



# Social dominance in rats is a determinant of susceptibility to stress

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Contributed by Richard G. M. Morris; received June 20, 2024; accepted March 15, 2025; reviewed by Eric Klann and Ron Stoop

Q:9 Establishing a dominance hierarchy in social organisms is important for access to resources. Stress has been proposed as a major factor influencing an animal's likely position in a social hierarchy. Although individual differences in vulnerability to stress are increasingly recognized, how social hierarchy affects vulnerability remains relatively understudied. Here, we examined how the social dominance status of adult male Sprague-Dawley rats influences their response to stress in interactions with a familiar animal living in the same cage, and separately when confronting an unfamiliar rat of uncertain dominance status from another cage, using the tube test. Having determined the relatively stable within-cage social dominance status of animals, half were subjected to a single episode of 2-h immobilization stress. Cagemates, both control and stressed rats, again faced one another 1 d and 10 d after stress. First, the predetermined hierarchical rank among familiar cagemates was relatively unaffected by stress. However, second, the same stress had a differential impact in competitions between unfamiliar rats. Socially dominant control rats continued to win competitions but subordinate control rats, that had previously lost, sometimes started to win against previously dominant rats that had been stressed. Strikingly, subordinate stressed rats displayed consistent submissive-like behavior and they alone showed differential effects on dendritic spine density in the amygdala. Thus, an individual's social rank can influence its response to stress, an effect that is detectable in interactions with unfamiliar animals. These findings add an additional dimension to animal models of stress used for exploring facets of social anxiety and withdrawal in stress-related psychiatric disorders.

stress | dominance | susceptibility | tube test | structural plasticity

Stressful events can trigger psychiatric disorders like depression and anxiety, with an impact on behavior and social dynamics (1–3). However, studies across species suggest that there are various coping mechanisms determining the degree of resilience or susceptibility to psychological stressors (4–7). Understanding the causes of differential susceptibility would help to identify targets of intervention for the treatment of stress-associated disorders. In addition to genetic and epigenetic alterations as well as early life stress itself, the social status of an animal could have an impact on resilience (6, 8, 9). For example, anxious subjects tend to exhibit reduced competitiveness under stress (10, 11) and such subjects may be socially submissive.

Social hierarchies are a fundamental organizing system of social animals (12–15). Being at the top of the hierarchy comes with advantages such as increased access to food, territory, and mating partners (16, 17). Several studies have explored whether social status has an impact on susceptibility to stress but, surprisingly, recent studies using mice have reported contradictory findings with respect to the impact of chronic stress (18–23). The current study examined laboratory rats rather than mice, and a milder form of stress by subjecting animals to a single 2-h episode of immobilization (24), rather than repeated exposures over days. This form of acute stress is known to cause measurable changes across biological scales (25).

The amygdala, a brain region essential for encoding emotionally salient information (26–29) regulates the stress response and maintains homeostasis in response to stressful stimuli (30). Even a single stressful event can leave a lasting effect on neuronal structure, with observations showing differential dendritic spine density in the basolateral amygdala (BLA) and changes in anxiety-like behavior (24, 31). Human imaging studies have also shown aberrant functional connectivity between the amygdala and its afferent/efferent regions in stress-related psychiatric disorders (32–35).

Beyond its involvement in mediating the stress response, the amygdala plays a significant role in the formation and maintenance of a dominance hierarchy. Research in both primates and other animals has identified the amygdala's importance in the formation of

## Significance

Although stress has significant impact on brain and behavior, its effects vary between individuals. Relatively little is known about how social status contributes to individual differences in stress vulnerability. We report that stress affects social dominance tests between unfamiliar animals (living in different cages), but not familiar animals (living together). Higher-ranked animals exhibited resilience to stress, whereas lower-ranked animals displayed enhanced submissiveness. Interestingly, poorer behavioral resilience caused by a combination of stress and lower social status was associated with structural changes in synaptic connectivity in the amygdala, a brain area that encodes emotional experiences. These findings add an additional dimension to animal models of stress used for exploring facets of social anxiety and withdrawal in stress-related psychiatric disorders.

Author contributions: D.J.S. and S.C. designed research; Q:6 D.J.S. performed research; D.S., V.K., and R.G.M.M. analyzed data; R.G.M.M. acted as consultant throughout the project; S.C. raised the funding; and D.J.S., R.G.M.M., and S.C. wrote the paper.

Reviewers: E.K., New York University; and R.S., Universite de Lausanne.

The authors declare no competing interest.

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This article contains supporting information online at <https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2412314122/-/DCSupplemental>.

Published XXXX.

117 dominance hierarchies and the expression of dominant or subor- 178  
118 dinate behavior (36–44). Moreover, the interaction between spe- 179  
119 cific pathways involving the amygdala and other brain areas can Q:1280  
120 bidirectionally influence social behavior and anxiety levels. For 181  
121 example, modulation of BLA-mPFC, and BLA-vCA1 projections 182  
122 can bidirectionally affect social behavior and anxiety (45, 46). 183  
123 Given its central role in stress response and dominance behaviors, 184  
124 this study aimed to explore how stress and dominance affect both 185  
125 behavior and structural plasticity at the level of dendritic spines 186  
126 in the BLA. 187

127 One of the standard tests for assessing dominance in rodents is 188  
128 the tube test (16, 47) which has been shown to be a reliable indi- 189  
129 cator of social dominance (16, 48) which correlates well with other 190  
130 dominance measures (49). It is done by placing animals, facing 191  
131 each other, at each end of a narrow tube leading to a desirable 192  
132 place of safety. The animals confront each other, unable to turn 193  
133 around or pass freely, until one animal either forces the other 194  
134 backward out of the tube or the other animal voluntarily with- 195  
135 draws. Both animals then enter either of the safe cages placed at 196  
136 each end of the tube, the interaction taking place within typically 197  
137 15 to 30 s. The “winner” of such contests is noted and, after num- 198  
138 ber of such tests, the animal winning the majority of such com- 199  
139 petitions is assigned the dominant rank. Once the dominance 200  
140 status of an animal in its own home cage has been established, 201  
141 and investigation of the impact of stress on that status within the 202  
142 home cage, separate competitions can be arranged between ani- 203  
143 mals living in different cages. In this case, the two animals are 204  
144 unfamiliar to each other and cannot know the social dominance 205  
145 status of the other. 206

146 This study explored the hypothesis that high social status may 207  
147 yield greater resilience to stress, whereas low social status greater 208  
148 susceptibility. To this end, we examined whether the dominance 209  
149 status of an animal influenced its response to stress with respect 210  
150 to the following questions: i) Does stress affect social dominance 211  
151 behavior when rats are confronted by their cage mate? ii) What 212  
152 happens when rats from different cages compete against each 213  
153 other? iii) Will prior acute stress impact competitions between 214  
154 unfamiliar animals living in different cages? iv) What will be the 215  
155 effect on stress-induced spine plasticity in the BLA of the combi- 216  
156 nation of prior acute stress and social dominance competitions? 217

## 157 Materials and Methods

158 **Animals.** Male Sprague-Dawley rats (N = 72, aged 55 to 60 d) were housed in 218  
159 pairs with ad libitum access to food and water on a 10- to 14-h light-to-dark cycle 219  
160 in a temperature-controlled environment. All maintenance and experimental 220  
161 procedures were approved by the Institutional Animal Ethics Committee, National 221  
162 Centre for Biological Sciences, India. 222

163 **Tube Test of Social Dominance.** The tube test protocol was adapted from (48). 224  
164 A transparent 8 cm diameter plastic tube was placed between two holding cages 225  
165 with fresh bedding. The tube size was selected such that only one rat could pass 226  
166 through the tube at any given time. Removable passage barriers were placed 227  
167 at the entrances on each side of the tube and at the center. The entire tube test 228  
168 assembly was placed on a table in a room with low lighting. Each session was 229  
169 video recorded for analysis. The protocol consisted of the following three steps: 230  
170 *Habituation, Intracage competitions, and Intercage tournament.* 231

171 **Habituation.** Animals were handled for 3 d and then allowed to explore the tube 232  
172 for 15 min over 3 d, with the apparatus cleaned with a 70% alcohol between trials. 233

173 **Intracage competitions.** Each test day began with a 3-min habituation phase 234  
174 followed by competition. Two cage mates were placed in their respective hold- 235  
175 ing cages at both ends of the tube. Both rats were then allowed to enter the 236  
176 tube from their respective ends by retracting the tube entrance barriers, and 237  
177 once they reached the center, the central barrier was lifted to trigger interac- 238  
178 tion. This typically consisted of mutual inspection, and then the beginnings of a

178 dominance competition, taking the form of one animal pushing the other. The rat 179  
179 that succeeded in pushing the opponent out of its way, or from whom the other 180  
180 animal withdrew, was designated as a *winner*; the retreating rat was designated Q:1280  
181 as a *loser*. There were four testing sessions across days each consisting of five 182  
182 consecutive daily trials. Entrance into the tube was counterbalanced left-to-right 183  
183 across trials and across days. The animal that won 3 out of 5 trials was considered 184  
184 the winner for that session. The animal that won most competitions was desig- 185  
185 nated as “dominant” and given the rank 1. The loser rat was given a rank of 2 and 186  
186 defined as “subordinate.” These two animals were then subject to stress (hereafter 187  
187 referred to as S), and further intracage competition held on day S + 1 and S + 10. 188

188 **Intercage tournament.** The primary difference in this phase of the study was that 189  
189 the pair of animals examined in any tube test competition were from different 190  
190 cages. They were therefore unfamiliar to each other and could not know each other’s 191  
191 dominance status before interacting. A different cohort of animals was used than 192  
192 for the intracage competitions. 1 d after stress (day S + 1), stressed rats competed 193  
193 against unstressed rats. Tournaments were established between the separate stress 194  
194 and control groups in an all-vs-all design, for which all specific pairings of rats were 195  
195 tested on each tournament day (this conducted on days S + 1 and S + 10). Prior to 196  
196 a social dominance tournament, the 4 rats of 2 distinct cages received the routine 197  
197 3-min habituation. With a tournament among four rats, there were four unique 198  
198 paired match combinations, for which each rat participated in two fight sessions 199  
199 each consisting of five trials. Importantly, the order of the fight sessions was random. 200  
200 A trial started when the rats were introduced to their respective holding cages at 201  
201 both ends of the tube and ended when one rat retreated voluntarily or was forced 202  
202 to retreat. A timer noted the exact moment when all four paws were in the starting 203  
203 cage from which it had started. The rat that successfully reached its goal cage was 204  
204 designated as the *winner* for that trial, and the other as the *loser* for that trial. The 205  
205 tournament was performed between the following pairs in a manner such that all 206  
206 10 stressed dyads competed against all 10 nonstressed dyads: 207

1. Stress dominant vs. control dominant 208
2. Stress subordinate vs. control subordinate 209
3. Stress dominant vs. control subordinate 210
4. Stress subordinate vs. control dominant 211

212 Thus, a total of 40 animals were used (10 animals per group). 213

214 To test the persistence of the social memory established on day S + 1, a second 215  
215 encounter in the tube test was conducted nine days after the first social conflict 216  
216 (day S + 10). 217

218 Detailed frame-by-frame video analysis was performed using behavior analy- 219  
219 sis software [BORIS v. 7.13.6; (50)]. We classified the tube test behaviors based on 220  
220 the classification of (19). We scored the occurrence of four predominant behaviors 221  
221 during the intercage tournament: push, resistance, retreat, and stillness. “Push” 222  
222 was when a rat initiated pushing the opponent by pushing its head under the 223  
223 opponent. “Resistance” was when both rats held or attempted to hold their posi- 224  
224 tion when being pushed. “Retreat” was when the animal retracted backward after 225  
225 being pushed or voluntarily withdrew upon meeting the opponent. “Stillness” 226  
226 was when the rats showed no movement, but it is an important component of 227  
227 tube test interactions as it is a time when an animal likely assesses the relative 228  
228 dominance of the other. 229

229 **Acute Immobilization Stress.** The acute stressor used was “immobilization 230  
230 stress”, as previously described (24). Briefly, the animals were immobilized in 231  
231 plastic conical bags with no access to food or water for 2 h (10 a.m. to 12 p.m.). 232  
232 Poststress, animals were returned to their respective home cages and had access 233  
233 to food and water ad libitum. Control rats were housed in a different room. 234

235 **Golgi-Cox Staining.** Following the second encounter (day S + 10), rats were 236  
236 killed under halothane anesthesia, and brains were collected for modified Q:1330  
237 Golgi-Cox staining. They were first incubated in the Golgi-Cox fixative solution 238  
238 containing mercuric chloride (Merck Ltd., Mumbai, India), potassium dichromate 239  
239 (Qualigens Fine Chemicals, Mumbai, India), and potassium chromate (Qualigens 240  
240 Fine Chemicals, Mumbai, India). 1 d later, the brains were transferred into 241  
241 fresh fixative for the remaining 14-d incubation period at room temperature. 242  
242 Subsequently, they were incubated in 5% sucrose in 0.5 M phosphate buffer for 243  
243 5 d. Finally, 120  $\mu$ m thick serial coronal sections were collected on gelatin and 244  
244 chrome-alum-coated slides using a Leica vibratome (VT-1200S). Sections were 245  
245 developed in 5% sodium carbonate, dehydrated in grades of ethanol (Merck Ltd., 246  
246 238

Mumbai, India), cleared in xylene (Qualigens Fine Chemicals, Mumbai, India), and mounted with DPX (Nice Chemicals, India).

**Dendritic Spine Density Analysis.** Coronal sections (120  $\mu\text{m}$ ) containing BLA (Bregma  $-2.16$  to  $-3.12$  mm) (51) were used to analyze spine density on the primary branches of the apical dendrites. Two experimenters, blinded to treatment conditions, quantified spine density (DS and VK) along 70  $\mu\text{m}$  of dendrites, averaging 6 to 8 dendrites per animal (10 animals per group). Spine density analysis was performed using NeuroLucida (MicroBrightField, Williston, VT) attached to an Olympus BX61 microscope (100 $\times$ , 0.95 NA).

**Statistical Analyses.** Data are presented as mean  $\pm$  SEM. Statistical analyses were conducted using GraphPad Prism (version 8), with details in the figure legends.

## Results

**Habituation.** Before determining the hierarchical status of rats within a cage, we first evaluated whether the rats crossed the tube from the starting cage to the goal cage reasonably quickly. The primary measures were latency (the time taken to enter the tube after introducing the rat in the starting cage) and the number of entries made during the 3 d of tube habituation (Fig. 1*B*). Rats made frequent successful crossovers by the end of the 3 d of habituation, taking successively less time to enter the tube (SI Appendix, Fig. S1*A*) and more entries on day 3 than on day 1 (SI Appendix, Fig. S1*B*).

**Stress Had No Effect on Within-Cage Dominance Status, but Affected Crossing Latency.** The next step was to assess dominance stability by conducting competitions between the same pairs over four consecutive days (Fig. 1*B*). Ranks were assigned to rats based on their frequency of winning. Dominance was considered stable only if the rat maintained the same rank across all 4 d of prestress testing (SI Appendix, Fig. S1*C*). After segregating the rats into dominant and subordinate populations, a 50% subset of rats was subjected to acute stress, the others (controls) being housed temporarily in a separate room (Fig. 1*B*). To assess the immediate effect of acute stress on the rats' behavior and their rank, we performed an "intracage" competition one day after stress exposure (S +1) (Fig. 1*B*). It is apparent that there was minimal change in relative dominance status. The only tangible change was that, stressed rats with subordinate social status made fewer crossovers during the 3 min habituation phase compared to before stress (Fig. 1*F* and SI Appendix, Fig. S1*D*) and relative to unstressed rats on S +1 (SI Appendix, Fig. S1*E*). Before they were separated into control and stress groups on session S -1, there was no difference in tube exploration (SI Appendix, Fig. S1*D*). After stress (S +1), in addition to diminished tube exploration, all stressed rats took longer to resolve the conflict during the intracage competition (Fig. 1*G*). They also took longer to complete the whole test session compared to unstressed groups (Fig. 1*H*). We speculate that the stressed "subordinates" may have taken longer to enter the tube during the intracage competition, based on the results shown in Fig. 1*F*, because subordinate rats made fewer entries during the habituation phase compared to stressed dominant rats. The additional time spent by stressed subordinates entering the tube between test trials, compared to stressed dominants (Fig. 1*F*), may explain why stressed rats took longer to complete the test session compared to control rats during the intracage competition on S +1.

However, the hierarchical status within the cage remained unchanged after stress exposure (Fig. 1*D* and SI Appendix, Fig. S1*H*). After a delay of 9 d, the intracage competition was repeated between the same test pairs (S +10) to test the long-term

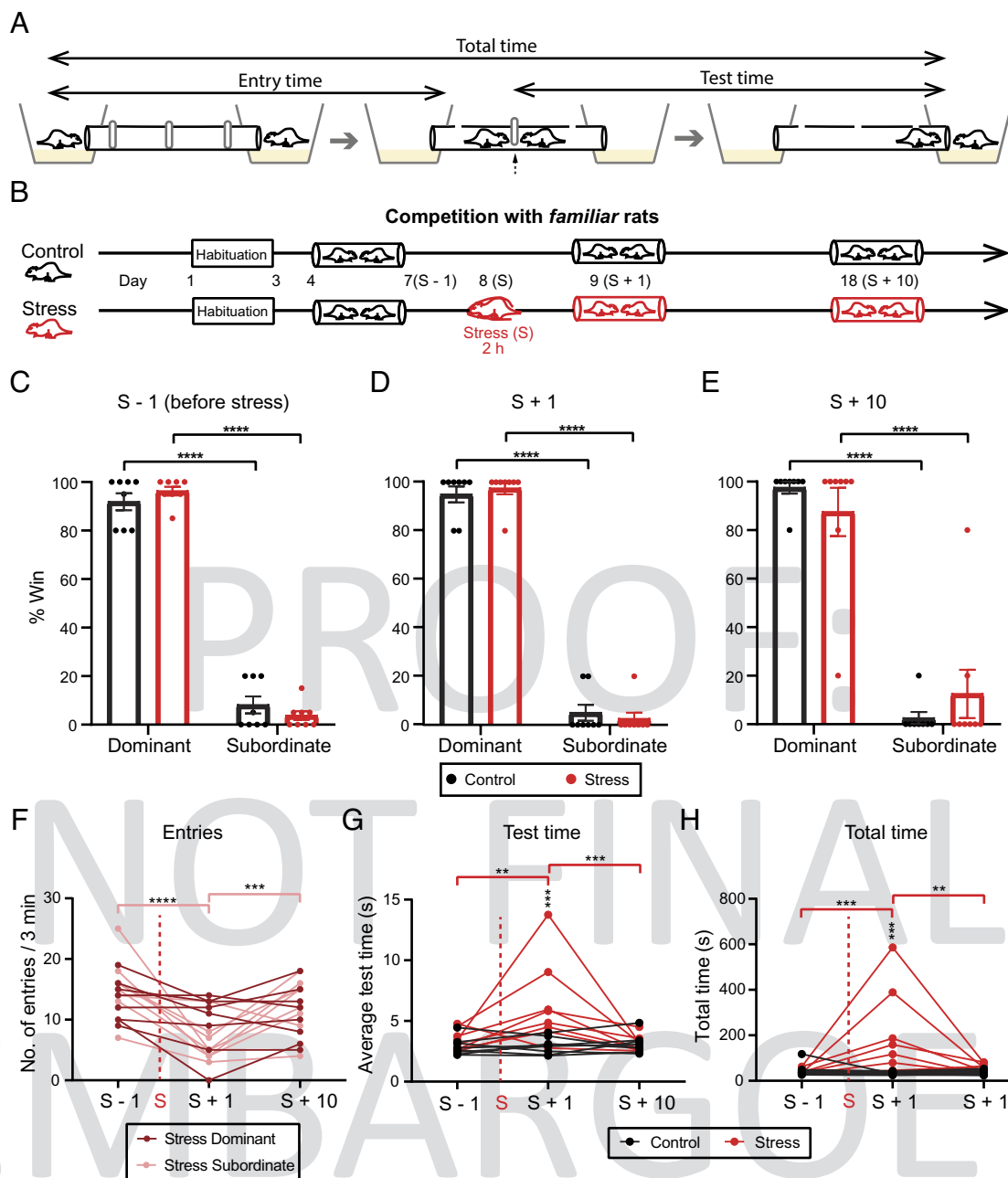
effects of acute stress (Fig. 1*B*). Unlike on S +1, the mild impact of stress affected tube exploration (Fig. 1*F* and SI Appendix, Fig. S1*F*), test time (Fig. 1*G*). Session time (Fig. 1*H*) was also diminished to being no longer observed on S +10. However, as observed on S +1, the hierarchical status in a cage was unaltered on S +10 (Fig. 1*E* and SI Appendix, Fig. S1*H*). No difference in tube exploration was observed between control dominant and subordinate rats on S -1 (SI Appendix, Fig. S1*D* and *G*), S +1 (SI Appendix, Fig. S1*E* and *G*), or S +10 (SI Appendix, Fig. S1*F* and *G*). In summary, while modest effects of stress were detectable, there was no impact on dominance status when animals were confronted by a known cage mate with which they lived.

**Social Dominance Status Influenced the Extent to Which Stress Affected Competitions Between Unfamiliar Animals.** The next step was to investigate the impact of stress on competitions when stressed rats competed against unfamiliar control rats 1 d after acute stress, S +1 (the first encounter). Such competitions are referred to hereafter as "intercage" competitions. As the history of winning can have a lasting impact on winning future conflicts (52–54), the tube test was also conducted 9 d later, denoted as S +10 (the second encounter) (Fig. 2*A*).

Intercage competitions, Fig. 2*B, C*, revealed several striking changes from the clear dominance patterns of Fig. 1*D* and *E*. First, subordinate rats subjected to stress exhibited a consistently lower chance of winning at both S +1 and S +10—around 10 to 15% compared to other groups. Second, the other groups showed changes consisting of both winning fewer competitions (dominant rats) or winning more competitions (control subordinate rats)—with competitions being won at between 50 and 75%. Whereas in intracage competitions, a dominant animal is always competing against its familiar subordinate, in intercage competitions, such animals would be competing against both dominant and subordinate animals from another cage. Interestingly, the stressed dominant rats showed a similar and only slightly lower winning ratio to that of control rats on both their first (S +1) and second (S +10) encounters (Fig. 2*B* and *C*); there was also no significant difference in the winning rate between control dominant and control subordinate rats in competitions against stressed rats of another cage (Fig. 2*B* and *C*).

We also checked whether the success of winning the competition was influenced by "intrinsic" factors such as body size or an animal's tendency to explore. No such relationship was observed. This is evidenced by the lack of influence of body size on the dominance status on both S +1 and S +10 (SI Appendix, Fig. S2*A* and *B*, respectively). In addition, the intrinsic likelihood of exploration observed during habituation had no impact either: Specifically, the number of entries made during the habituation phase on days 7 (S -1), 9 (S +1), and 18 (S +10) did not affect the rats' winning rate (SI Appendix, Fig. S2*C–E*, respectively).

**Unstressed Subordinates Display High Risk-Taking Behavior When Competing with Unfamiliar Rats.** To evaluate the impact of social recognition memory and stress on winning a conflict, we compared intracage and intercage competitions on day S +1. Unsurprisingly, unstressed dominants largely maintained their prior within-cage dominance status when competing against unfamiliar rats, the proportion of wins dropping only from circa 95 to 75% (SI Appendix, Fig. S3*A, Left*). This small drop was largely made up of competitions between the control dominant and stressed dominant animals. Similarly, stressed subordinates showed a consistently lower winning rate during all the conflicts, irrespective of their familiarity with the opponent (SI Appendix, Fig. S3*A, Left-Middle*). The larger and highly significant changes

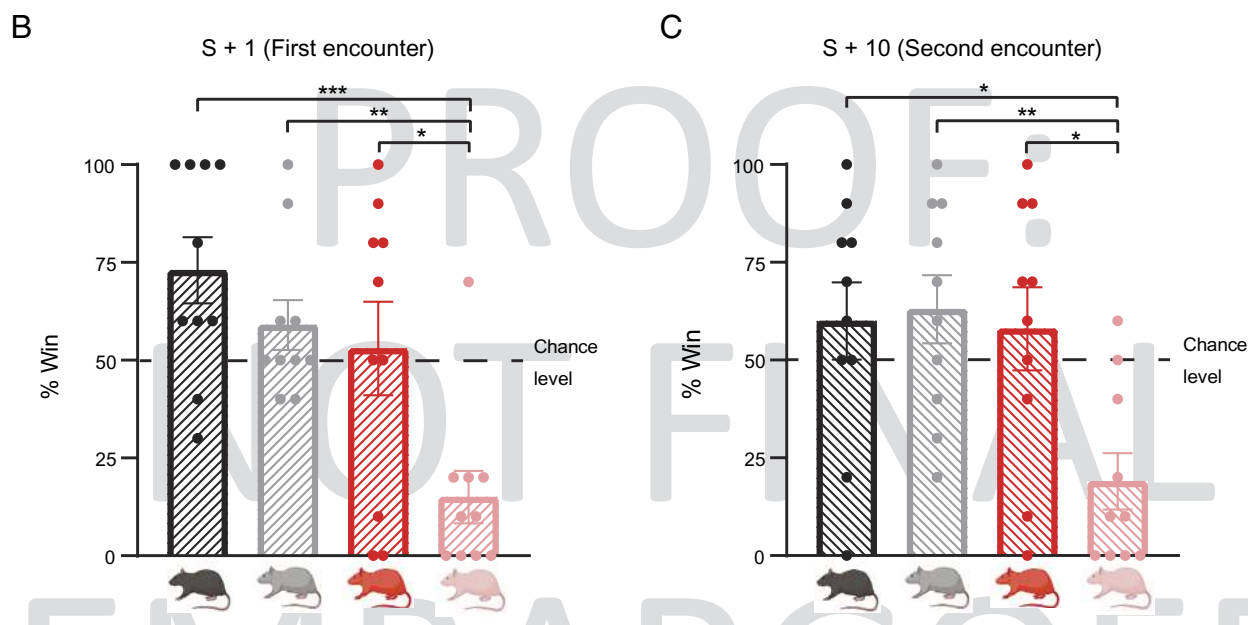
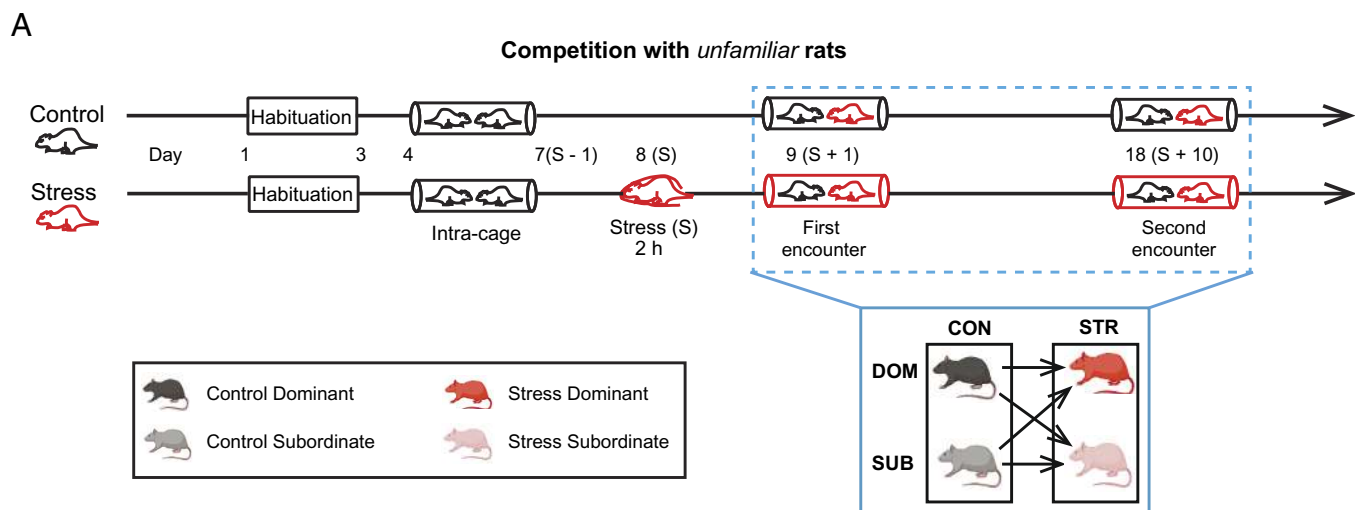


**Fig. 1.** Acute stress has a detectable but minimal effect on a preestablished social hierarchy among familiar animals. (A) Schematic of a dominance tube test showing competition between two rats in a cylindrical tube. *Entry time*: time taken to enter the tube in each test trial. *Test time*: time taken to resolve the conflict. *Total time*: entry time + test time for all the trials. (B) Timeline of the behavioral study. After habituation, an *intracage* competition between cagemates was conducted to determine the dominance status of the rats over 4 d. 24 h after the last testing day, half the rats were subjected to stress while the other half served as controls (day 8). A further competition was conducted 1 d after stress (S + 1) to assess the immediate effects of stress and a second test nine days later (S + 10) to evaluate the stability of the dominance hierarchy. (C–E) Winning percentages of control and stress rats (Ns = 8) before (S – 1) and after (S + 1) and (S + 10) respectively. The preestablished hierarchical rank within a cage remained unchanged by stress. (F) Stress did cause several changes in behavior, notably reduced tube exploration by stressed subordinate rats compared to unstressed rats on S + 1, with a significant interaction between Dominance and Sessions contributed primarily by the stress subordinate animals. By S + 10, however, both dominant and subordinate rats exhibited similar tube exploration patterns (Two-way RM ANOVA, Sidak's multiple comparisons test, Dominance:  $F(1, 14) = 0.01, P = 0.93$ , Days:  $F(2, 28) = 19.11, P < 0.001$ , Dominance X Days:  $F(2, 28) = 4.66, P = 0.02$ ; Ns = 8). (G) The test time increased immediately after stress (S + 1), with stressed rats taking longer to complete the test trials on S + 1. There was a significant Stress x Sessions interaction. (Two-way RM ANOVA, Sidak's multiple comparisons test, Days:  $F(2, 28) = 5.31, P < 0.05$ ; Stress:  $F(1, 14) = 6.97, P < 0.05$ ; Stress X Days:  $F(2, 28) = 5.78, P < 0.01$ ; N = 8 rats per group). (H) Stressed rats took a longer Total time to complete the five trials of each session (Two-way RM ANOVA, Sidak's multiple comparisons test, Days:  $F(2, 28) = 4.76, P < 0.05$ ; Stress:  $F(1, 14) = 5.60, P < 0.05$ ; Stress X Days:  $F(2, 28) = 5.91, P < 0.01$ ; N = 8 rats per group). All values are mean  $\pm$  SEM.

seen between intracage and intercage contests were apparent in the competitions in which the unstressed subordinate controls and the stressed dominants competed against others. Unstressed subordinate controls, who previously lost almost all their contests, now won over half the time (*SI Appendix, Fig. S3A, Right-Middle*); and stressed dominant rats, who previously won most of their

contests, showed a decline to circa 50% winning (*SI Appendix, Fig. S3A, Right*).

Two facets of these data should be noted. First, unstressed subordinates, typically considered “losers” in the home cage, exhibited increased motivation to fight back when facing opponents of unknown status, winning a considerable number of such



**Fig. 2. Stress causes changes in the success of encounters between unfamiliar animals.** (A) Experimental design. After determining the dominance status of the rats within their cages (Days 4 to 7), half of a separate group of rats ( $N = 40$ ) were subject to immobilization stress. After 24 h ( $S + 1$ , first encounter), stressed rats competed against control, unstressed, stranger rats (from different cages). Dominance behavior was then reevaluated after 9 d ( $S + 10$ , second encounter). CON: control; STR: stress; DOM: dominant; SUB: subordinate. (B) Winning percentages of stress and control groups on  $S + 1$  (first encounter). There was a clear result with subordinate rats winning fewer encounters than dominant rats, and stress also causing less success in social dominance encounters (Two-way ANOVA, Tukey's multiple comparisons test, Rank:  $F(1, 36) = 9.03, P < 0.01$ , Stress:  $F(1, 36) = 13.67, P < 0.001$ , Rank X Stress:  $F(1, 36) = 1.92, P > 0.05$ .  $N = 10$  rats per group). (C) The pattern for the effect of stress on  $S + 10$  (second encounter) was different. The interaction between dominance status and stress was now significant. (Two-way ANOVA, Tukey's multiple comparisons test, Rank:  $F(1, 36) = 3.83, P = 0.058$ , Stress:  $F(1, 36) = 6.25, P < 0.05$ , Rank X Stress:  $F(1, 36) = 5.21, P < 0.05$ ). All values are mean  $\pm$  SEM.

competitions. In effect, they displayed risk-taking behavior against an unknown opponent. Second, whereas subordinates could take risks in this way, a short period of acute stress prevented this from happening. Analysis of the changes in winning percentage of individual rats between the intracage prestress and intercage poststress encounters bears out these claims (*SI Appendix, Fig. S3B*).

**Not Knowing the Status of the Opponent Slows Down the Time Taken in Social Encounters.** *SI Appendix, Fig. S4A* shows that the time taken to resolve competitions was longer when the competition involved two unfamiliar rats compared to when the competition was with a known cagemate. *SI Appendix, Fig. S4B* illustrates that during the first encounter with unfamiliar rats ( $S + 1$ ), the time taken to resolve the conflict was longer when the two competing rats had equal dominance status compared to

when they had mixed dominance. This difference declines by the time of the second competition on day  $S + 10$ .

**Stressed Dominant Rats Display Resilient-Like Behavior, Whereas Subordinate Rats Exhibit Submissive-Like Behaviors.** To conduct a detailed behavior analysis during the intercage tournament, the following behaviors were scored for each testing pair: push, resistance, retreat, and stillness (these designations are explained in *SI Appendix, Fig. S5A*). Overall, during the intercage tournament on  $S + 1$ , control dominant rats generated more and longer pushes compared to stressed dominants and subordinates. Unstressed control subordinates also generated longer pushes than stressed subordinates. Stressed subordinate rats displayed more frequent and longer retreats, as expected, but stressed dominant rats displayed more resistance when being pushed by an opponent in their first unfamiliar social encounter,  $S + 1$  (*Fig. 3A and B and SI Appendix, Fig. S5 B–D*).

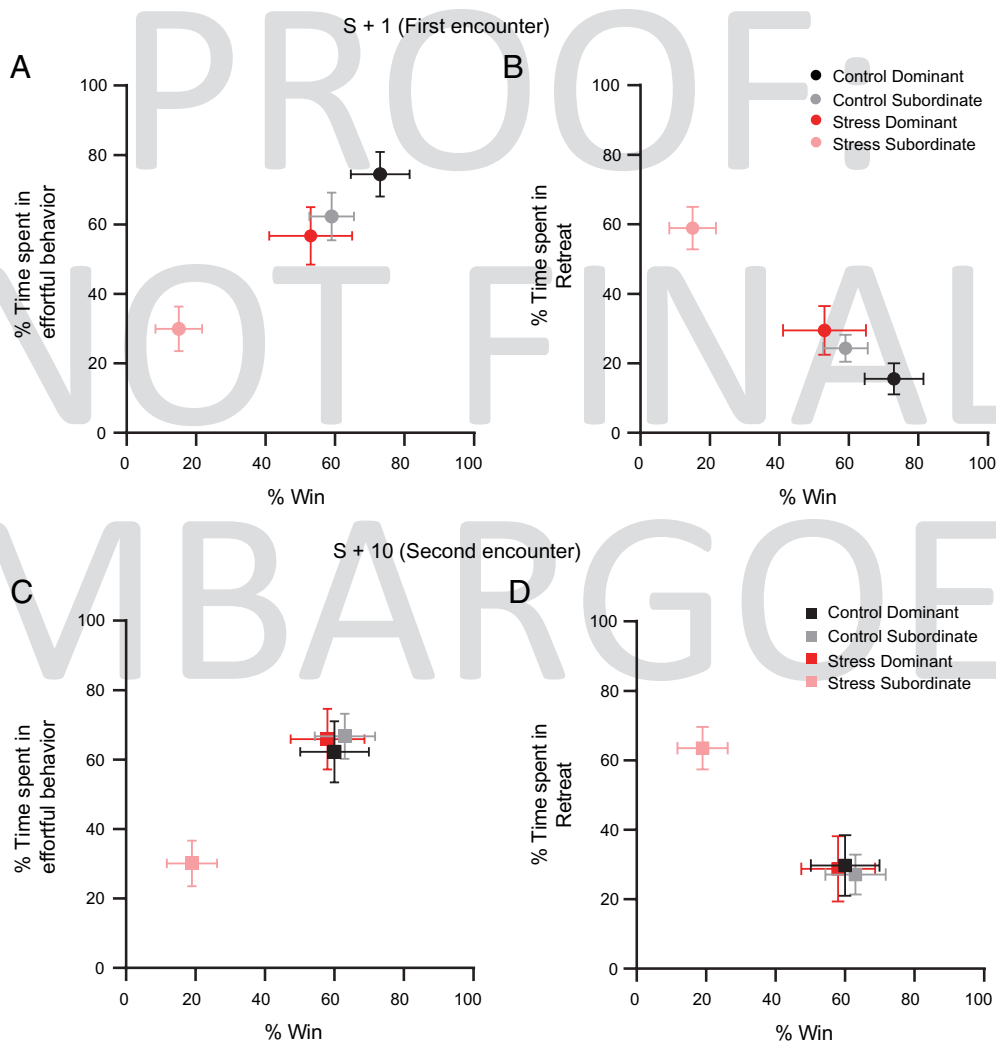
This finding points to social dominance being permissive of resilient behavior in the face of stress. No significant differences were observed in the behavior patterns of control dominants and subordinates during their first social encounter (Fig. 3 A and B and *SI Appendix, Fig. S5 B–D*). Pairwise comparisons on S + 1 revealed that similar behavior patterns, such as increased resistance and increased pushing, emerged during conflicts between control subordinates and stressed dominants (*SI Appendix, Fig. S6C*). This pattern reflects their respective risk-taking and resilience. Likewise, stressed dominant rats pushed and resisted more when competing against control dominants (*SI Appendix, Fig. S6A*).

However, and strikingly, stressed subordinate rats showed more retreat, resisted less, and generated fewer pushes during their first encounter with both control dominants and subordinates (*SI Appendix, Fig. S6 B and D*). During the second social encounter (S + 10), stressed subordinates continued to display fewer pushes, less resistance, and more retreat, while stressed dominants exhibited similar behaviors to control dominants and subordinates (Fig. 3 C and D, *SI Appendix, Fig. S7 A–D*). Again, pairwise comparisons revealed that stressed dominants maintained similar

behavior patterns against both control dominants and subordinates (*SI Appendix, Fig. S7 A and B*). Stressed subordinates consistently showed more retreat and less resistance when competing against control rats (*SI Appendix, Fig. S7 C and D*). These results suggest that stressed dominant rats display resilience-like behaviors by pushing more frequently and showing increased resistance during both social conflicts after a single episode of stress. Control rats, both dominants and subordinates, as well as stressed dominants, may have learned from their initial social conflict and exhibited more winning-prone behaviors in subsequent encounters.

### Dominant and Subordinate Rats Show Differential Structural Remodeling in the Amygdala After Acute Stress Exposure.

Previous studies have demonstrated that acute stress increased the number of dendritic spines on principal neurons of the BLA (24, 31), when observed 10 d later. However, the impact of dominance on neuronal morphology has not yet been reported. Here, the animals were killed immediately after the second social encounter, and their brains were collected for dendritic spine

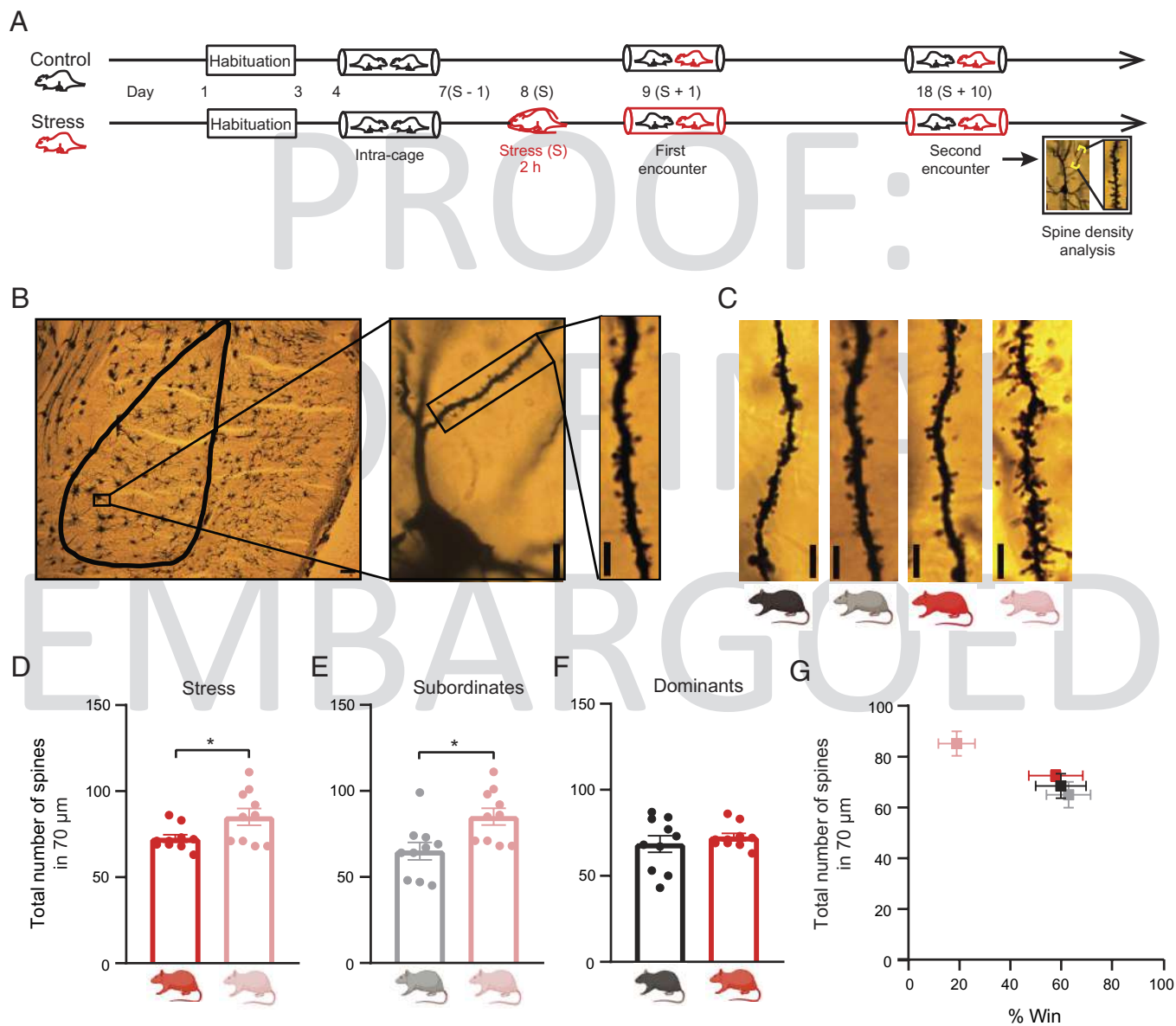


**Fig. 3.** Microdissection of behavioral patterns reveals a basis for the impact of acute stress (A–D) Correlation plots show that stressed subordinates have a lower chance of winning and exhibit fewer push and retreat when competing against unstressed rats during their first (A and B) and second (C and D) encounters, while stressed dominants display behavioral patterns similar to those of control dominant and subordinate rats (Pearson correlation. S + 1: Control dominant: % Win vs effortful behavior -  $r = 0.77$ ,  $P < 0.01$ ; % Win vs retreat -  $r = -0.96$ ,  $P < 0.0001$ . Control subordinate: % Win vs effortful behavior -  $r = 0.88$ ,  $P < 0.001$ ; % Win vs retreat -  $r = -0.88$ ,  $P < 0.001$ . Stress dominant: % Win vs effortful behavior -  $r = 0.55$ ,  $P = 0.10$ ; % Win vs retreat -  $r = -0.96$ ,  $P < 0.0001$ . Stress subordinate: % Win vs effortful behavior -  $r = 0.81$ ,  $P < 0.01$ ; % Win vs retreat -  $r = -0.87$ ,  $P < 0.001$ . S + 10: Control dominant: % Win vs effortful behavior -  $r = 0.96$ ,  $P < 0.0001$ ; % Win vs retreat -  $r = -0.96$ ,  $P < 0.0001$ . Control subordinate: % Win vs effortful behavior -  $r = 0.96$ ,  $P < 0.0001$ ; % Win vs retreat -  $r = -0.99$ ,  $P < 0.001$ . Stress dominant: % Win vs effortful behavior -  $r = 0.93$ ,  $P < 0.0001$ ; % Win vs retreat -  $r = -0.94$ ,  $P < 0.0001$ . Stress subordinate: % Win vs effortful behavior -  $r = 0.88$ ,  $P < 0.001$ ; % Win vs retreat -  $r = -0.82$ ,  $P < 0.01$ ).

density analysis in BLA principal neurons (Fig. 4A). The key finding was that stressed subordinates exhibited a significantly higher number of dendritic spines on the primary apical dendrites of BLA neurons compared to stressed dominants (Fig. 4C and D). Additionally, there was elevated spinogenesis in stressed subordinates in BLA principal neurons compared to control subordinates (Fig. 4E). No difference in spine density was observed between stressed and unstressed dominant animals (Fig. 4F). Thus, consistent with our behavioral results, the differential effect of stress on dendritic spines in dominant and subordinate rats suggests that subordinates are more vulnerable to stress (Fig. 4G). These findings underscore the importance of considering social hierarchy in understanding the neural responses to stress.

## Discussion

The two key findings of this study are that social dominance status interacts with stress to cause differential effects 1) on familiar versus unfamiliar pairs of animals with respect to any stress-induced changes in social dominance behavior, and 2) stress-induced changes in the number of dendritic spines in the BLA. Specifically, while only modest effects of acute stress were detectable in competitions between animals living in the same cage who would have known each other's social status, much more striking changes were seen in the intercage competitions between animals unfamiliar with one another. High social dominance permitted resilience in the face of acute stress, whereas low social status animals subject to ostensibly same stress displayed enhanced



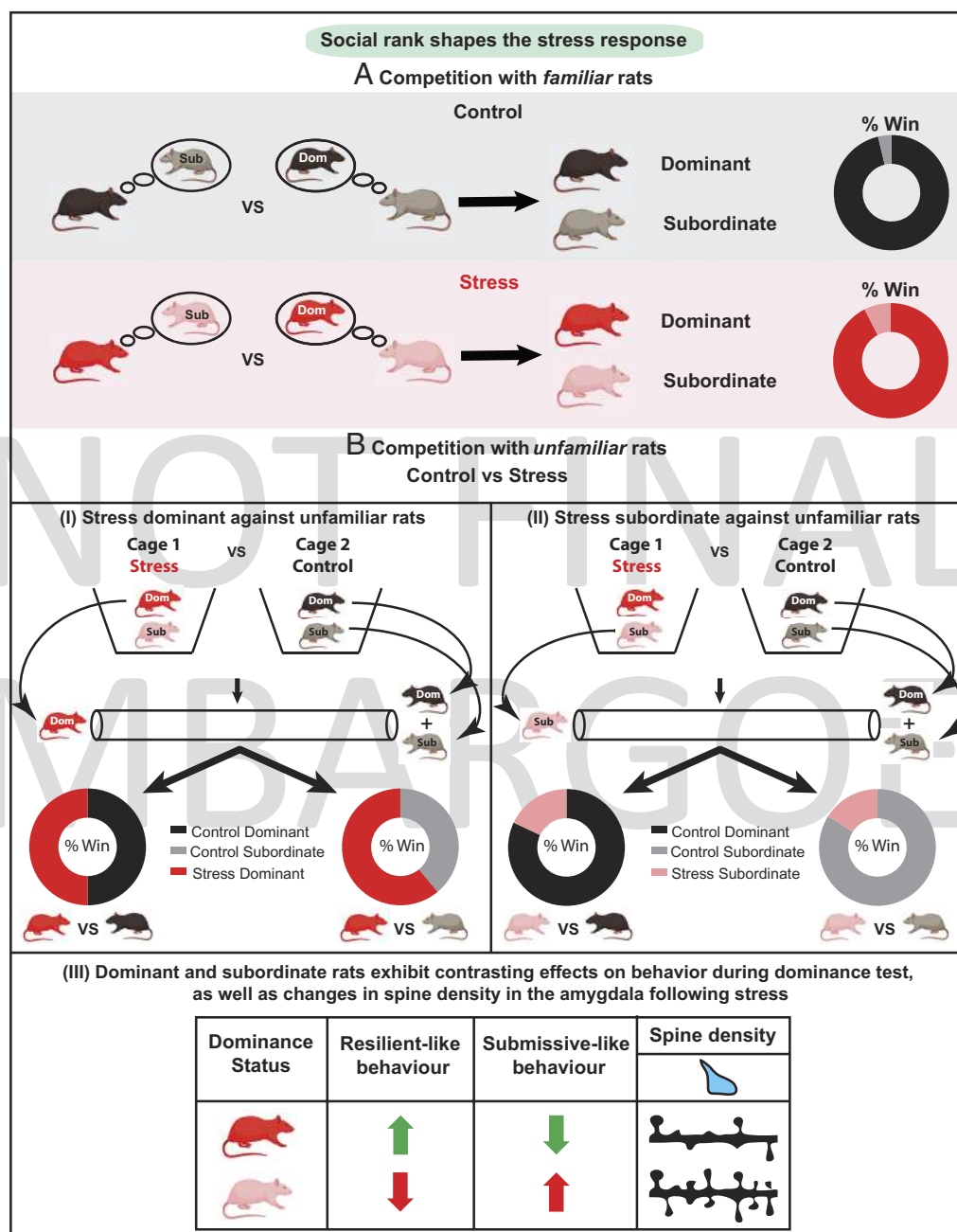
**Fig. 4.** Dominant and subordinate rats show differential stress-induced effects on dendritic spines in the BLA. (A) Experimental design. The rats of the second experiment were killed after the 2nd intercage competition (S + 10) for spine density analysis in principal neurons of the BLA. (B) Representative image showing a Golgi-stained coronal section of a rat amygdala (Left, Scale bar, 200 μm), a neuron in the amygdala (Middle, Scale bar, 20 μm), and a representative image of dendritic spines (Right, Scale bar, 10 μm). (C) Representative images of dendritic spines from the experimental groups (Scale bar, 10 μm). Note visibly striking increase in spine density in the stressed subordinate rats. (D) Acute stress induces contrasting spine density patterns between stressed dominant and subordinate rats in the BLA (unpaired *t* test,  $P < 0.05$ ). (E) Stressed subordinate rats exhibit increased spine density compared to unstressed subordinate rats (unpaired *t* test,  $P < 0.05$ ). (F) No significant difference in spine density between stressed and unstressed dominant rats was observed (unpaired *t* test,  $P > 0.05$ ). (G) The correlation plot illustrates the relationship between dominance status and stress-induced changes in BLA spine density. Increased spine density in stressed subordinates highlights the influence of stress and dominance on anxiety-like behavior. *Ns* = 10. All values are mean ± SEM.

submissiveness. In the absence of prior stress, behavior akin to risk-taking behavior was observed in subordinate animals. Experiencing acute stress and having low social status in the home cage was associated with significant growth in the number of BLA dendritic spines along with poorer behavioral resilience.

Our hypothesis was that social dominance status would cause differential effects with respect to the impact of stress, measured in dominance tests between familiar (living together) and unfamiliar animals (living in different cages). Our first observation was twofold: that stress does *not* affect a stable preestablished within-cage dominance hierarchy (Fig. 5A), with the only detectable changes being in the frequency of spontaneous entries into the tube in competitions and the duration that such tests took.

In contrast, the experience of acute stress *does* affect social interactions with unfamiliar animals. The duration of group housing profoundly affects the maintenance of the hierarchy, as social hierarchies in group-living animals form early in life and stabilize over time (55, 56). Living together allows rodents to both convey and learn social signals to and from their cagemates and establish dominance (57–59). Despite the dynamic nature of larger social groups, where individuals may dominate some and be subordinate to others, recognizing social status is crucial for forming appropriate social bonds and reducing aggression (37).

In the first phase of our study, the ostensibly stable social status of rats that lived together in a cage was unaffected by exposure to stress when evaluated 1 d and 10 d after acute stress (S +1 and



**Fig. 5.** Subordinate rats are more susceptible to stress. (A) Stress does not alter the preestablished dominance status within a cage. The absence of changes in dominance status in both control and stress groups indicates that rats maintain a stable hierarchy within a known group once established. (B) Intercage tournament among stress and control rats. Stressed dominant rats exhibit a similar winning pattern when competing against both unstressed dominant and subordinate rats (B-I). However, stressed subordinates have a lower chance of winning and display lasting retreat behavior when competing against unstressed groups. The pie charts illustrate the average win percentage for the first and second social encounters (B-II). The increased submissive-like behavior and spine density in the amygdala of subordinate rats compared to dominant rats after stress demonstrate that subordinate rats are more vulnerable to stress (B-III).

S +10, Fig. 5A). This outcome was not the only one that could have occurred, as acute stress might instead have caused a radical change in the relative dominance of the two animals living together. However, only a modest effect of stress was apparent, with the subordinate rats subject to stress showing only a modestly reduced motivation to explore the arena and an unwillingness to compete against their dominant counterpart (Fig. 1 C–E). Moreover, this stress-induced effect was transient, as the observed effects a day after stress (S +1) were substantially diminished 10 d later (S +10, Fig. 1 C–E).

A very different pattern emerged when the animals competed against unfamiliar animals from a different cage in the second experiment of our study. In this case, not only was the other animal unfamiliar, its social status was also not known to its opponent. Notably, these intercage competitions allowed observation of encounters between animals that had been subjected to stress with animals that had not. The overall pattern (Fig. 2) was for stressed subordinate animals to display relatively consistent losing behavior, even as long as 10 d after stress (Fig. 5 B, II). On the other hand, dominant animals sometimes won and sometimes lost, depending on whether they were competing against an animal that was submissive or dominant in its home cage (Fig. 5 B, I and II). Importantly, subordinate animals that had not been subjected to stress now won just over half of their competitions (Fig. 5 B, I and II).

The formation and maintenance of a dominance hierarchy are relative phenomena influenced by the competing animals' behavior. Brain activity in the medial prefrontal cortex reflects one animal's interpretation of the other's actions (60, 61). To assess behavioral contributions, we performed a frame-by-frame analysis of interanimal behaviors: push, resistance, retreat, and stillness (SI Appendix, Fig. S5A). When faced with unfamiliar rats, stressed dominant rats won using a more effortful strategy, displaying more pushes and resistance behavior, whereas stressed subordinates showed more frequent and longer bouts of retreating. Control dominant animals won most competitions, losing only occasionally to stressed dominants. Strikingly, control subordinates also now won more competitions, partly because they usually won against stressed subordinate animals, but also because they sometimes won against stressed dominant animals (SI Appendix, Fig. S3).

It is important to note that the high-risk behaviors observed in control subordinates might have differed if their opponents had not been subjected to stress. Unstressed subordinates might have lost conflicts against unfamiliar unstressed dominant animals, as has been observed in weanling mice (62). Our study focused on the impact of stress on the winning probabilities of stressed rats facing unfamiliar, unstressed opponents. The increased win rate of control subordinates, coupled with the decreased win rate of stressed dominants, indicates that these effects were influenced not only by unfamiliarity but also by stress exposure.

As in experiments using familiar animals, this outcome was not a foregone conclusion, as animals that had been subjected to stress and were now facing an unfamiliar animal might have always behaved in a submissive manner. This did not occur. Together, these results suggest that dominant animals display resilient-like behavior, despite exposure to stress, whereas subordinate animals, subjected to the same stress, show submissive behavior. These findings are in broad agreement with an earlier report showing passive coping, manifested by submission, freezing, and immobility, correlated with susceptibility (63). This raises the intriguing possibility that, when subjected to stress, active coping, characterized by the assumption of defensive or aggressive behaviors, as well as exploratory activity, is positively correlated with resilience, as seen in stressed dominant animals in this study.

Experiments conducted by Laborit and colleagues in the 1970s demonstrated that rats subjected to inescapable electric shocks developed chronic hypertension, whereas those that could avoid the shocks or engage in fights with a cagemate did not (64). This raised the intriguing possibility that dominant rats might alleviate stress by displaying dominant, aggressive behaviors toward submissive cagemates, an interpretation that would support the notion that dominance may confer a protective effect against stress. Thus, our results offer insights into how social dominance may influence coping mechanisms.

Our second key observation was the divergent effect of acute stress on dendritic spine density in principal neurons of the BLA. Subordinate rats subjected to stress exhibited enhanced spine density (SI Appendix, Fig. S6). Specifically, BLA spine density in stressed subordinate animals was higher than both control subordinate (SI Appendix, Fig. S6F) and stressed dominant animals (SI Appendix, Fig. S6D). In other words, only in animals of similar lower social rank (i.e. subordinate), enhanced BLA spinogenesis was seen after stress exposure (Fig. 5 B, III). Conversely, dominant animals under the same stress did not show increased BLA spine density (Fig. 5 B, III) suggesting resilience to stress. Moreover, stressed subordinate animals, exhibiting enhanced BLA spinogenesis, were behaviorally more susceptible to stress and displayed submissive behaviors (Fig. 5B). The change in BLA spine numbers was measured 10 d after the exposure to acute stress and thus parallels the lasting effects on submissive behavior observed 10 d after the same stress. This aligns with earlier evidence linking BLA spinogenesis to stress-induced increase in anxiety-like behavior in rodents (25). While previous studies have examined stress effects on BLA spine density and anxiety-like behavior, our results add an additional dimension with respect to social hierarchy. Future research would benefit from further investigations of vulnerability to stress, as our data suggest (but do not definitively establish) an association between amygdala dendritic remodeling and submissive behavior. It is tempting to suppose that exposure to acute stress, coupled with persistent submissive behavior, collectively can cause an increase in BLA dendritic spines.

The BLA has also been implicated in regulating social anxiety, with increased activity correlating with reduced sociability (46, 65, 66) and reduced social novelty (66). Previous research has demonstrated that dominant mice spend more time interacting with a stranger mouse during the three-chamber task, indicating a positive correlation between dominance and social motivation (67). On the other hand, chronic stress has been shown to impair sociability and social novelty in mice during the same task (22). In our study, we observed that dominant rats displayed greater resilience to stress compared to subordinate rats, who were more susceptible to its adverse effects. Based on these findings, we speculate that dominant rats subjected to stress may exhibit increased sociability in the three-chamber test compared to stressed subordinate rats. Thus, examining additional measures of social behavior could provide further insights into how social dominance interacts with stress.

This study focused exclusively on male rats. However, sex differences in stress responses and social behaviors could influence the parameters examined in our study. Acute stress is known to induce delayed increase in anxiety-like behavior in males but not in females (68). Social hierarchies also differ by sex—males exhibit a steep, aggressive hierarchy, while females have a less linear and less aggressive social structure (69). Moreover, dominant males show increased locomotion and exploration, whereas females do not. Stress responses also vary by gender, with subordinate males exhibiting more locomotion and dominant females showing reduced anxiety (19). Therefore, it would be interesting to explore

sex differences in future studies to better understand the impact of social dominance on differential susceptibility to stress.

In conclusion, the wider implication of these results points to social dominance, as evidenced by the winning ratio, being one of the variables that is responsible for differential susceptibility to stressful exposure and that knowledge of social standing is essential when considering the treatment of stress-related psychiatric disorders.

**Data, Materials, and Software Availability.** All study data are included in the article and/or supporting information.

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**ACKNOWLEDGMENTS.** We are grateful to excellent animal care by the staff at NCBS and funding for this study from CHINTA.

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