski et al., 2018 [↗](#)), extending this paradigm to female mice—with careful control for estrous cycles—will provide a more comprehensive understanding of how hierarchy and social context shape defensive strategies.

In conclusion, our findings reveal that social context does not simply suppress fear but selectively tunes defensive and social behaviors according to both rank and threat type. This adaptive flexibility allows groups to manage risk while maintaining cohesion, paralleling collective coping strategies observed in humans and other animals. By providing a behavioral framework for probing the neural basis of social modulation of innate fear, our work opens new avenues for dissecting the interplay between social and defensive circuits. Given that maladaptive fear and social dysfunction often co-occur in neuropsychiatric disorders, these insights may inform mechanistic models of social-affective dysfunction and inspire new translational approaches.

Methods

All experimental procedures complied with animal welfare guidelines and were approved by the Institutional Animal Care and Use Committee at the Chinese Institute for Brain Research, Beijing.

Animal

Male C57BL/6J mice (8–12 weeks old) were pair-housed under a 12-h light/12-h dark cycle. Male Long-Evans rats (6 months old) were housed under the same light-dark cycle. All experiments were performed during the light phase. A total of 64 mice were used.

Arena design

The looming-exposure arena (48 (L)×48 (W) × 25 (H) cm) was constructed from infrared-transparent acrylic, with an overhead monitor (53 ×30 cm) to present visual stimuli. A prism-shaped shelter (20× 15 ×12 cm) was placed in one corner with its opening facing the monitor. Illumination was provided by two diagonally positioned infrared lamps. For real-time tracking, an OpenMV camera was placed 80 cm below the arena. A second infrared camera (LBAS-U350-74M, LUSTER LightTech) was placed 75 cm below the arena to record animal behavior at 30 Hz.

The rat-exposure arena (38.5 ×21× 40 cm) had three opaque acrylic walls and one infrared-transparent wall for video recording. The rat holding cage (18 ×14 ×35 cm) was made from transparent acrylic, with the wall facing the mouse perforated to allow visual, olfactory, and

auditory cues. Behavior was recorded using two cameras: one overhead (75 cm above the arena floor) and one lateral (50 cm away from the infrared-transparent wall).

Tube Test

Social rank within each mouse pair was determined using the tube test. To minimize stress, each pair was co-housed for at least two weeks with a 15-cm tube placed in their home cage. Before testing, mice were trained to traverse a 30-cm tube over two consecutive days (10 trials per day), alternating entry from either end. On the test day, each mouse pair competed in up to seven trials using the same 30-cm tube, and the first mouse to win four trials was designated as the dominant. Social rank stability was reassessed one day after threat exposure, and only pairs with consistent ranks were included in subsequent analyses.

Threat Exposure

Looming exposure was conducted over two consecutive days using a counterbalanced design of single and paired trials. Half of the mice were exposed under single conditions on day 1 and paired conditions on day 2, with the order reversed for the other half. Each mouse underwent one 30-minute session per day, beginning with 10 minutes of free exploration. Looming stimuli were presented on a monitor suspended 25 cm above the arena. Each stimulus consisted of a dark disk (98% contrast on a grey background) that expanded from 1° to 20° of visual angle at 40°/s and remained at 20° for 0.25 s. Stimuli were triggered when a mouse entered a 13×13 cm zone beneath the monitor center. Each mouse could trigger up to 10 stimuli per session, with a minimum inter-stimulus interval of two minutes to prevent habituation to the looming. A total of 40 mice were used.

Rat exposure followed the same two-day, counterbalanced design. Each session began with 5 minutes of habituation in the arena, followed by 5 minutes of exposure to a live rat. A total of 24 mice were used.

Animal tracking

In the looming paradigm, seven key points on each mouse were tracked using Deeplabcut (Mathis et al., 2018 [DOI](#)): nose, left ear, right ear, neck, spine, tail base, and tail middle. The model was trained on a dataset of 1000 labeled frames. Only data points with a likelihood greater than 0.9 and instantaneous speed below 200 cm/s were used for analysis, and missing values were linearly interpolated. In the rat paradigm, mouse coordinates were extracted from recorded videos using EthoVisionXT (Noldus).

Heatmap of time allocation and speed distribution

The arenas were divided into 2×2 cm grid cells. For each cell, the animal's velocity vectors, speed, and occupancy time were calculated and then averaged across all animals. For visualization, grid cells with a total occupancy time of less than 0.17 s or fewer than five visits per mouse were excluded.

Behavioral zones were identified using a data-driven approach. In the looming arena, the edge zone width was defined at the elbow point of the “time in edge zone” curve (Figure S2A [DOI](#)). In the rat arena, the far zone boundary was set at the elbow point of the “time in far zone” curve (Figure S3A [DOI](#)), while the near zone boundary was determined at the stable point of the “near-middle zone transition” curve (Figure S3B [DOI](#)). Time allocation and speed were subsequently calculated for each zone.

Behavioral annotation

Behavioral annotation was performed using BENTO (Segalin et al., 2021 [DOI](#)). Four behaviors were annotated for both paradigms: freezing (complete stillness for ≥0.5 s), sniffing (nose twitching while investigating the environment), grooming (using mouth or paws to clean the fur or skin), and tail rattling (vigorous tail shaking in a crouched or alert posture). Paradigm-specific behaviors

were also annotated. For the looming exposure paradigm, we annotated up-stretch (extending the body toward the screen with stationary hind limbs), rearing (lifting forelimbs off the ground while facing the screen), and escape (fleeing away from the looming stimulus). For the rat exposure paradigm, we annotated approach (moving toward the rat), investigation (nose contact with the rat cage or remaining within 5 cm), withdrawal (retreating from the rat), and stretch-attend (extending the body toward the rat while stationary). Additionally, in paired conditions, five social behaviors were annotated: approaching partner, sniffing partner (nose-directed investigation of the partner's face, body, or tail), grooming partner, following partner, and huddling (remaining close to the partner without engaging in other social behaviors). The remaining unannotated behaviors were grouped as other neutral solitary behaviors.

Behavior transitions

The sequences of annotated behaviors were extracted for each animal. A transition was defined when two consecutive behaviors occurred within < 1 s; intervals ≥ 1 s were classified as transitions to other neutral solitary behaviors. Individual transition matrices were computed for each animal and then averaged to generate the overall transition matrices (Figures 1D [↗](#), S1C [↗](#), and S1D [↗](#)). Transition difference matrices (Figure 1E [↗](#)) were obtained by subtracting transition probabilities in the single condition from those in the paired condition and averaging across animals.

In Figures 1D [↗](#) and 1F [↗](#), node size increased with average bout duration (T) of each behavior according to the equation $r = 0.01 + 0.05 \ln(1 + T)$ (with $T > 0$), and edge thickness indicated the transition probability.

Behavioral analysis

Freezing subtypes in rat exposure

To examine the relationship between freezing stability and social interaction, freezing behavior was classified into three mutually exclusive subtypes (Figure S2I [↗](#)). Type 1 freezing was characterized by the absence of any overlap between mouse A's freezing and mouse B's social behavior, and without initiation of social behavior by mouse A within 1 s after freezing termination. Type 2 freezing was characterized by an overlap between mouse A's freezing and mouse B's social behavior. Type 3 freezing denoted cases in which mouse A engaged in social behavior within 1 s after its freezing ended.

Quantification of social interactions

To quantify the social interactions, we first merged individual social behaviors occurring within 1 s into social episodes. A social interaction was then defined as a pair of social episodes—one from each mouse—that either overlapped or were separated by a gap of ≤ 1 s. Based on the temporal sequence of behavior initiation, interactions were classified into six types reflecting asynchronous initiation, with the initiating mouse designated as the proactive individual (Figure 4F [↗](#)). The proactive bout response rate was calculated as the proportion of proactive bouts that received a response.

Quantification and statistical analysis

No statistical methods were used to predetermine the sample size. Data normality was assessed using the Shapiro-Wilk test. For normally distributed data, paired t-tests were applied; otherwise, Wilcoxon signed-rank tests were used. ANOVA with post-hoc tests was applied to assess the effects of time and social variables. Details of the statistical tests are provided in the figure legends and the Results section.

Resource availability

Materials availability

This study did not generate new unique reagents.

Supplemental information

Additional details related to the Results and Methods sections are provided below.

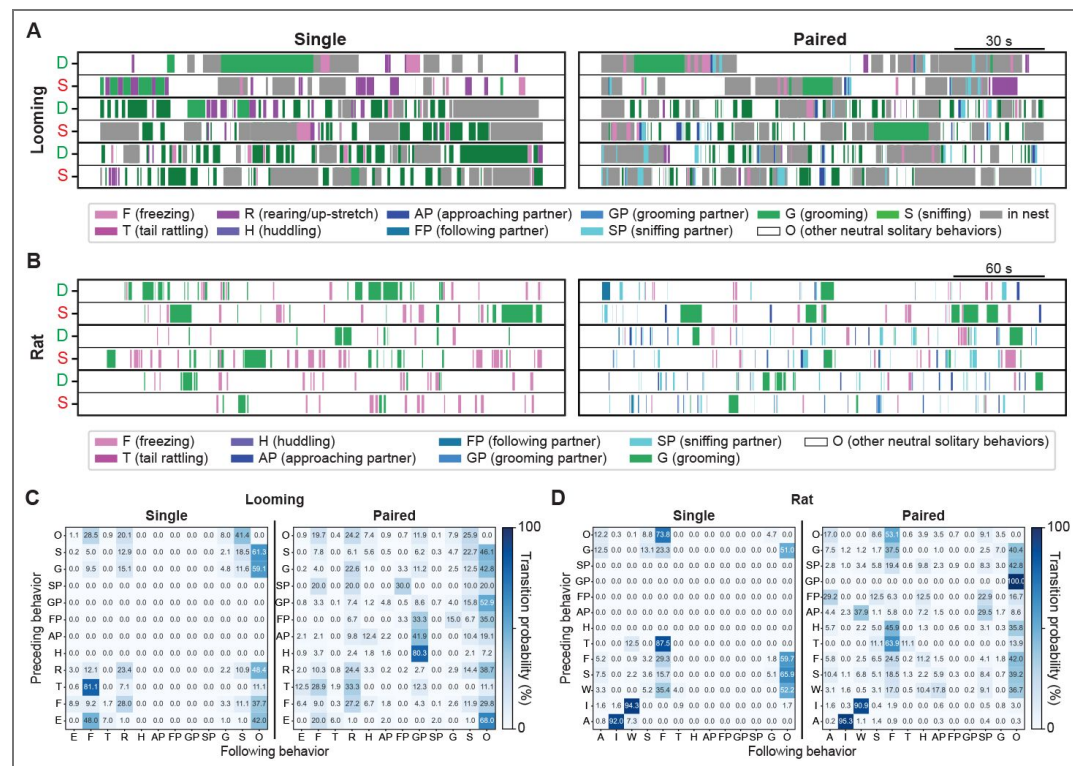


Figure S1. Behavior during habituation and additional information related to transition maps. (A-B)

Representative raster plots of behaviors during the habituation phase in single and paired exposures. (C-D)

Transition probabilities between behavior pairs. Looming, $N = 40$ mice; rat, $N = 22$ mice.

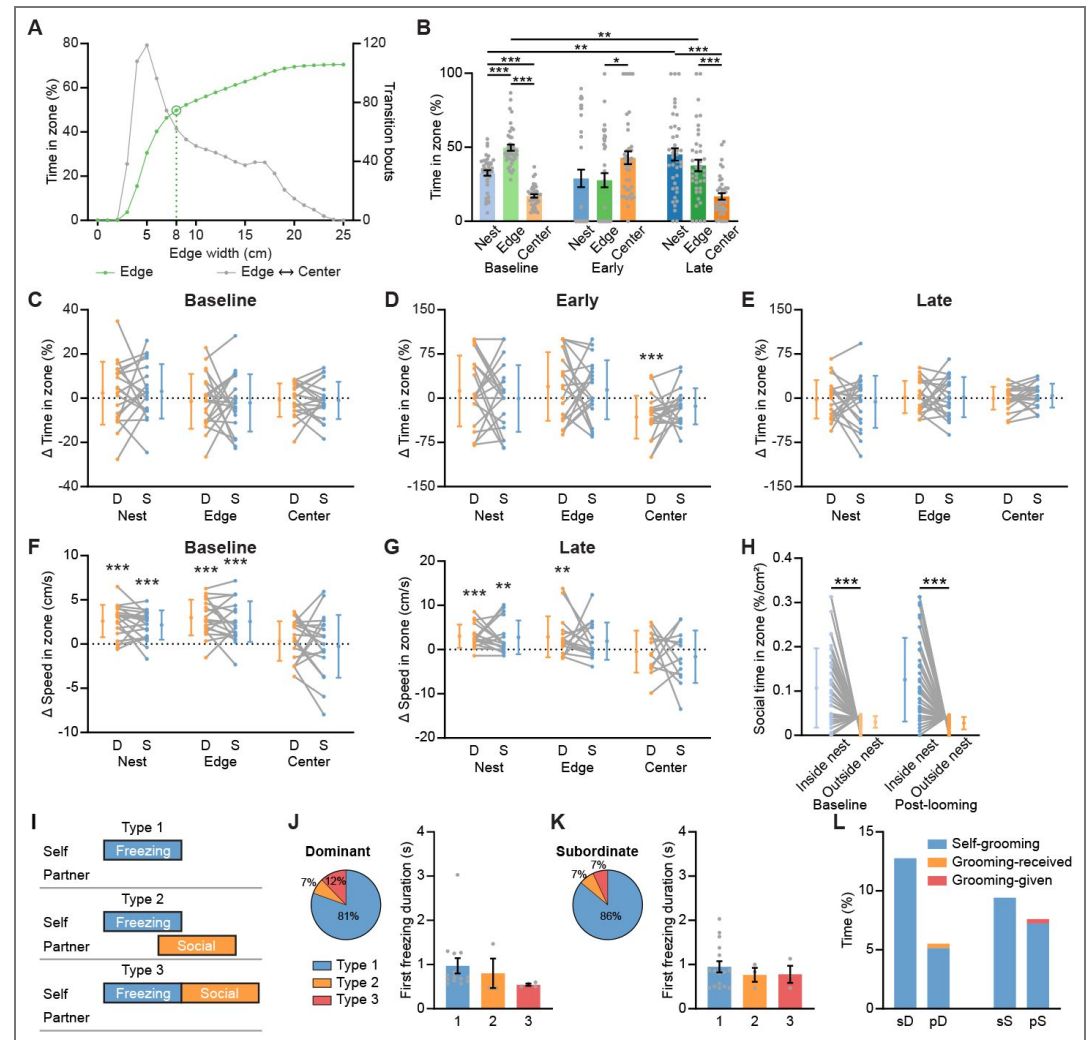


Figure S2. Characterization of looming-evoked defensive behaviors. (A) Time in the edge zone (green) and transitions between edge and center zones (gray) plotted against edge width. The green circle marks the elbow point, which was selected as the edge width for analysis in Figure 2. $N = 39$ mice. (B) Percentage of time spent in each zone across different time windows. (C-E) Difference (pair - single) of time spent in each zone during baseline (C), early (D), and late (E) phases. $N = 20$ mice for both dominant and subordinate groups. (F, G) Difference (pair - single) of speed in each zone during baseline and late phases. F: $N = 20$ mice; G: $N = 17, 16, 13$ mice. (H) Distribution of social time inside and outside the nest zones. $N = 40, 39$ for baseline and post-looming, respectively. (I) Illustration of three freezing types. Type 1 illustrates isolated freezing; type 2 illustrates freezing that overlapped with the partner's social behavior; type 3 illustrates freezing that was followed by the animal's own social behavior. (J, K) Percentage and duration of the three freezing types during the first freezing episode in dominant and subordinate mice. $N = 14, 3, 4$ mice in J and $N = 16, 3, 3$ mice in K. (L) Time allocation for three types of grooming. $N = 20$ mice for all groups. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

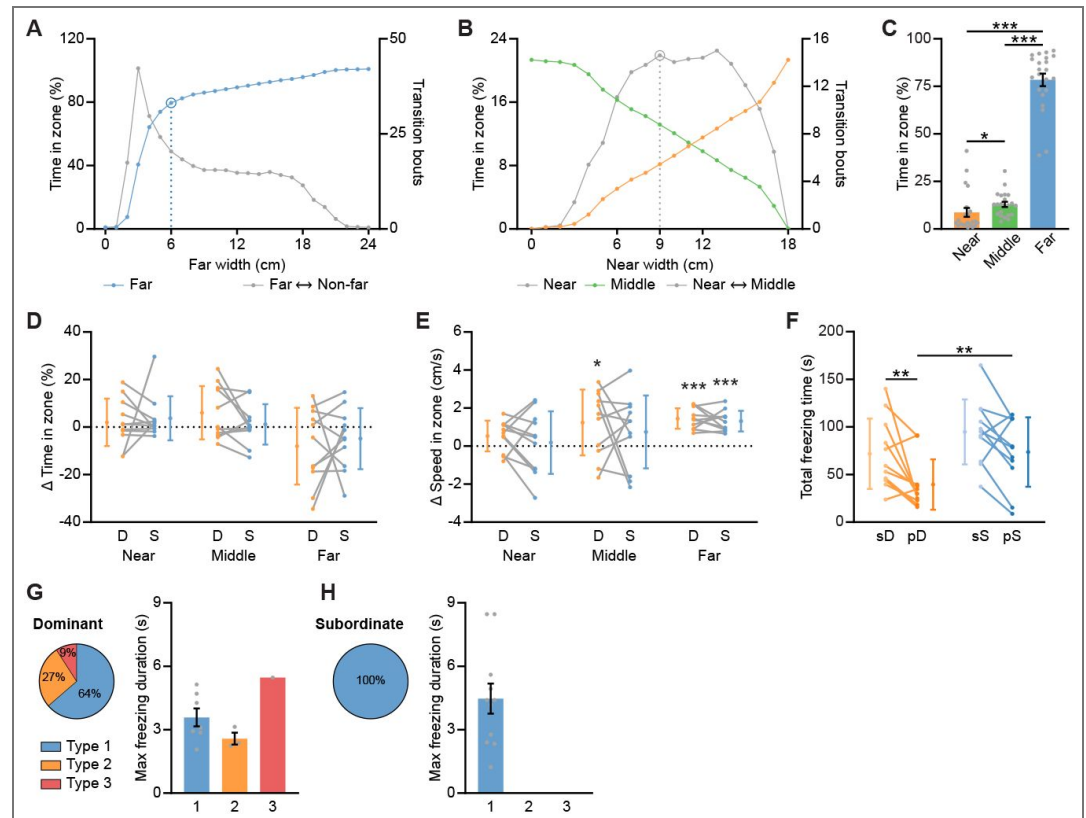


Figure S3. Characterization of rat evoked defensive behaviors. (A) Time in the far zone (blue) and transitions between the far and the non-far zones (gray) plotted against far zone width. The blue circle marks the elbow point, which was selected as the far zone width for analysis in Figure 3. $N = 22$ mice. (B) Time in the near (orange) and middle (green) zones and transitions between near and middle zones (gray) plotted against near zone width. The gray circle marks the stable point, which was selected as the near zone width for analysis in Figure 3. $N = 22$ mice. (C) Percentage of time spent in each zone during rat exposure. $N = 22$ mice. (D, E) Difference (pair - single) in time spent and speed across zones during rat exposure. $N = 11$ mice for each group. (F) Total freezing time. $N = 11$ mice for each group. (G-H) Percentage and duration of the three freezing types during the maximum freezing in dominant and subordinate mice. $N = 7, 3, 1$ mice in G and $N = 11$ mice in H. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Data availability

Data and code will be available in a public repository upon acceptance of the manuscript.

Acknowledgements

Ling-yun Li is supported by the Natural Science Foundation of Beijing Municipality (5244028), the National Natural Science Foundation of China (32471071), and the R&D Program of Beijing Municipal Education Commission (1240030201). Ya-tang Li is supported by the National Natural Science Foundation of China (32271060), the Natural Science Foundation of Beijing Municipality (IS23073), and the start-up fund from CIBR.

Additional information

Author contributions

Ling-yun Li and Ya-tang Li supervised the project; Ling-yun Li, Xinjian Gao, and Ya-tang Li designed the experiments; Xinjian Gao collected the data; Xinjian Gao and Ling-yun Li analyzed the data; Ling-yun Li and Ya-tang Li wrote the manuscript.

Funding

Funder	Grant reference number	Author
北京市科学技术委员会 Natural Science Foundation of Beijing Municipality (北京市自然科学基金)	5244028	Ling-yun Li
MOST National Natural Science Foundation of China (NSFC)	(32471071	Ling-yun Li
MOST National Natural Science Foundation of China (NSFC)	32271060	Ya-tang Li

Author ORCID iDs

Ling-yun Li: <https://orcid.org/0009-0005-3495-2602>

Ya-tang Li: <https://orcid.org/0000-0003-2763-1534>

References

- Adolphs R.** (2013) The biology of fear. *Current biology* **23**:R79-R93
- Benavidez N. L.,** Bienkowski M. S., Zhu M., Garcia L. H., Fayzullina M., Gao L., Bowman I., Gou L., Khanjani N., Cotter K. R., *et al.* (2021) Organization of the inputs and outputs of the mouse superior colliculus. *Nature Communications* **12**:4004
- Blanchard D. C.,** Blanchard R. J. (1990) Behavioral correlates of chronic dominance-subordination relationships of male rats in a seminatural situation. *Neuroscience and Biobehavioral Reviews* **14**:455-462
- Blanchard D. C.,** Sakai R. R., McEwen B., Weiss S. M., Blanchard R. J. (1993) Subordination stress: behavioral, brain, and neuroendocrine correlates. *Behavioural Brain Research* **58**:113-121
- Blanchard D. C.,** Shepherd J. K., Carobrez De Padua, Blanchard R. J. (1991) Sex effects in defensive behavior: baseline differences and drug interactions. *Neuroscience and Biobehavioral Reviews* **15**:461-468
- Blanchard R. J.,** Blanchard D. C. (1989) Antipredator defensive behaviors in a visible burrow system. *Journal of Comparative Psychology* **103**:70-82
- Bowen M. T.,** Kevin R. C., May M., Staples L. G., Hunt G. E., McGregor I. S. (2013) Defensive aggregation (huddling) in *rattus norvegicus* toward predator odor: individual differences, social buffering effects and neural correlates. *PloS One* **8**:e68483
- Canteras N. S.** (2002) The medial hypothalamic defensive system: Hodological organization and functional implications. *Pharmacology Biochemistry and Behavior* **71**:481-491
- Cisek P.,** Green A. M. (2024) Toward a neuroscience of natural behavior. *Current Opinion in Neurobiology* **86**:102859
- Coan J. A.,** Schaefer H. S., Davidson R. J. (2006) Lending a hand: social regulation of the neural response to threat. *Psychological Science* **17**:1032-1039

- Cooper William E., Blumstein D. T. (2015) *Escaping from predators: an integrative view of escape decisions* Cambridge: Cambridge University Press.
- Davis J. F., Krause E. G., Melhorn S. J., Sakai R. R., Benoit S. C. (2009) Dominant rats are natural risk takers and display increased motivation for food reward. *Neuroscience* **162**:23-30
- Davitz J. R., Mason D. J. (1955) Socially facilitated reduction of a fear response in rats. *Journal of Comparative and Physiological Psychology* **48**:149-151
- de Gelder B., Snyder J., Greve D., Gerard G., Hadjikhani N. (2004) Fear fosters flight: a mechanism for fear contagion when perceiving emotion expressed by a whole body. *Proceedings of the National Academy of Sciences* **101**:16701-16706
- Dennis E. J., Hady A. E., Michael A., Clemens A., Tervo D. R. G., Voigts J., Datta S. R. (2021) Systems neuroscience of natural behaviors in rodents. *Journal of Neuroscience* **41**:911-919
- Evans D. A., Stempel A. V., Vale R., Branco T. (2019) Cognitive control of escape behaviour. *Trends in Cognitive Sciences* **23**:334-348
- Evans D. A., Stempel A. V., Vale R., Ruehle S., Lefler Y., Branco T. (2018) A synaptic threshold mechanism for computing escape decisions. *Nature* **558**:590-594
- Fan Z., Zhu H., Zhou T., Wang S., Wu Y., Hu H. (2019) Using the tube test to measure social hierarchy in mice. *Nature Protocols* **14**:819-831
- Faustino A. I., Tacão-Monteiro A., Oliveira R. F. (2017) Mechanisms of social buffering of fear in zebrafish. *Scientific Reports* **7**:44329
- Ferreira C. H., Heinemans M., Farias M., Gonçalves R., Moita M. A. (2022) Social cues of safety can override differences in threat level. *Frontiers in Ecology and Evolution* **10**
- Grammer J., Zelikowsky M. (2022) Neuroscience: the sting of social isolation. *Current Biology* **32**:R572-R574
- Gross C. T., Canteras N. S. (2012) The many paths to fear. *Nature Reviews Neuroscience* **13**:651-658
- Gutzeit V. A., Ahuna K., Santos T. L., Cunningham A. M., Sadsad Rooney M., Muñoz Zamora A., Denny C. A., Donaldson Z. R. (2020) Optogenetic reactivation of prefrontal social neural ensembles mimics social buffering of fear. *Neuropsychopharmacology* **45**:1068-1077
- Hein A. M., Gil M. A., Twomey C. R., Couzin I. D., Levin S. A. (2018) Conserved behavioral circuits govern high-speed decision-making in wild fish shoals. *Proceedings of the National Academy of Sciences* **115**:12224-12228
- Hintiryan H., Zhu M., Zhao P., Zhang M., Barry J., Nanda S., Rudd M., Wong A., Miller S., Gou L., *et al.* (2025) Neural networks of the mouse visceromotor cortex. *Nature* <https://doi.org/10.1038/s41586-025-09360-w>
- Kennedy A., Kunwar P. S., Li L.-y., Stagkourakis S., Wagenaar D. A., Anderson D. J. (2020) Stimulus-specific hypothalamic encoding of a persistent defensive state. *Nature* **586**:730-734
- Kikusui T., Winslow J. T., Mori Y. (2006) Social buffering: relief from stress and anxiety. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361**:2215-2228
- Kingsbury L., Huang S., Wang J., Gu K., Golshani P., Wu Y. E., Hong W. (2019) Correlated neural activity and encoding of behavior across brains of socially interacting animals. *Cell* **178**:429-446.e16
- Kiyokawa Y., Honda A., Takeuchi Y., Mori Y. (2014) A familiar conspecific is more effective than an unfamiliar conspecific for social buffering of conditioned fear responses in male rats. *Behavioural Brain Research* **267**:189-193
- Kiyokawa Y., Kikusui T., Takeuchi Y., Mori Y. (2004) Partner's stress status influences social buffering effects in rats. *Behavioral Neuroscience* **118**:798-804
- Krause J., Ruxton G. D. (2002) *Living in groups* (1st) New York: Oxford University Press, Oxford.
- Kunwar P. S., Zelikowsky M., Remedios R., Cai H., Yilmaz M., Meister M., Anderson D. J. (2015) Ventromedial hypothalamic neurons control a defensive emotion state. *eLife* **4**:e06633 <https://doi.org/10.7554/eLife.06633>

- Lima S. L., Dill L. M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology* **68**:619-640
- Lungwitz E. A., Stuber G. D., Johnson P. L., Dietrich A. D., Schartz N., Hanrahan B., Shekhar A., Truitt W. A. (2014) The role of the medial prefrontal cortex in regulating social familiarity-induced anxiolysis. *Neuropsychopharmacology* **39**:1009-1019
- Marek R., Strobel C., Bredy T. W., Sah P. (2013) The amygdala and medial prefrontal cortex: partners in the fear circuit. *The Journal of Physiology* **591**:2381-2391
- Mathis A., Mamidanna P., Cury K. M., Abe T., Murthy V. N., Mathis M. W., Bethge M. (2018) DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nature Neuroscience* **21**:1281-1289
- Mobbs D., Wise T., Tashjian S., Zhang J., Friston K., Headley D. (2024) Survival in a world of complex dangers. *Neuroscience & Biobehavioral Reviews* **167**:105924
- Morozov A., Ito W. (2019) Social modulation of fear: Facilitation vs buffering. *Genes, Brain, and Behavior* **18**:e12491
- Morris W. N., Worchel S., Bois J. L., Pearson J. A., Rountree C. A., Samaha G. M., Wachtler J., Wright S. L. (1976) Collective coping with stress: group reactions to fear, anxiety, and ambiguity. *Journal of Personality and Social Psychology* **33**:674-679
- Mumenthaler C., Sander D. (2012) Social appraisal influences recognition of emotions. *Journal of Personality and Social Psychology* **102**:1118-1135
- Oh S. W., Harris J. A., Ng L., Winslow B., Cain N., Mihalas S., Wang Q., Lau C., Kuan L., Henry A. M., et al. (2014) A mesoscale connectome of the mouse brain. *Nature* **508**:207-14
- Olsson A., Phelps E. A. (2007) Social learning of fear. *Nature Neuroscience* **10**:1095-1102
- Ozbay F., Johnson D. C., Dimoulas E., Morgan C., Charney D., Southwick S. (2007) Social support and resilience to stress. *Psychiatry (Edmont)* **4**:35-40
- Peek M. Y., Card G. M. (2016) Comparative approaches to escape. *Current Opinion in Neurobiology* **41**:167-173
- Pentkowski N. S., Litvin Y., Blanchard D. C., Blanchard R. J. (2018) Effects of estrus cycle stage on defensive behavior in female long-evans hooded rats. *Physiology & Behavior* **194**:41-47
- Preston E. F. R., Thompson F. J., Kyabulima S., Croft D. P., Cant M. A. (2011) The dynamics of social cohesion in response to simulated intergroup conflict in banded mongooses. *Ecology and Evolution* **11**:18662-18675
- Qi Y., Bruch D., Krop P., Herrmann M. J., Latoschik M. E., Deckert J., Hein G. (2021) Social buffering of human fear is shaped by gender, social concern, and the presence of real vs virtual agents. *Translational Psychiatry* **11**:641
- Rosen J. B. (2004) The neurobiology of conditioned and unconditioned fear: a neurobehavioral system analysis of the amygdala. *Behavioral and Cognitive Neuroscience Reviews* **3**:23-41
- Segalin C., Williams J., Karigo T., Hui M., Zelikowsky M., Sun J. J., Perona P., Anderson D. J., Kennedy A. (2021) The mouse action recognition system (MARS) software pipeline for automated analysis of social behaviors in mice. *eLife* **10**:e63720 <https://doi.org/10.7554/eLife.63720>
- Shang C., Liu Z., Chen Z., Shi Y., Wang Q., Liu S., Li D., Cao P. (2015) A parvalbumin-positive excitatory visual pathway to trigger fear responses in mice. *Science* **348**:1472-1477
- Silva B. A., Gross C. T., Gräff J. (2016) The neural circuits of innate fear: detection, integration, action, and memorization. *Learning & Memory* **23**:544-555
- Sotres-Bayon F., Quirk G. J. (2010) Prefrontal control of fear: more than just extinction. *Current Opinion in Neurobiology* **20**:231-235
- Swanson L. W. (2000) Cerebral hemisphere regulation of motivated behavior. *Brain Research* **886**:113-164

- Taylor G. T.** (1981) Fear and affiliation in domesticated male rats. *Journal of Comparative and Physiological Psychology* **95**:685-693
- Testard C.**, Tremblay S., Parodi F., DiTullio R. W., Acevedo-Ithier A., Gardiner K. L., Kording K., Platt M. L. (2024) Neural signatures of natural behaviour in socializing macaques. *Nature* **628**:381-390
- Top D. N.**, Stephenson K. G., Doxey C. R., Crowley M. J., Kirwan C. B., South M. (2016) Atypical amygdala response to fear conditioning in autism spectrum disorder. *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* **1**:308-315
- Vogt J. L.**, Coe C. L., Levine S. (1981) Behavioral and adrenocorticoid responsiveness of squirrel monkeys to a live snake: is flight necessarily stressful?. *Behavioral and Neural Biology* **32**:391-405
- von Dawans B.**, Fischbacher U., Kirschbaum C., Fehr E., Heinrichs M. (2012) The social dimension of stress reactivity: acute stress increases prosocial behavior in humans. *Psychological Science* **23**:651-660
- Wang F.**, Zhu J., Zhu H., Zhang Q., Lin Z., Hu H. (2011) Bidirectional control of social hierarchy by synaptic efficacy in medial prefrontal cortex. *Science* **334**:693-697
- Wei P.**, Liu N., Zhang Z., Liu X., Tang Y., He X., Wu B., Zhou Z., Liu Y., Li J., *et al.* (2015) Processing of visually evoked innate fear by a non-canonical thalamic pathway. *Nature Communications* **6**:6756
- Yilmaz M.**, Meister M. (2013) Rapid innate defensive responses of mice to looming visual stimuli. *Current Biology* **23**:2011-2015

Peer reviews

Reviewer #1 (Public review):

Summary:

This study presents an interesting behavioral paradigm and reveals interactive effects of social hierarchy and threat type on defensive behaviors. However, addressing the aforementioned points regarding methodological detail, rigor in behavioral classification, depth of result interpretation, and focus of the discussion is essential to strengthen the reliability and impact of the conclusions in a revised manuscript.

Strengths:

The paper is logically sound, featuring detailed classification and analysis of behaviors, with a focus on behavioral categories and transitions, thereby establishing a relatively robust research framework.

Weaknesses:

Several points require clarification or further revision.

(1) Methods and Terminology Regarding Social Hierarchy:

The study uses the tube test to determine subordinate status, but the methodological description is quite brief. Please provide a more detailed account of the experimental procedure and the criteria used for determination.

The dominance hierarchy is established based on pairs of mice. However, the use of terms like "group cohesion" - typically applied to larger groups - to describe dyadic interactions seems overstated. Please revise the terminology to more accurately reflect the pairwise experimental setup.

(2) Criteria and Validity of Behavioral Classification:

The criteria for classifying mouse behaviors (e.g., passive defense, active defense) are not sufficiently clear. Please explicitly state the operational definitions and distinguishing features for each behavioral category.

How was the meaningfulness and distinctness of these behavioral categories ensured to avoid overlap? For instance, based on Figure 3E, is "active defense" synonymous with "investigative defense," involving movement to the near region followed by return to the far region? This requires clearer delineation.

The current analysis focuses on a few core behaviors, while other recorded behaviors appear less relevant. Please clarify the principles for selecting or categorizing all recorded behaviors.

(3) Interpretation of Key Findings and Mechanistic Insights:

Looming exposure increased the proportion of proactive bouts in the dominant zone but decreased it in the subordinate zone (Figure 4G), with a similar trend during rat exposure. Please provide a potential explanation for this consistent pattern. Does this consistency arise from shared neural mechanisms, or do different behavioral strategies converge to produce similar outputs under both threats?

(4) Support for Claims and Study Limitations:

The manuscript states that this work addresses a gap by showing defensive responses are jointly shaped by threat type and social rank, emphasizing survival-critical behaviors over fear or stress alone. However, it is possible that the behavioral differences stem from varying degrees of danger perception rather than purely strategic choices. This warrants a clear description and a deeper discussion to address this possibility.

The Discussion section proposes numerous brain regions potentially involved in fear and social regulation. As this is a behavioral study, the extensive speculation on specific neural circuitry involvement, without supporting neuroscience data, appears insufficiently grounded and somewhat vague. It is recommended to focus the discussion more on the implications of the behavioral findings themselves or to explicitly frame these neural hypotheses as directions for future research.

<https://doi.org/10.7554/eLife.109571.1.sa3>

Reviewer #2 (Public review):

Summary:

The authors investigate how dominance hierarchy shapes defensive strategies in mice under two naturalistic threats: a transient visual looming stimulus and a sustained live rat. By comparing single versus paired testing, they report that social presence attenuates fear and that dominant and subordinate mice exhibit different patterns of defensive and social behaviors depending on threat type. The work provides a rich behavioral dataset and a potentially useful framework for studying hierarchical modulation of innate fear.

Strengths:

(1) The study uses two ecologically meaningful threat paradigms, allowing comparison across transient and sustained threat contexts.

(2) Behavioral quantification is detailed, with manual annotation of multiple behavior types and transition-matrix level analysis.

(3) The comparison of dominant versus subordinate pairs is novel in the context of innate fear.

(4) The manuscript is well-organized and clearly written.

(5) Figures are visually informative and support major claims.

Weaknesses:

Lack of neural mechanism insights.

<https://doi.org/10.7554/eLife.109571.1.sa2>

Reviewer #3 (Public review):

Summary:

This study examines how dominance hierarchy influences innate defensive behaviors in pair-housed male mice exposed to two types of naturalistic threats: a transient looming stimulus and a sustained live rat. The authors show that social presence reduces fear-related behaviors and promotes active defense, with dominant mice benefiting more prominently. They also demonstrate that threat exposure reinforces social roles and increases group cohesion. The work highlights the bidirectional interaction between social structure and defensive behavior.

Strengths:

This study makes a valuable contribution to behavioral neuroscience through its well-designed examination of socially modulated fear. A key strength is the use of two ethologically relevant threat paradigms - a transient looming stimulus and a sustained live predator, enabling a nuanced comparison of defensive behaviors. The experimental design is robust, systematically comparing animals tested alone versus with their cage mate to cleanly isolate social effects. The behavioral analysis is sophisticated, employing detailed transition maps that reveal how social context reshapes behavioral sequences, going beyond simple duration measurements. The finding that social modulation is rank-dependent adds significant depth, linking social hierarchy to adaptive defense strategies. Furthermore, the demonstration that threat exposure reciprocally enhances social cohesion provides a compelling systems-level perspective. Together, these elements establish a strong behavioral framework for future investigations into the neural circuits underlying socially modulated innate fear.

Weaknesses:

The study exhibits several limitations. The neural mechanism proposed is speculative, as the study provides no causal evidence.

<https://doi.org/10.7554/eLife.109571.1.sa1>

Author response:

Public Reviews:

Reviewer #1 (Public review):

Summary:

This study presents an interesting behavioral paradigm and reveals interactive effects of social hierarchy and threat type on defensive behaviors. However, addressing the aforementioned points regarding methodological detail, rigor in behavioral classification, depth of result interpretation, and focus of the discussion is essential to strengthen the reliability and impact of the conclusions in a revised manuscript.

Strengths:

The paper is logically sound, featuring detailed classification and analysis of behaviors, with a focus on behavioral categories and transitions, thereby establishing a relatively robust research framework.

Weaknesses:

Several points require clarification or further revision.

(1) Methods and Terminology Regarding Social Hierarchy:

The study uses the tube test to determine subordinate status, but the methodological description is quite brief. Please provide a more detailed account of the experimental procedure and the criteria used for determination.

We will add more details about how the tube test was performed in the revised manuscript.

The dominance hierarchy is established based on pairs of mice. However, the use of terms like "group cohesion" - typically applied to larger groups - to describe dyadic interactions seems overstated. Please revise the terminology to more accurately reflect the pairwise experimental setup.

Thanks for the comment. We agree that the term "group cohesion" can be misleading and will replace it with "social engagement".

(2) Criteria and Validity of Behavioral Classification:

The criteria for classifying mouse behaviors (e.g., passive defense, active defense) are not sufficiently clear. Please explicitly state the operational definitions and distinguishing features for each behavioral category.

Passive defense was defined as an immobility-based defensive strategy characterized by suppression of locomotor activity. This category included freezing and tail rattling, which in our study involved minimal body displacement aside from rapid tail vibration. Active defense was defined as movement- or posture-dependent defensive strategy, encompassing behaviors that involved locomotor engagement or spatial repositioning relative to the threat, including approach, investigation, withdrawal, and stretch-attend. We will clarify this in the revised manuscript.

How was the meaningfulness and distinctness of these behavioral categories ensured to avoid overlap? For instance, based on Figure 3E, is "active defense" synonymous with "investigative defense," involving movement to the near region followed by return to the far region? This requires clearer delineation.

Defensive behaviors in the rat exposure paradigm were grouped into two categories: passive and active defense, each comprising distinct behaviors. All the manually annotated behaviors were mutually exclusive; that is, each video frame was assigned a single behavioral label to avoid overlap across behaviors. Active defense includes four behaviors: approach, investigation, withdrawal, and stretch-attend. We will clarify these points in the revised manuscript.

The current analysis focuses on a few core behaviors, while other recorded behaviors appear less relevant. Please clarify the principles for selecting or categorizing all recorded behaviors.

Thank you for pointing this out. In the current study, we focused primarily on defensive and social behaviors. We also included several neutral solitary behaviors related to anxiety and defensive state, such as sniffing, grooming, and rearing, which were consistently expressed across animals and closely linked to our main findings. We will clarify this rationale in the revised manuscript.

(3) Interpretation of Key Findings and Mechanistic Insights:

Looming exposure increased the proportion of proactive bouts in the dominant zone but decreased it in the subordinate zone (Figure 4G), with a similar trend during rat exposure. Please provide a potential explanation for this consistent pattern. Does this consistency arise from shared neural mechanisms, or do different behavioral strategies converge to produce similar outputs under both threats?

Thanks for bringing up this important question. The consistent increase in proactive bouts in dominant mice across both paradigms suggests a consistent rank-dependent reorganization of dyadic interaction under threats. We propose that this convergence reflects a shared neural mechanism that links defensive state with social-rank information, potentially mediated by overlapping hypothalamic and prefrontal circuits. We will expand the Discussion to incorporate this explanation.

(4) Support for Claims and Study Limitations:

The manuscript states that this work addresses a gap by showing defensive responses are jointly shaped by threat type and social rank, emphasizing survival-critical behaviors over fear or stress alone. However, it is possible that the behavioral differences stem from varying degrees of danger perception rather than purely strategic choices. This warrants a clear description and a deeper discussion to address this possibility.

We thank the reviewer for this insightful comment. We agree that, in principle, behavioral differences could arise from variations in perceived danger rather than strategic choice. In humans, decisions can sometimes reflect value-based strategies that override perceived danger. In contrast, under naturalistic threat conditions, mice likely rely predominantly on danger perception to make behavioral decisions, and such responses are expected to be consistent with value-based strategies shaped by natural selection. In the revised manuscript, we will expand the Discussion to address the role of threat perception and its relationship to decision-making in our behavioral paradigms.

The Discussion section proposes numerous brain regions potentially involved in fear and social regulation. As this is a behavioral study, the extensive speculation on specific neural circuitry involvement, without supporting neuroscience data, appears insufficiently grounded and somewhat vague. It is recommended to focus the discussion more on the implications of the behavioral findings themselves or to explicitly frame these neural hypotheses as directions for future research.

We will revise the Discussion to focus more directly on behavioral findings and add explicit neural hypotheses as potential future directions.

Reviewer #2 (Public review):

Summary:

The authors investigate how dominance hierarchy shapes defensive strategies in mice under two naturalistic threats: a transient visual looming stimulus and a sustained live rat. By comparing single versus paired testing, they report that social presence attenuates fear and that dominant and subordinate mice exhibit different patterns of defensive and social behaviors depending on threat type. The work provides a rich behavioral dataset and a potentially useful framework for studying hierarchical modulation of innate fear.

Strengths:

(1) The study uses two ecologically meaningful threat paradigms, allowing comparison across transient and sustained threat contexts.

(2) Behavioral quantification is detailed, with manual annotation of multiple behavior types and transition-matrix level analysis.

(3) The comparison of dominant versus subordinate pairs is novel in the context of innate fear.

(4) The manuscript is well-organized and clearly written.

(5) Figures are visually informative and support major claims.

Weaknesses:

Lack of neural mechanism insights.

The current study focused on behavior. In the revised manuscript, we will incorporate a discussion of potential neural mechanisms and highlight this as an important direction for future work.

Reviewer #3 (Public review):

Summary:

This study examines how dominance hierarchy influences innate defensive behaviors in pair-housed male mice exposed to two types of naturalistic threats: a transient looming stimulus and a sustained live rat. The authors show that social presence reduces fear-related behaviors and promotes active defense, with dominant mice benefiting more prominently. They also demonstrate that threat exposure reinforces social roles and increases group cohesion. The work highlights the bidirectional interaction between social structure and defensive behavior.

Strengths:

This study makes a valuable contribution to behavioral neuroscience through its well-designed examination of socially modulated fear. A key strength is the use of two ethologically relevant threat paradigms - a transient looming stimulus and a sustained live predator, enabling a nuanced comparison of defensive behaviors. The experimental design is robust, systematically comparing animals tested alone versus with their cage mate to cleanly isolate social effects. The behavioral analysis is sophisticated, employing detailed transition maps that reveal how social context reshapes behavioral sequences, going beyond simple duration measurements. The finding that social modulation is rank-dependent adds significant depth, linking social hierarchy to adaptive defense strategies. Furthermore, the demonstration that threat exposure reciprocally enhances social cohesion provides a compelling systems-level perspective. Together, these elements establish a strong behavioral framework for future investigations into the neural circuits underlying socially modulated innate fear.

Weaknesses:

The study exhibits several limitations. The neural mechanism proposed is speculative, as the study provides no causal evidence.

Establishing causal evidence for neural mechanisms is beyond the scope of the current behavioral study. We highlight this as an important direction for future work.

<https://doi.org/10.7554/eLife.109571.1.sa0>