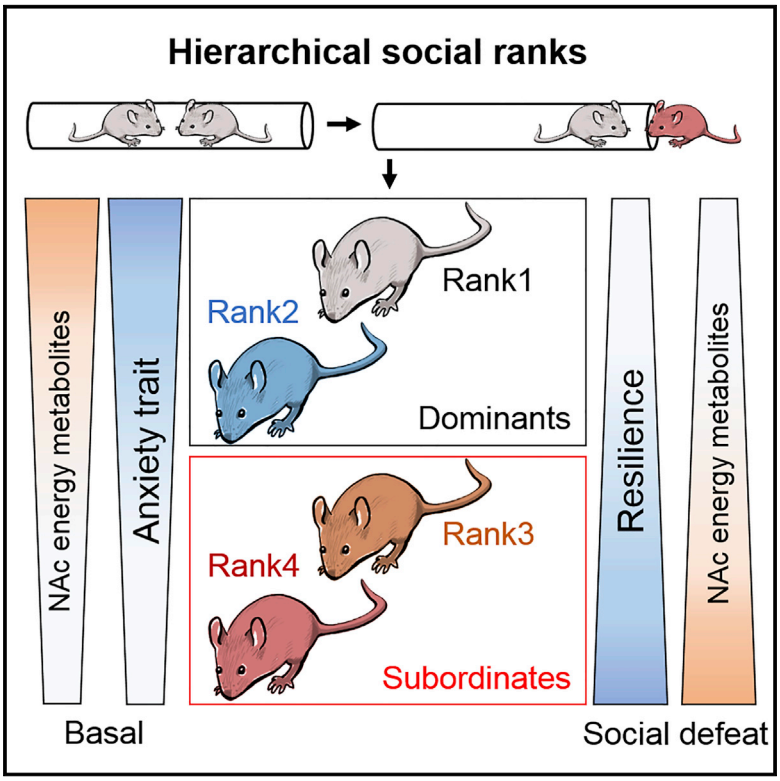
Report

Current Biology

Hierarchical Status Predicts Behavioral Vulnerability and Nucleus Accumbens Metabolic Profile Following Chronic Social Defeat Stress

Graphical Abstract



Highlights

- Socially naive inbred mice were segregated into dominant and subordinate mice
- Dominant mice exhibited high trait anxiety compared to subordinate mice
- Social rank predicts vulnerability versus resilience to social defeat stress
- Metabolic profile in the NAc relates to social status and vulnerability to stress

Authors

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In Brief

Larrieu et al. show that dominant mice are the ones susceptible to develop depression-like behaviors after social defeat. Spectroscopy reveals that the metabolic profile in the NAc relates to both social status and vulnerability to stress. This study identifies non-invasive risk factors predictive of vulnerability to stress and metabolic changes.

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Hierarchical Status Predicts Behavioral Vulnerability and Nucleus Accumbens Metabolic Profile Following Chronic Social Defeat Stress

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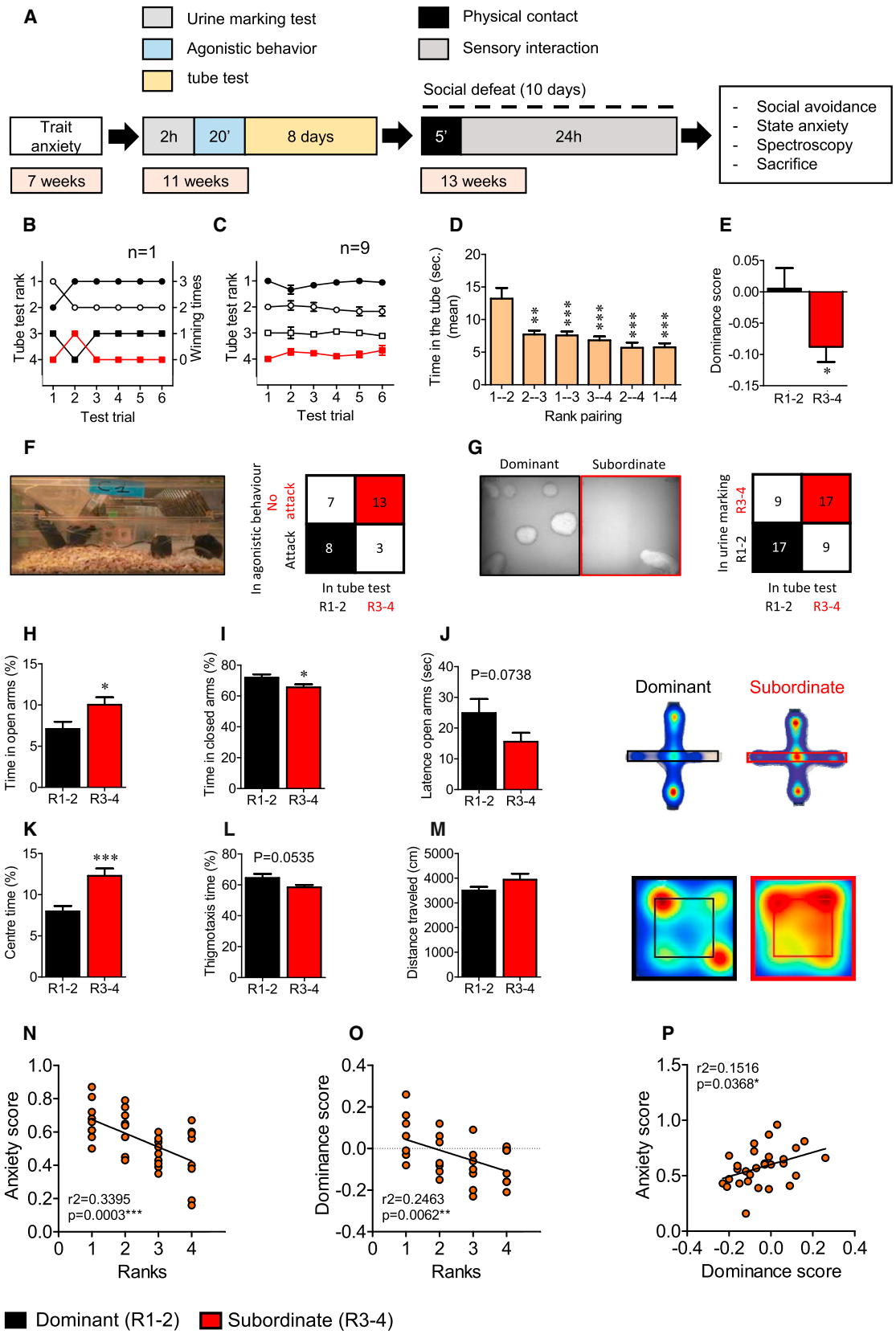
SUMMARY

Extensive data highlight the existence of major differences in individuals' susceptibility to stress [1–4]. While genetic factors [5, 6] and exposure to early life stress [7, 8] are key components for such neurobehavioral diversity, intriguing observations revealed individual differences in response to stress in inbred mice [9–12]. This raised the possibility that other factors might be critical in stress vulnerability. A key challenge in the field is to identify non-invasively risk factors for vulnerability to stress. Here, we investigated whether behavioral factors, emerging from preexisting dominance hierarchies, could predict vulnerability to chronic stress [9, 13–16]. We applied a chronic social defeat stress (CSDS) model of depression in C57BL/6J mice to investigate the predictive power of hierarchical status to pinpoint which individuals will exhibit susceptibility to CSDS. Given that the high social status of dominant mice would be the one particularly challenged by CSDS, we predicted and found that dominant individuals were the ones showing a strong susceptibility profile as indicated by strong social avoidance following CSDS, while subordinate mice were not affected. Data from ^{1}H-NMR spectroscopy revealed that the metabolic profile in the nucleus accumbens (NAc) relates to social status and vulnerability to stress. Under basal conditions, subordinates show lower levels of energy-related metabolites compared to dominants. In subordinates, but not dominants, levels of these metabolites were increased after exposure to CSDS. To the best of our knowledge, this is the first study that identifies non-invasively the origin of behavioral risk factors predictive of stress-induced depression-like behaviors associated with metabolic changes.

RESULTS AND DISCUSSION

Segregation of Naive C57BL6/J Inbred Mice into Dominant and Subordinate Populations

As we aimed to investigate the impact of chronic social defeat stress (CSDS) in a well-established social hierarchy, groups of four C57BL6/J 6-week-old male mice were left together in the same homecage for 7 weeks before stress exposure (Figure 1A). We applied a social-confrontation tube test in mice [17] after at least 4 weeks of cohabitation (Figure S1A). Following individual habituation and training to cross through the tube (Figures S1B and S1C), mice were tested pairwise using a round robin design in which each mouse was daily paired with the three other cagemates. We considered social rank as stable only when mice adopted the same rank position within their homecage for 4 consecutive days (see Figure 1B for an example from a single cage and Figure 1C for the average of n = 9 cages). Importantly, the social rank assessed in the tube test was not linked to the time spent in the tube during the 2-day habituation phase (Figures S1B and S1C) or to the body weight displayed by mice before or after the tube test (Figures S1D–S1F). From this point, for statistical and representation purposes, mice attaining ranks 1 and 2 were defined as dominant and those with ranks 3 and 4 as subordinate. It is noteworthy that the results we present here were recapitulated when specifically comparing mice from rank 1 and rank 4 (Figure S2). To further validate the social hierarchy, we compared the results obtained in the tube test with those obtained by agonistic behavior (Figures 1E and 1F) and the territory urine-marking test (Figure 1G). We found that dominant mice exhibit a higher dominance score than subordinate mice in agonistic behaviors. In addition, both tests correlated with dominance evaluated in the tube test (Figures 1F and 1G), supporting the results from the social confrontation tube test as reliable indices of the social hierarchy within the homecage [17, 18].



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To gather further information about the behavioral profile related to the determined social rank, we subjected mice to tests for anxiety-like behaviors and exploration outside the homecage prior to starting the social-confrontation tube test. In both the elevated plus maze (Figures 1H–1J) and the open-field (Figures 1K and 1L) tests, dominant mice exhibited higher basal anxiety-like and less risk-taking (data not shown) behaviors than subordinate mice. Finally, social rank linearly correlated with anxiety-related behavior and dominance (Figures 1N and 1O). Overall, these findings are in line with the positive relationship observed between aggression and/or dominance and anxiety-like behavior in male mice [19–21] (Figure 1P). Importantly, social rank was not linked to differences in total locomotion (Figure 1M).

Dominant, but Not Subordinate, Mice Are Susceptible to CSDS

Then, we studied whether social rank can predict susceptibility to CSDS. For this purpose, high and low social rank mice were subjected to a daily bout of social defeat by an aggressive CD1 male mouse over 10 consecutive days [22] followed by a social interaction test 24 hr later (Figure 2A). Dominant and subordinate control mice were housed in pairs and separated by a perforated Plexiglas divider for the duration of each sensory contact session undergone by defeated mice (see Supplemental Information). A high social avoidance score following CSDS was only observed in dominant mice (Figure 2B). In fact, the social avoidance score of defeated subordinates was similar to that observed in undefeated mice revealing a resilience phenotype. Importantly, social status did not influence the total duration of submissive behavior produced (Figure 2C) or aggression received (Figure 2D) during social defeat. The latency to defeat decreased over sessions regardless of social rank (Figure 2E). We then conducted a parallel analysis to ease the comparison between our social avoidance data and the existing literature on susceptible and resilient mice. By using the social avoidance score [23], we segregated defeated mice into two groups of mice, "susceptible" representing 56% of defeated mice with a score > 0 and "resilient" representing 44% of defeated mice with a score ≤ 0 (Figures 2F and 2G). A 2 × 2 contingency table analysis shows that the proportion of dominant mice that become resilient to CSDS is lower (1 out of 9) than subordinate individuals (7 out of 9) (Figure 2H). Our identification of dominant mice as the ones that show vulnerability to CSDS might appear counterintuitive at first glance given the broad literature indicating that subordinate individuals tend to have reduced fitness [24, 25]. A possible explanation of this finding is that high social status of dominant mice is particularly challenged by CSDS while subordinate animals might be used to be defeated during social hierarchy establishment, making them more resilient to subsequent social stress. Indeed, our findings reinforce the view that defeat is more pertinent to depression than social subordination [26–28]. In the future, it will be important to study whether the susceptibility to CSDS observed in dominant mice can be generalized to other stress protocols, such as chronic restraint stress. Our data demonstrate the critical importance of the homecage social hierarchy in predicting the susceptibility (89% and 22% of dominants and subordinates, respectively) of inbred C57BL6/J to CSDS. Therefore, we reveal that this susceptibility is rank specific and apparent in an inbred population of mice.

Dominant and Subordinate Mice Displayed Distinct Phenotypes after CSDS

Increases in state anxiety have been reported in both susceptible and resilient sub-groups after CSDS, while body weight changes were only apparent in susceptible individuals [9]. Consistently, both dominant and subordinate individuals exhibited an increase in state-anxiety-like behavior after CSDS, spending less time in the light compartment of a light-dark box (Figures 3A–3C). In addition, only dominant mice displayed a significant increase in body weight when compared to undefeated dominant mice (Figure 3D), while no difference was observed between undefeated and defeated subordinate mice (Figure 3E). Dominant and subordinate mice displayed a similar attenuated stress response in free plasma corticosterone levels (Figure 3F), suggesting that the difference observed in social avoidance between high and low social ranks is unlikely due to hypothalamic-pituitary-adrenal axis alteration. When measuring free plasma testosterone levels, we found a main effect of social rank revealed by higher levels in subordinate than dominant mice, regardless of the stress condition. We also found a main effect

Figure 1. Hierarchical Rank Using a Social Confrontation Tube Test

(A) Illustration of the general timeline of the study.

(B) Example of one cage representing the tube test ranks and winning times as a function of tube test trials.

(C) Summary for nine cages over the 6-day test trials.

(D) Time spent in the tube (s) as a function of the rank pairing ($F_{5,48} = 9.78$, $p < 0.001$, one-way ANOVA; $^{**}p < 0.01$, $^{***}p < 0.001$, Bonferroni's test, n = 9 per rank pairing).

(E) Dominance score after agonistic behaviors in the homecage ($t_{28} = 2.30$, $^{*}p < 0.05$, unpaired t test, two-tailed n = 15 per group).

(F) 2 × 2 contingency table for correlation between agonistic behaviors and tube test ranks (Fisher's exact test, two-tailed, $p = 0.050$).

(G) Left: picture representing typical urine marks profile of dominant and subordinate mice revealed by a UV light source. Right: 2 × 2 contingency table for correlation between urine marking test and tube test ranks (Fisher's exact test, two-tailed, $p = 0.026$, n = 26 pairs).

(H) Percentage of the time spent in the open arms ($t_{34} = 2.33$, $^{*}p < 0.05$, unpaired t test, two-tailed n = 18 per group).

(I) Percentage of the time spent in the closed arms ($t_{34} = 2.19$, $^{*}p < 0.05$, unpaired t test, two-tailed n = 18 per group).

(J) Latency for entering in the open arms ($U = 105$, $p = 0.07$, Mann-Whitney test, two-tailed n = 18 per group).

(K) Percentage of the time spent in the center ($t_{34} = 3.89$, $^{***}p < 0.001$, unpaired t test, two-tailed n = 18 per group).

(L) Percentage of the time spent exploring the wall (thigmotaxis) ($t_{34} = 1.86$, $p = 0.053$, unpaired t test, two-tailed n = 18 per group).

(M) Total distance traveled ($t_{34} = 1.56$, $p > 0.05$, unpaired t test, two-tailed n = 18 per group). Right: representative heatmaps.

(N–P) Pearson correlation coefficient (r) was calculated to establish relationships between individual social rank and basal anxiety score (N), between individual social rank and dominance score (O), and between basal anxiety and dominance behaviors (P).

Data are displayed as mean ± SEM. See also Figures S1 and S2.

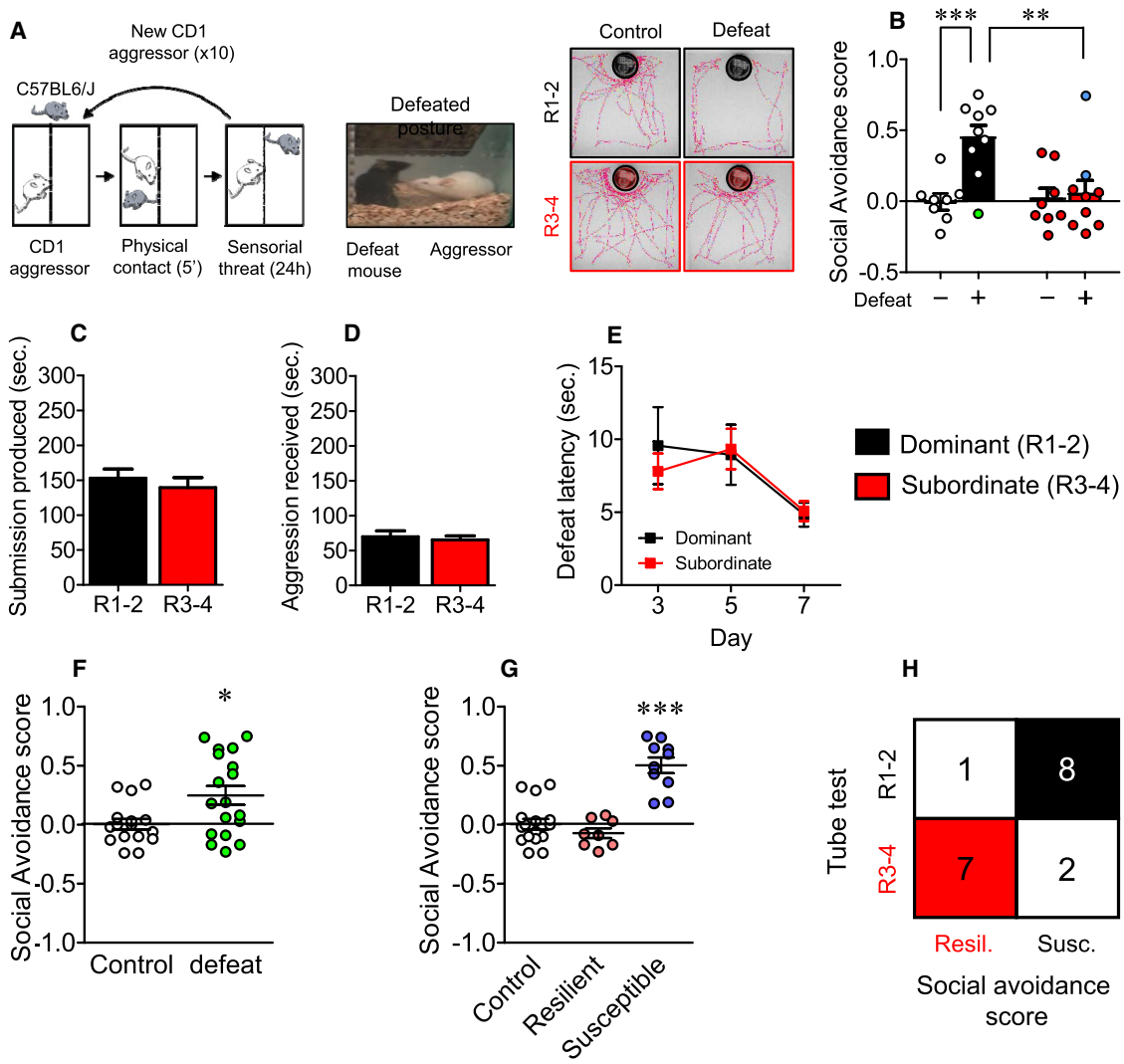

Figure 2. Dominant Mice Exhibit Susceptible Phenotype after 10 Days of Social Defeat

(A) Experimental design and representative tracking information of the time spent exploring a social target in control versus defeated dominant and subordinate mice.

(B) Social avoidance scores for control and defeated dominant versus subordinate mice ($F_{1,30} = 6.64$, $p < 0.05$, two-way ANOVA; $^{**}p < 0.01$, $^{***}p < 0.001$, Bonferroni's test, n = 8–9 per group).

(C–E) Social status did not alter (C) the total duration of submissive behavior that defeated mice produced ($t_{18} = 0.70$, $p > 0.05$, unpaired t test, two-tailed n = 10 per group), (D) the total duration of aggression that defeated mice received ($t_{18} = 1.22$, $p > 0.05$, unpaired t test, two-tailed n = 10 per group), and (E) the latency to display defeat posture over the days (interaction: $F_{2,20} = 0.26$, $p > 0.05$; day effect: $F_{2,20} = 3.85$, $p < 0.05$; rank effect: $F_{2,20} = 0.08$, $p > 0.05$, two-way ANOVA).

(F) Scores are calculated for each behavioral outcome. Social interaction ratios and time spent in interaction zone are multiplied by −1 so that higher scores reflect more social avoidance. The average of all four scores is used as the social avoidance score ($t_{32} = 2.56$, $p = 0.015$, unpaired t test, two-tailed n = 16–18 per group).

(G) Social avoidance score after segregation into resilient and susceptible defeated mice (stress effect: $F_{2,33} = 31.47$, $p < 0.0001$, one-way ANOVA; $^{***}p < 0.001$ versus control group, Bonferroni's test).

(H) 2 × 2 contingency table analysis shows the proportion of dominant and subordinate mice that become resilient to CSDS (Fisher's exact test, two-tailed $^{*}p = 0.0152$).

Data are displayed as mean ± SEM. See also Figure S2.

of stress reflected by a decrease in free plasma testosterone regardless of the social rank in defeated mice (Figure 3G). In addition, the plasma testosterone/corticosterone ratio measured after CSDS tended to be higher in subordinate compared to dominant mice (Figure 3H). To further validate these findings, we evaluated levels for corticosterone and testosterone in a second experiment and found similar results (corticosterone: dominant, 44.89 ± 6.889, n = 6; subordinate, 16.36 ± 4.966, n = 5; $t_9 = 3.22$, $p = 0.0103$, unpaired t test, two-tailed; testosterone: dominant, 0.5260 ± 0.1748, n = 5; subordinate, 5.968 ± 3.636, n = 5; $t_9 = 1.49$, $p = 0.170$, unpaired t test, two-tailed). Although these data might appear inconsistent with several studies showing a positive association between testosterone and dominance [29–35], the link between

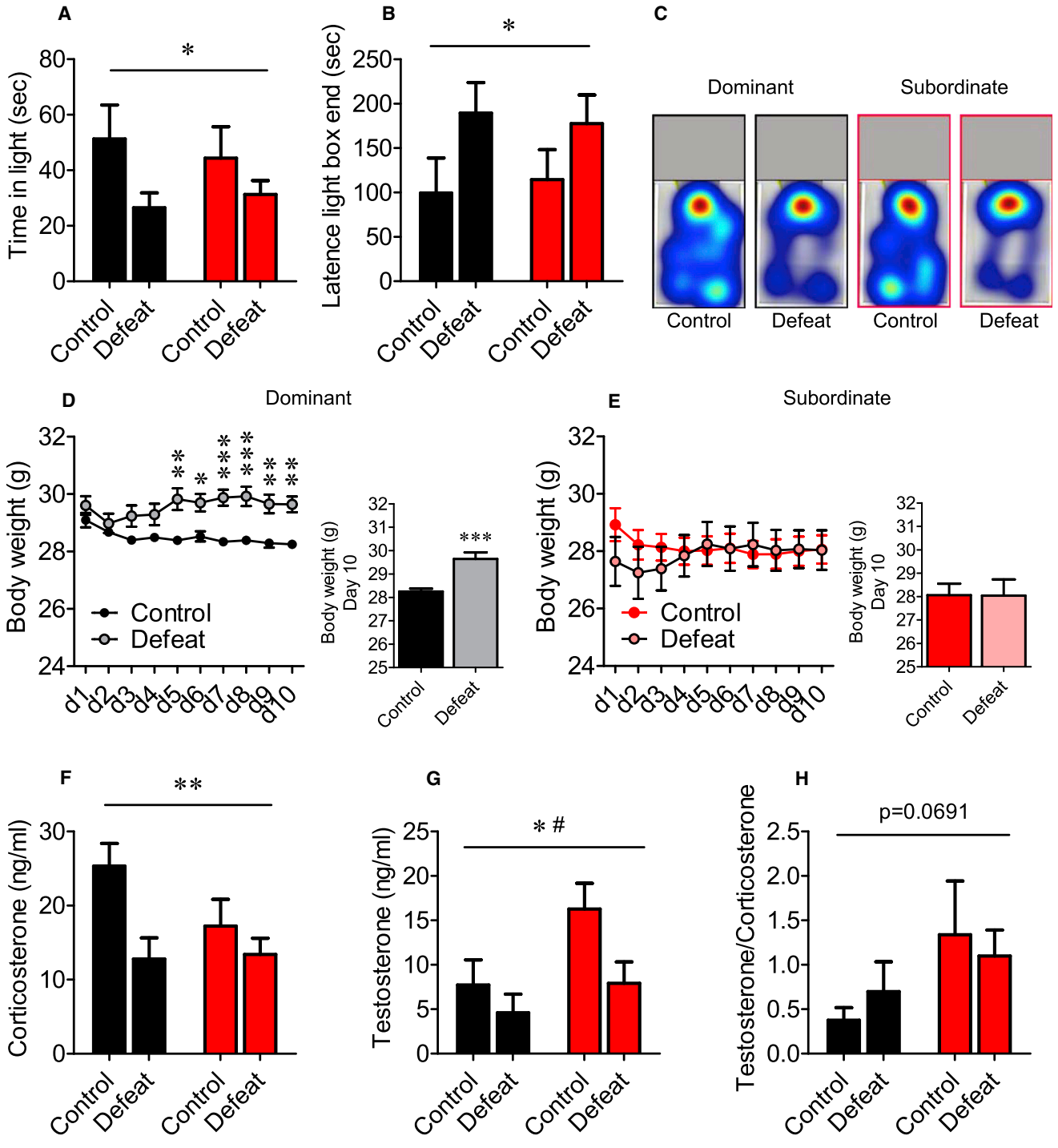

Figure 3. Dominant and Subordinate Mice Exhibit Similar State Anxiety after CSDS

(A) Time spent in the lit compartment of a light-dark box (interaction: $F_{1,30} = 0.44$, $p > 0.05$; stress effect: $F_{1,30} = 4.70$, $^{*}p < 0.05$; rank effect: $F_{1,30} = 0.01$, $p > 0.05$, two-way ANOVA, n = 8–9 per group).

(B) Latency to enter the lit compartment of a light-dark box (interaction: $F_{1,30} = 0.14$, $p > 0.05$; stress effect: $F_{1,30} = 4.80$, $^{*}p < 0.05$; rank effect: $F_{1,30} = 0.0018$, $p > 0.05$, two-way ANOVA, n = 8–9 per group).

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testosterone and social status seems to be complex. Indeed, several other studies in mice reported either no relationship or the opposite association between these factors [36–39]. Differences in findings across studies might be accounted by differences in methodology, as social behaviors depend on a range of interacting environmental, individual, and social factors [39].

Neurochemical and Metabolic Profiles in the NAc, but Not in the mPFC, Relate to Social Status and Vulnerability to CSDS

Finally, we examined whether defeat-induced social avoidance is associated with differences in metabolic profile in high and low social rank mice by using ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy. Typical spectra and neuroanatomical images of medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) acquired from a mouse are presented in Figure 4A. To identify metabolite patterns from the ^{1}H-NMR spectroscopy data, we used an objective and unbiased multivariate factor analysis (FA) approach. FA applied separately to the metabolites measured in each of the brain regions studied identified two main factors that jointly accounted for 53% and 60% of the total variance in the data from the NAc and the mPFC, respectively (Tables S1 and S2).

Accumbal metabolite loadings for factor 1 include a strong positive contribution from phosphocreatine (PCr), creatine (Cr), glutamate (Glu), glutamine (Gln), aspartate (Asp), myo-inositol (Ins), N-acetyl-aspartate (NAA), and taurine (Tau) (all individual metabolite loadings above 0.6) (Figure 4B and Table S1), indicating that low concentrations of these metabolites will contribute to a lower score for factor 1 and vice versa. This factor was able to discriminate all the groups, as revealed by an interaction between rank and stress (Figure 4B). In control animals, social rank is associated with differential levels of factor 1 in the NAc, with greater levels in high than in low social rank individuals. Interestingly, while accumbal metabolic profile in defeated subordinate animals significantly increased after CSDS, stress did not induce a significant change in factor 1 in dominant mice. In addition, we found that results from factor 1 in the NAc were mainly due to changes in the concentration of Glu, PCr, NAA, and Tau as well as Glx (Glu + Gln) and tCr (Cr + PCr) (Figure S3A). Although factor 2 identified correlations between PCr, glucose (Glc), N-acetylaspartylglutamate (NAAG), ascorbate (Asc), and Cr, the two-way ANOVA showed that the variance summarized in this factor did not differ across groups (Figure 4C and Table S1). Among the remaining metabolites not loading in any of the two considered factors or loading below 0.6, only alanine (Ala), and tCho (GPC + PCho) showed higher levels in control dominant mice than their subordinates. Similarly, these two metabolites increased after CSDS only in subordinate mice with no further changes in dominant mice (Figure S3B). Interestingly, we also found that individual tube test ranks (from R1 to R4) linearly and positively correlated with levels of some energy-related metabolites (Ala, PCr, tCr, PCr/Cr, Glu, Glx, NAA, tCho, and Tau) in the NAc specifically after CSDS (Figure S4). In the mPFC, a two-way ANOVA showed that the variance summarized in both factor 1 (Figure 4D and Table S2) and factor 2 (Figure 4E and Table S2) did not differ across groups. This was confirmed when analyzing individually these metabolites from the mPFC (Figures S3C and S3D). Furthermore, no significant correlations were observed between individual social rank and metabolite levels in the mPFC (data not shown). Altogether, these findings are in line with the recent implication of energy metabolism and mitochondrial function in the NAc on attainment of social dominance following a dyadic competition in rats [40] and with previous observations in tree shrews linking social subordination with a reduction in tCr and NAA as measured in the hippocampus [41]. Total creatine, NAA, as well as Glx are well-known markers of cellular energy metabolism and neuronal activity and/or integrity [42] and to be modified by exposure to psychogenic stressors [43]. The observed increases of these metabolites in the NAc after CSDS may reflect NAc integrity in subordinate compared to dominant mice. Moreover, several of the metabolites found to be increased in subordinate mice following CSDS (e.g., Ala, Cr, PCr, Tau) have been reported to reduce oxidative stress [44, 45]. This is particularly relevant as there is evidence that links vulnerability to CSDS in rodents with oxidative stress and its reversal by antioxidants [46]. Finally, our spectroscopy results suggest that the metabolic machinery in the NAc of resilient subordinate mice is more likely to be able to cope with the increased energetic demand induced by CSDS by producing the necessary energy-related metabolites. Therefore, our data highlight NAc metabolism as a potential index of stress-induced adaptations and raise the question as to whether the observed changes in brain energy metabolism could occur before the onset of the behavioral symptoms. Longitudinal ^{1}H-NMR studies are warranted to establish whether preexisting differences in brain energy metabolism are related to differential stress vulnerability.

Conclusions

Although we cannot discard the existence of a priori differences that could have predisposed individuals to attain a specific social rank order (e.g., trait anxiety [47]), our data strongly suggest that individual susceptibility to develop social avoidance after CSDS might result from mice social organization, such as preexisting dominance hierarchies. We expect that this new insight will

(C) Representative heatmaps of time spent in the lit compartment of a light-dark box in control and defeated dominant versus subordinate mice.
(D) Defeated dominant mice display an increase in the body weight during CSDS (interaction: $F_{9,135} = 5.46$, $p < 0.0001$, two-way ANOVA; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Bonferroni's test, n = 8–9 per group). Dominant body weight on day 10 ($t_{15} = 4.33$, ***$p < 0.001$, unpaired t test, two-tailed n = 8–9 per group).
(E) Body weight from defeated subordinate mice does not differ from control subordinate mice during CSDS (stress effect: $F_{1,144} = 0.06$, $p > 0.05$, two-way ANOVA, n = 8–10 per group). Subordinate body weight on day 10 ($t_{16} = 0.02$, $p > 0.05$, unpaired t test, two-tailed n = 8–10 per group).
(F) Dominant and subordinate mice show similar free corticosterone response after CSDS (interaction: $F_{1,30} = 2.29$, $p > 0.05$; stress effect: $F_{1,30} = 8.13$, **$p < 0.01$; rank effect: $F_{1,30} = 1.69$, $p > 0.05$; two-way ANOVA, n = 8–10 per group).
(G) Free plasma testosterone after CSDS (interaction: $F_{1,30} = 1.05$, $p > 0.05$; stress effect: $F_{1,30} = 5.06$, *$p < 0.05$; rank effect: $F_{1,30} = 5.37$, *$p < 0.05$; two-way ANOVA, n = 8–10 per group).
(H) Testosterone/Corticosterone ratio after CSDS (interaction: $F_{1,30} = 0.61$, $p > 0.05$; stress effect: $F_{1,30} = 0.01$, $p > 0.05$; rank effect: $F_{1,30} = 3.56$, $p = 0.069$; two-way ANOVA, n = 8–10 per group).
Data are displayed as mean ± SEM. See also Figure S2.

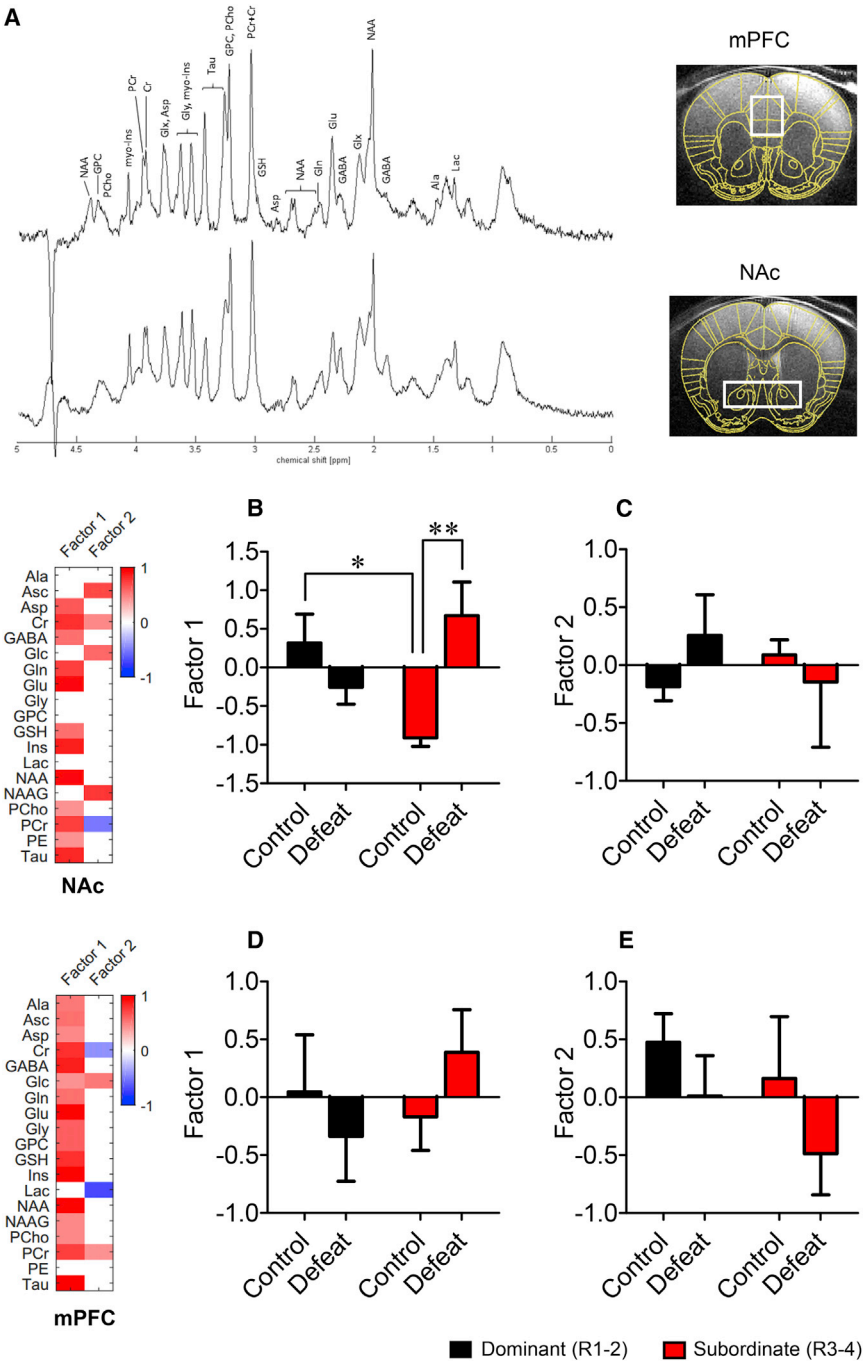

Figure 4. Factor Analysis Identified Two Main Factors that Account for the Variance in the Metabolic Data

(A) Typical spectra and neuroanatomical images of mPFC and NAc with respective voxel localization acquired from a mouse. Spectra are shown with 2-Hz line broadening and two spectra are from the same mouse. Signals arising from the proton resonances used in this analysis are labeled as follows: N-acetylaspartate (NAA), myo-inositol (Ins), phosphocreatine (PCr), creatine (Cr), glutamate (Glu), glutamine (Gln), Glu + Gln (Glx), taurine (Tau), glycerophosphorylcholine (GPC), phosphorylcholine (PCho), total choline-containing compounds (tCho), γ-aminobutyrate (GABA), aspartate (Asp), glucose (Glc), ascorbate (Asc), N-acetylaspartylglutamate (NAAG), alanine (Ala), lactate (Lac), glycine (Gly), phosphatidylethanolamine (PE), and glutathione (GSH).

(B) In the NAc, factor 1 represents a linear combination that summarizes metabolic changes found in metabolites with strong (above 0.6: PCr, Asp, Gln, Glu, Tau, Ins, Cr, and NAA) and moderate (0.4–0.6: GABA, PCho, PE, and GSH) contribution (interaction: $F_{1,23} = 10.38$, $p < 0.01$, two-way ANOVA; *$p < 0.05$, **$p < 0.01$, Bonferroni's test, n = 6–8 per group).

(C) In the NAc, factor 2 represents a linear combination that summarizes metabolic changes found in metabolites with strong (above 0.6: NAAG and Asc) and moderate (0.4–0.6: PCr, Cr, and Glc) contribution (interaction: $F_{1,23} = 0.73$, $p > 0.05$; stress effect: $F_{1,23} = 0.07$, $p > 0.05$; rank effect: $F_{1,23} = 0.02$, $p > 0.05$; two-way ANOVA, n = 6–8 per group).

(D) In the mPFC, factor 1 represents a linear combination that summarizes metabolic changes found in metabolites with strong (above 0.6: PCr, GABA, GPC, GSH, Glu, Tau, Ins, Cr, and NAA) and moderate (0.4–0.6: Ala, Glc, Asp, Gln, NAAG, PE, and Asc) contribution (interaction: $F_{1,23} = 1.41$, $p > 0.05$; stress effect: $F_{1,23} = 0.04$, $p > 0.05$; rank effect: $F_{1,23} = 0.41$, $p > 0.05$; two-way ANOVA, n = 6–8 per group).

(E) In the mPFC, factor 2 represents a linear combination that summarizes metabolic changes found in metabolites with strong (above 0.6: Lac and Glc) and moderate (0.4–0.6: GPC) contribution (interaction: $F_{1,23} = 0.05$, $p > 0.05$; stress effect: $F_{1,23} = 2.09$, $p > 0.05$; rank effect: $F_{1,23} = 1.10$, $p > 0.05$; two-way ANOVA, n = 6–8 per group).

Data are displayed as mean ± SEM. See also Figure S3 and Tables S1 and S2.

greatly facilitate progress on the identification of the neurobiological mechanisms inherent to vulnerability to stress and those that foster resilience.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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- METHOD DETAILS
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  - Open-field test
  - Anxiety and dominance score
  - Territory urine marking assay
  - Agonistic behavior
  - Social-confrontation tube test
  - Chronic social defeat stress

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2017.06.027.

AUTHOR CONTRIBUTIONS

Conceptualization, T.L. and C.S.; Methodology, T.L., A.C., A.D., H.L., R.G., and C.S.; Investigation, T.L., A.C., and A.D.; Formal Analysis, T.L., A.C., A.D., J.R., and C.S.; Writing, T.L. and C.S.; Funding Acquisition, C.S.; Supervision, C.S.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical Commercial Assays		
Corticosterone ELISA kit	Enzo Life Sciences	ADI-901-097
Testosterone ELISA kit	Enzo Life Sciences	ADI-901-065
Experimental Models: Organisms/Strains		
Mouse: C57BL6/J	Charles River Laboratory	Crl:C57BL6/J
Mouse: CD1	Charles River Laboratory	Crl:CD1(ICR)
Software and Algorithms		
Ethovision 11.0 XT	Noldus, Information Technology	http://www.noldus.com/
Observer 11.0	Noldus, Information Technology	http://www.noldus.com/
GraphPad prism 5	GraphPad	http://www.graphpad.com/
SPSS version 21	IBM	https://www.ibm.com/analytics/fr/fr/technology/spss/
Other		
Lithium heparinized tubes	Sarstedt Microvette	CB300LH

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Carmen Sandi (carmen.sandi@epfl.ch).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

All experiments were performed with the approval of the Cantonal Veterinary Authorities (Vaud, Switzerland) and carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609EEC). All experiments were performed on C57Bl6/J mice obtained from Charles River Laboratories. After arrival, animals were housed four per cage and allowed to acclimate to the vivarium for one week. All animals were subsequently handled for 1 min per day for a minimum of 3 days. Animals were weighted upon arrival as well as weekly to ensure good health. Mice were maintained under standard housing conditions on corn cob litter in a temperature- (23 ± 1°C) and humidity (40%) -controlled animal room with a 12-h light/dark cycle (0700–1900 hr), with ad libitum access to food and water. Retired CD1 breeders used as the resident aggressors in the chronic social defeat experiments were obtained from Charles River laboratories. All tests were conducted during the light period.

METHOD DETAILS

General experimental design

One week after their arrival, mice were tested at 7 week-old in an elevated plus maze and open-field tests for their basal anxiety between 8000 and 9000 hr. After 5 weeks of cohabitation with their cagemates, urine-marking assay and agonistic behavior were monitored prior to starting social confrontation tube test at 11 week-old at 1700 hr. Thirteen week-old dominant and subordinates mice were then exposed to chronic social defeat stress for 10 days followed by a social interaction test and metabolite measurements by spectroscopy. Defeat sessions were conducted at 1700 hr, 2 hr before the light-off. Animals were finally sacrificed at 1400 hr by decapitation. Trunk blood was collected and brains were rapidly dissected out, frozen at −30°C in isopentane and stored at −80°C until further processing.

Elevated plus maze test

The test was performed as previously described in [48]. The apparatus was made from black PVC with a white floor. The apparatus consisted of a central platform (5 × 5 cm) elevated from the ground (65 cm) from which two opposing open (30 × 5 cm) and two opposing (30 × 5 × 14 cm) close arms emanated. Light conditions were maintained at 14–15 lx in the open arms, and 3–4 lx in the closed arms. At the start of the test, animals were placed at the end of the closed arms faced to the wall, after which the animals were allowed to freely explore the apparatus for 5 min. Mice were tracked (Ethovision 11.0 XT, Noldus, Information Technology) to measure the time spent in the open-arms, closed arms and, the risk zones (edge of the open arms). The number of head dipping was

manually counted by an experimenter blind to the conditions. The basal anxiety score was calculated as the algebraic sum of standardized scores ((x - min value) / (max value - min value)) of each of the 2 main analyzed parameters (time in the open-arms and the center of an EPM and OF, respectively) of the two anxiety-related behavior tests.

Open-field test

The test was performed as previously described in [48]. The apparatus consisted of a rectangular Plexiglas arena (50 × 50 × 40 cm) that was illuminated with dimmed lights (5–6 lx). The floor was cleaned between each trial to avoid olfactory clues. Mice were introduced face to the wall of the arena and allowed to freely explore the arena for 10 min. A virtual center zone (25 × 25 cm), in the middle of the arena was included for the behavioral analysis as indicator for anxiety-like behavior. A video tracking system (Ethovision 11.0 XT, Noldus, Information Technology) recorded the exact track of each mouse as well as total distance traveled (cm) and the time spent exploring each zone was calculated.

Anxiety and dominance score

Anxiety score was calculated as previously described [12] as algebraic sum of standardized scores of each of the 2 main analyzed parameters of the two anxiety-related behavior tests (open-field (time spent in the center (%)), and elevated plus maze (Time spent in the open arms (%)). Standardization consisted in subtracting the value of each animal to the minimum value of the whole population and then dividing this number by the maximum value of the whole population minus minimum value of the whole population: (x – min value) / (max value – min value). This procedure yields scores which are distributed along a scale from 0 to 1, 1 reflecting high anxiety. The same was applied for the dominance score. These scores were used in order to get a global portrait of anxiety-like as well as dominance behaviors encompassing respectively the EPM and the OF and Sniffing, chasing, attacking and submissive data.

Territory urine marking assay

Using a round-robin design, all mice from the same homecage were tested pairwise leading to 6 possible pairs per cage of four mice. Mice were placed in pairs within an empty cage similar to that of their home cage, with one mouse per side separated by a perforated Plexiglas divider for the duration of each sensory interaction session. Pieces of sheets of filter paper were arranged below each cage to collect urine deposited by the mice. After two hours of sensory contact, mice were returned in their homecage and filter papers were analyzed with a UV light source. The number and/or the distance of urine marks from the divider were analyzed to identify dominant-subordinate pattern.

Agonistic behavior

Right after body weighted and tail-marked mice from the same cage, we videotaped the mice in their homecage in the housing room for assessing agonistic activities for 20 min. The duration of offensive behaviors (sniffing, chasing, attacking) as well as the duration of submissive behaviors (flight, freezing and submissive posture reflected by limp forepaws, head angled up, and retracted ears) were analyzed from video-recorded events using the Observer program (version 11.0, Noldus Information Technology). The dominance score was calculated as the algebraic sum of standardized scores ((x - min value) / (max value - min value)) of each of the six analyzed parameters of the two behaviors (offensive and submissive). For the defeat sessions, mice were videotaped and we quantified the behavior of subjects using Noldus Observer software (version 11.0, Noldus Information Technology). We quantified the total duration of the following parameters: submissive/defensive (flight, avoid, freezing, and submissive postures); and aggressive (chasing, attack including bite, upright and side offensive postures) behaviors.

Social-confrontation tube test

The test was performed as previously described in [17]. The tube test was performed in mice that have been living together for 5 weeks. Each mouse was trained to move forward out of a clear Plexiglas tube (diameter, 3cm; length, 30cm) five time from each end of the tube for 2 consecutive days. The size of the diameter is just sufficient to permit an adult mouse to move through the tube without reversing its direction. If the mouse retreated or stopped moving for a certain amount of time, it was gently push by touching its tail with a plastic stick. The tube was cleaned and dried between each trial with 70% ethanol solution to remove odor, urine or feces. After the two-day-habituation phase, social ranks were evaluated for 6 consecutive days. Before starting the confrontation, each mouse was trained again to go through the tube once from each end. Using a round-robin design, all mice from the same social group were pairwise tested leading to 6 confrontations per cage of four mice. Two mice were hold by the tail and guided simultaneously at the opposite ends of the tube until they reached the middle of the tube. Then the tail was released and the time spent into the tube was recorded until one of the two mice forced its cagemate to go backward out from the tube. The mouse that retreated from the tube is designated as the 'loser' of that specific trial. From trial to trial, the same mouse was placed in the tube from each end alternatively. The percentage of the winning time was calculated as an index of social dominance and mice were then ranked from 1 to 4 with ranks 1 and 2 as the most dominant mice and ranks 3 and 4 as the most subordinate mice.

Chronic social defeat stress

CSDS was conducted as previously described in [22]. Briefly, adult male C57BL/6J mice 13 weeks old were individually exposed to an aggressive CD1 mouse for 5 min/day, during which they were attacked and displayed subordinate posturing. Each episode of

stress was followed by 24 hr of protected sensory contact with their aggressor. Mice were exposed to a different resident each day for 10 days in order to prevent any habituation to the resident aggressor. Control mice were housed in pairs within a home cage setup identical to that of defeated mice, with one control mice per side separated by a perforated Plexiglas divider for the duration of each sensory contact session. All control mice are rotated on a daily basis in a manner similar to that of mice undergoing defeat. Twenty-four hours after the last session of social defeat, we conducted between 8000 and 9000 hr a social avoidance test consisting of 2 consecutive sessions of 2.5 min. During the first session, the open-field contained an empty wire mesh. During the second session, a social target animal (unfamiliar CD1 male mouse) was introduced into the cage and the time spent in the interaction and corner zones was recorded to assess social avoidance after CSDS. A social avoidance score was calculated according to the following behavioral parameters [23]: 1) the absolute time spent in the interaction zone when the target was present; 2) the absolute time spent in the corner zones when the target was present; 3) social interaction ratio calculated as the time spent in the interaction zone when the target is present divided by the time spent in the interaction zone when the target is absent; and 4) corner zone ratio calculated as the time spent in the corner zone when the target is present divided by the time spent in the corner zone when the target is absent. In a parallel analysis, we used a social avoidance score as the threshold for dividing defeated mice into the susceptible (ratio above 0) and resilient (ratio equal or below 0) categories.

Light Dark Test

The LDT was performed as previously described in [12]. The LD box test uses a 44 × 21 × 21 cm high Plexiglas box divided into a dark (14 × 21 × 21 cm) and a light (30 × 21 × 21 cm; 200 lux illuminated) compartments separated by an open door (5 × 5 cm) located in the center of the partition at floor level. Each mouse was placed into the dark chamber and the door was opened 5 s later. The door is used in order to avoid that mice escape from the experimenter in the light side. Mice were allowed to freely explore the apparatus for five minutes. The Noldus Ethovision 11.0 XT software (Noldus Information Technology) was used to analyze anxiety-like behavior by calculating the time spent in zones.

^{1}H-NMR spectroscopy

All spectroscopic measurements were performed on animals after at least one week of acclimation upon arrival. Animals were anesthetized with 3% isoflurane for induction and fixed on an in-house-built holder with biting piece and ear bars. Animal physiology was maintained stable under 1.3%–1.5% isoflurane in a 1:1 air/oxygen mixture and was monitored for breathing (small animal monitor system: SA Instruments Inc., New York, NY, USA) and rectal temperature (circulating water bath). Body temperature was maintained at 36.5 ± 0.4°C and breathing rate ranged between 70 – 100 rpm. Maximal time under anesthesia was 2h for each animal. Mice were scanned with a horizontal 14.1T/26 cm Varian magnet (Agilent Inc., USA) and a homemade ^{1}H surface coil in quadrature consisting of two geometrically decoupled loops used as radio frequency (RF) transceiver. Coronal T_2-weighted fast spin echo (FSE) images were obtained (15 × 0.4mm slices, TE_{eff} /TR = 50/2000ms, averages = 2) for volumes of interest (VOIs) localization. VOIs included medial prefrontal cortex (mPFC) (1.7x1.4x1.2 mm^3) and bilateral nuclei accumbens (NAc) (1.4x4.1x1 mm^3). Field homogeneity was adjusted using first- and second order shims obtained using the FASTMAP protocol [49] to reach a water linewidth under 20Hz. The VAPOR module was used for water suppression and outer volume suppression was performed to avoid spectra artifacts. Spectra were obtained using the spin echo full intensity acquired localized (SPECIAL) sequence on the target VOIs (TE/TR = 2.8/4000ms) [50] in blocks of 16 averages. Scan time was adjusted in order to obtain a satisfactory SNR (i.e.>10) and was in average around 20 min for NAc and 25 min for mPFC. Spectra were frequency corrected using the Creatine (Cr) frequency peak at 3.03 ppm as reference and blocks were summed for quantification. The LCModel [51] method, which is based on a linear combination of metabolite resonance peaks, was used to quantify the spectra in the frequency domain. For each animal, nineteen individual metabolites together with the macromolecule signals were quantified [alanine (Ala), ascorbate (Asc), aspartate (Asp), gamma-amino butyric acid (GABA), N-acetylaspartate (NAA), N-acetyl-aspartate glutamate (NAAG), glutathione (GSH), Cr, phosphocreatine (PCr), glutamate (Glu), glutamine (Gln), lactate (Lac), taurine (Tau), myoinositol (Ins), glycine (Gly), phosphorylcholine (PCho), glycerophosphocholine (GPC), glucose (Glc), phosphorylethanolamine (PE)]. An unsuppressed water spectrum was acquired before each MRS scan and was used as a reference for metabolite absolute concentration determination assuming 80% water content in the brain. Fitting reliability was determined using the Cramér-Rao lower bound errors (CRLB) [52]. A threshold of CRLB $\leq$ 20% was chosen for high concentrated metabolites and CRLB $\leq$ 50% for low concentration metabolites. Similarity in macromolecule content was used to control for reliable metabolite quantification between groups as these molecules were assumed to be constant.

Hormone analyses

Mice were sacrificed by decapitation. Trunk blood was collected and centrifuged at 15,000 g to isolate plasma. 6μL of plasma sample were then prepared according to manufacturer's instructions to measure corticosterone and testosterone concentrations using an ELISA kit (Enzo Life Sciences, ADI-901-097 for corticosterone, ASI-901-065 for testosterone). The method plots the standards versus hormone concentrations using linear (y) and log (x) axes and performs a 4-parameter logistic fit. Concentration of samples were then calculated from the fit. To further validate our findings, we evaluated levels for corticosterone and testosterone in a second experiment.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

All values are given as mean ± s.e.m. Results obtained in social-confrontation tube test experiment were all analyzed by a one-way analysis of variance (ANOVA), with social rank or rank pairing as fixed factors. Analyses were followed by the Bonferroni post hoc test when appropriate. Results obtained in agonistic behavior experiment (attacking, chasing sniffing, submissive behavior duration and, dominance score) were all analyzed by an unpaired t test. A 2x2 contingency table with a Fisher's exact test was calculated for correlations between agonistic behavior, urine marking assay and tube test ranks as well as between social avoidance score and tube test ranks. Results obtained in trait-anxiety experiments (elevated plus maze and open-field tests) were all analyzed by an unpaired t test. Results obtained in chronic social defeat stress experiment (social interaction, light-dark tests and hormone analyses) were all analyzed by a two-way analysis of variance (ANOVA), with stress and social rank as fixed factors. Analyses were followed by the Bonferroni post hoc test when appropriate. Results obtained in the spectroscopy scan after chronic stress (neurotransmitters and energy metabolism) were all analyzed by a two-way analysis of variance (ANOVA), with stress and social rank as fixed factors. Analyses were followed by the Bonferroni post hoc test when appropriate. Pearson correlation coefficient (r) was calculated to establish relationships between metabolites and individual social rank for both control and defeat group. All statistical tests were performed with GraphPad Prism (GraphPad software, San Diego, CA, USA) using a critical probability of $p < 0.05$. Statistical analyses performed for each experiment are summarized in each figure legend with the chosen statistical test, sample size 'n' and P values, as well as degree of freedom and F/t values.

Factor analysis

Factor analysis was employed with IBM SPSS Statistics version 21 to allow statistical tests using the metabolite's latent variables as dependent variables, in both the mPFC and the NAc. This statistical analysis is particularly relevant for studies including numerous variables as it makes it possible to generate linear combinations of variables reducing 'noise' caused by other measured irrelevant variables. Mean value imputation was used for missing values before the computation of correlation matrices, to ensure positive definiteness. For the sake of simplicity in the ensuing statistical tests, and after analyzing the scree plots, a total of two factors was chosen for both brain regions, using principal axis factoring. This resulted in a total percentage of variance explained of 60% for the mPFC and 53% for the NAc. In Figure 4 and Tables S1 and S2 we depict how metabolites load into these factors, without rotation and omitting coefficients below 0.4.

DATA AND SOFTWARE AVAILABILITY

Raw data and analysis will be provided upon request by the Lead Contact, Carmen Sandi (carmen.sandi@epfl.ch).