Affective bonding explains post-traumatic behavioral development in adult mice

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Abstract: Despite a broad research focus on the diverse symptoms of post-traumatic stress disorder (PTSD), the gradual emergence of symptoms following trauma exposure remains as a major conceptual challenge. We identified and examined behavioral development in mice isolated after observing a traumatic event inflicted upon their pair-housed partners. Compared to mice isolated without traumatic experience, fine-scale behavioral analysis showed that anxiety-related reactions gradually increased through isolation, while the early phases were independent of acute stress reactions. Instead of social avoidance, traumatized mice presented long-term social apathy to strangers. These behaviors were not observed if the traumatic event happened to strangers. Additionally, traumatized mice showed rebound responses to their partners after a long separation. Concluding, a single common factor, affectional bonding, underlies the rather independent development of PTSD-like behaviors in mice. The observed psychological homology between

mice and humans emphasizes the potential of rodent models to untangle subtle psychobehavioral development in humans.

Introduction

PTSD is one of the most prevalent mental health disorders and has received serious clinical and research attention (DSM-III, 1980;DSM-5, 2013). Still, it remains as one of the most poorly understood and controversial presentations of psychiatric disorders (McFarlane, 2010). The debates have even questioned the existent of PTSD (McHugh and Treisman, 2007;Walton et al., 2017), centered on its diversity, inconsistency, and delayed onset of symptoms even after a protracted symptom-free period (Andrews et al., 2007;Pai et al., 2017). Trauma- and Stressor-Related Disorders, as the latest classification introduced in the current diagnostic manual (DSM-5, 2013), further reflect this difficult situation that the resulting disorders inconsistently embrace a wide array of symptoms even though they are common in the defining feature which includes the involvement of an adverse event (Pai et al., 2017). Since the combination of psychological therapy and pharmacotherapy does not provide a more efficacious treatment than psychological therapy alone (Hetrick et al., 2010;Mataix-Cols et al., 2017), a primary question has been how a psychopathological model could account for PTSD development (McFarlane, 2010). Several psychological programs were demonstrated as efficacious first-line treatments for PTSD (Beck, 2011;Schnyder and Cloitre, 2015), but not during the incubation period. Additionally, the risk of worsening PTSD symptoms remains (Monson et al., 2012;Bryant et al., 2013). Although the mechanisms underlying these psychological therapies are largely speculative and rather not amenable to scientific inquiry (Schnyder and Cloitre, 2015), a core belief shared across various hypotheses is to view PTSD as an association between trauma-induced states such as fear memory, increased anxiety, and social withdrawal (Ehlers and Clark, 2000;Siegmund and Wotjak,

45 2006;Hayes et al., 2012;Zoladz and Diamond, 2013;Bryant et al., 2017). This view has further
46 been presumed in explaining PTSD development over the incubation period (Ehlers and Clark,
47 2000;Siegmund and Wotjak, 2006;Bryant et al., 2017). Complex developmental trajectories of
48 PTSD symptoms were demonstrated with associated physiological and environmental regulators
49 (Bryant et al., 2013), yet the scientific support for underlying psychological mechanism is still
50 absent.

51 To identify psychopathological mechanism underlying stress incubation requires an
52 experimental assay with purely psychosocial manipulations on controlled subjective experiences
53 and a homogeneous genetic background. Laboratory rodents are widely researched model systems
54 with rich empirical evidence of their validity in neuroscience, genetics, and pharmacology of stress
55 (Cryan and Holmes, 2005;Kaouane et al., 2012;Calhoon and Tye, 2015;Tovote et al., 2015).
56 Behavioral paradigms that simulate PTSD were established to expose the mechanistic and causal
57 insights of long-term fear memory following acute physical stress (Balogh et al., 2002;Philbert et
58 al., 2011) and learned depression after a prolonged period of repeated social defeat, either directly
59 (Golden et al., 2011) or through witnessing (Patki et al., 2014;Sial et al., 2016). While similar
60 observations of a delayed period before showing substantial PTSD-like behaviors were reported
61 in a relatively small number of experimental paradigms (Davis, 1989;Pamplona et al.,
62 2011;Warren et al., 2013;Tsuda et al., 2015), there have been no studies that systematically and
63 quantitatively address this important developmental process. On the other hand, behavioral studies
64 revealed that laboratory rodents engage in advanced social behaviors within their cognitive
65 capacities and share similar psychological states as human (Bartal et al., 2011;Bartal et al.,
66 2014;Burkett et al., 2016;Smith et al., 2016). Yet, the capability of these animal model systems to
67 imply human complex psychobehavioral development in psychiatry remains unclear. By

68 combining behavioral scenarios, analytic methods, and theoretical hypotheses, here we scoped for
69 a novel research approach to achieve implications about human PTSD psychological developments
70 from psychobehavioral experiments in laboratory mice.

71 **Results**

72 ***Evidence of stress development revealed by fine-scale behavioral analysis***

73 To identify post-traumatic behavioral development with minimal physical impact and peri-
74 traumatic contribution, we developed the following assay of acute witnessing trauma (Figure 1A):
75 Partnership between the male focal mouse and its male partner was established by housing them
76 together for 3 weeks (Day-21 – Day0). During this pair-housing, the mice slept together in a
77 single nest they built, and no aggressive interaction was observed. On Day0 (trauma induction),
78 the focal mouse observed its partner being attacked by 5 different aggressor mice in succession
79 (aggressive encounter), and stayed together with the attacked partner between each aggressive
80 encounter (stress infiltration and resting). Importantly, the focal mouse only experienced the
81 traumatic event by witnessing the attacks and via social communication, but not through any direct
82 physical threat, such as attack bites and pursuits from either the aggressors or its partner. After
83 the last aggressive encounter, the focal mouse (Traumatized mouse) was socially isolated for 4
84 weeks (Day0 – Day28). Behavior was tested on Days -7, 1, 7, and 28.

85 To differentiate behavioral consequences from the trauma induction and the effects of
86 isolation, adaptation to the tests, and aging, a control group of mice were isolated from their
87 partners on Day0 without trauma induction (Control mice). We found that Traumatized mice (n =
88 47) built nests with significantly higher walls than those constructed by Control mice (n = 47) after
89 isolation (Figure 1B, top panel). Traumatized mice also increased their body masses in the late
90 phase of the study (Figure 1B, bottom panel). While these differences could be alternatively

91 explained [e.g. having better emotional stability and therefore nicer nests and better appetites
92 *versus* manifesting protective responses of anxiety by hiding behind higher nest walls and
93 engaging in anxiety-induced binge eating (Goto et al., 2014;Otabi et al., 2017)], they clearly
94 indicated a long-term psychological effect of trauma.

95 To investigate the emotional states of Traumatized mice, we used standard behavioral
96 testing setups. Although the general beliefs in identifying rodent emotionality in standard
97 behavioral tests have been criticized (Ramos, 2008), spontaneous behavior in a steady environment
98 is arguably a representation of internal states such as emotion (Archer, 1973). In humans, anxious
99 behaviors show when facing unexpected events or uncertain situations (Grupe and Nitschke, 2013).
100 Behavioral tests for rodent anxiety-like reactions were therefore designed to evaluate their stress
101 reaction against their willingness to explore (e.g. light-dark box and elevated plus-maze) or their
102 activity under conditions with a gradient of uncertainty (e.g. open field). We first examined
103 spontaneous behaviors in the light-dark box test (Figure 1C; n = 8 mice for each group), where the
104 stressor was a natural aversion to brightly lit areas (Kumar et al., 2013). While the time spent in
105 the light area did not differ significantly between Control and Traumatized mice on Day1,
106 Traumatized mice spent more time in the light area than Control mice on Days 7 and 28 (Figure
107 1C, left panels). This result, as the first glance, suggests delayed expression of a chronic behavioral
108 difference.

109 This result raised the following questions: (i) Did this behavioral difference start to develop
110 immediately after the traumatic event or only after a delay? (ii) What comparative emotionality
111 does this behavioral difference indicate? To answer the first question, we examined the fine-scale
112 positions of the mice in the light-dark box. Emotion dynamically maps internal states and external
113 stimuli toward a set of corresponding behaviors, which should be represented in the probability

114 distribution of behaviors that are shown. Based on spatial symmetry, we analyzed T(x), the
115 cumulative probability distribution of time that the mouse spent at positions along the axis of the
116 light-dark box (Figure 1C, top-right scheme and the equation, and middle panels for the results).
117 We calculated significance levels [presented as -log of *p*-values, -log(p)] of T(x) between
118 Traumatized and Control populations by computing position-dependent population means and
119 applying a two-tailed, two-sample Student's *t*-test (Figure 1C, bottom-middle panels). Already on
120 Day1, Traumatized mice showed differences in their spatial distribution, as they spent less time
121 than Control mice at the far end of the dark area. This tendency increased with time: On Day7,
122 Traumatized mice spent more time in the light area close to the door compared to Controls, and
123 then on Day 28 additionally on the far side of the light area. Collapsed -log(p) distributions reveal
124 the overall gradual increase in spatial preference differences (Figure 1C, right panel). Additionally,
125 Traumatized mice showed more transfers between the boxes and shorter latencies until their first
126 transfers from Day1 (Figure 1-figure supplement 1), and maintained a higher locomotor speed
127 compared to the gradually decreasing speed of Control mice (Figure 1-figure supplement 2). These
128 results indicate an onset and gradual increase of behavioral differences immediately following the
129 trauma.

130 ***Development of different psychological components untangled in standard behavioral tests***

131 These differences may reflect the hypo-sensitivity to sensory harm and hyper-activity, as
132 characteristic of somatic anxiety in PTSD patients (Cloninger, 1988;Adler et al., 2004;Mostoufi et
133 al., 2014); however, they may also simply indicate that Traumatized mice became braver and more
134 excited. To determine whether the differences could be attributed to chronic anxiety, we examined
135 their behaviors in the elevated plus-maze test (Figure 2A; n = 8 mice for each group). In this test,
136 stressors included fear of falling and exposure, which accompanied both sensation and cognitive

137 interpretation (Kumar et al., 2013). Reactions of cognitive anxiety are expected to be opposite of
138 those shown in somatic anxiety (Cloninger, 1988). After first exposure on Day-7 and separation,
139 mice spent only a fraction of the time in the open arms of the maze, but with no significant
140 difference between Control and Traumatized mice (Figure 2A, left panels). However, Traumatized
141 mice spent increasingly more time in the far end of the closed arms (Figure 2A, middle panels)
142 and moved more slowly in the elevated plus-maze after trauma induction (Figure 2-figure
143 supplement 1) with longer periods of freezing and fewer entries to the central platform from the
144 closed arms (Figure 2-figure supplement 2). Together, this indicates a higher anxiety level and
145 supports the hypothesis that behavioral differences are the result of a chronic anxiety. Gradually
146 increasing differences between Control and Traumatized mice (Figure 2A, right panel; Figure 2-
147 figure supplement 1) further confirm that stress incubation of anxiety caused the observed
148 differences.

149 To date, the controversy at the core of PTSD development that has not been directly
150 addressed is the nature and source of internal psychological development. Rather than exploring
151 determinants of a developmental phenotype or asking whether and how a behavioral output is
152 generated via a conjoint process of multiple interpreting and evaluating systems (Hayes et al.,
153 2012;Calhoon and Tye, 2015), the fundamental question asked for an internal psychological
154 development is whether and how the alternation of an interpreting or evaluating system is
155 dependent on the state of another interpreting or evaluating system. This dependence can be
156 examined by comparing the observed results with the predictions according to possible causal
157 scenarios for a pair of trauma-induced states in a unified framework (Sapolsky, 2002;Oizumi et
158 al., 2016). To explore the developmental process of different psychological components, we next
159 investigated the emotional basis further revealed in fine-scale behavioral distributions. In both

160 tests, key incubation features were consistently more obvious within stressor-free zones (dark area
161 and closed arms) than within stressor zones (light area and open arms), and thus the features of the
162 behavioral response might change depending upon whether mice were under the stressor. To
163 quantitatively investigate the differences between zones, we analyzed locomotor speed separately
164 in stressor and stressor-free zones. As expected, two different behavioral patterns developed in
165 the two zones: While speed differences consistently increased in stressor-free zones (Figure 2B,
166 right panels; Figure 2-figure supplement 3; Figure 2-figure supplement 4), speed differences in
167 stressor zones only showed acute increases on Day1 (Figure 2B, left panels; Figure 2-figure
168 supplement 5; Figure 2-figure supplement 6). These results suggest that different psychological
169 features were measured in the two zones, allowing further identification of association among
170 different trauma-induced states.

171 In humans, fear is a response to a known threat with a magnitude that increases with the
172 certainty of the threat, whereas anxiety is a response to uncertainty with a magnitude that increases
173 with the uncertainty of a situation (Grupe and Nitschke, 2013). Together with the presented
174 demonstration of anxiety incubation (Figure 1C and 2A; Figure 1-figure supplement 1; Figure 2-
175 figure supplement 2), we hypothesized that behavioral differences in stressor-free zones (closed to
176 stressor) are the result of anxiety reactions to uncertainty, while acute stress reactions in stressor
177 zones (under stressor) are the result of lasting fear responses from trauma induction. We examined
178 mouse locomotor speed in uncertain and familiar environments separately using the open field test
179 (Figure 2C; n = 8 mice for each group) and the locomotor activity test (Figure 2D; n = 8 mice for
180 each group), respectively. In the open field test, mice were exposed in a dimly lit environment
181 with a gradient of spatial uncertainty from the field center (high uncertainty) to field boundaries
182 (low uncertainty). In the locomotor activity test, mice were habituated in a dark environment with

183 familiar cues of bedding materials from their homecages indicative of less environmental stress.
184 Traumatized mice moved faster in the center region of the open field (Figure 2-figure supplement
185 7), compared to Controls, and slower in the periphery (Figure 2-figure supplement 8). The
186 difference between groups increased gradually and was greater in the center than in the periphery
187 (Figure 2D). The avoidance of spatial uncertainty by Traumatized mice was also reflected in the
188 shorter time they spent near the center (Figure 2-figure supplement 9, left panel), but showed a
189 shorter latency to the first rearing during the test (Figure 2-figure supplement 9, right panel), which
190 together suggests anxiety, instead of depression-like reactions with low exploratory motivation, as
191 the causal emotional state. In contrast, Traumatized mice moved significantly more slowly on
192 Day1 in the locomotor activity test, and recovered on Days 7 and 28 (Figure 2D; Figure 2-figure
193 supplement 10). Notably, the two developmental patterns showed a consistent uncertainty-
194 dependent manner and were measured across various behavioral tests. The patterns raised together
195 in the acute phase, but one vanished when the other continued to increase in the later phase. These
196 results support the hypothesized psychological features and further imply that delayed anxiety
197 expression is not simply an expanded, sustained response of acute stress, thereby refuting a general
198 belief (DSM-III, 1980;Ehlers and Clark, 2000;Siegmund and Wotjak, 2006;Andrews et al.,
199 2007;Pamplona et al., 2011;Hayes et al., 2012;DSM-5, 2013;Zoladz and Diamond, 2013;Schnyder
200 and Cloitre;Bryant et al., 2017).

201 ***PTSD-like heterogeneous picture of psychology between emotionality and sociality***

202 Emotional responses simplify and speed up animal reactions to complex external cues and
203 are critical in social interactions (Anderson, 2016). Impairment in social functioning is also
204 considered in PTSD diagnosis (DSM-5, 2013). To test potential effects of anxiety on sociality, we
205 examined mouse social motivation in a two-session social test (Figure 3A, a non-social session

206 followed by social session; n = 5 mice for each group). The social stimulus was either a female or
207 a male stranger mouse. During the social session, Traumatized mice spent less time in social
208 approaches of nose poking toward both female and male strangers, starting from Day1, and
209 remained less social compared to Controls during the post-traumatic period (Figure 3B, top panels).
210 In addition, less social vocalization was recorded during the female stranger test for Traumatized
211 mice (Figure 3-figure supplement 1). The time they spent in the interaction zone around the social
212 target, however, did not differ significantly from that of Control mice (Figure 3B, bottom panels).
213 These results indicated a general and chronic social apathy that developed after the trauma (Figure
214 3-video supplement 1). These observations are comparable to the diminished responsiveness in
215 interpersonal relationships that begins soon after traumatic events as reported in PTSD patients
216 (DSM-III, 1980), rather than social avoidance, as expected in case of chronic anxiety generally
217 considered in PTSD social withdrawal (DSM-III, 1980;DSM-5, 2013). In addition, onset of
218 chronic social apathy from Day1 preceded the notable drop of spontaneous locomotor speed during
219 the non-social session on Days 7 and 28 (Figure 3C; Figure 3-figure supplement 2 and 3). These
220 results additionally suggest that post-traumatic social apathy is hard to explain in terms of
221 emotional development due to its early onset, indicating that emotional and social consequences
222 may arise independently from trauma induction.

223 ***Relationship-dependent behaviors suggesting affectional mechanism***

224 To explore the key factor that determined the development of both emotionality and
225 sociality required several control experiments (Figure 4-figure supplement 1). First, we compared
226 behavioral developments of Control mice with a second control group of mice (Stranger-exposed
227 mice) where the aggressor mice for trauma induction were replaced by non-aggressive strangers.
228 Stranger-exposed mice did not show the developments of behavioral differences (Figure 4-figure

229 supplement 3–6), suggesting that social aggression is necessary to induce stress developments. As
230 Traumatized mice experienced the traumatic event purely psychosocially, partnership between
231 mice served as a candidate variable with which to alter the emotional impact and coping strategy
232 (Sandi and Haller, 2015). Thus, we analyzed a third control group of mice (Stranger-observing
233 mice) as each of them observed different stranger mice being attacked by different aggressors and
234 stayed together with each stranger between aggressive encounters before isolation. Stranger-
235 observing mice did not experience any physical stress from strangers or aggressors. Two notable
236 behavioral differences during trauma induction were observed: While tail rattling during
237 aggressive encounters and hiding under bedding material with the partner during resting were
238 observed in 83% (n = 39 out of 47 mice) and all (n = 47 out of 47 mice) Traumatized mice (Figure
239 4-video supplement 1), respectively, no such behaviors were shown by Stranger-observing mice
240 (n = 0 out of 20 mice). In Traumatized mice, the frequency of tail rattles dropped progressively
241 during aggressive encounters (Figure 4A), suggesting that it represents a rapid phasic
242 psychological reaction during trauma induction. These results suggest that social relationship
243 rapidly modulated emotional impact and social reactions during trauma induction. Moreover,
244 chronic behavioral differences were not observed in Stranger-observing mice (Figure 4B; Figure
245 4-figure supplement 2), which further excluded potential effects from salient, non-specific
246 environmental manipulation (e.g. rotation through aggressors' homecages) and sensory shock (e.g.
247 olfactory cues from urine and vocalization indicating fear) in trauma induction. To further
248 characterize the effects of social relationship, we examined the impact of social support on
249 behavioral developments by a fourth control group of mice (Buffered mice) as each of them was
250 kept pair-housed with its attacked partner after trauma induction. Developments of the differences
251 in emotionality of stress did not occur in Buffered mice (Figure 4-figure supplement 3–5), but

252 developments that reflected their sociality showed the differences of Traumatized mice (Figure 4-
253 figure supplement 6). The observation suggests that Buffered mice showed an emotional recovery
254 from experiences of distress when being together with their partners, which further supports that
255 emotionality and sociality during stress incubation were separately regulated. Taken together, the
256 results of these control experiments reveal that social relationship constitutes a critical
257 psychological factor that determined post-traumatic psychobehavioral development.

258 An important question remained: Why was stress incubated, but not attenuated? As
259 traumatic consequences were modulated by social relationship, we hypothesized that acute
260 traumatic induction yielded chronic stress experience through partnership memory during social
261 isolation, similar to human tending to recall relatively vivid memories of the past relationship after
262 a traumatic relationship dissolution (Chung et al., 2003). We examined the persistence of social
263 memory in a partner-revisiting test on Day28 (Figure 4C; n = 5 mice for each group). Social
264 stimuli were the previous partner and a stranger mouse, both immobilized to allow enough social
265 cues to be attractive, but no active interaction with the focal mouse. To avoid possible influences
266 of social cues from socially defeated mice, stranger mice used to test Control and Stranger-
267 observing mice were partners of Traumatized mice. Strikingly, Traumatized mice spent three
268 times as much time allogrooming or pushing their previous partners as did Control and Stranger-
269 observing mice (Figure 4D; Figure 4-video supplement 2). This amount of allogrooming or
270 pushing previous partners is similar in Buffered mice which were kept pair-housed with their
271 attacked partners until the partner-revisiting test (Figure 4-figure supplement 7). The preference
272 of Traumatized mice to their previous partners, together with the findings of chronic anxiety
273 (Figure 1 and 2) and social apathy (Figure 3) after separation, suggests affectional bonding as a
274 key mechanism of stress incubation in this paradigm (Figure 4-figure supplement 8).

275 **Discussion**

276 We present a novel approach to interpret previously challenging and inconclusive
277 behavioral development, centered on the study of PTSD-like internal psychological development
278 in laboratory mice. These results demonstrate the first system-level view of experimentally
279 disentangled components, processes, and determinants in post-traumatic stress development.
280 While our data suggest that laboratory mice and PTSD patients share similar post-traumatic
281 reactions and psychological substrate, we found little support for the common view that a complex
282 association among trauma-induced psychological states govern the developmental process (DSM-
283 III, 1980;Ehlers and Clark, 2000;Siegmund and Wotjak, 2006;Pamplona et al., 2011;Hayes et al.,
284 2012;DSM-5, 2013;Zoladz and Diamond, 2013;Schnyder and Cloitre;Bryant et al., 2017) (Figure
285 4-figure supplement 8). Instead, we propose a unitary view where the alternation of a single
286 cognitive source is sufficient for triggering changes of diverse and rather independent
287 psychological states. Accordingly, we expect that behavioral signs of PTSD development could
288 also be detected in humans already shortly after the traumatic event, which are likely to be
289 independent of acute stress reactions. The fine-scale behavioral analyses introduced here provide
290 a simple, non-invasive analytic tool to capture informative behavioral details and are not limited
291 to laboratory animals, opening a new window for early detection and prediction to prevent a
292 development into PTSD.

293 While much remains to be considered before clinical applications, including a
294 corresponding study in humans, our study establishes a solid basis to uncover psychological
295 theories for therapeutic strategies (Schnyder and Cloitre, 2015). More than leading toward a better
296 efficacy and lower risk in conducting psychological treatments, this foundation provides a platform
297 to test the role of biological substrates in a system-level view that integrates multiple mechanisms

underlying highly diverse behavioral consequences (Figure 4-figure supplement 8). For instance, a path toward brain physiology can be to test how the potentially slow and global change of brain dynamics arises from an altered dynamic in social bonding circuits through their interconnected nodes (Lee et al., 2014;Lee et al., 2015;Funamizu et al., 2016;Amadei et al., 2017;Ko, 2017). The efficacy of pharmacotherapies could therefore be improved. Beyond PTSD, our research approach to imply human complex psychology by quantitatively and experimentally inferring intertwined psychological qualities in laboratory mice opens a wide range of possibilities in psychiatry.

Materials and Methods:

Study design

Sample size. No statistical methods were used to predetermine sample sizes. Animal numbers were determined based on previous studies.

Rules for stopping data collection. Partner mice were expected to get minor injuries from aggressor mice during aggressive encounters, which normally should be through attack bites on the dorsal side of posterior trunk (Takahashi et al., 2015). The aggressive encounter and all further experiments were terminated once (i) the partner mouse showed severe bleeding or ataxia, or (ii) the aggressor mice showed abnormal attack bites on any other body part. Partner mice that reached the criteria (i) were euthanized. Aggressor mice that reached the criteria (ii) were terminated its usage in any further experiments. If any aggressive sign (sideways threat, tail rattle, pursuit, and attack bite) was shown by the partner mouse, all further experiments with the partner mouse, aggressor mouse, and observer mouse were terminated.

Data inclusion/exclusion criteria. No data were excluded.

Outliers. No outliers were defined.

320 *Selection of endpoints.* The endpoints were prospectively selected. The criteria were approved by
321 the Institutional Animal Care and Use Committee (IACUC) in the animal facility at the Okinawa
322 Institute of Science and Technology (OIST) Graduate University, accredited by the Association
323 for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

324 *Replicates.* All behavioral tests were conducted in quintuplicate to octuplicate sampling replicates.
325 All behavioral tests were conducted in single experimental cohorts. All other records were
326 conducted in quadruplicate to septuplicate experimental cohorts.

327 *Research objectives.* The goal of this work is to identify diverse psychological aspects, temporal
328 patterns, and associations of behavioral development in mice after a single trauma induction. Pre-
329 specified hypotheses stated that (i) internal psychological development is already represented in
330 behavioral details during early post-traumatic phase, while (ii) an association among trauma-
331 induced states such as fear memory, increased anxiety, and social withdrawal explains PTSD-like
332 behavioral development. Our data support the first pre-specified hypothesis, but not the second.
333 All other hypotheses were suggested after initiation of the data analyses.

334 *Research subjects or units of investigation.* 8-week-old male C57BL/6J mice, 16-week-old female
335 C57BL/6J mice, and 20-week-old or older male Slc:ICR mice were used.

336 *Experimental design.* We approached the research goal by developing a novel mouse model of
337 psychosocial trauma under highly controlled conditions (psychosocial manipulations, subjective
338 experiences, and genetic background) and by applying fine-scale analysis to standard behavioral
339 tests. To induce acute witnessing trauma, a pair-housed mouse observed how its partner got bullied
340 by a larger, aggressive mouse on the day of trauma induction. After this trauma, the observer
341 mouse was isolated and developed behavioral differences compared to control mice in the ensuing
342 weeks. The control groups included (i) mice isolated without experiencing trauma induction, (ii)

343 mice isolated after observing how a stranger mouse got bullied by a larger, aggressive mouse, (iii)
344 mice isolated after exposed to a non-aggressive stranger mouse, and (iv) mice which were not
345 isolated after observing how its partner got bullied by a larger, aggressive mouse. The behavioral
346 tests included the light-dark box test, elevated plus-maze test, open field test, locomotor activity
347 test, active social contact test to a female stranger, active social contact test to a male stranger, and
348 partner-revisiting test.

349 *Randomization.* Mice were from different litters. Mice were randomly paired. A focal mouse
350 was randomly selected from each pair of mice. Mice were randomly allocated into experimental
351 groups. Testing order among groups was counterbalanced. Strangers and aggressors were
352 randomly assigned.

353 *Blinding.* The investigator was blinded to behavioral outcomes but not to group allocation during
354 data collection and/or analysis.

355 ***Overview***

356 All experimental procedures were approved by IACUC in the animal facility at the OIST
357 Graduate University, accredited by AAALAC. In total, 336 male C57BL/6J mice (CLEA Japan,
358 Inc.), 36 female C57BL/6J mice (CLEA Japan, Inc.), and 28 male Slc:ICR mice (Japan SLC, Inc.;
359 retired from used for breeding) were used in this study. In CLEA Japan, nursing females were
360 individually housed (CL-0103-2; 165 × 234 × 118 mm), while pups were separated on P21
361 according to gender and housed ≤15 mice per cage (CL-0104-2; 206×317×125 mm). Pups were
362 re-arranged on P28 according to their weights and housed ≤13 mice per cage (CL-0104-2). Mice
363 were shipped in boxes each with 10 – 30 mice to the OIST Animal Facility. In the OIST Animal
364 Facility, mice were housed in 380×180×160-mm transparent holding cages (Sealsafe Plus Mouse
365 DGM - Digital Ready IVC; Tecniplast Inc., Quebec, Canada) bedded with 100% pulp

366 (FUJ9298101; Oriental Yeast Co., Ltd., Tokyo, Japan) under a 12-hr dark/light cycle (350-lux
367 white light) at a controlled temperature of 22.7 – 22.9 ºC, humidity of 51 – 53%, and differential
368 pressure of -14 – -8 Pa with food and water available *ad libitum*. Circadian time (CT) is defined
369 to start at mid-light period and described in a 24-hr format, i.e. light off at CT 6:00.

370 The experimenter and caretakers wore laboratory jumpsuits, lab boots, latex gloves, face
371 masks, and hair nets when handling mice and performing experiments. Handling of mice during
372 the dark cycle was done under dim red light and mice were transported in a lightproof carrier
373 within the animal facility. For mice in experimental and control groups tested on the same dates,
374 the testing order was alternated. Surfaces of experimental apparatuses were wiped with 70%
375 ethanol in water and dry paper tissues after testing each mouse to remove olfactory cues. Each
376 mouse was only used for one behavioral test (in total 4 records with intervals of 6 – 21 days) to
377 avoid confounded results due to cross-testing and to minimize measurement effects on its
378 psychological development (Krishnan et al., 2007).

379 ***Pre-traumatic period (Day-21 to Day0)***

380 To establish partnerships between mice, a male C57BL/6J mouse (focal mouse; 8 weeks)
381 was pair-housed with another male C57BL/6J mouse (partner mouse; 8 weeks) for 3 weeks (Day-
382 21 to Day0, with trauma induction on Day0). The partner was initially marked by ear punching.
383 The holding cage was replaced once per week, with the last change 3 days before the traumatic
384 event (Day-3).

385 To establish the territory of an aggressor mouse in its homecage, an Slc:ICR mouse
386 (aggressor mouse; ≥20 weeks) was pair-housed with a female C57BL/6J mouse (female mouse;
387 16 weeks) for 3 weeks (Day-21 to Day0). The holding cage was replaced with a clean one once a
388 week, with the last change one week before the traumatic event (Day-7).

389 Aggression level of aggressors was screened on Days -5, -3, -1 through intruder encounters
390 (Miczek and O'Donnell, 1978) toward different screening mice to determine appropriate
391 aggressors to be used for trauma induction on Day0. Aggression screening was carried out in the
392 behavior testing room at 22.4 – 23.0 ºC, 53 – 58% humidity, -4 – -3 Pa differential pressure, and
393 57.1 dB(C) ambient noise level during the light period (CT 4:00 – 6:00) with 350-lux white light.
394 After the female and pups with the aggressor were taken out of their homecage and kept in a clean
395 holding cage in the behavior testing room, a 3-min aggression screening was started after a male
396 C57BL/6J mouse (screening mouse; 10 weeks) was brought into the homecage of the aggressor,
397 followed by covering the cage with a transparent acrylic lid. During screening, the aggressor freely
398 interacted with the screening mouse. The aggressor was brought back to the holding room after
399 the screening mouse was taken away from the aggressor's homecage and the female and pups were
400 brought back to its homecage right after screening. Aggressors were selected for trauma induction
401 on Day0 if they showed biting attacks on all of these screening days and the latencies to the initial
402 bites on Day-3 and Day-1 were less than 20 s.

403 ***Trauma induction (Day0)***

404 The following experimental assay emotionally introduced an acute traumatic experience in
405 mice through a social process. The setup was the aggressor's homecage, divided into an 80×180-
406 mm auditorium zone and a 300×180-mm battle arena by the insertion of a stainless-steel mash
407 with 8×8-mm lattices. The cage was covered with a transparent acrylic lid. The behavioral
408 procedure was carried out in the behavior testing room during CT 4:00 – 6:00, with 3 – 5
409 experiments done in parallel.

410 After the female and pups with the aggressor were taken out of their homecage, a divider
411 was inserted into the aggressor's homecage, allowing the aggressor to freely behave in the battle

412 arena, but not to enter the auditorium zone. A 5-min aggression encounter session started after the
413 focal mouse was brought to the auditorium zone and its partner to the battle arena. Tail rattling
414 counts of the focal mouse during aggressive encounter were recorded by experimenter. The
415 aggressive encounter session was followed by a 5-min stress infiltration session, in which the
416 partner was brought to the focal mouse in the auditorium zone, while the aggressor remained in
417 the battle arena. Right after the stress infiltration session, both focal mouse and its partner were
418 brought back to their homecage in the behavior testing room for a 10-min resting period. The
419 procedure was repeated 5 times with different aggressors. During each resting session, the
420 aggressor stayed in its homecage without the divider before its next intruder encounter. Each
421 aggressor had 3 – 5 encounters with resting periods of 10 – 30 min. After the 5th aggression
422 encounter session, the focal mouse was placed back in its homecage where the nest had been razed,
423 and brought back to the holding room. Partners from different pairs were brought to a new holding
424 cage and housed in groups of 3 – 5 per cage. Right after the last intruder encounter for each
425 aggressor, the female and pups were brought back to the homecage and returned to the holding
426 room together with the aggressor.

427 ***Post-traumatic period (Day0 to Day28)***

428 To investigate the behavior of focal mice after trauma induction (now called Traumatized
429 mice), they were housed individually for 4 weeks after the procedure (Day0 to Day28). No
430 environmental enrichment was provided and the holding cage was not changed during social
431 isolation.

432 ***Control experiments***

433 To differentiate behavioral consequences of the emotionally traumatic experience from
434 consequences of social isolation, a control group of mice had their partners taken away and their

435 nests razed during body weighing on Day0 without trauma induction (Control mice, Figure 4-
436 figure supplement 1A).

437 To test the influence of social relationship on the emotionally traumatic experience, a
438 control group of mice witnessed the traumatic events toward stranger mice of the same strain,
439 gender, and age instead (Stranger-observing mice, Figure 4-figure supplement 1B). In each
440 iteration of the aggression encounter, stress infiltration, and resting period, a different stranger
441 mouse was presented.

442 To identify the impacts of aggression during trauma induction, a control group of mice
443 experienced exposure to strangers of the same strain, gender, and age, instead of the aggressor
444 mice for trauma induction (Stranger-exposed mice, Figure 4-figure supplement 1C).

445 To examine the effects of social support on the emotionally traumatic experience, a control
446 group of mice was kept pair-housed with their attacked partners after trauma induction (Buffered
447 mice, Figure 4-figure supplement 1D).

448 ***Body mass and nest wall height***

449 In the holding room, body masses of all individuals were recorded on Days -7, 0, 1, 7, 28,
450 while the heights of nest walls built by each individual were recorded on Days 1, 7, 28. The height
451 of the nest wall was measured with 5-mm resolution using a transparent acrylic ruler, while the
452 mouse was weighed with 10-mg resolution on a balance. Mice were placed back in their
453 homecages right after recording.

454 ***Light-dark box test***

455 The light-dark box test is an experimental assay to measure anxiety in rodents (Crawley
456 and Goodwin, 1980), designed to evaluate their natural aversion to brightly lit areas against their

457 temptation to explore. The light-dark box setup consisted of two connected 200×200×250-mm
458 non-transparent PVC boxes, separated by a wall with a 50×30-mm door. The boxes were covered
459 with lids with white and infrared LED light illumination for the light and dark areas, respectively,
460 and CCD cameras in the centers (4-chamber system; O'Hara & Co., Ltd., Tokyo, Japan). The
461 floors of the boxes were white, while the walls of the boxes were white for the light area and black
462 for the dark area. Uniform illumination in the light area was 550 lux. Behavioral tests were carried
463 out on Days -7, 1, 7, and 28 in the behavior testing room at 22.7 – 23.0 ºC, 51 – 54% humidity, -
464 11 – -9 Pa differential pressure, and 53.6 dB(C) ambient noise level during dark period (CT 6:00
465 – 8:00).

466 After habituation for 10 min individually in the homecage in the behavior testing room in
467 darkness, the focal mouse was transferred to the dark area through a 50×50-mm side door. A 5-
468 min behavior record was started right after the side door of dark area was closed and the door
469 between light and dark areas was opened. Locomotion was recorded 2-dimensionally at 15 Hz
470 from top-view with CCD video cameras. Right after recording, the mouse was returned to its
471 homecage, and brought back to the holding room.

472 ***Elevated plus-maze test***

473 The elevated plus-maze test is an experimental assay to measure anxiety in rodents (Pellow
474 et al., 1985), designed to evaluate their natural fear of falling and exposure against their temptation
475 to explore. The elevated plus-maze setup consisted of a gray PVC platform raised 500 mm above
476 the ground (single maze system; O'Hara & Co., Ltd.). The platform was composed of a 50×50-
477 mm square central platform, two opposing 50×250-mm open arms, and two opposing 50×250-
478 mm closed arms with 150-mm semi-transparent walls. Each of the two open arms emanated at
479 90° to each of the two closed arms, and *vice versa*. The apparatus was installed in a soundproof

480 box with white fluorescent lamp illumination (20 lux) and ventilators. Behavioral tests were
481 carried out on Days -7, 1, 7, 28 in the behavior testing room at 22.8 – 23.0 ºC, 53 – 56% humidity,
482 -13 – -11 Pa differential pressure, and 52.1 dB(C) ambient noise level during dark period (CT 8:00
483 – 10:00).

484 After habituation for 10 min individually in the homecage in the behavior testing room in
485 darkness, the focal mouse was brought to the central platform of the elevated plus-maze, facing
486 the open arm on the opposite side from the door of the soundproof box. A 5-min behavior
487 recording was started right after the door of the soundproof box was closed. Locomotion was
488 recorded 2-dimensionally at 15 Hz from top-view with a CCD video camera installed above the
489 center of the central platform. Delineated entrances to open and closed arms were defined at 50
490 mm from the center of the central platform. Right after recording, the mouse was placed back in
491 its homecage, and brought back to the holding room.

492 ***Open field test***

493 The open field test is an experimental assay to measure anxiety in rodents (Hall and
494 Ballachey, 1932), designed to evaluate their spontaneous activity under a gradient of spatial
495 uncertainty (high in the field center and low along the walls and at the corners of the field). The
496 open field setup consisted of a 400×400×300-mm non-transparent gray PVC box with no cover,
497 installed in a soundproof box with white LED light illumination and ventilators (2-chamber system;
498 O'Hara & Co., Ltd.). Behavioral tests were carried out on Days -7, 1, 7, 28 in the behavior testing
499 room at 22.8 – 23.0 ºC, 53 – 56% humidity, -13 – -11 Pa differential pressure, and 56.7 dB(C)
500 ambient noise level during dark period (CT 8:00 – 10:00).

501 After habituation for 10 min individually in the homecage in the behavior testing room in
502 darkness, the focal mouse was brought to the center of the open field arena under 20-lux uniform

503 illumination, facing the wall on the opposite side from the door of the soundproof box. A 5-min
504 behavior recording was started right after the door of the soundproof box was closed. Locomotion
505 was recorded 2-dimensionally at 15 Hz from top-view with a CCD video camera installed above
506 the center of the open field arena. Vertical activity of exploratory rearing behavior was recorded
507 by the blocking of invisible infrared beams created and detected by photocell emitters and
508 receptors, respectively, positioned 60 mm high on the walls of the open field box. A delineated
509 center region was defined as the central 220×220 mm area. Right after recording, the mouse was
510 placed back in its homecage, and returned to the holding room.

511 ***Locomotor activity test***

512 The locomotor activity test is an experimental assay to measure spontaneous activity of
513 rodents in an environment without an experimentally designed stressor. The locomotor activity
514 setup consisted of a 200×200×250 mm non-transparent covered PVC box with infrared LED
515 illumination and a CCD camera in the center (the dark area of the light-dark box setup, while the
516 door between the light and dark areas was closed and fixed). The floor of the box was embedded
517 with bedding material from the homecage of the focal mouse, while the walls of the box were
518 black. Behavioral test was carried out on Days -7, 1, 7, 28 in the behavior testing room at 22.7 –
519 23.0 ºC, 51 – 54% humidity, -11 – -9 Pa differential pressure, and 53.6 dB(C) ambient noise level
520 during dark period (CT 6:00 – 8:00).

521 After habituation for 30 min individually in the behavior testing box, a 1-hr behavior
522 recording was started. The behavior testing box was not covered completely in order to allow air
523 circulation. Locomotion was recorded 2-dimensionally at 15 Hz from top-view with the CCD
524 video camera. Right after recording, the mouse was returned to its homecage, and brought back
525 to the holding room.

526 ***Active social contact test***

527 The active social contact test is a 2-session experimental assay to measure social motivation
528 in rodents (Berton et al., 2006). The setup consists of a 400×400×300-mm non-transparent gray
529 PVC box with no cover, installed in a soundproof box with 20-lux white LED illumination and
530 ventilators. A 60×100×300-mm stainless-steel chamber with wire grid sides was placed in the
531 center of the wall on the opposite side from the door of the soundproof box. The wire grid had
532 8×8 mm lattices at a height of 10 – 60 mm from the bottom. An ultrasound microphone
533 (CM16/CMPA; Avisoft Bioacoustics, Glienicke, Germany) with an acoustic recording system
534 (UltraSoundGate; Avisoft Bioacoustics) was hung outside the chamber, 100 mm above the ground.
535 Behavioral tests were carried out on Days -7, 1, 7, 28 in the behavior testing room at 22.8 – 23.0
536 ºC, 53 – 56% humidity, -13 – -11 Pa differential pressure, and 56.7 dB(C) ambient noise level
537 during dark period (CT 8:00 – 10:00).

538 The social target used for active social contact tests was either a male or a female C57BL/6J
539 mouse (18 weeks), pair-housed with a partner of the same strain, gender, and age for more than 2
540 weeks before the tests. The social target was adapted to the experimental protocol one day before
541 the tests in the behavior testing room during dark period (CT 8:00 – 9:00): After habituation for 5
542 min individually in the homecage in the soundproof box under 20-lux uniform illumination, the
543 social target was brought into the chamber in the open field arena under 20 lux uniform
544 illumination. A male C57BL/6J mouse (11–16 weeks; from partners of Control mice in previous
545 experiment) was then brought to the open field arena for a 2.5-min spontaneous exploration and
546 interaction with the social target. The social target was then brought back to its homecage in the
547 soundproof box under 20-lux uniform light for a 5-min rest. The social interaction procedure was
548 repeated with a different male C57BL/6J mouse right afterward. After the social target had

549 interacted with 4 different mice, it was returned to its homecage and brought back to the holding
550 room.

551 On testing days, after 10-min habituation individually in its homecage in the behavior
552 testing room in darkness, the first session of the active social contact test started by placing the
553 focal mouse at the center of the open field arena under 20-lux uniform light, facing the empty
554 chamber. A 2.5-min behavior recording started right after the door of the soundproof box was
555 closed. Locomotion was recorded 2-dimensionally at 15 Hz from top-view with a CCD video
556 camera installed above the center of the open field arena. Ultrasonic vocalization was recorded at
557 250 kHz. In the second session of the active social contact test, which followed the first session,
558 the social target was brought into the chamber. Another 2.5-min behavior recording started as soon
559 as the door of the soundproof box was closed. Right afterward, the focal mouse was returned to
560 its homecage and brought back to the holding room.

561 The focal mouse experienced active social contact tests with different social targets on
562 different recording days (Days -7, 1, 7, 28), while different focal mice were tested with the same
563 social target on the same recording day (5 – 10 records). The social target remained in its
564 homecage in a soundproof box under 20-lux uniform illumination before and between each test.
565 A delineated interaction zone was taken as the region within 80 mm of the edges of the chamber.
566 Social approaches of the focal mouse poking its nose toward the social target were recorded
567 manually using the event recording software, tanaMove ver0.09 (http://www.mgrl-
568 lab.jp/tanaMove.html).

569 ***Partner-revisiting test***

570 The partner-revisiting test is a memory-based experimental assay to measure social
571 bonding in rodents. The partner-revisiting setup was the uncovered homecage of the focal mouse,

572 installed in a soundproof box with white LED illumination and ventilators (O'Hara & Co., Ltd.,
573 Tokyo, Japan). The long sides of the homecage were parallel to the door of soundproof box. The
574 partner-revisiting test was carried out on Day28 in the behavior testing room at 22.8 – 23.0 ºC, 53
575 – 56% humidity, -13 – -11 Pa differential pressure, and 56.7 dB(C) ambient noise level during light
576 period (CT 4:00 – 6:00) with 350-lux light intensity.

577 The previously separated partner of the focal mouse, being a social target in the test, was
578 initially sedated with 3%v/v isoflurane in oxygen, and then anesthetized by intraperitoneal (i.p.)
579 injection of a mixture of medetomidine (domitor, 3%v/v in saline, 0.3 mg/kg), midazolam
580 (dormicum, 8%v/v in saline, 4 mg/kg), and butorphanol (vetorphale, 10%v/v in saline,5 mg/kg).
581 Also, a stranger mouse (15 weeks; a separated partner of a Traumatized or Buffered mouse for
582 testing a Control, Stranger-observing, or Stranger-exposed mouse, and *vice versa*) was
583 anesthetized as an alternative social target. Both anesthetized mice were kept on a heating pad at
584 34ºC (B00O5X4LQ2; GEX Co., Ltd., Osaka, Japan) to maintain their body temperatures before
585 the test.

586 The focal mouse was brought to a clean, uncovered holding cage in the soundproof box
587 under 50-lux uniform illumination for 5-min habituation, while its homecage was placed in another
588 soundproof box under 50-lux uniform light. During habituation of the focal mouse, the
589 anesthetized social targets were injected with atipamezole hydrochloride (antisedan; 6%v/v in
590 saline for 0.3 mg/kg, i.p.) to induce recovery from anesthesia. During the waking-up period, the
591 social targets were still immobilized and not able to actively interact with the focal mouse during
592 the following recording, but showed enough social cues to be attractive for the focal mouse. The
593 immobilized social targets were then placed in the homecage of the focal mouse with their nose
594 pointing toward the center of the short side of the wall (10 mm of nose-to-wall distance) with their

595 bellies facing the door of the soundproof box. After habituation, the focal mouse was brought to
596 the center of its homecage, facing the long side of the homecage wall on the opposite side from
597 the door of soundproof box. A 10-min behavior record started right after the door of the soundproof
598 box was closed. Locomotion was recorded 2-dimensionally at 15 Hz from top-view with a CCD
599 video camera installed above the center of the homecage. Right after recording, social targets were
600 taken out of the focal mouse's homecage and the focal mouse was brought back to the holding
601 room.

602 Social contacts including sniffing, allogrooming, and pushing of the focal mouse toward
603 each of the social targets were recorded manually using the event recording software, tanaMove
604 ver0.09 (http://www.mgrl-lab.jp/tanaMove.html).

605 ***Video image process***

606 Video image data was processed using custom scripts written in MATLAB R2015b
607 (MathWorks). Each video frame from a recorded AVI video file was read as a 2-dimensional
608 matrix with an 8-bit gray scale. Each of these matrices was then divided by a background matrix
609 read from a TIF image file of the background taken before bringing the test mouse to the setup.
610 The centroid of the area with non-one values in each matrix ratio was taken as the position of the
611 mouse at this specific time point. Speed was calculated as the distance between temporally
612 adjacent positions multiplied by 15 (15-Hz recording). Freezing periods were sorted out if the area
613 of the mouse body between temporally adjacent frames was less than 20 mm^2.

614 ***Audio signal process***

615 Audio signal data was processed with custom scripts written in MATLAB R2015b
616 (MathWorks). Each recorded WAV audio file was read and transformed into a spectrogram using

617 fast Fourier transform with non-overlapping 0.4-ms time windows. To identify the time segments
618 with ultrasonic vocalization signals, recordings were thresholded at a power spectral density (PSD)
619 ≥-75 dB/Hz, and time segments with averaged PSD between 0–50 kHz higher than that between
620 50–120 kHz were removed. The duration of remaining time segments was calculated.

621 ***Statistical analysis***

622 Numerical data were analyzed with custom scripts written in MATLAB R2015b
623 (MathWorks). Statistical significance of the difference between 2 mean values was estimated with
624 two-tailed, two-sample Student's *t*-test. Statistical significance of the difference between 2 median
625 values (vocalization analysis; Figure 3-figure supplement 1) was estimated using one-tailed Mann–
626 Whitney *U* test.

627 To capture fine-scale behavioral details of location within the light-dark box and the
628 elevated plus-maze (Figure, 1C and 2A), we computed T(x), the cumulative probability of finding
629 position ≤x, for each individual (light traces) for all measured locations (a collection of locations
630 from all mice for the statistics). We then show the average across the control group (bold blue
631 trace) and the Traumatized group (bold red trace). We compared the averages of each group with
632 a two-tailed, two-sample Student's *t*-test and plot the resulting p-values, presented as -log(p), the
633 negative logarithm of *p*-values. We also show the box plot (the minimum, lower quartile, median,
634 upper quartile, and maximum) of -log(p) values collapsed across all measured locations. To
635 capture the fine- scale behavioral details of speed, we followed a similar procedure as above, but
636 with U(v), the cumulative distribution function of finding speed ≤v.

637 To evaluate the uncertainty of the percentage for each tail rattle count (Figure 4A), we
638 created 10,000 bootstrapped data sets where each sample was randomly picked with replacement
639 from the original data set. Each bootstrapped data set had the same sample size as the original

data set. The standard error was taken as the standard deviation of the bootstrapped percentages for a tail rattle count. A similar procedure was carried out to evaluate the standard error of mean for the percentage of time spent in each behavior in the partner-revisiting test (Figure 4D), where each sample of the bootstrapped data sets was a set of the percentages of the three classified behaviors (partner concern, stranger concern, and non-social activity/sniffing at social targets) from a mouse record. Standard errors of means for other results were estimated with the formula $\sigma/\sqrt{n}$, where σ is the sample standard deviation and n is the sample size.

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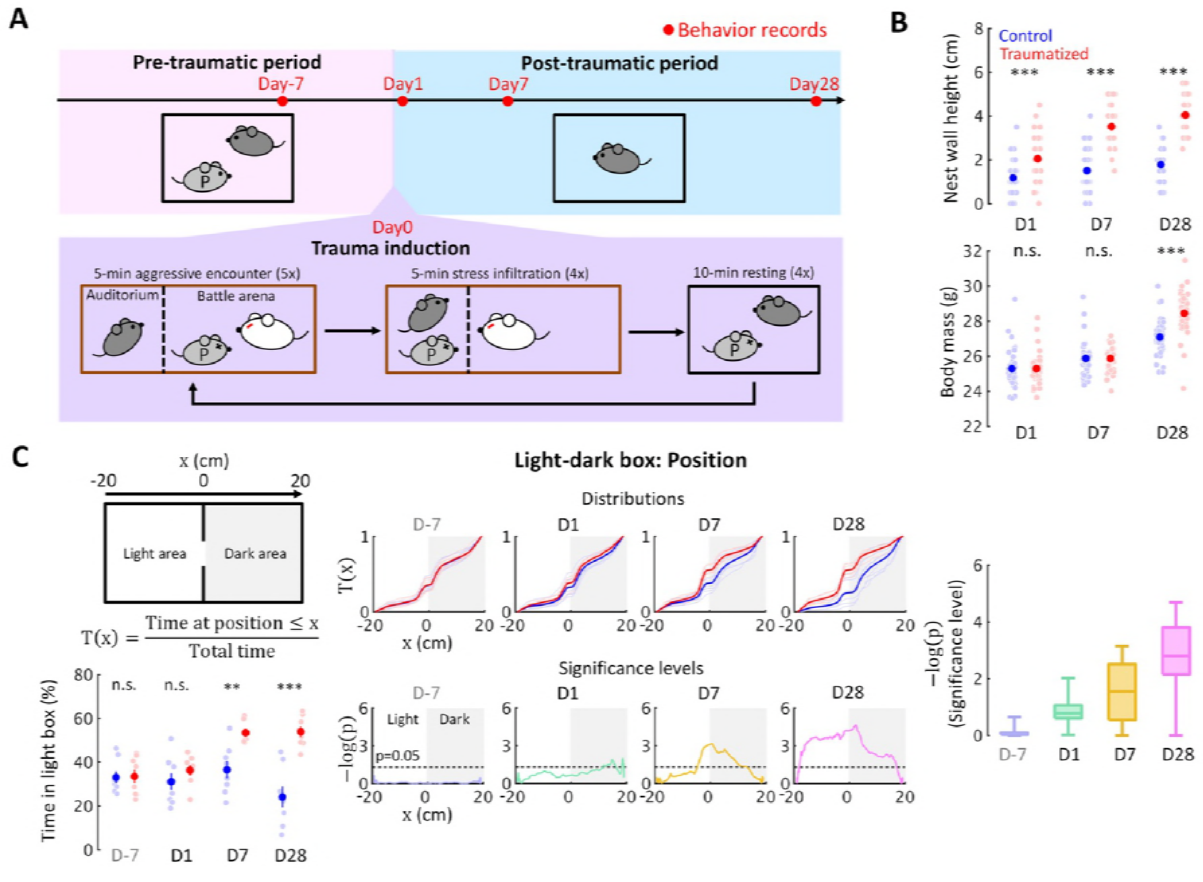


Figure 1. **Post-traumatic behavioral development is revealed by acute induction of psychosocial trauma and spatially fine-scale behavioral analysis.** **(A)** Paradigm inducing acute psychosocial trauma in mice. Focal mouse (dark gray), partner mouse (light gray P), aggressor mouse (white), focal mouse's homecage (black), aggressor's homecage (brown), and wire-meshed divider (dashed line). **(B)** Nest wall heights (top) and body masses (bottom) show significant differences after trauma induction. **(C)** Light-dark box test quantified through the cumulative position probability T(x) along the light-dark axis (left). On average, Traumatized mice (red) spent more time in the light area than Control mice (blue) during the late post-traumatic period [T(0), bottom-left]. Spatially fine-scale behavioral analysis reveals significant differences between Traumatized and Control populations already in the early post-traumatic period (middle). For each position, we compute the mean T(x) across the Control and Traumatized populations and compute

statistical significance through a two-population Student's *t*-test. These differences gradually increased, as evidenced by significance distributions collapsed across all positions (right; box plots show the minima, lower quartiles, medians, upper quartiles, and maxima). Light dots or lines, data of individual mice; deep dots or lines, means across populations; deep vertical lines, standard errors in the means; n.s., $p \geq 0.05$; **, $0.001 \leq p < 0.01$; ***, $p < 0.001$; -log(p), negative logarithm of the p-value; statistical significance of the difference in mean values was determined with a two-tailed, two-sample Student's *t*-test. The following supplements are available for Figure 1: figure supplement 1–2.

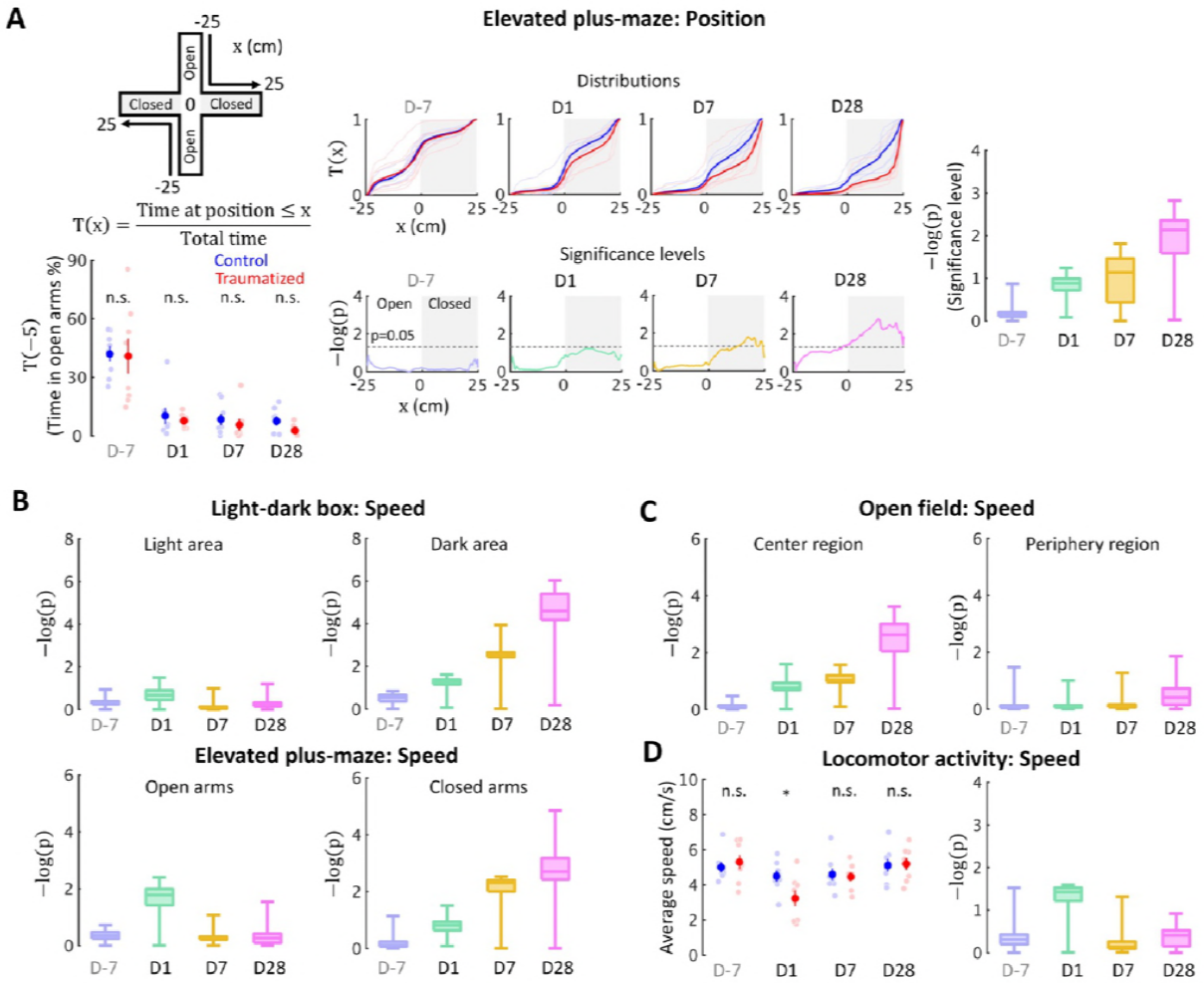
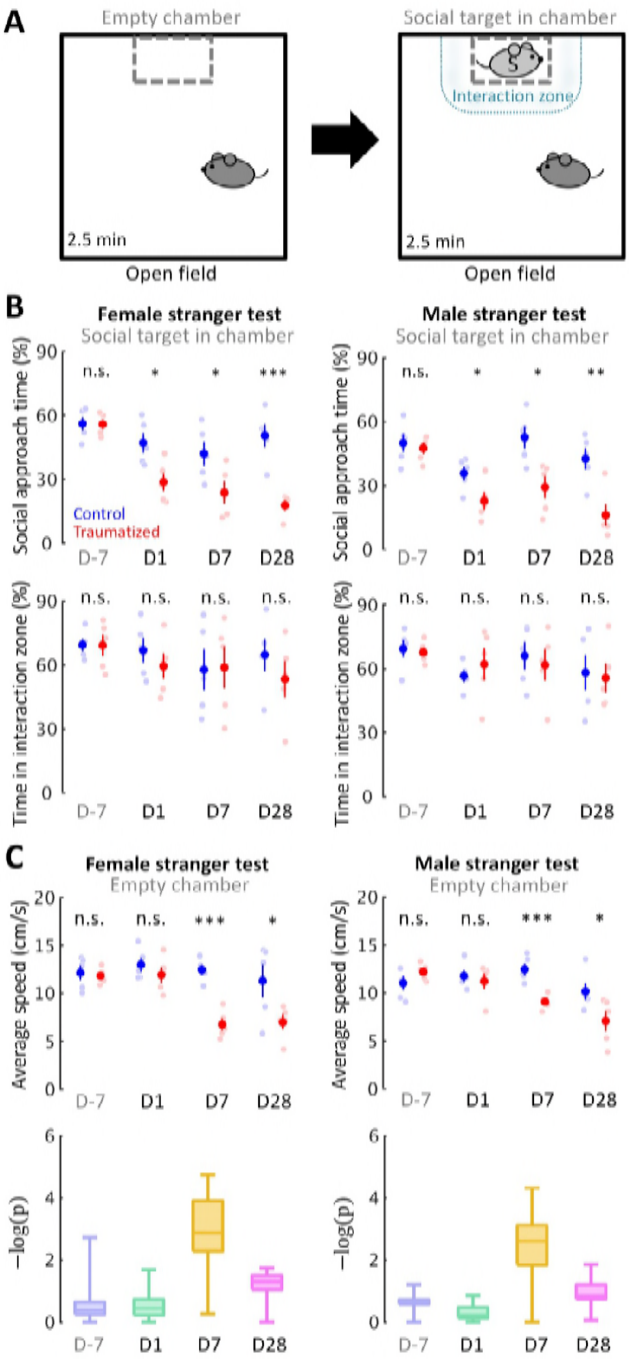


Figure 2. Acute psychosocial trauma induces both gradually increasing anxiety and acute stress. **(A)** In the elevated plus-maze test, Traumatized and Control mice did not differ significantly in the time they spent in opened arms; however, spatial distributions show differences in preferred location between Traumatized and Control mice in the closed arms, which increased with time. **(B)** Locomotion speed shows acute difference only in stressor zones (light area and open arms), but incubated differences in stressor-free zones (dark area and closed arms). We similarly quantified speed using the fine-scale cumulative distribution U(v) of having speed ≤v and we show the statistical analysis of population differences in U(v). **(C)** Anxiety is evident in the open field test through a delayed onset of locomotor speed differences in the stressor zone

847 (center region). **(D)** In the locomotor activity test without stressors, acute effects of activity
848 reduction recovered in the later post-traumatic period. *, $0.01 \leq p < 0.05$. The following
849 supplements are available for Figure 2: figure supplement 1–10.



850

851 **Figure 3. Acute psychosocial trauma decreases social interest. (A)** Consecutive non-social and
852 social behavior tests. During the social phase, motivation for social contact toward a stranger
853 mouse (light gray S) was evaluated as the time spent for social approaches of nose poking and the
854 time spent in the delineated interaction zone. **(B)** Traumatized mice made fewer social approaches

855 (top) to both female (left) and male (right) strangers, but there was no significant difference in the
856 time spent in the interaction zone (bottom). **(C)** Traumatized mice showed a delayed onset of
857 reduction in locomotion speed during the non-social phase. The following supplements are
858 available for Figure 3: figure supplement 1–3; video supplement 1.

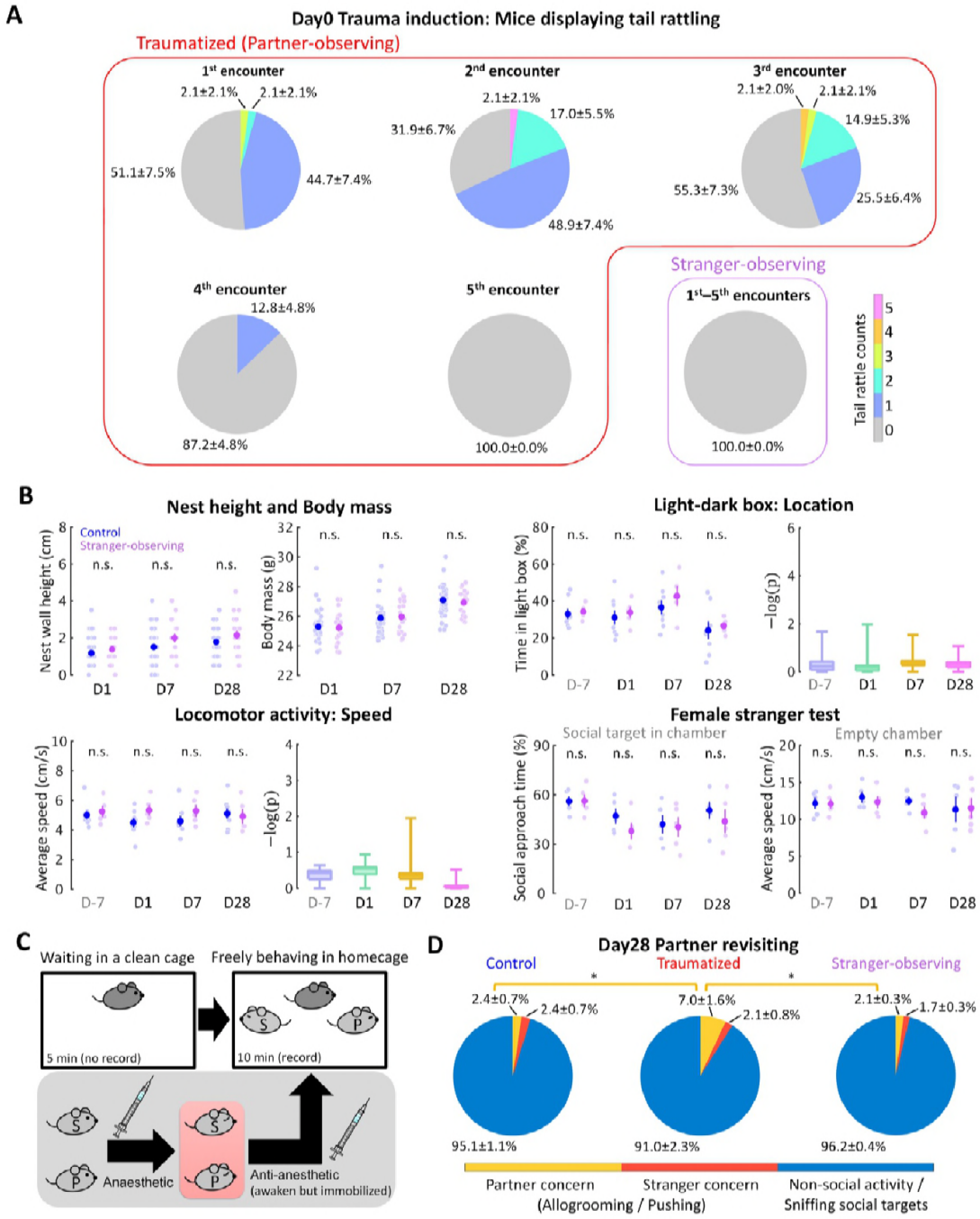


Figure 4. Social relationship in trauma induction determines stress development. (A) Social relationship increased emotional impact, evidenced by tail rattling behavior during trauma induction. **(B)** No significant chronic difference was found in Stranger-observing mice. **(C)** The partner-revisiting test. A stranger mouse (light gray S) and the previously pair-housed partner

(light gray P), both immobilized, were presented as social targets. Pink rectangle, heating pad. **(D)** Traumatized mice showed significantly longer allogrooming or pushing their partners (yellow, % of time spent in partner concern behavior) than either Control or Stranger-observing mice. Standard errors were calculated from bootstrapped data. The following supplements are available for Figure 4: figure supplement 1–8; video supplement 1–2.

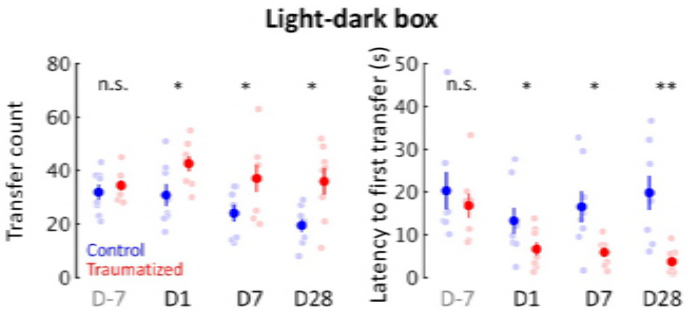
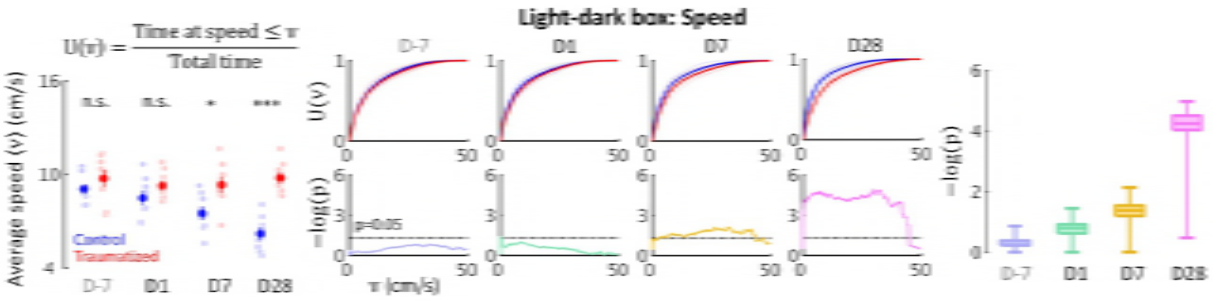


Figure 1-figure supplement 1. Behavioral repertoires in the tests demonstrate that mice experience anxiety. These results, together with those demonstrated in the main text, support anxiety-like reactions in Traumatized mice. In the light-dark box task, higher transfer counts and shorter latency to the first transfers in Traumatized mice suggest their higher activity and exploratory motivation, respectively.



875

876 **Figure 1-figure supplement 2. Increasing difference of locomotion speed in the light-dark**

877 **box suggest increased anxiety in Traumatized mice.** Cumulative distribution functions (U(v)

878 of having speed ≤v) and corresponding significance distributions show a gradually increasing

879 differences in behavior.

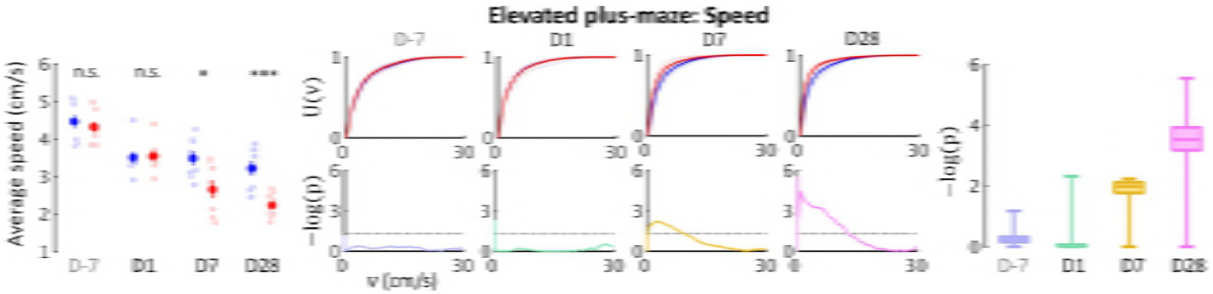


Figure 2-figure supplement 1. Increasing difference of locomotion speed in the elevated-plus maze tests suggest increased anxiety in Traumatized mice. Cumulative distribution functions (U(v) of having speed ≤v) and corresponding significance distributions show a gradually increasing differences in behavior.

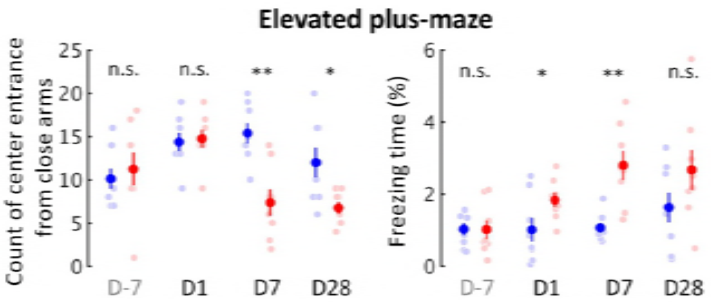


Figure 2-figure supplement 2. Behavioral repertoires in the tests demonstrate that mice experience anxiety. These results, together with those demonstrated in the main text, support anxiety-like reactions in Traumatized mice. In the elevated plus-maze, less exploration from close arms to platform center and longer freezing time in Traumatized mice suggest their stronger stress reactions.

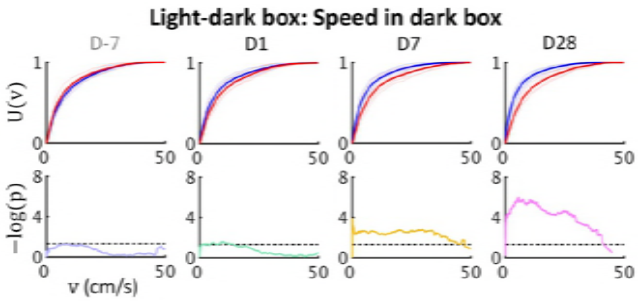


Figure 2-figure supplement 3. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2B, top-right panel.

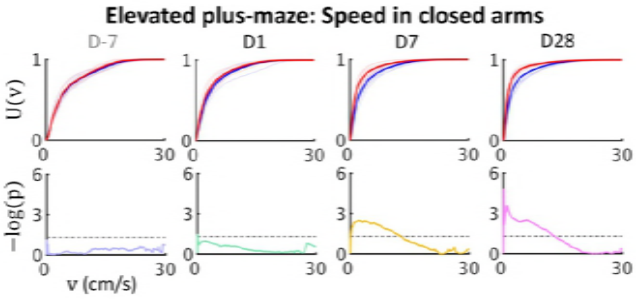


Figure 2-figure supplement 4. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2B, bottom-right panel.

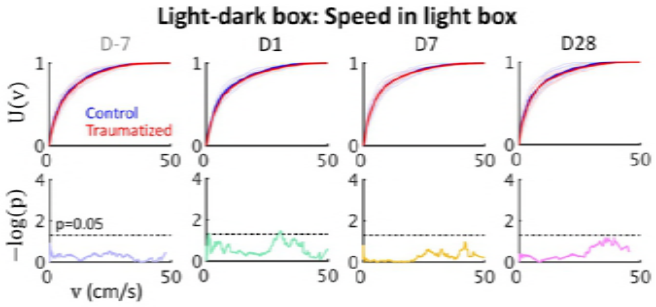


Figure 2-figure supplement 5. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2B, top-left panel.

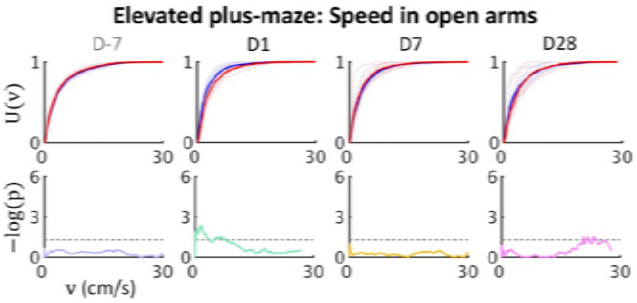


Figure 2-figure supplement 6. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2B, bottom-left panel.

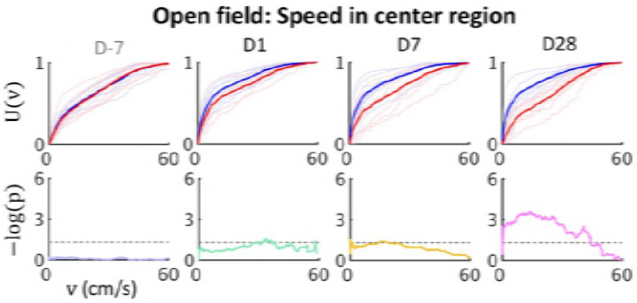


Figure 2-figure supplement 7. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2C, left panel.

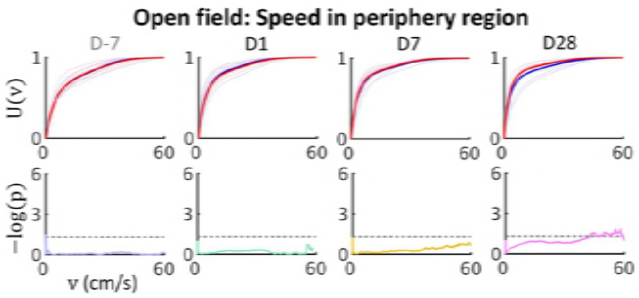


Figure 2-figure supplement 8. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2C, right panel.

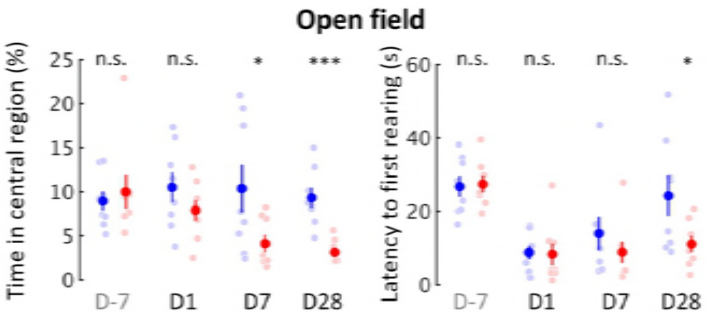


Figure 2-figure supplement 9. Behavioral repertoires in the tests demonstrate that mice experience anxiety. These results, together with those demonstrated in the main text, support anxiety-like reactions in Traumatized mice. In the open field, less time spent in the central region by Traumatized mice suggests their avoidance of a region with high special uncertainty, while shorter latency to their first rearing indicates their higher exploratory motivation.

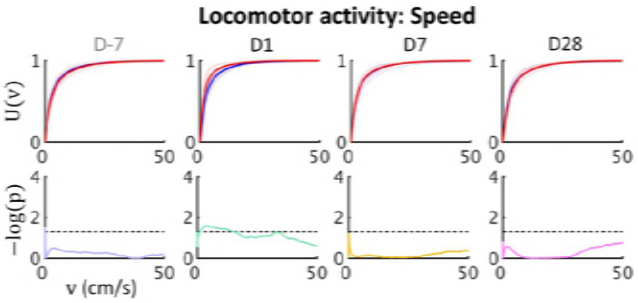


Figure 2-figure supplement 10. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 2D.

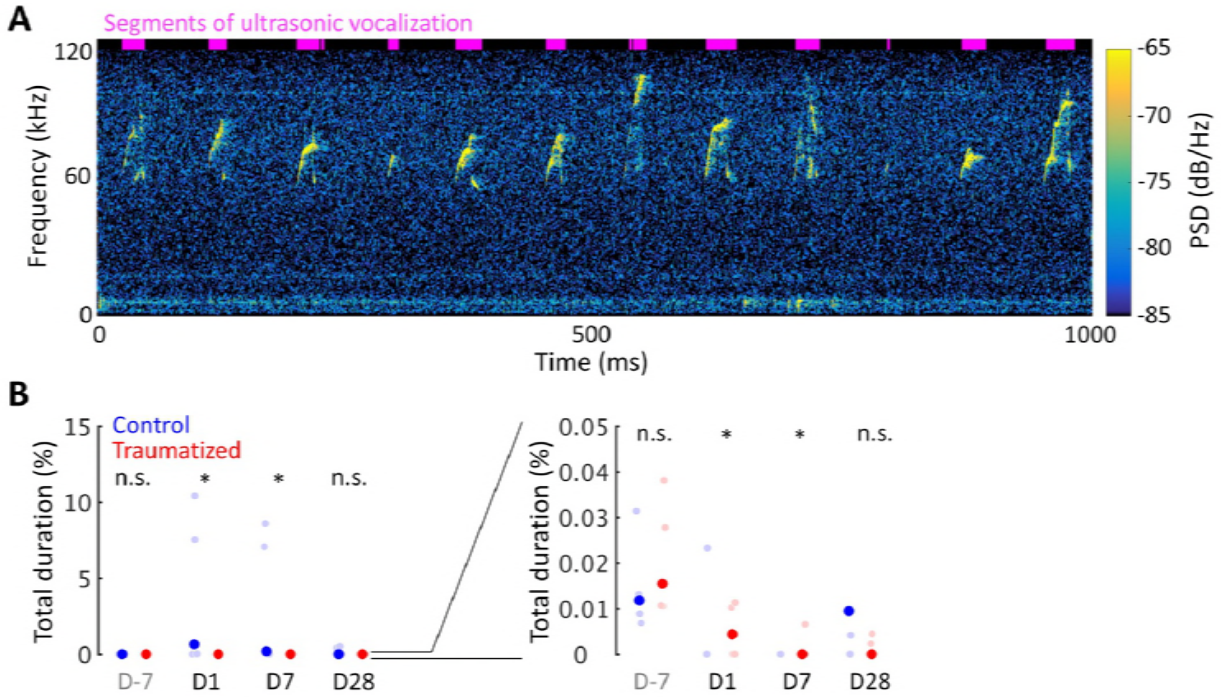


Figure 3-figure supplement 1. Reduced ultrasonic vocalization during the social session of the female stranger test in Traumatized mice attests to diminished social communication. (A) Spectrogram of ultrasonic vocalizations during the social session in the female stranger test of a Control mouse on Day1. **(B)** More vocalization was recorded from Control mice than Traumatized mice on Days 1 and 7. Data points and median, one-tailed Mann-Whitney *U* test; PSD, power spectral density.

Figure 3-figure supplement 2. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 3C, left panels.

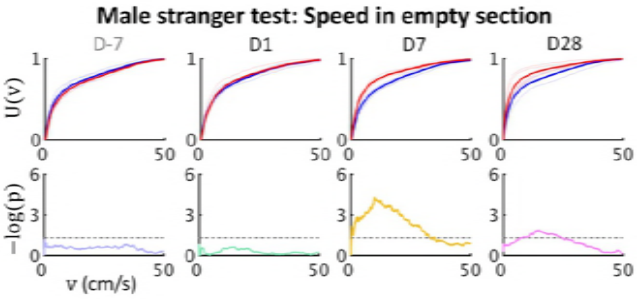


Figure 3-figure supplement 3. Locomotor speeds of Traumatized and Control mice in various tests indicate that Traumatized mice develop diverse behavioral differences. Cumulative distributions and corresponding *p*-value distributions for Figure 3C, right panels.

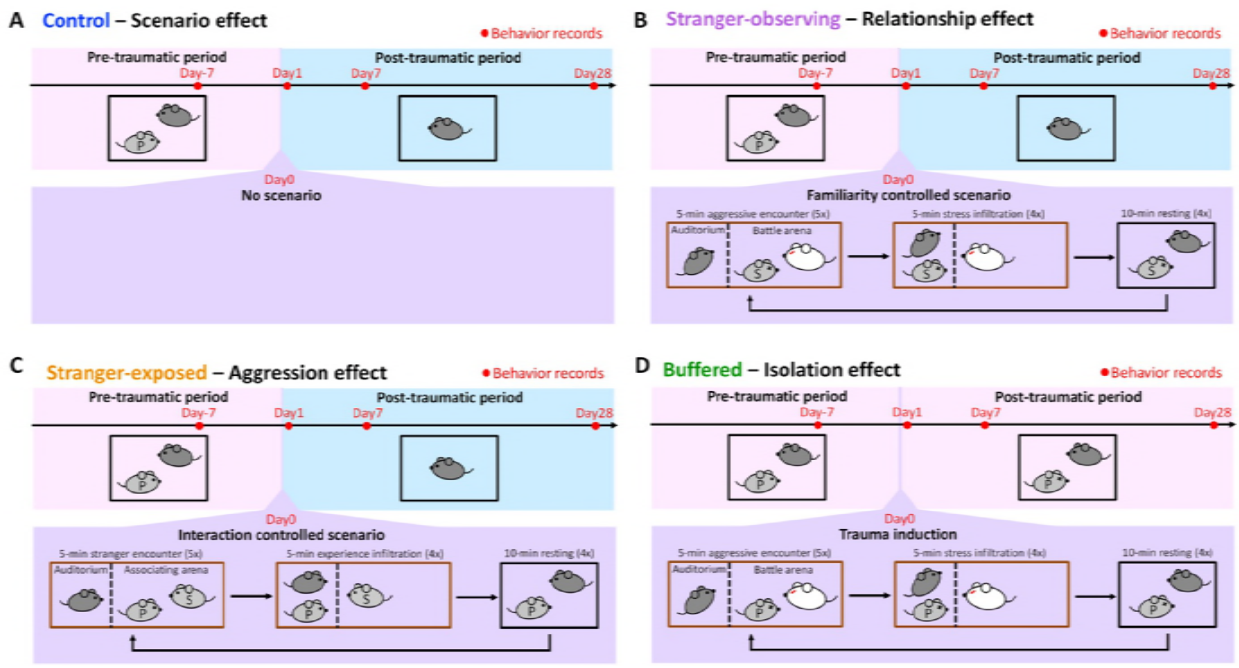


Figure 4-figure supplement 1. Control experiments demonstrate psychosocial factors that determined the behavioral developments. **(A)** Experiments of Control mice which had no experience of trauma induction identified the scenario effect in the behavioral paradigm. **(B)** Experiments of Stranger-observing mice which had witnessing experience of trauma that happened to strangers, rather than to their pair-housed partners, identified the relationship effect in the behavioral paradigm. **(C)** Experiments of Stranger-exposed mice which had experience of social interactions without witnessing stress from strangers identified the aggression effect in the behavioral paradigm. **(D)** Experiments of Buffered mice which were pair-housed with their partners after trauma induction identified the isolation effect in the behavioral paradigm.

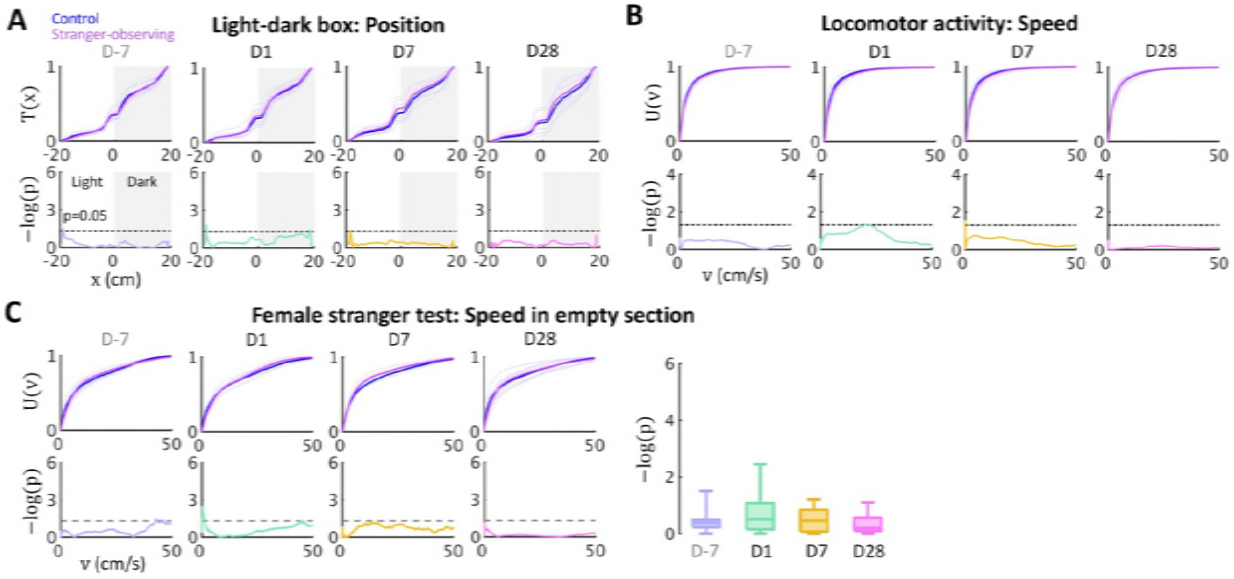


Figure 4-figure supplement 2. Stranger-observing and Control mice in various tests indicate that Stranger-observing mice did not develop the behavioral differences of Traumatized mice. Cumulative distributions and corresponding *p*-value distributions for **(A)** Figure 4B, top-right panels, **(B)** Figure 4B, bottom-left panels, **(C)** Figure 4B, bottom-right panels, Empty chamber.

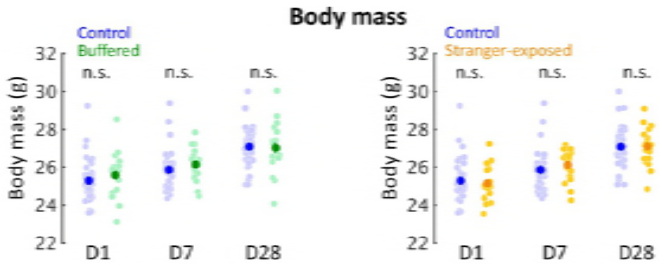
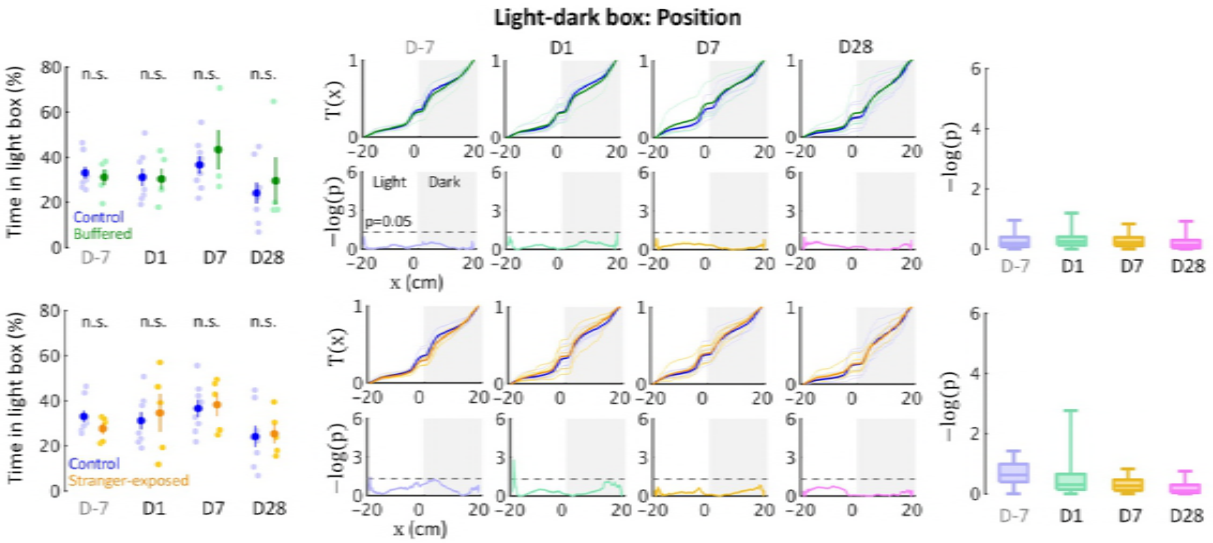


Figure 4-figure supplement 3. Body masses of Buffered, Stranger-exposed, and Control mice indicate that Buffered and Stranger-exposed mice did not develop the physiological differences of Traumatized mice.



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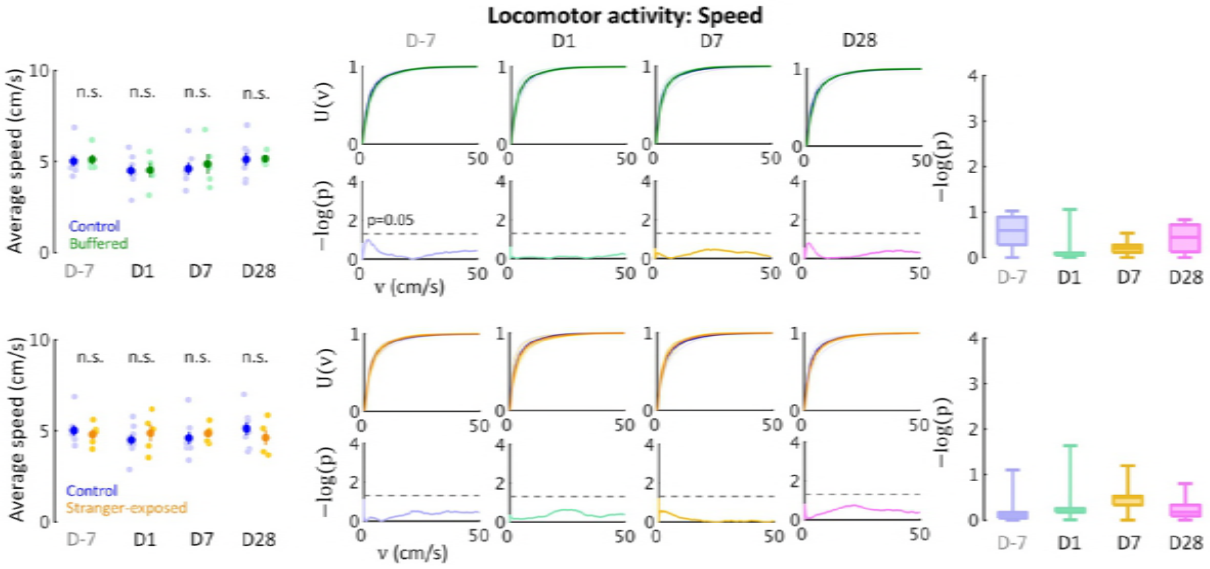
961 **Figure 4-figure supplement 4. Positions of Buffered, Stranger-exposed, and Control mice in**

962 **the light-dark box test indicate that Buffered and Stranger-exposed mice did not develop the**

963 **chronic stress reactions of Traumatized mice.**



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965 **Figure 4-figure supplement 5. Locomotor speeds of Buffered, Stranger-exposed, and Control**

966 **mice indicate that Buffered and Stranger-exposed mice did not develop the acute stress**

967 **reactions of Traumatized mice.**

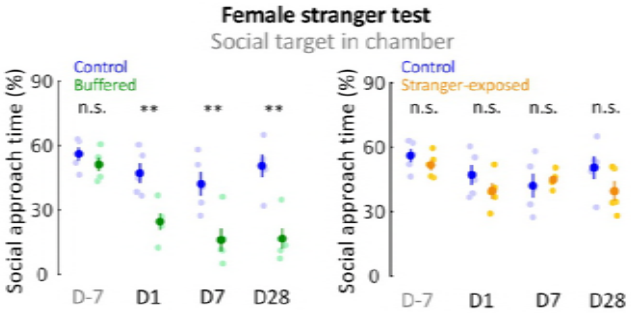

Figure 4-figure supplement 6. Social approach times of Buffered, Stranger-exposed, and Control mice in the female stranger test indicate that Buffered mice did, but Stranger-exposed mice did not develop the social differences of Traumatized mice.

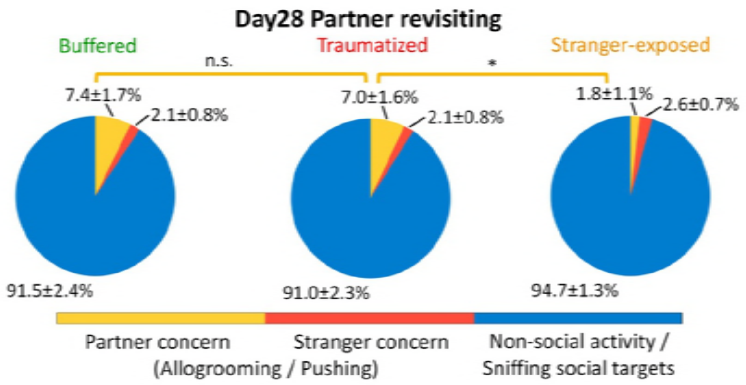

Figure 4-figure supplement 7. Social contacts of Buffered, Stranger-exposed, and Traumatized mice in the partner-revisiting test demonstrate that Traumatized mice are affectively bonded with their partners. Buffered mice and Traumatized mice spent longer time on the behaviors reflecting partner concern. The observation of more contacts with a familiar social target in Traumatized and Buffered mice is opposite to the lower contacts with familiar social targets in a three-chamber social novelty test where curiosity to novelty dominates the behavior (Okuyama et al., 2016). In addition, social isolation generally disrupts mouse capacities for social recognition and social memory measured in curiosity-based behavioral tests (Kogan et al., 2000;Okuyama et al., 2016). Together with the findings of gradually increasing separation distress after trauma induction in Traumatized mice, the results provide empirical evidence supporting that the partner revisiting test is a memory-based assay to measure social bonding in mice. Therefore, we conclude that Traumatized and Buffered mice show a stronger affectional bonding to their previously pair-housed partners compared to Control, Stranger-observing, and Stranger-exposed mice.

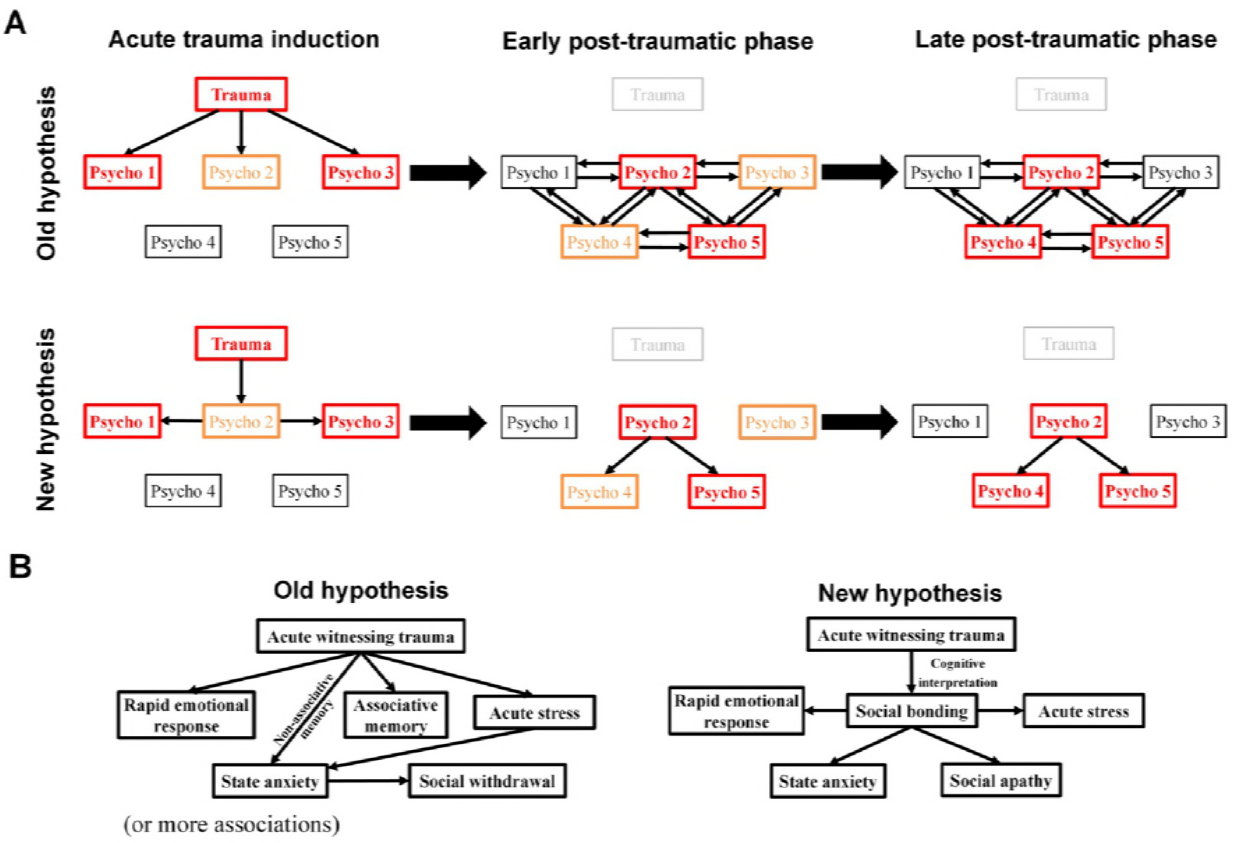

Figure 4-figure supplement 8. Conceptual models explain PTSD development. (A) Causal relations (arrows) among different psychological elements (Psycho #, a type of emotion or cognition) for stress development in different phases in the views of old (top) and new (bottom) hypotheses. Orange and red represent medium and strong trauma-induced differences, respectively. From a neuroscience perspective, for an example, each psychological element can be considered as a corresponding neural circuit, and the trauma-induced difference can be taken as the difference between its post-traumatic and pre-traumatic dynamical states. **(B)** Associations among psychological elements in these conceptual models for the behavioral paradigm in this study. Our data show little support for an associating view speculated in previous hypotheses (left), but support a unitary view (right) where diverse PTSD-like reactions independently developed via a common cognitive source ("Social bonding"). This new view suggests a psychological substrate

999 in system-level that integrates multiple mechanisms underlying highly diverse behavioral
1000 consequences, as a specific clinical target in the full mechanistic picture.

1001 **Figure 3-video supplement 1. Social apathy is an observed behavioral characteristic of**
1002 **Traumatized mice.** Examples of 30-s recordings during the social session in the female stranger
1003 test on Day1. First scene, Control mouse; Second scene, Traumatized mouse.

1004 **Figure 4-video supplement 1. Illustration of behavioral characteristics that were specific to**
1005 **observer mice during trauma induction when their partners were attacked.** First scene, tail
1006 rattling during aggressive encounter; Second scene, tail rattling during aggressive encounter (4x
1007 slower); Third scene, hiding under bedding material with the partner during resting. These
1008 behaviors were not observed if a stranger mouse got attacked.

1009 **Figure 4-video supplement 2. Illustration of rebound reaction of a Traumatized mouse to its**
1010 **previously pair-housed partner during the partner-revisiting test**.