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Title

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Permalink

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Journal

eNeuro, 13(7)

ISSN

2373-2822

Authors

Lee, Hyo Gun

Lu, Ju

Zuo, Yi

Publication Date

2026-06-29

DOI

10.1523/eneuro.0441-25.2026

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Adolescent Social Isolation Induces Persistent Impairments in Emotional Discrimination and Helping Behavior

Hyo Gun Lee,¹ Ju Lu,² and Yi Zuo¹

¹Department of Molecular, Cell and Developmental Biology, University of California Santa Cruz, Santa Cruz, California 95064 and ²Department of Biological Sciences, Lehigh University, Bethlehem, Pennsylvania 18015

Abstract

Prosocial behaviors, helpful actions directed toward the well-being of others, are an essential component of social behavior. Adolescence is a particularly sensitive period for the development of social behaviors, yet how social experience during this window shapes prosocial behavior remains poorly understood. Here, we used behavioral assays to examine how social isolation at different ages affects emotional discrimination and affiliative helping behavior in mice of both sexes. We found that adult mice raised in social isolation since weaning failed to allogroom distressed conspecifics and were unable to distinguish stressed from unstressed mice in an emotional discrimination assay. Remarkably, 2 weeks of post-weaning social isolation was already sufficient to impair both emotional discrimination and prosocial allogrooming, and these deficits persisted despite 2 months of resocialization. In contrast, 2 weeks of adult social isolation spared emotional discrimination but impaired allogrooming toward stressed conspecifics. These findings reveal a dissociation between emotional discrimination and prosocial helping behavior. Overall, this work identifies adolescence as a sensitive period for the development of emotional state recognition and provides a behavioral framework for investigating how early social experience sculpts neural circuits underlying empathy and stress buffering.

Key words: adolescence; allogrooming; emotional discrimination; prosocial behavior; social isolation

Received Nov. 23, 2025; revised May 21, 2026; accepted May 26, 2026.

The authors declare no competing financial interests.

Author contributions: H.G.L. and Y.Z. designed research; H.G.L. performed research; H.G.L. and J.L. analyzed data; H.G.L., J.L., and Y.Z. wrote the paper.

We thank Dr. Shaorong Ma for critical comments on the manuscript. This work was supported by grants from National Institute of Mental Health (R01MH127737; R01MH136381) to Y.Z.

Correspondence should be addressed to Yi Zuo at yizuo@ucsc.edu.

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Significance Statement

Prosocial behaviors are essential for social cohesion and emotional well-being in social animals, but what shapes their development remains poorly understood. We show that social isolation during adolescence impairs the mouse's ability to recognize the emotional state of stressed conspecifics, which cannot be rescued by subsequent resocialization. In contrast, social isolation in adulthood impairs prosocial actions without disrupting emotional discrimination. These findings identify adolescence as a critical period for the development of socio-affective behavior and reveal a dissociation between emotional discrimination and prosocial helping behavior. Our study provides a behavioral framework for investigating how early social experience sculpts neural circuits underlying empathy and stress-buffering and establishes a foundation for mechanistic studies of adolescent vulnerability to social and affective disorders.

Introduction

Interactions with conspecifics are an essential part of the life of social animals. Many species, from insects to humans, display a rich repertoire of prosocial behaviors, including helping, sharing, and comforting (Penner et al., 2005; Cronin, 2012; Decety et al., 2016; Wu and Hong, 2022). A common feature of these behaviors is that they aim at benefiting other individuals. For instance, primates provide affiliative contact to victims of aggression (Romero et al., 2010), and elephants console distressed companions with trunk touch (Plotnik and

de Waal, 2014). Rodents, too, touch and groom those in distress or pain and exhibit rescue-like behaviors toward unresponsive conspecifics (Burkett et al., 2016; Wu et al., 2021; Zhang et al., 2024; F. Sun et al., 2025; W. Sun et al., 2025).

Allogrooming, the act of one animal grooming another conspecific, is one of the best-characterized prosocial behaviors in rodents. It often increases when the conspecific partner is in pain or distress, which makes it a robust, ethologically relevant readout of socio-affective sensitivity. A classic work on prairie voles shows that they groom recently stressed cagemates significantly more than unstressed cagemates; this “consolation-like” behavior depends on oxytocin and buffers the recipient’s anxiety and corticosterone levels (Burkett et al., 2016). Similarly, laboratory mouse and rat display consolation-like touch (allogrooming/allolicking) toward distressed familiars, strengthening the case that prosocial touch is a conserved mammalian strategy for social buffering of stress (Lu et al., 2018; Wu et al., 2021). Consistent with a buffering function, stressed rodents that receive allogrooming show reduced anxiety-like behavior compared with those that do not, which links the behavior to measurable improvements in affect (Wu et al., 2021). The ability to recognize and differentiate others’ affective states is a prerequisite for appropriately directing prosocial behavior toward individuals in need. This can be assessed in rodents with an emotional discrimination test (Ferretti et al., 2019).

Adolescence is widely recognized as a crucial period for social development. During adolescence, humans acquire social-cognitive skills such as perspective-taking, emotional regulation, and peer interaction strategies, all of which are essential for a successful adult social life (Andrews et al., 2021). Many psychiatric disorders that disrupt social interaction, including depression and schizophrenia, also emerge during adolescence, underscoring its importance in socio-emotional development (Kessler et al., 2007; Paus et al., 2008; Andrews et al., 2021). Insufficient social interaction during adolescence has been linked to hormonal imbalance, depressive symptoms, reduced cognitive capacity, and increased mortality risk (Cacioppo et al., 2015; Holt-Lunstad et al., 2015; Elovainio et al., 2017). The COVID-19 pandemic further highlighted such vulnerability, as prolonged social distancing disproportionately affected children and adolescents, resulting in heightened anxiety and depressive symptoms (Racine et al., 2021; Wang et al., 2022; Gohari et al., 2024). Together, these findings emphasize that adolescent social interaction is essential for healthy socio-emotional development.

In rodents, the post-weaning period, which begins approximately postnatal day (P) 21, corresponds to adolescence and represents a sensitive window for the maturation of social behaviors (Nardou et al., 2019). Social isolation during this period has been widely used as an experimental model to study the consequences of early social deprivation. Previous research has shown that social isolation during adolescence leads to persistent alterations in social behaviors such as aggression and social recognition in adulthood (Kercmar et al., 2011; Li et al., 2021; Takahashi, 2025), but its impact on emotional discrimination and prosocial behavior remains less understood.

In this study, we investigated how social isolation in adolescent mice affects two complementary aspects of social behavior in adulthood: (1) prosocial allogrooming toward stressed conspecifics and (2) emotional discrimination between stressed and unstressed individuals. We found that social isolation from weaning to adulthood impaired both behaviors. Moreover, a brief (2 weeks) social isolation imposed immediately post-weaning sufficed to impair emotional discrimination; the detrimental effects persisted despite prolonged subsequent resocialization. In contrast, 2-week social isolation in adulthood had no detectable effect on emotional discrimination but affected prosocial allogrooming. These results suggest that emotional discrimination and prosocial helping behavior are dissociable and that adolescent social experience is critical for the maturation of socio-emotional functions.

Materials and Methods

Experimental animals. C57BL/6J mice (<https://www.jax.org/strain/000664>) were obtained from The Jackson Laboratory. Both male and female mice were used in this study. Unless otherwise noted, mice were group housed with littermates (2–5 per cage) under standard laboratory conditions [21°C, 50% humidity, 12 h light/dark cycle, zeitgeber time (ZT) 0 = 6:00 A.M.] with *ad libitum* access to food and water. Mice were randomly assigned to experimental groups. All animal procedures were conducted in accordance with the regulations of University of California Santa Cruz Institutional Animal Care and Use Committee (IACUC).

Social isolation and resocialization. Mice were randomly assigned to social isolation (SI, individual housing in standard cages) or group housing. In adolescent social isolation, mice were isolated at weaning (P21) and remained singly housed for 2 weeks (SI^{P21–35}) or until adulthood (~3 months old, SI^{P21-adult}). In adult social isolation, mice were isolated starting on P90 and remained singly housed for 2 weeks (SI^{P90–104}).

For resocialization, SI^{P21–35} mice were transferred to a cage containing at least two age- and sex-matched mice that had been group housed together. Most previously isolated mice began sleeping alongside their new cagemates within 1–2 d after resocialization began.

Prosocial interaction assay. The observer and the demonstrator mouse were weight- and sex-matched cagemates (i.e., housed in the same cage since birth) or non-cagemates. Except for experiments in Figure 1D and E, novel conspecifics were used as demonstrators. All tests were conducted in the observer’s home cage. Both observer and demonstrator were habituated to the experimenter’s handling for at least 3 d before testing. The observer was also habituated to the test environment by placing its home cage (with the observer only) in the behavioral room for 30 min per day for 3 consecutive days. During these sessions, the observer’s cagemates (for group-housed observers) were temporarily transferred to a clean holding cage.

On the test day, both observer and demonstrator cages were transferred to the behavioral room at least 30 min before testing. The observer's cage was placed in the test arena without food or cage lid for 30 min to allow for acclimation. During this period, the control demonstrator was singly housed in a clean cage containing a small amount of bedding from its home cage to minimize novelty-induced stress, while the stressed demonstrator underwent 30 min of restraint stress in a perforated 50 ml conical tube. The observer and the demonstrator were kept in separate rooms during this 30 min separation. The demonstrator was then introduced into the observer's home cage, and social interactions were recorded for 15 min using a CMOS camera (acA1300-60gm, Basler; 1,280 × 960 pixels, 30 Hz frame rate). The same observer always encountered the control demonstrator first and then the stressed demonstrator on the next day. Key behavioral events including sniffing, allogrooming, and self-grooming were manually annotated using BORIS v7.10.2 (Friard and Gamba, 2016). Experiments on cagemates and non-cagemates were conducted using different cohorts of animals.

Emotion discrimination test. Sex- and age-matched non-cagemate mice served as demonstrators for each observer. For all experiments, novel conspecifics were used as demonstrators. The observer and the demonstrators were habituated to the experimenter's handling prior to testing. The observer was further habituated to the test chamber for 30 min per day for 3 consecutive days before the test. On the test day, both observer and demonstrator cages were transferred to the behavioral room at least 30 min before testing. The emotional discrimination test was conducted in an opaque plexiglass chamber (38 × 28 × 23 cm). Two cylindrical wire cups (height, 10 cm; bottom diameter, 10 cm) were placed in opposite corners of the chamber. The test consisted of three sequential sessions—habituation, baseline, and discrimination—separated by a 30 min stress manipulation between the baseline and the discrimination phase.

In the habituation session, the observer was placed in the chamber containing two empty wire cups for 20 min for acclimation to the test environment. During the baseline session, two demonstrators were placed in the wire cups, and the observer was introduced into the chamber and allowed to interact freely for 5 min.

Following the baseline session, one randomly selected demonstrator was subjected to 30 min of restraint stress, while the other demonstrator remained in a clean cage containing a small amount of bedding from its home cage. During this stress period, the observer was returned to its home cage in a separate room to prevent exposure to auditory, visual, or olfactory cues from demonstrators. In the discrimination session, both demonstrators were returned to their previous wire cups, and the observer was reintroduced into the chamber for 5 min. The chamber and the wire cups were cleaned with 70% ethanol and air-dried between each session. Behaviors were recorded using a CMOS camera (acA1300-60gm, Basler; 1,280 × 960 pixels, 30 Hz frame rate) positioned above the chamber, and the observer's interactions with the wire cups were manually annotated using BORIS v7.10.2. We quantified the interaction number and time with each demonstrator and the discrimination index (DI), defined as $DI = (T_{\text{animal 1}} - T_{\text{animal 2}}) / (T_{\text{animal 1}} + T_{\text{animal 2}})$, where T denotes the time spent interacting with each demonstrator. We also analyzed the total freezing time of the observer during both baseline and discrimination phases. The freezing behavior refers to the transient immobility of the observer in front of either wired cup containing a demonstrator and was quantified as the total amount of time freezing in front of both cups (control and stressed demonstrators) across both baseline and discrimination phases. This behavior was only observed in SI mice.

Open field test. The open field test arena was a 44 cm × 44 cm × 44 cm opaque Plexiglas cage. During the test, the subject mouse was placed at the center of the arena and allowed to roam freely and uninterrupted for 10 min. Behavior was recorded using a CMOS camera (acA1300-60gm, Basler; 1,280 × 960 pixels, 30 Hz frame rate) and analyzed with MouseActivity, an open-source MATLAB program (Zhang et al., 2020). Distance traveled and time spent in the arena center versus periphery were measured following the protocol described previously (Zhang et al., 2020).

Statistics. All behavioral data were analyzed with the analyst blinded to experimental conditions. All statistical analyses were performed with GraphPad Prism 10 (GraphPad Software). We used Mann–Whitney test to compare two independent samples, Wilcoxon matched-pairs signed rank test to compare two paired samples, and two-sided one-sample t test to compare the mean of a sample to a specified value. To compare more than two independent samples, we used one-way ANOVA followed by Tukey's multiple-comparisons test if the samples passed normality test; otherwise, we used Kruskal–Wallis test followed by Dunn's multiple-comparisons test. To determine the effect of two independent factors (e.g., demonstrator status and test phase) with repeated measures on the same subject, we used two-way repeated-measures ANOVA followed by uncorrected Fisher's LSD post hoc test. The sample size n refers to the number of mice. We indicated the sex of individual animals by distinct symbols for males and females in figures wherever appropriate. We report the statistical tests used and the p values in the figure legends. Statistical significance is defined as $p < 0.05$.

Results

Mice perform prosocial allogrooming toward stressed conspecifics, independent of sex or familiarity

Allogrooming, the act of one animal grooming another, is a key component of social behavior, serving both affiliative and consoling functions (Wu et al., 2021). We asked whether mice would preferentially direct this behavior toward stressed conspecifics. We started by separating pairs of adult mice (~3 months old) that had been reared together since birth (group housed or GH). One mouse was designated as the “observer” and the other as the “demonstrator.” While the observer

stayed in the home cage, the demonstrator was temporarily removed from the home cage and either placed in another empty cage (control demonstrator) or subjected to 30 min restraint stress (RS demonstrator). The demonstrator was then returned to the home cage, and interactions between the observer and the demonstrator were recorded for 15 min (Fig. 1A,B).

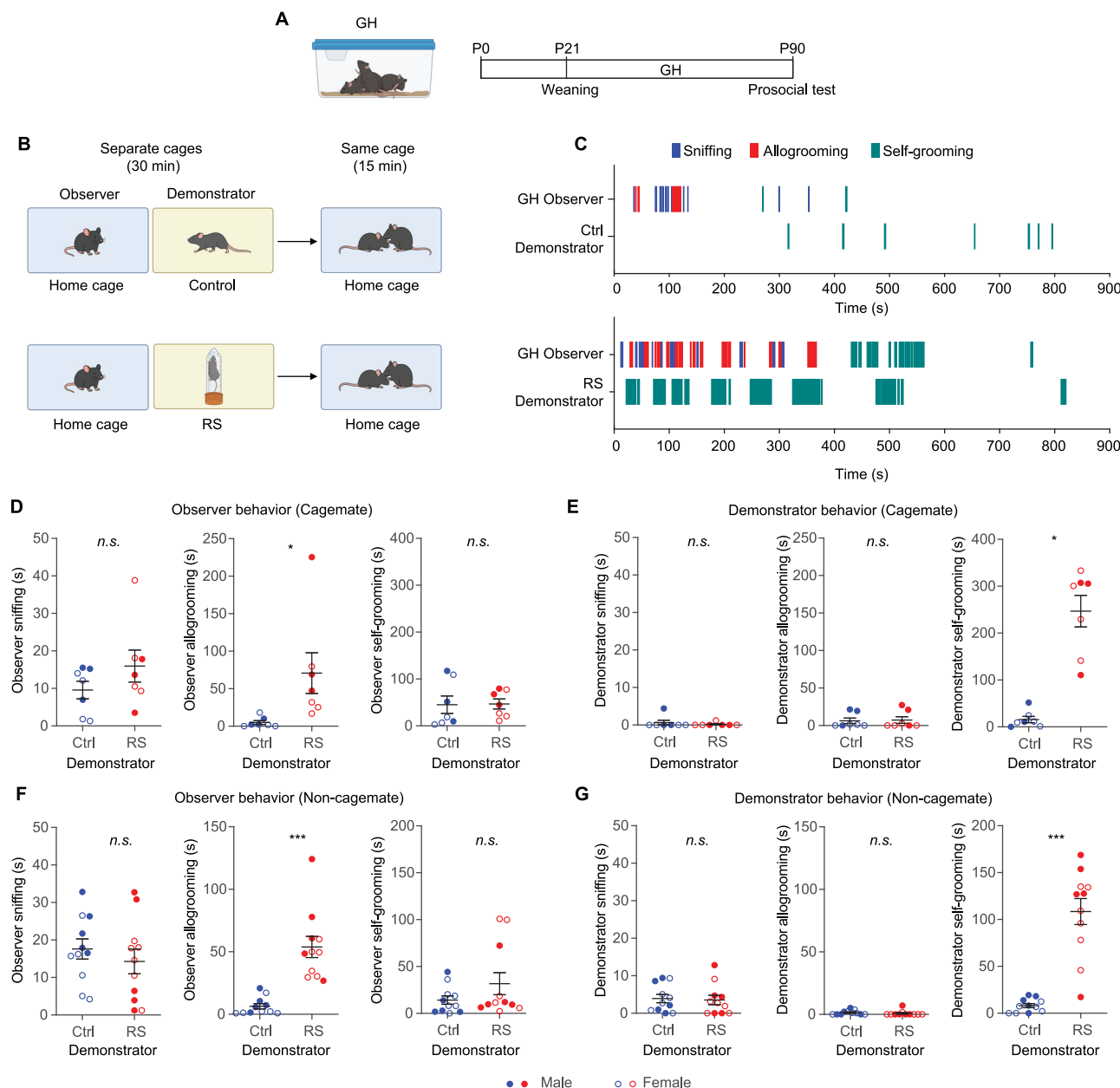
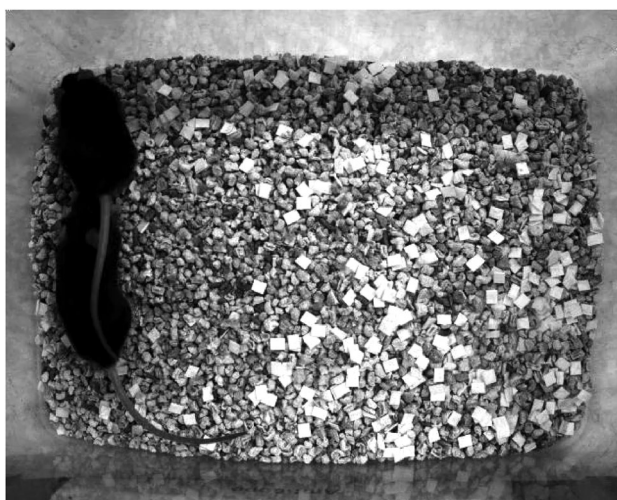


Figure 1. GH adult mice exhibit increased allogrooming toward recently stressed demonstrators. **A**, Schematic of group housing and timeline of the prosocial test. **B**, Experimental design for the prosocial test. In the control group, the observer and the demonstrator mouse are separated for 30 min, followed by a 15 min social interaction. In the stress group, the demonstrator is subjected to restraint stress (RS) during the 30 min separation period. **C**, Representative raster plots illustrating sniffing, self-grooming, and allogrooming of a GH observer and a control (Ctrl) demonstrator (top) or a RS demonstrator (bottom) during social interaction. **D**, The total time spent on different behaviors by GH observers during interaction with Ctrl versus RS cagemate demonstrators. Sniffing $W = 12$, $p = 0.3750$; allogrooming $W = 28$, $p = 0.0156$; self-grooming $W = 4$, $p = 0.8125$. **E**, Total time spent on different behaviors by Ctrl versus RS demonstrators during interaction with GH cagemate observers. Sniffing $W = -1$, $p > 0.9999$; allogrooming $W = 4$, $p = 0.6250$; self-grooming $W = 28$, $p = 0.0156$. **F**, Total time spent on different behaviors by GH observers during interaction with Ctrl versus RS non-cagemate demonstrators. Sniffing $W = -24$, $p = 0.3203$; allogrooming $W = 66$, $p = 0.0010$; self-grooming $W = 24$, $p = 0.3203$. **G**, Total time spent on different behaviors by Ctrl versus RS demonstrators during interaction with GH non-cagemate observers. Sniffing $W = -7$, $p = 0.7344$; allogrooming $W = -14$, $p = 0.2969$; self-grooming $W = 66$, $p = 0.0010$. Wilcoxon matched-pairs signed rank test for all comparisons. Hereinafter data are presented as mean $\pm$ SEM; each data point represents one mouse, with males and females represented by distinct symbols. n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$. Extended Data Figures 1-1 and 1-2 are associated with Figure 1.

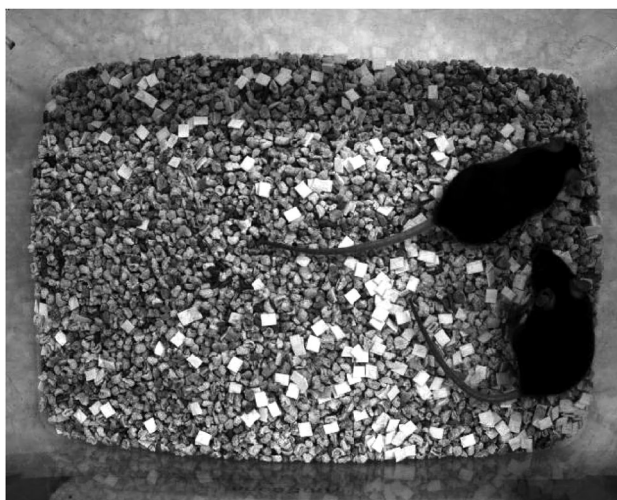
We quantified three types of behaviors during observer–demonstrator interactions: anogenital sniffing, self-grooming, and allogrooming (Movies 1–3). We found that observers spent similar amount of time sniffing or self-grooming regardless of demonstrator status (Fig. 1*D*). In contrast, they spent significantly more time allogrooming RS demonstrators than control demonstrators (Fig. 1*C,D*). On the other hand, both types of demonstrators showed little allogrooming (Fig. 1*E*) and sniffed observers less frequently than observers sniffed them (Extended Data Fig. 1-1*A,B*). Notably, RS demonstrators self-groomed markedly more than controls (Fig. 1*C,E*), consistent with stress-induced anxiety (Fernandez-Teruel and Estanislau, 2016).

To determine whether the increase in allogrooming toward stressed conspecifics depends on social familiarity, we repeated the same assay with non-cagemate demonstrators. Observers displayed minimal allogrooming toward control non-cagemate demonstrators but significantly more toward RS non-cagemates (Fig. 1*F*). The amount of observer's allogrooming toward RS non-cagemates was comparable with that toward RS cagemates (Extended Data Fig. 1-1*C*). However, cagemate RS demonstrators self-groomed significantly more than non-cagemate RS demonstrators did (Extended Data Fig. 1-1*D*). Both observer's allogrooming and demonstrator's self-grooming were comparable between male and female pairs (Extended Data Fig. 1-1*E,F*). Thus, the increase in allogrooming is contingent upon the demonstrator's stress state, but independent of the observer's sex or prior social familiarity with the demonstrator.

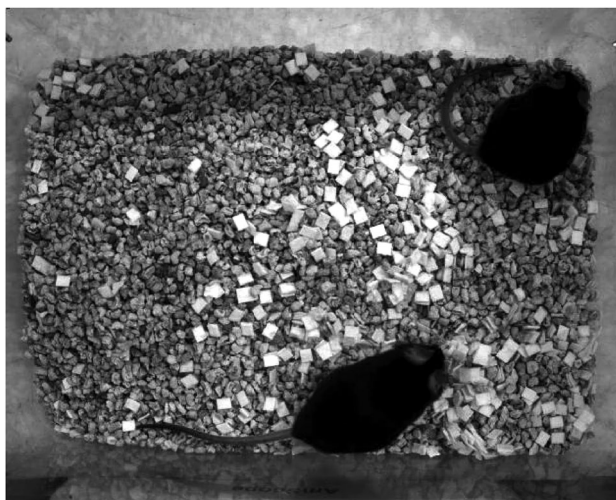
Next, we investigated whether the demonstrator's self-grooming served as a cue for observer allogrooming by examining their temporal relationship. We found that demonstrator self-grooming was not a prerequisite for the



Movie 1. An example of sniffing behavior. [[View online](#)]



Movie 2. An example of self-grooming. [[View online](#)]



Movie 3. An example of allogrooming. [View online]

initiation of observer allogrooming. Specifically, 14 of 18 observers initiated at least one bout of allogrooming before the demonstrator exhibited any self-grooming (80 of 246 total allogrooming bouts). This occurred in 4 of 7 observers with cagemate demonstrators (11 of 86 bouts) and 10 of 11 observers with non-cagemate demonstrators (69 of 160 bouts). Furthermore, only a small fraction of observer allogrooming bouts were initiated during demonstrator self-grooming: 30 of 246 episodes total (12.2%), with similar rates across cagemate (11 of 86 bouts, 12.8%) and non-cagemate (19 of 160 bouts, 11.9%) demonstrator groups. We also quantified how often observer allogrooming occurred shortly after demonstrator self-grooming onset. Only 28 of 246 observer allogrooming bouts (11.4%) occurred within 5 s, and 47 of 246 bouts (19.1%) within 10 s, of demonstrator self-grooming onset. Moreover, the average latency between the onset of observer allogrooming and the onset of the most recent demonstrator self-grooming bout exceeded 1 min (64.9 s for cagemate demonstrators and 62.6 s for non-cagemate demonstrators). Collectively, these data suggest that demonstrator self-grooming is neither a necessary nor a primary cue driving observer allogrooming or emotional discrimination.

Finally, we examined whether allogrooming would alleviate stress-induced anxiety in demonstrators. Adult mice were subjected to 30 min RS and then either singly housed for 15 min or paired with another mouse for 15 min to allow allogrooming. Anxiety-like behavior was subsequently assessed using the open field test. Compared with unstressed controls, RS mice traversed similar distances but spent significantly more time in the periphery of the arena. However, RS demonstrators that received allogrooming spent similar amount of time in the center of the arena as unstressed controls (Extended Data Fig. 1-2).

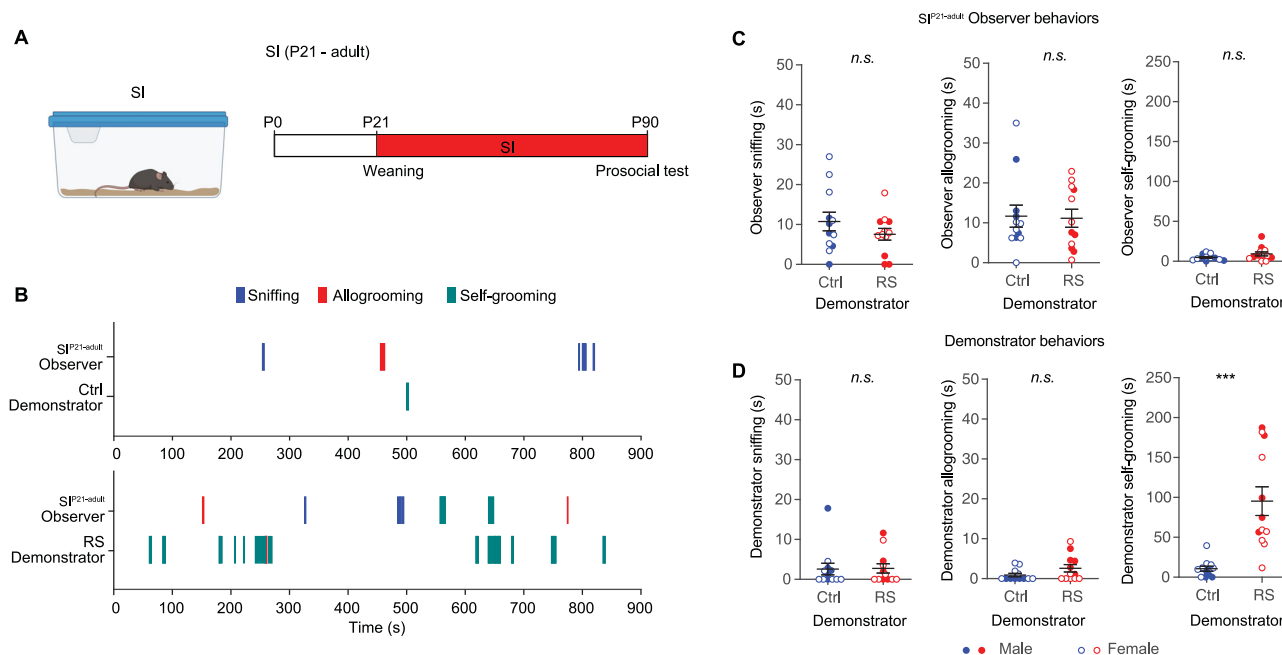
Together, these results indicate that mice of both sexes exhibit prosocial allogrooming toward stressed conspecifics, independent of prior familiarity, which alleviates stress-induced anxiety of the recipient.

Social isolation since weaning impairs prosocial allogrooming

Next, we asked how early social experience shapes adult prosocial behavior. We reared mice either in social isolation (SI) from weaning (P21) or with standard GH (Fig. 2A) and examined their prosocial behavior in adulthood (P90). We found that SI^{P21-adult} observers behaved similarly as GH observers when interacting with control demonstrators (sniffing, allogrooming, and self-grooming; Extended Data Fig. 2-1A–C). However, in the presence of RS demonstrators, SI^{P21-adult} observers showed normal sniffing (Fig. 2C, Extended Data Fig. 2-1D) but did not exhibit increased allogrooming as GH observers (Fig. 2C, Extended Data Fig. 2-1E). As expected, RS demonstrators self-groomed more than controls, regardless of whether their partner was a GH or SI^{P21-adult} observer (compare Figs. 2D, 1G). Furthermore, both observer's allogrooming and demonstrator's self-grooming were comparable between male and female pairs (Extended Data Fig. 2-2). Taken together, these findings indicate that early social experience is critical for the development of prosocial grooming.

Social isolation since weaning impairs emotional discrimination

To determine whether impaired allogrooming in SI^{P21-adult} mice reflects deficits in recognizing conspecific stress, we performed an emotional discrimination test (Ferretti et al., 2019; Fig. 3A). In this test, an observer mouse was presented with two age- and sex-matched non-cagemate demonstrator mice confined in wired cups. After a 5 min baseline phase, one demonstrator was temporarily moved to a new empty cage (control demonstrator) and the other underwent 30 min



restraint stress (RS demonstrator). In the discrimination phase, both demonstrators were returned to the wired cups and the observer freely interacted with them for another 5 min. We measured the total interaction time with each demonstrator and calculated the discrimination index (DI), defined as $DI = (T_{RS} - T_{Ctrl}) / (T_{RS} + T_{Ctrl})$, where T denotes the time spent interacting with each demonstrator.

During the baseline phase, both adult GH and SI^{P21}-adult observers spent comparable time with the two demonstrators, yielding DI values near zero (Fig. 3B,C). In the discrimination phase, GH observers preferentially interacted with the RS demonstrator (DI significantly greater than zero; Fig. 3B), whereas SI^{P21}-adult observers showed no preference (DI ≈ 0 ; Fig. 3C). Total interaction time during discrimination was lower in SI^{P21}-adult than GH observers (Fig. 3D). Moreover, we found that GH observers spent significantly less time interacting with control demonstrators during discrimination than baseline but comparable time with RS demonstrators during the two phases (Fig. 3E; note that we labeled a demonstrator as “RS” even during the baseline, if it underwent RS prior to the discrimination phase). In contrast, SI^{P21}-adult mice spent less time interacting with demonstrators during discrimination than at the baseline, irrespective of whether the demonstrator was stressed or not (Fig. 3F). The number of interaction bouts was similar across groups and demonstrator status (Fig. 3G). The preference of GH observers to interact with RS demonstrators during discrimination arose from longer bouts on average rather than more frequent approaches (Fig. 3H). While GH mice showed no sex difference in either the discrimination index or interaction time (Extended Data Fig. 3-1A,B), SI^{P21}-adult mice exhibited a weak sex difference in interaction time, but not in the discrimination index (Extended Data Fig. 3-1C,D). These results show that SI^{P21}-adult mice fail to discriminate between stressed and control conspecifics.

Brief social isolation during adolescence suffices to impair emotional discrimination and allogrooming

We next investigated how the timing of social isolation affects emotional discrimination. To assess the effect of adolescent isolation, we raised mice either under GH or under SI for 2 weeks starting at weaning (SI^{P21-35}) and then tested them at P35 using the emotional discrimination assay. During the baseline phase, both GH and SI^{P21-35} mice showed comparable, approximately zero DI (Fig. 4A,B). However, during the discrimination phase, GH observers exhibited a significantly positive DI, reflecting a clear preference for interacting with the RS demonstrator (Fig. 4A), whereas SI^{P21-35} observers showed no such preference (Fig. 4B). Total interaction time during the discrimination phase did not differ between the two observer groups (Fig. 4C). Comparing the interaction time with the same mouse during baseline and discrimination, P35 GH mice, like adult GH mice, showed a decrease in interaction with control demonstrators but not RS demonstrators (Fig. 4D). However, SI^{P21-35} mice exhibited reduced interaction with both control and RS demonstrators (Fig. 4E). P35 GH mice

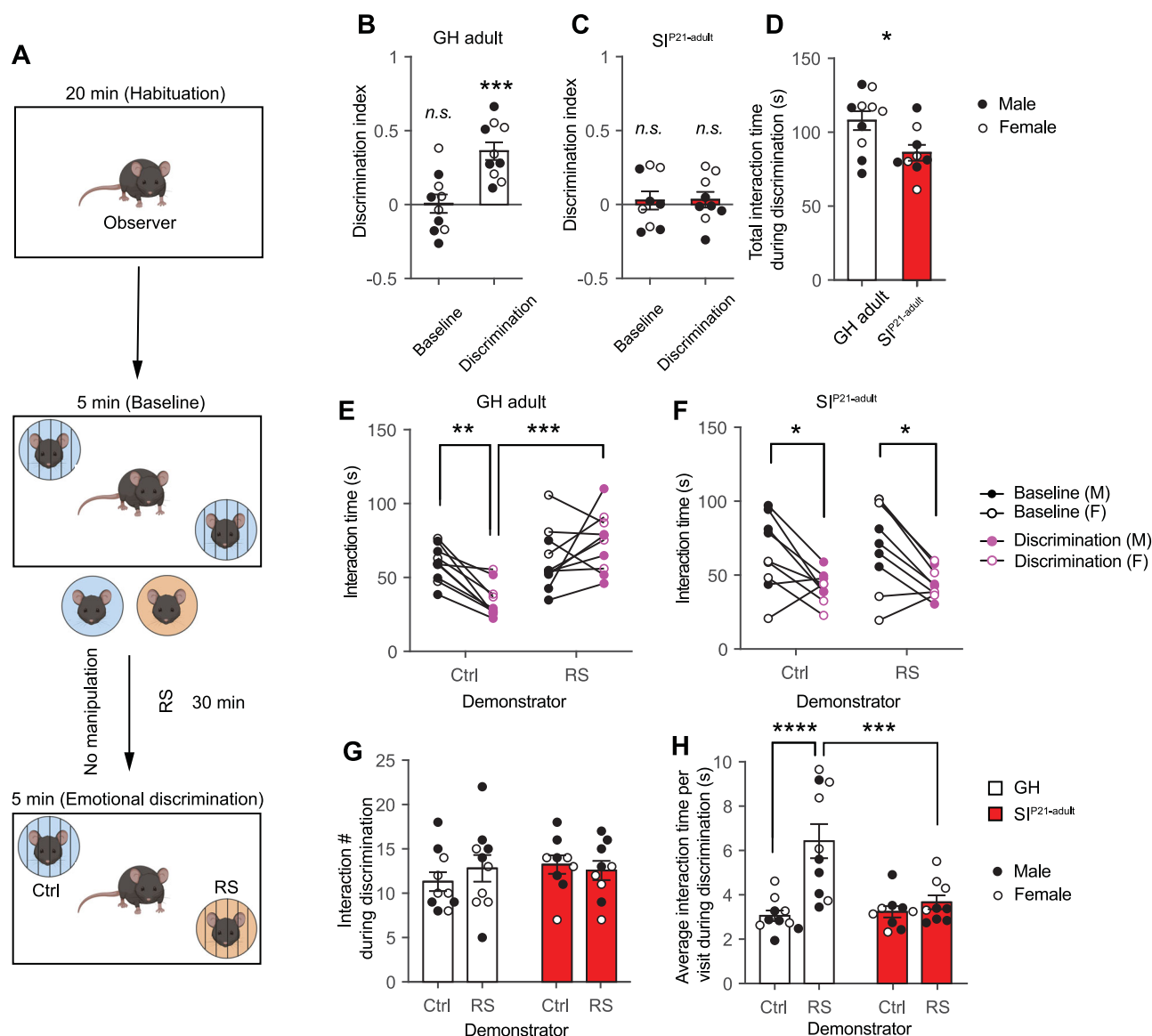


Figure 3. Adult GH mice preferentially interact with recently stressed demonstrators in an emotional discrimination test, while SI^{P21-adult} mice fail to do so. **A**, Experimental setup of the emotional discrimination test. **B**, Adult GH mice preferentially interact with RS mice during discrimination. The discrimination index $DI = (T_{RS} - T_{Ctrl}) / (T_{RS} + T_{Ctrl})$, T denotes the time spent interacting with the demonstrator. The demonstrator is designated Ctrl or RS based on its status during discrimination. One-sample t test compared with 0: baseline $t_{(9)} = 0.0913$, $p = 0.9292$; discrimination $t_{(9)} = 6.063$, $p = 0.0002$. **C**, Adult SI^{P21-adult} mice do not show interaction preference during discrimination. One-sample t test compared with 0: baseline $t_{(9)} = 0.4339$, $p = 0.6759$; discrimination $t_{(9)} = 0.5988$, $p = 0.5659$. **D**, Total interaction time during discrimination in GH versus SI^{P21-adult} mice. Mann–Whitney test, $U = 17$, $p = 0.0220$. **E**, Interaction time of GH adult with Ctrl versus RS demonstrators during baseline and discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,18)} = 13.69$, $p = 0.0016$; main effect of experiment phase: $F_{(1,18)} = 2.136$, $p = 0.1611$; interaction between demonstrator status and experiment phase: $F_{(1,18)} = 10.58$, $p = 0.0044$. Uncorrected Fisher’s LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0015$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.1155$; baseline interaction (Ctrl vs RS demonstrators) $p = 0.7554$; discrimination phase interaction (Ctrl vs RS demonstrators) $p = 0.0001$. **F**, Interaction time of SI^{P21-adult} mice with Ctrl and RS demonstrators during baseline and discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,16)} = 0.6393$, $p = 0.4357$; main effect of experiment phase: $F_{(1,16)} = 8.166$, $p = 0.0114$; interaction between demonstrator status and experiment phase: $F_{(1,16)} = 0.0898$, $p = 0.7684$. Uncorrected Fisher’s LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0262$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.0130$. **G**, Total interaction number of GH and SI^{P21-adult} mice toward Ctrl versus RS demonstrators during discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,17)} = 0.1586$, $p = 0.6954$; main effect of observer housing condition: $F_{(1,17)} = 0.3892$, $p = 0.5410$; interaction between demonstrator status and observer housing condition: $F_{(1,17)} = 1.072$, $p = 0.3149$. **H**, Average interaction time per visit of GH and SI^{P21-adult} mice toward Ctrl versus RS demonstrators during discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,17)} = 21.58$, $p = 0.0002$; main effect of observer housing condition: $F_{(1,17)} = 6.089$, $p = 0.0245$; interaction between demonstrator status and observer housing condition: $F_{(1,17)} = 12.93$, $p = 0.0022$. Uncorrected Fisher’s LSD: interaction with Ctrl demonstrator (GH vs SI^{P21-adult}) $p = 0.7858$; interaction with RS demonstrator (GH vs SI^{P21-adult}) $p = 0.0002$; GH observer interaction (Ctrl vs RS demonstrators) $p < 0.0001$; SI^{P21-adult} observer interaction (Ctrl vs RS demonstrators) $p = 0.4797$. Extended Data Figure 3-1 is associated with Figure 3.

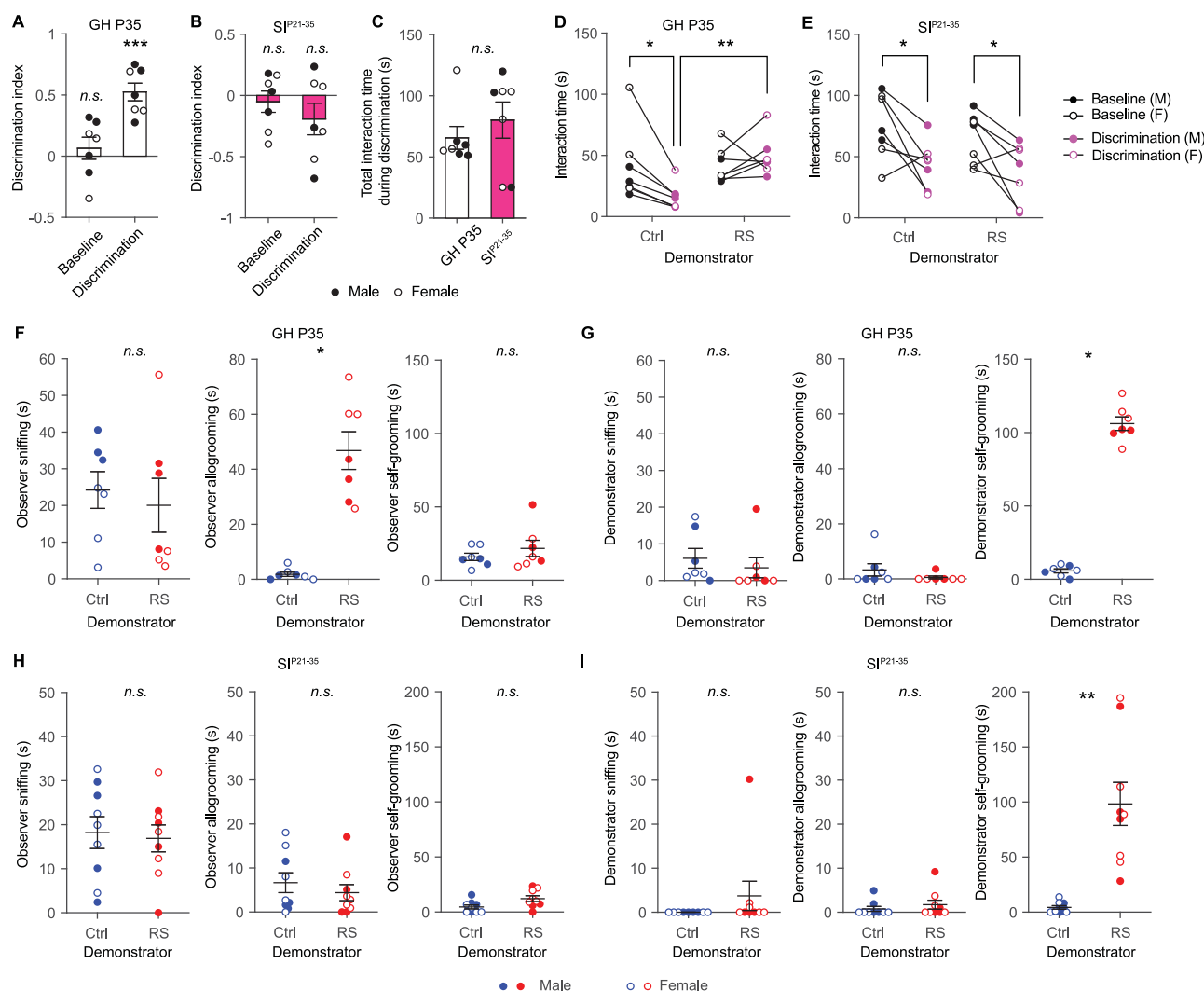


Figure 4. Two-week social isolation starting from weaning is sufficient to impair emotional discrimination and prosocial allogrooming. **A**, P35 GH mice preferentially interact with RS demonstrators during the discrimination phase. One-sample t test compared with 0: baseline $t_{(6)} = 0.7171$, $p = 0.5002$; discrimination $t_{(6)} = 7.302$, $p = 0.0003$. **B**, SI^{P21-35} mice do not show interaction preference during discrimination. One-sample t test compared with 0: baseline $t_{(6)} = 0.5858$, $p = 0.5794$; discrimination $t_{(6)} = 1.497$, $p = 0.1851$. **C**, Total interaction time during discrimination is comparable between GH and SI^{P21-35} mice at P35. Mann–Whitney test, $U = 19$, $p = 0.5350$. **D**, Interaction time of P35 GH with Ctrl versus RS observers during baseline and discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,12)} = 9.306$, $p = 0.0101$; main effect of experiment phase: $F_{(1,12)} = 1.031$, $p = 0.3299$; interaction between demonstrator status and experiment phase: $F_{(1,12)} = 9.132$, $p = 0.0106$. Uncorrected Fisher's LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0215$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.4692$; baseline interaction (Ctrl vs RS demonstrators) $p = 0.9842$; discrimination phase interaction (Ctrl vs RS demonstrators) $p = 0.0010$. **E**, Interaction time between SI^{P21-35} mice and RS demonstrators (both Ctrl and RS) is less during discrimination than during baseline. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,12)} = 1.101$, $p = 0.3148$; main effect of experiment phase: $F_{(1,12)} = 9.524$, $p = 0.0094$; interaction between demonstrator status and experiment phase: $F_{(1,12)} = 0.041$, $p = 0.8429$. Uncorrected Fisher's LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0159$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.0271$; baseline interaction (Ctrl vs RS demonstrators) $p = 0.3935$; discrimination phase interaction (Ctrl vs RS demonstrators) $p = 0.5605$. **F**, Total time spent on different behaviors by P35 GH observers during interaction with Ctrl versus RS demonstrators. Sniffing $W = -14$, $p = 0.2656$; allogrooming $W = 28$, $p = 0.0156$; self-grooming $W = 6$, $p = 0.6875$. **G**, Total time spent on different behaviors by Ctrl versus RS demonstrators during interaction with P35 GH observers. Sniffing $W = -9$, $p = 0.5000$; allogrooming $W = -6$, $p = 0.3750$; self-grooming $W = 28$, $p = 0.0156$. **H**, Total time spent on different behaviors by SI^{P21-35} observers during interaction with Ctrl versus RS demonstrators. Sniffing $W = -15$, $p = 0.4102$; allogrooming $W = -11$, $p = 0.5703$; self-grooming $W = 25$, $p = 0.1641$. **I**, Total time spent on different behaviors by Ctrl versus RS demonstrators during interaction with SI^{P21-35} observers. Sniffing $W = 6$, $p = 0.2500$; allogrooming $W = 8$, $p = 0.2500$; self-grooming $W = 45$, $p = 0.0039$. Wilcoxon matched-pairs signed rank test for all comparisons in **F–I**. Extended Data Figure 4-1 is associated with Figure 4.

showed an increase in both interaction number and average interaction time toward RS demonstrators during the discrimination phase, both of which were impaired in the SI^{P21-35} mice (Extended Data Fig. 4-1). Furthermore, unlike GH P35 mice, SI^{P21-35} mice failed to increase allogrooming toward RS demonstrators (Fig. 4F–I). Together, these results suggest that 2-week adolescent social isolation is sufficient to impair the normal development of emotional discrimination and prosocial behavior.

Brief social isolation during adulthood spares emotional discrimination but impairs prosocial allogrooming

To determine whether social isolation has similar effects in adulthood, we socially isolated mice for 2 weeks beginning at P90 (SI^{P90-104}) and then tested them using the same assay. SI^{P90-104} observers preferentially interacted with RS demonstrators during discrimination (DI significantly greater than zero; Fig. 5A), with a total interaction time comparable with that of GH adults (Fig. 5B). Just as GH adults, SI^{P90-104} observers interacted with control demonstrators less during discrimination than baseline (Fig. 5C). However, unlike SI^{P21-35} and SI^{P21-adult} mice, which show decreased interaction with RS demonstrators during discrimination (Figs. 4E, 3F), SI^{P90-104} mice show increased interaction with RS demonstrators during discrimination (Fig. 5C). Although this pattern might suggest that SI^{P90-104} mice have stronger differential responses to stressed versus non-stressed conspecifics, direct comparison between adult GH and SI^{P90-104} mice revealed no significant difference in either interaction time or DI (Extended Data Fig. 5-1). Unlike in GH adults, the increase in DI during the discrimination phase exhibited by SI^{P90-104} mice was driven by both longer interaction time per visit and more frequent interactions (Fig. 5D,E). Surprisingly, SI^{P90-104} mice failed to increase allogrooming toward RS demonstrators (Fig. 5F), despite elevated self-grooming of RS demonstrators (Fig. 5G). Thus, adolescent social isolation disrupts both emotional discrimination and prosocial allogrooming, whereas adult isolation selectively impairs prosocial allogrooming without abolishing emotional discrimination.

Resocialization fails to rescue emotional discrimination or prosocial deficits induced by adolescent social isolation

We next asked whether subsequent social experience could alleviate the social behavior deficits caused by adolescent isolation. SI^{P21-35} mice were resocialized with at least two age- and sex-matched mice for at least 2 months, and their social behaviors were tested in adulthood (Fig. 6A). Resocialization failed to restore the ability to discriminate between RS and control demonstrators (Fig. 6B,C). Like all other groups, resocialized mice spent less time interacting with control

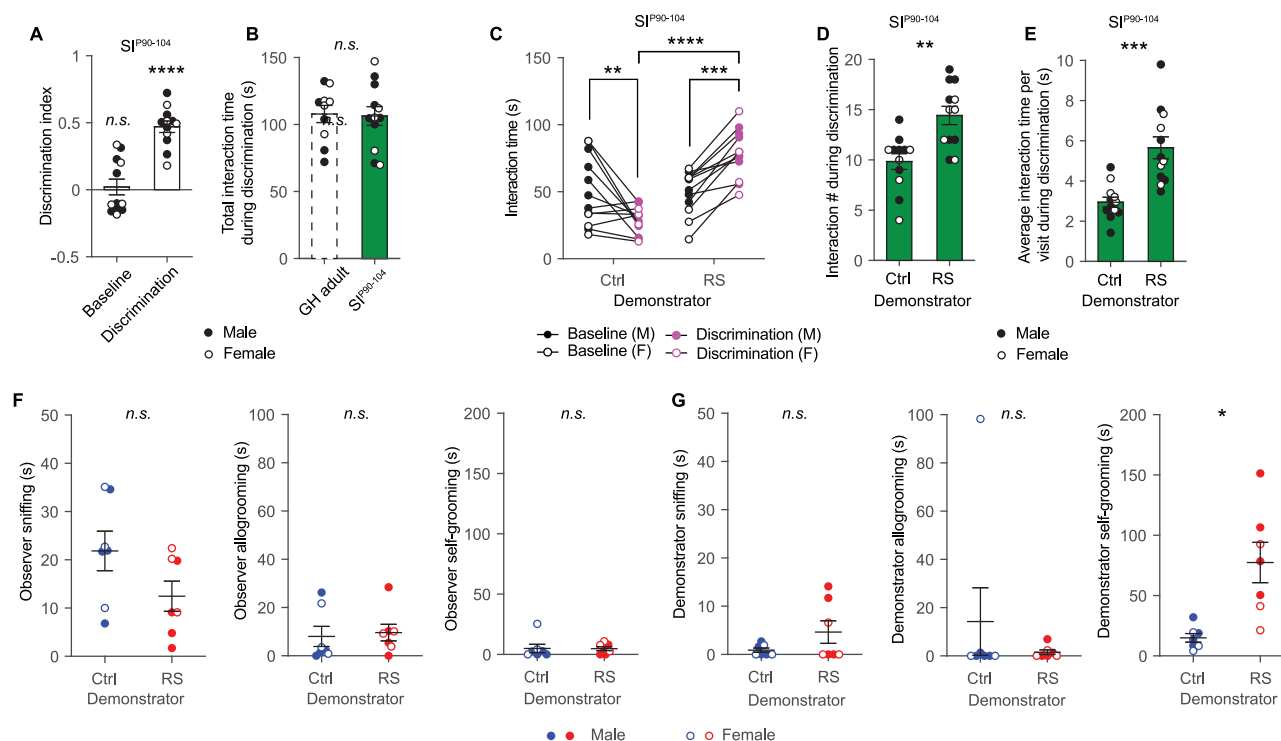


Figure 5. Two-week SI in adulthood does not affect emotional discrimination but impairs prosocial allogrooming. **A**, SI^{P90-104} mice preferentially interact with RS mice during discrimination. One-sample *t* test compared with 0: baseline $t_{(11)} = 0.3506$, $p = 0.7325$; discrimination $t_{(11)} = 10.97$, $p < 0.0001$. **B**, Total interaction time during discrimination is comparable between adult GH and SI^{P90-104} mice. Mann-Whitney test, $U = 53$, $p = 0.6744$. GH data are the same as in Figure 3D. **C**, Interaction time of SI^{P90-104} mice with Ctrl versus RS demonstrators during baseline and discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,22)} = 40.03$, $p < 0.0001$; main effect of experiment phase: $F_{(1,22)} = 0.2883$, $p = 0.5967$; interaction between demonstrator status and experiment phase: $F_{(1,22)} = 42.62$, $p < 0.0001$. Uncorrected Fisher's LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0066$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.0005$; baseline interaction (Ctrl vs RS demonstrator) $p = 0.8879$; discrimination phase interaction (Ctrl vs RS demonstrator) $p < 0.0001$. **D**, SI^{P90-104} mice interact more frequently with RS demonstrators than Ctrl demonstrators during discrimination. $W = 73$, $p = 0.0020$. **E**, SI^{P90-104} mice show significantly longer average interaction time per visit toward RS demonstrators than Ctrl demonstrators during discrimination. $W = 78$, $p = 0.0005$. **F**, Total time spent on different behaviors by SI^{P90-104} observers during interaction with Ctrl versus RS demonstrators. Sniffing $W = -22$, $p = 0.0781$; allogrooming $W = 6$, $p = 0.6875$; self-grooming $W = -1$, $p > 0.9999$. **G**, Total time spent on different behaviors by Ctrl versus RS demonstrators during interaction with SI^{P90-104} observers. Sniffing $W = 9$, $p = 0.3125$; allogrooming $W = 2$, $p = 0.8750$; self-grooming $W = 28$, $p = 0.0156$. Wilcoxon matched-pairs signed rank test for **D-G**. Extended Data Figure 5-1 is associated with Figure 5.

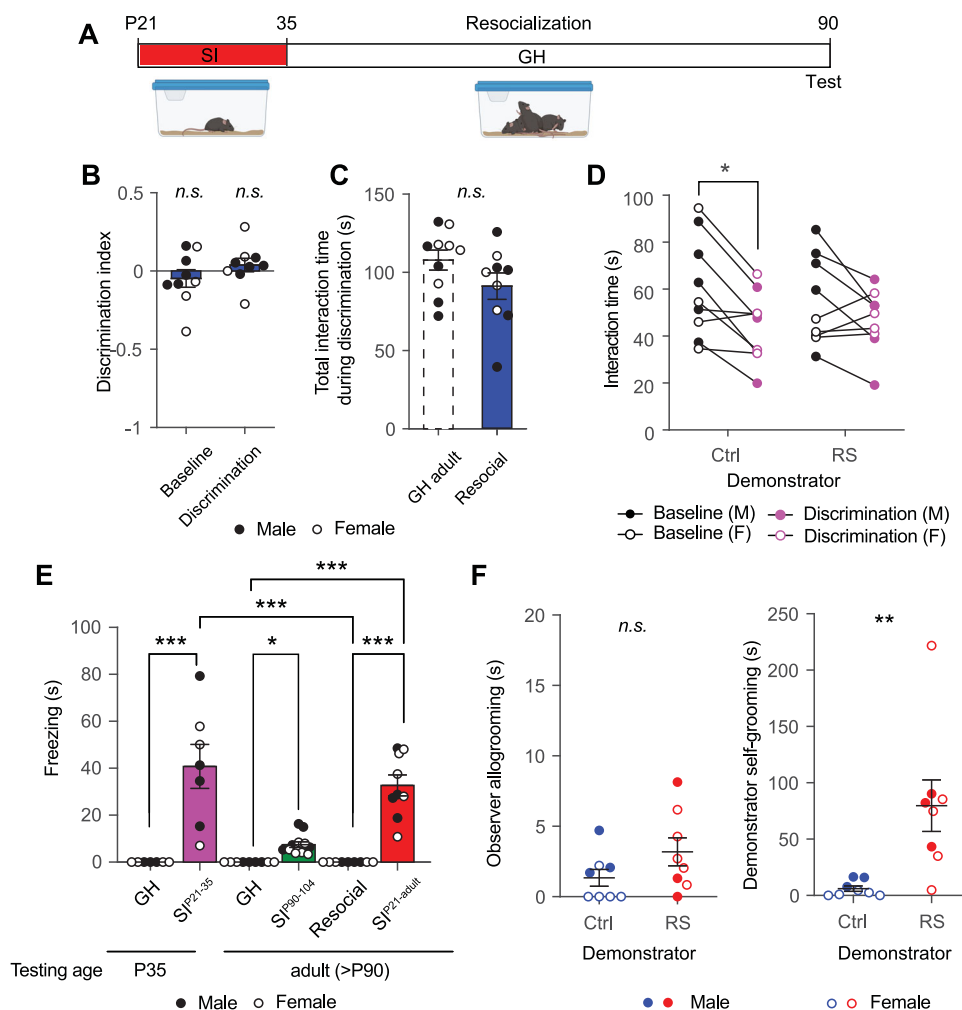
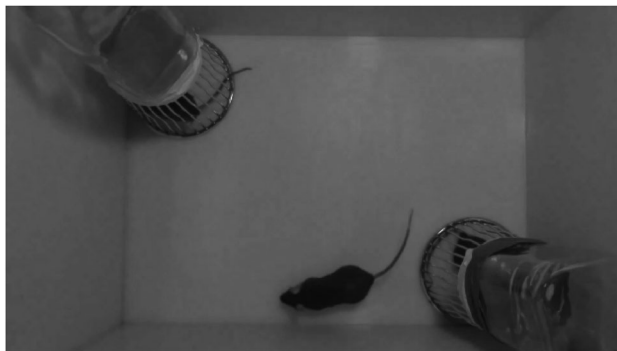


Figure 6. Resocialization of SI^{P21-35} mice from P35 to adult fails to restore emotional discrimination and prosocial allogrooming but reduces freezing during social interaction. **A**, Timeline of the resocialization experiment. **B**, DI during baseline and discrimination of resocialized mice. One-sample t test compared with 0: baseline $t_{(8)} = 0.8541$, $p = 0.4179$; discrimination $t_{(8)} = 0.8858$, $p = 0.4016$. **C**, Total interaction time during discrimination is comparable between adult GH and resocialized mice. Mann–Whitney test, $U = 24$, $p = 0.0947$. GH data are the same as in Figure 3D. **D**, Interaction time of resocialized mice with Ctrl versus RS demonstrators during baseline and discrimination. Two-way repeated-measures ANOVA, main effect of demonstrator status: $F_{(1,16)} = 0.1336$, $p = 0.7195$; main effect of experiment phase: $F_{(1,16)} = 2.990$, $p = 0.1030$; interaction between demonstrator status and experiment phase: $F_{(1,16)} = 1.191$, $p = 0.2912$. Uncorrected Fisher’s LSD: interaction with Ctrl demonstrator (baseline vs discrimination) $p = 0.0493$; interaction with RS demonstrator (baseline vs discrimination) $p = 0.3474$; baseline interaction (Ctrl vs RS demonstrator) $p = 0.3182$; discrimination phase interaction (Ctrl vs RS demonstrator) $p = 0.6148$. **E**, Resocialization reduces freezing in all three SI groups. Kruskal–Wallis statistic = 48.76, $p < 0.0001$. Dunn’s multiple-comparisons test: GH P35 versus SI^{P21-35} $p = 0.0004$; GH adult versus $SI^{P21-adult}$ $p < 0.0001$; GH adult versus $SI^{P90-104}$ $p = 0.0440$; GH adult versus resocialization $p > 0.9999$; $SI^{P21-adult}$ versus resocialization $p < 0.0001$; SI^{P21-35} versus resocialization $p = 0.0001$. **F**, Allogrooming of resocialized observers toward Ctrl versus RS demonstrators (left), $W = 24$, $p = 0.1094$; self-grooming of Ctrl versus RS demonstrators in the presence of resocialized observers (right), $W = 36$, $p = 0.0078$; Wilcoxon matched-pairs signed rank test. Extended Data Figure 6-1 is associated with Figure 6.

demonstrators during discrimination than baseline (Fig. 6D). Interestingly, while their interaction time with RS mice was comparable between baseline and discrimination like GH adults (Extended Data Fig. 6-1), the DI was not rescued (phenocopying SI^{P21-35} and $SI^{P21-adult}$, Fig. 6B vs Figs. 3C, 4B). Moreover, SI mice ($SI^{P21-adult}$, $SI^{P90-104}$, and SI^{P21-35}) showed a freezing-like behavior during emotional discrimination, i.e., a transient immobility in front of the wired cup containing the demonstrator; this behavior was not observed in GH mice (P35 and adult; Fig. 6E; Movies 4, 5). We quantified the total freezing time during the emotional discrimination task (i.e., freezing in front of both cups) and found that resocialization alleviated the freezing phenotype observed in SI^{P21-35} and $SI^{P21-adult}$ mice (Fig. 6E). Resocialization also failed to restore prosocial allogrooming toward RS demonstrators (Fig. 6F).

Discussion

Prosocial behavior is broadly defined as any intentional action that benefits another individual (Eisenberg et al., 2007). Such behaviors encompass a wide range of forms and motivations, making them inherently complex to study (Witek and



Movie 4. An example of GH mouse's behavior during the emotional discrimination test, highlighting its interaction with the demonstrator. [View online]



Movie 5. An example of SI mouse's behavior during the emotional discrimination test. Note the freezing behavior when the observer approaches the cup containing the demonstrator. [View online]

Bekkers, 2015; Pfattheicher et al., 2022). The Perception–Action Mechanism (PAM) model of empathy (Preston and de Waal, 2002; de Waal and Preston, 2017) provides a useful framework to distinguish between the recognition or perception of another's emotional state (e.g., distress) and the action taken in response (e.g., grooming and comforting). Most rodent work on prosocial behavior has focused on action, but much less is known about discrimination or perception.

In our work, the emotional discrimination assay taps primarily the perception side, i.e., the ability to distinguish a stressed versus unstressed conspecific, while allogrooming toward stressed conspecifics indicates more of the actional side of prosocial behavior. Combining both tests enabled us to dissociate the perceptual and actional components. We found that adolescent social isolation impairs both, whereas adult isolation disrupts the latter but leaves the former intact. Thus, intact emotional state recognition is not sufficient to ensure prosocial helping.

Furthermore, our study revealed a clear temporal sensitivity to social isolation. The inability to rescue the social deficit in SI^{P21-35} mice with resocialization highlights the lasting nature of adolescent social experience in shaping socio-affective capacity. This developmental vulnerability aligns with the concept of a sensitive period, a window during which experience exerts disproportionately strong and lasting effects on brain organization (Knudsen, 2004; Blakemore and Mills, 2014; Nelson et al., 2016; Cheng et al., 2024). A classic work demonstrated that rats isolated between P25 and P45—roughly corresponding to adolescence—exhibited persistent abnormalities in exploratory behavior, which could not be reversed by resocialization (Einon and Morgan, 1976, 1977). Our findings extend this framework to socio-affective processing, indicating that adolescence represents a sensitive period for establishing emotional discrimination abilities (Schneider, 2013).

Why is adolescence uniquely sensitive? One possibility is that critical neural circuits subserving affective recognition and prosocial behavior, such as the medial prefrontal cortex, undergo major developmental reorganization during this period (Caballero et al., 2016; Juraska and Drzewiecki, 2020). Social interaction may help calibrate these circuits to detect and respond to others' affective states. Isolation may lead to maladaptive tuning, reduced synaptic pruning, or altered connectivity. Our data therefore supports a developmental programming view: early social experience sculpts the neural foundations of prosocial/empathic capacities. Furthermore, social play, which is abundant during adolescence but markedly reduced in adulthood, may provide unique experiential input (reciprocity, emotional cue-learning, affiliative touch) that trains recognition/action coupling (Burke et al., 2017; Kellman and Radwan, 2022; Achterberg and Vanderschuren, 2023). Deprivation of peer play may thus remove a key training signal during the sensitive window. In contrast, the persistence of allogrooming deficits after adult isolation suggests that the execution of affiliative helping behavior remains plastic and vulnerable beyond adolescence, even when emotional discrimination is preserved.

Finally, the freezing phenotype provides additional insight into the consequences of social isolation. The transient immobility displayed by isolated mice in front of the demonstrator-containing cup likely reflects social fear and is associated with reduced social investigation (Toth and Neumann, 2013). Its disappearance after resocialization indicates that some isolation-induced abnormalities remain reversible.

In summary, our data identify adolescence as a critical period for the development of prosocial behavior in mice. Brief social isolation during this period suffices to induce persistent deficits in emotional discrimination and affiliative responses, deficits that are not rescued by later social experience. In contrast, adult isolation selectively impairs affiliative helping without abolishing emotional discrimination. These results establish a behavioral framework for investigating how early social experience sculpts neural circuits underlying empathy, prosociality, and stress buffering.

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