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Cumulative effects of social stress on reward-guided actions and prefrontal cortical activity

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Abstract

BACKGROUND: When exposed to chronic social stress, animals display behavioral changes that are relevant to depressive-like phenotypes. However, the cascading relationship between incremental stress exposure and neural dysfunctions over time remains incompletely understood.

METHODS: We characterize the longitudinal effect of social defeat on goal-directed actions and prefrontal cortical activity in mice, using a novel head-fixed sucrose preference task and two-photon calcium imaging.

RESULTS: Behaviorally, stress-induced loss of reward sensitivity intensifies over days. Motivational anhedonia, the failure to translate positive reinforcements into future actions, requires multiple sessions of stress exposure to become fully established. For neural activity, individual layer 2/3 pyramidal neurons in the Cg1 and M2 subregions of the medial prefrontal cortex have heterogeneous responses to stress. Changes in ensemble activity differ significantly between susceptible and resilient animals after the first defeat session, and continue to diverge following successive stress episodes before reaching persistent abnormal levels.

CONCLUSIONS: Collectively, these results demonstrate that the cumulative impact of an ethologically relevant stress can be observed at the level of cellular activity of individual prefrontal neurons. The distinct neural responses associated with resilience versus susceptibility raises the hypothesis that the negative impact of social stress is neutralized in resilient animals, in part through an adaptive reorganization of prefrontal cortical activity.

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DISCLOSURES

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Keywords

Chronic stress; social defeat; goal-directed behavior; reward; prefrontal cortex; pyramidal neurons

INTRODUCTION

Stress is associated with increased risk for multiple neuropsychiatric disorders, including major depression (1). A prevailing framework for stress-related disorders is the idea of allostatic load, which posits that although acute stress may promote adaptation, repeated exposures over a prolonged period of time can lead to persistently heightened reactions and maladaptations (2, 3). Accordingly, there is growing evidence that acute and chronic stress are associated with distinct alterations in the mammalian brain (4, 5). However, effects of stress on behavior and neural functions are generally sampled at the beginning and end of chronic stress exposure, whereas the adaptations across successive stress episodes are not understood. One difficulty is that individuals may exhibit different degrees of resilience, and therefore their time-dependent responses to repeated stress may be variable. Another difficulty is that longitudinal measurements are needed to track the dynamic relationship. For example, depressive-like phenotypes in rodents are thought to arise only after chronic stress, yet the timing of when the dysfunction is precipitated remains poorly understood.

In this study, we characterize the time course of stress effects on motivated behavior and prefrontal cortical activity in mice. Motivated behavior – specifically the capacity to select appropriate actions based on reinforcing outcomes – is an essential aspect of learning. Deficits in reward-guided behavior can be quantified precisely using instrumental decision-making tasks in multiple animal species and therefore have translational significance (6). For example, in humans, stress blunts goal-directed actions and promotes habitual responses (7). In agreement, rodents subjected to chronic stress paradigms exhibit a diminished ability to modify actions flexibly in an outcome-dependent manner (8-11).

A classic assay to measure reward-guided behavior in stressed rodents is the two-bottle test of sucrose preference, which is reduced following chronic stress (12, 13). However, reduced preference can arise for at least two reasons. First, animals could be impaired in their ability to sense or gain pleasure from sucrose. This would relate to the emotional reactivity to the reward. Second, the sucrose may remain desirable, but animals could fail to translate that positive reinforcement into future actions. These two components of reward-guided behavior are well-known, and the corresponding deficits have been termed ‘appetitive anhedonia’ and ‘motivational anhedonia’ (14, 15). Unfortunately, the conventional sucrose preference metric does not dissociate these two forms of reward processing deficits (16), so a more sophisticated paradigm is necessary to dissect the stress-induced behavioral alterations.

Reward-guided behavior is thought to involve the distributed cortical-basal ganglia network including the prefrontal cortex (6). In addition to deficits in reward processing, the effects of chronic stress on the prefrontal cortex can be detected at many levels, including the morphological, molecular, and cellular (4, 5). For example, in rodents, prolonged stress exposure causes structural atrophy, including dendritic retraction and synapse loss (8, 17, 18), and disrupts synaptic plasticity and synaptic transmission in the medial prefrontal cortex

(19, 20). Although it is obvious that the structural and synaptic alterations must compromise prefrontal cortical function, how stress exposure modifies neural activity *in vivo* is less clear. There have been several studies of stress effects on cortical activity patterns (21-24), but they provide few details on the effect of escalating stress burden over time.

Given the gaps in our current knowledge, the main goal of this study is to determine how social stress influences reward-guided behavior and prefrontal cortical activity over time. Using a novel head-fixed sucrose preference test and longitudinal two-photon calcium imaging, we show a progressive emergence of motivational anhedonia and heterogeneous neural activity maladaptations as a function of cumulative social stress and resilience.

METHODS AND MATERIALS

Animals

We used male adult (2–6-month-old) mice. Behavioral experiments were performed using wild type C57BL/6J mice (#000664, Jackson Laboratory). Imaging experiments were performed using heterozygous CaMKIIa-Cre mice (#005359, Jackson Laboratory) or C57BL/6J mice. For social defeat, aggressive residents were male retired breeder CD-1 mice (CrI:CD1(ICR), #022, Charles River).

Surgery

Supplement and previous publications (25, 26) provide details of the surgery for head plate implant and imaging window installation. Briefly, we targeted the Cg1 and M2 regions of the medial prefrontal cortex (AP=+1.5 mm and ML=+0.4 mm relative to bregma). To express genetically encoded calcium indicator in pyramidal neurons, we injected pAAV(AAV1)-hSyn1-Flex-mRuby2-GSG-P2A-GCaMP6s-WPRE-pA (Addgene) in CaMKII-Cre mice. In a subset of experiments, we injected pAAV(AAV1)-CaMKII-GCaMP6f-WPRE-SV40 (Penn Vector Core or Addgene) in C57BL/6J mice.

Self-paced, instrumental sucrose preference task

The head-fixed mouse was placed on a treadmill and positioned in front of a lick spout made by soldering together 3 blunted 20-gauge hypodermic needles. The spout could deliver independently 4 μ L of one of three solutions: 0% sucrose (i.e., tap water), 3% sucrose (w/v), and 10% sucrose. During the task, the fluid-restricted mouse must make 10 licks onto the spouts (FR10) to receive 4 μ L of fluid reinforcement. Following the fluid delivery, there was a 5-second timeout period (FI5) during which licks do not count towards next FR10. The reinforcement type cycled from 0%, 3%, 10%, to 3% in a block design with 1-minute blocks. For details of the task design and analyses of behavioral data, see Supplement.

Repeated social defeat

We followed published procedures to induce social defeat stress (27). Note that this protocol was milder than social defeat protocols in other studies (12, 28), because the time for sensory interaction was shorter. For details, see Supplement.

Two-photon imaging

The laser-scanning two-photon microscope was equipped with a resonant scanner unit for imaging at a frame rate of 29.8 Hz (Movable Objective Microscope, Sutter Instrument). Supplement provides details of the imaging setup and analyses.

Data availability

The behavioral and imaging data, as well as MATLAB code, are available on GitHub [link to be added at the time of publication].

RESULTS

A self-paced, instrumental sucrose preference task for the head-fixed mouse

We designed a self-paced, instrumental sucrose preference task to characterize reward-directed actions. We had three major criteria for the task: Actions are self-paced to engage voluntary behavior; Sucrose reinforcements are varied to manipulate the motivational level; Animal is head-fixed to enable cellular-resolution optical imaging, but mobile to minimize stress during behavioral testing. To meet these criteria, we incorporated concepts from prior studies involving ratio schedules (29), incentive contrast (30, 31), and treadmill (32). During the task, the fluid-restricted mouse makes tongue licks on a spout and is reinforced on a tandem fixed-ratio 10, fixed-interval 5 schedule (FR10-FI5; Fig.1A-B). The FI5 schedule is effectively a 5 s timeout after the reinforcement to avoid counting consummatory licks towards the subsequent FR10 schedule. The spout can independently deliver 4 μ L of water, 3% sucrose, or 10% sucrose solution. For simplicity, we will refer to water as 0% sucrose solution. The task employs a block design (Fig.1C). Each block lasts 60 s and is associated with one fluid type, always going from 0% to 3% to 10% to 3%, and then the sequence repeats again. Cycling through the fluid types allows the study of relative reward values, because a 3%-block preceded by a 0%-block has different successive incentive contrast compared to a 3%-block preceded by a 10%-block. We refer to these two kinds of 3%-blocks as 3% positive contrast (3%pc) and 3% negative contrast (3%nc) blocks. Overall, the self-paced, instrumental sucrose preference task allows us to measure how an animal adjusts its actions in response to differences in absolute and relative reward values.

Mice are sensitive to sucrose concentration and incentive contrast

Figure 1D shows a typical behavioral session for an adult male C57BL/6J mouse. Plotting the number of rewards earned on a block-by-block basis revealed a sawtooth-shaped modulation, which matched the rises and falls of the sucrose concentration (Fig.1E) The mouse could suppress intake when they anticipate more palatable fluids in the near future (33), and therefore responded most vigorously for the 10%-blocks. Summarizing performance from 39 mice, animals earned 201 ± 10 reinforcements per session (mean, s.e.m.; Fig.1F). Mice were motivated to complete more FR10 schedule to obtain more reinforcements during blocks with more palatable fluids ($P=4 \times 10^{-20}$, main effect of block type, one-way ANOVA; Fig.1G), such that they obtained $33 \pm 5\%$ more rewards during 10%-blocks relative to 0%-blocks ($P=4 \times 10^{-9}$, post hoc Tukey-Kramer test). Animals also responded significantly more during 3%pc-blocks than 3%nc-blocks ($P=0.0011$, post hoc

Tukey-Kramer test). Therefore, both absolute and relative differences in reward values were relevant for performance in this task. Because ratio schedule is a well-known approach to induce goal-directed actions (29), these results emphasize the motivational component of responding.

Mice licked in bouts with a pause between successive reinforcements (Fig.1H). A typical bout would therefore include 10 licks to complete the fixed-ratio schedule, and then additional consummatory licks after reinforcement (11.0 ± 0.5 licks for water). Animals had more consummatory licks as the concentration of sucrose was increased ($P=6 \times 10^{-8}$, main effect of block type, one-way ANOVA; Fig.1I). There is an extensive literature indicating that the number of consummatory licks is directly related to the emotional reaction to reinforcement (34, 35). Results from two task variants (Fig.1J-M; see Supplement for details) confirmed that differences in consummatory licking arises from the appetitive component of responding, and excluded caloric content as the factor underlying performance.

Motivational anhedonia in mice susceptible to social defeat stress

Although it is known that stress can induce anhedonia in rodents, the extent to which behavioral alterations can be attributed to the motivational versus appetitive components, as well as the time course of deficits, is not known. Social defeat stress has ethological relevance for animals (12) and is associated with maladaptations in the rodent frontal cortex (13, 23, 36). In these experiments (Fig.2A), C57BL/6J mice received 10 days of social defeat, and were tested on the self-paced, instrumental sucrose preference task at 2-day intervals before, during, and after the defeat episodes.

The cohort tested included 28 stressed and 14 control animals. Results from social interaction tests confirmed that the defeat procedures led to a stress-dependent behavioral outcome (Fig.2B-C). We analyzed data from the self-paced, instrumental sucrose preference task during pre-stress (day -1 and 1), during-stress (day 5, 7, and 9), and post-stress (day 11 and 13) stages. As expected, for control mice, rewards earned increased with sucrose concentration, and this motivational effect was stable across days ($P=1 \times 10^{-8}$, main effect of reward type; ANOVA with block type and stage as fixed factors and subject as random factor; Fig.2D). By contrast, susceptible mice had diminished sensitivity to both absolute and relative reward differences that persisted into the post-stress stage ($P=7 \times 10^{-8}$, main effect of reward type; $P=0.003$, main effect of stage; Fig.2E). Resilient mice also had reduced reward sensitivity, but the decrease was transient, as sucrose preference quickly returned to pre-stress level following the last defeat session (Fig.2F). Therefore, animals subjected to repeated social defeat displayed motivational anhedonia, with the susceptible subtypes displaying a more prolonged phenotype.

Delayed emergence of motivational anhedonia from cumulative effects of stress

To gain insight into the factors that drove the motivational anhedonia, we applied a computational model of response vigor (37, 38). The total cost for performing a lick bout is a sum of the energetic cost of physical effort in performing actions in quick succession (37) and the opportunity cost of forgoing a reward by not performing an action (Fig.3A).

Opportunity costs grow linearly as a function of latency between actions, with a rate for water rewards (k_o) and scaling factors to account for accelerated rates for other reinforcement types (c_{3nc} , c_{3pc} , c_{10}). Satiety is captured by marginal utility with exponentially diminishing returns (α), which occurs after a certain number of rewards (R_o). At any moment during the task, the goal of the animal is to select a latency for the next lick bout to minimize the total cost.

The model was useful for distilling the session-by-session variations in behavioral performance into a few parameters. For each session, we fit the model to the behavioral data (Fig.3B-C). Focusing on susceptible animals, reward sensitivity was significantly modulated by reward type and day ($P=3 \times 10^{-4}$, main effect of reward type; $P=3 \times 10^{-6}$, main effect of day; ANOVA with block type and day as fixed factors and subject as random factor; Fig.3D-F). Notably, the motivational deficit did not emerge immediately following the first few stress sessions, but rather became detectable starting on day 7. This stress-related blunting of motivational action was severe in susceptible animals, relative to the more variable effects in resilient animals (Fig.3G). By contrast, the threshold for marginal utility also increased after stress but did not depend on susceptibility (Fig.3H-J; see Supplement for details). Appetitive anhedonia, as measured by consummatory licks, were induced by stress but only transiently (Fig.4; see Supplement for details). Overall, for motivational anhedonia, the delayed emergence and protracted time course indicate that it is a consequence of the cumulative impact of the chronic stressor.

Prefrontal Cg1/M2 region: Imaging the time course of neural activity changes at cellular resolution

The behavioral analysis and particularly the modeling suggest a progressive emergence of motivational anhedonia. Where should one look for the accompanying neural alterations? In rodents, the cingulate (Cg1) and medial secondary motor (M2) subregion, which constitutes the most dorsal aspect of medial prefrontal cortex (39), is important for the production of self-initiated (40, 41) and reward-guided actions (26, 42-44). Furthermore, Cg1/M2 is a target of stress, as identified by brain-wide mapping of neuronal activation in a rodent model of depression (45), and its response to the fast-acting antidepressant ketamine (46-48).

Neuronal firing leads to somatic calcium transients in pyramidal neurons (49), which could be visualized using a fluorescent calcium indicator. We used a recently developed bicistronic vector (50) to express the genetically encoded calcium indicator GCaMP6s along with the fluorophore mRuby2 in pyramidal neurons in Cg1/M2 (Fig.5A-C; see Supplement for experimental considerations). Using a two-photon microscope and relying on static mRuby2 fluorescence to identify cell assemblies, we could reliably track the same layer 2/3 neurons for up to 7 sessions spanning 13 days (Fig.5D-E).

Social defeat stress initially elevates the activity of prefrontal pyramidal neurons

To determine the short-term effect of social stress on Cg1/M2, we compared the spontaneously occurring calcium transients in layer 2/3 pyramidal neurons in awake mice, before versus after a single defeat (Fig.6A). Social interaction tests were used to classify the imaging animals into susceptible and resilient subtypes (Fig.6B). To infer activity rates from

somatic fluorescence signals, we used a peeling algorithm based on template matching (51, 52). Prior to stress, cells from the different animals had no appreciable difference in their spontaneous activity (Fig.6C). After a single defeat, susceptible animals had elevated spontaneous activity, relative to control animals (control versus susceptible: $P=0.002$, Wilcoxon rank-sum test; Fig.6D). Intriguingly, resilient and control animals were indistinguishable by this measure of median activity change (control versus resilient: $P=0.3$, Wilcoxon rank-sum test). However, when we plotted the distribution of activity changes, a more nuanced picture arose. The distribution of the activity change was more dispersed for susceptible and resilient animals (interquartile range: 0.27 for controls, 0.54 for susceptible, 0.47 for resilient; Fig.6E). For resilient animals, this means that although the median did not change, there were more cells with greater decrease or increase in spontaneous activity following stress relative to controls (control versus resilient: $P=0.005$, Kolmogorov-Smirnov test). Collectively, these results demonstrate that a single stress episode already had a detectable impact on the activity of layer 2/3 pyramidal neurons in Cg1/M2. Moreover, there is a resilience-specific compensation that dampened the overall activity after stress to be comparable to control animals.

Social defeat stress exerts heterogeneous long-term effects on prefrontal cortical activity

We continued to image the stressed animals and, for a majority of the cells, tracked their activity throughout and beyond the social defeat manipulation (Fig.7a). Using heatmaps, we could visualize and compare how the spontaneous activity levels of the same layer 2/3 pyramidal neurons in Cg1/M2 changed relative to pre-stress baseline (Fig.7B). We applied hierarchical clustering to agglomerate cells with similar time courses of stress-induced activity changes, which segregated into four major clusters: Type 1, 2, 3, and 4 (Fig.7C-E; Supplemental Fig. 1). Type 1 cells included neurons with an initial elevation of spontaneous activity, followed by decreases for the next two sessions down to a sustained level below the baseline. This was the most frequently observed type of time course; cells with this profile could be found in both susceptible and resilient animals ($P=0.49$, chi-square test). Type 2 cells had a monotonic decline in spontaneous activity in response to repeated stress. The decrease continued across the initial stress sessions before stabilizing to a steady below-baseline level. Notably, Type 2 cells were significantly more common in resilient animals ($P=0.002$, chi-square test). By contrast, Type 3 and Type 4 cells were more abundant in the susceptible animals, although the comparisons did not reach statistical significance likely because there were few cells with these time courses (Type 3: $P=0.05$, Type 4: $P=0.20$, chi-square test). Type 3 and Type 4 cells had a time course that was either steady near the baseline or had prolonged heightened spontaneous activity. In particular, Type 4 cells might be the most related to the stress-induced motivational anhedonia, as they were predominantly found in susceptible animals (6/7 cells) and mirrored the progression of the behavioral deficit. The pre-stress activity levels of these functional cell types did not appear to predispose an animal to be susceptible (Fig.7F). Together, these results show the stress-induced emergence of heterogeneous neural adaptations in prefrontal pyramidal neurons.

DISCUSSION

In this study, we measured the longitudinal effects of repeated social defeat on reward-directed actions and determined the concomitant profiles of activity changes for layer 2/3 pyramidal neurons in Cg1/M2. There are two main findings. First, behavioral and neural deficits in response to social defeat build over multiple stress episodes. This suggests that the response to repeated stress is not a unitary behavioral condition, but rather represents a continuum where accumulating exposure progressively deteriorates reward processing. The progression was observed in behavior as well as in activity of individual neurons. Second, resilience is associated with neural activity adaptations that are not only distinct from susceptible mice, but also dissimilar from control animals. This finding raises the possibility that resilience involves the capacity to reorganize prefrontal cortical activity adaptively to counteract the negative impact of social stress.

On average across all cells, we showed an initial elevation and then subsequent reduction of spontaneous activity in prefrontal pyramidal neurons in susceptible animals. These results match well with what we know in terms of stress-induced glutamatergic dysfunction in the prefrontal cortex (53). Specifically, acute restraint stress leads to an immediate increase in extracellular glutamate recorded in the medial prefrontal cortex, which peaks 20 min after the termination of stress but continues to be elevated up to the latest data point measured at 80 min post-stress (54). This initial hyperexcitation may underlie the increased surface levels of glutamatergic receptors, as well as potentiated synaptic responses following acute exposure to stress (55). Studies of chronic stress exposure have generally showed the opposite trend of prefrontal cortical hypoactivity. Mice subjected to 10 days of social defeat have reduced glutamate levels in the prefrontal cortex (36). A number of cellular mechanisms may underlie the reduced glutamatergic signaling, including the loss of AMPA and NMDA receptors (19), structural atrophy such as dendritic retraction and synapse loss (17, 18), and maladaptive synaptic inhibition (56).

The novelty of this study is that we have measured the heterogeneous changes in cellular activity as the animal experiences successive stress episodes and develops a behavioral response to that stress. Previous studies have hinted at heterogeneous neural responses on a short-term time scale. For example, it has been reported that prefrontal cortical neurons can have either transient or protracted firing responses to restraint (57). Moreover, stress-related excitatory synaptic modifications may be selective to a subset of prefrontal cortical neurons (20). In the current study, we used hierarchical clustering to identify several major profiles of stress-induced activity changes in prefrontal cortical neurons. The most numerous neuronal subtype (Type 1) display bidirectional activity changes involving initial hyperactivity followed by sustained hypoactivity. This time course might be expected based on the glutamatergic dysfunction associated with acute and chronic stress. Interestingly, both susceptible and resilient animals had a large number of Type 1 neurons, suggesting that this functional cell type may not be the distinguishing feature of susceptibility versus resilience. Instead, Type 2, 3, and 4 neurons have a monotonic progression of activity changes over time, and their proportions differ depending on the animal's reactivity to social stress. The implication is that a minor fraction of prefrontal cortical neurons may be particularly relevant for mediating the stress-induced behavioral deficits.

The progressive alterations of prefrontal cortical activity would be accompanied by the structural retraction of dendrites that have been widely documented in rodent mPFC including the Cg1/M2 areas (58, 59). There is evidence to suggest that the functional and structural maladaptations may occur in lockstep. On the one hand, structural atrophy is expected to reduce dendritic excitability and diminish the firing rates of pyramidal neurons (60). On the other hand, perturbations in glutamatergic neurotransmission could induce stress-related structural remodeling in mPFC (61). The bidirectional relationship may be the basis for a positive feedback loop that promotes functional and structural neural maladaptations. Moreover, just as we showed that pyramidal neurons exhibit different types of activity changes in response to repeated social defeat, it has also been reported that stress-related dendritic remodeling can be heterogeneous across cell types. For example, among layer 2/3 pyramidal neurons in the mPFC, repeated immobilization stress caused dendritic retraction in the entorhinal cortex-projecting subpopulation, but had no effect on the amygdala-projecting subpopulation (62).

In addition to the long-term differences in time courses, we demonstrated distinct features in neural activity changes following a single defeat between susceptible and resilient animals. Intriguingly, resilient animals were different from both susceptible and control animals. At the ensemble level, resilient animals were characterized by a wide range of activity changes, yielding a median activity change that is comparable to control animals but significantly lower than susceptible mice. These observations suggest that resilience is associated with adaptations of prefrontal cortical activity that brings the overall equilibrium to a normal level. By contrast, susceptibility to social stress may represent a failure to reorganize neural activity appropriately to counteract the stress effects, leading to a hyperactive state. More experiments will be needed to relate these findings to other molecular and network mechanisms that have been suggested to confer vulnerability and resilience to social stress (63, 64). The idea that resilience is not the same as unstressed, but rather is ‘stressed plus neural compensations’, is in agreement with the current perspective for how brain structure and connectivity in humans may adapt to early-life stress (65). Our cellular-resolution investigation provides clues for how the network-level reorganization may take shape.

To sum, the results presented here demonstrate a progressive development of behavioral and neural deficits in response to an ethologically relevant social stress. The data provide direct support for the allostatic load hypothesis, which posits that maladaptations arise from the cumulative burden of repeated stress (2). Looking ahead, a number of studies have shown that depressive-like phenotypes in rodents may be reversed by inducing synaptic plasticity in the medial prefrontal cortex. This may be achieved by controlling firing rates using optogenetics (13, 66) or by the administration of fast-acting antidepressants such as ketamine (21, 47, 67). Except in a few cases (68, 69), to date most studies have manipulated prefrontal pyramidal neurons indiscriminately. By showing that individual cells can have distinct profiles of activity adaptation in response to social stress, our findings suggest that neural interventions that target functional subtypes of pyramidal neurons may approximate more closely the physiological process of resilience and therefore be effective at promoting behavioral modifications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Kendler KS, Karkowski LM, Prescott CA (1999): Causal relationship between stressful life events and the onset of major depression. *Am J Psychiatry*. 156:837–841. [PubMed: 10360120]
2. McEwen BS (2004): Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann N Y Acad Sci*. 1032:1–7. [PubMed: 15677391]
3. McEwen BS, Stellar E (1993): Stress and the individual. Mechanisms leading to disease. *Arch Intern Med*. 153:2093–2101. [PubMed: 8379800]
4. Arnsten AF (2009): Stress signalling pathways that impair prefrontal cortex structure and function. *Nat Rev Neurosci*. 10:410–422. [PubMed: 19455173]
5. Hollon NG, Burgeno LM, Phillips PE (2015): Stress effects on the neural substrates of motivated behavior. *Nat Neurosci*. 18:1405–1412. [PubMed: 26404715]
6. Griffiths KR, Morris RW, Balleine BW (2014): Translational studies of goal-directed action as a framework for classifying deficits across psychiatric disorders. *Front Syst Neurosci*. 8:101. [PubMed: 24904322]
7. Schwabe L, Wolf OT (2009): Stress prompts habit behavior in humans. *J Neurosci*. 29:7191–7198. [PubMed: 19494141]
8. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, et al. (2009): Chronic stress causes frontostriatal reorganization and affects decision-making. *Science*. 325:621–625. [PubMed: 19644122]
9. Cerqueira JJ, Mailliet F, Almeida OF, Jay TM, Sousa N (2007): The prefrontal cortex as a key target of the maladaptive response to stress. *J Neurosci*. 27:2781–2787. [PubMed: 17360899]
10. Der-Avakian A, Mazei-Robison MS, Kesby JP, Nestler EJ, Markou A (2014): Enduring deficits in brain reward function after chronic social defeat in rats: susceptibility, resilience, and antidepressant response. *Biol Psychiatry*. 76:542–549. [PubMed: 24576687]
11. Donahue RJ, Muschamp JW, Russo SJ, Nestler EJ, Carlezon WA Jr., (2014): Effects of striatal DeltaFosB overexpression and ketamine on social defeat stress-induced anhedonia in mice. *Biol Psychiatry*. 76:550–558. [PubMed: 24495460]
12. Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, et al. (2007): Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*. 131:391–404. [PubMed: 17956738]
13. Covington HE, 3rd, Lobo MK, Maze I, Vialou V, Hyman JM, Zaman S, et al. (2010): Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. *J Neurosci*. 30:16082–16090. [PubMed: 21123555]
14. Treadway MT, Zald DH (2011): Reconsidering anhedonia in depression: lessons from translational neuroscience. *Neurosci Biobehav Rev*. 35:537–555. [PubMed: 20603146]
15. Salamone JD, Correa M (2012): The mysterious motivational functions of mesolimbic dopamine. *Neuron*. 76:470–485. [PubMed: 23141060]
16. Chaouloff F (2013): Social stress models in depression research: what do they tell us? *Cell Tissue Res*. 354:179–190. [PubMed: 23532563]

17. Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, et al. (2006): Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cereb Cortex*. 16:313–320. [PubMed: 15901656]
18. Cook SC, Wellman CL (2004): Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol*. 60:236–248. [PubMed: 15266654]
19. Yuen EY, Wei J, Liu W, Zhong P, Li X, Yan Z (2012): Repeated stress causes cognitive impairment by suppressing glutamate receptor expression and function in prefrontal cortex. *Neuron*. 73:962–977. [PubMed: 22405206]
20. Wang M, Perova Z, Arenkiel BR, Li B (2014): Synaptic modifications in the medial prefrontal cortex in susceptibility and resilience to stress. *J Neurosci*. 34:7485–7492. [PubMed: 24872553]
21. Moda-Sava RN, Murdock MH, Parekh PK, Fetcho RN, Huang BS, Huynh TN, et al. (2019): Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. *Science*. 364.
22. Wilber AA, Walker AG, Southwood CJ, Farrell MR, Lin GL, Rebec GV, et al. (2011): Chronic stress alters neural activity in medial prefrontal cortex during retrieval of extinction. *Neuroscience*. 174:115–131. [PubMed: 21044660]
23. Kumar S, Hultman R, Hughes D, Michel N, Katz BM, Dziras K (2014): Prefrontal cortex reactivity underlies trait vulnerability to chronic social defeat stress. *Nat Commun*. 5:4537. [PubMed: 25072279]
24. Abe R, Okada S, Nakayama R, Ikegaya Y, Sasaki T (2019): Social defeat stress causes selective attenuation of neuronal activity in the ventromedial prefrontal cortex. *Sci Rep*. 9:9447. [PubMed: 31263153]
25. Ali F, Shao L-X, Gerhard DM, Sweasy K, Pothula S, Pittenger C, et al. (2020): Inhibitory regulation of calcium transients in prefrontal dendritic spines is compromised by a nonsense Shank3 mutation. *bioRxiv*.
26. Siniscalchi MJ, Wang H, Kwan AC (2019): Enhanced Population Coding for Rewarded Choices in the Medial Frontal Cortex of the Mouse. *Cereb Cortex*. 29:4090–4106. [PubMed: 30615132]
27. Mineur YS, Fote GM, Blakeman S, Cahuzac EL, Newbold SA, Picciotto MR (2016): Multiple Nicotinic Acetylcholine Receptor Subtypes in the Mouse Amygdala Regulate Affective Behaviors and Response to Social Stress. *Neuropsychopharmacology*. 41:1579–1587. [PubMed: 26471256]
28. Golden SA, Covington HE 3rd, Berton O, Russo SJ (2011): A standardized protocol for repeated social defeat stress in mice. *Nat Protoc*. 6:1183–1191. [PubMed: 21799487]
29. Dickinson A, Nicholas DJ, Adams CD (1983): The Effect of the Instrumental Training Contingency on Susceptibility to Reinforcer Devaluation. *The Quarterly Journal of Experimental Psychology Section B*. 35:35–51.
30. Flaherty CF (1982): Incentive Contrast - a Review of Behavioral-Changes Following Shifts in Reward. *Animal Learning & Behavior*. 10:409–440.
31. Parent MA, Amarante LM, Liu B, Weikum D, Laubach M (2015): The medial prefrontal cortex is crucial for the maintenance of persistent licking and the expression of incentive contrast. *Front Integr Neurosci*. 9:23. [PubMed: 25870544]
32. Dombeck DA, Khabbaz AN, Collman F, Adelman TL, Tank DW (2007): Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron*. 56:43–57. [PubMed: 17920014]
33. Flaherty CF, Checke S (1982): Anticipation of Incentive Gain. *Animal Learning & Behavior*. 10:177–182.
34. Dwyer DM (2012): Licking and liking: the assessment of hedonic responses in rodents. *Q J Exp Psychol (Hove)*. 65:371–394. [PubMed: 22404646]
35. Davis JD, Smith GP (1992): Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. *Behav Neurosci*. 106:217–228. [PubMed: 1554433]
36. Veeraiyah P, Noronha JM, Maitra S, Bagga P, Khandelwal N, Chakravarty S, et al. (2014): Dysfunctional glutamatergic and gamma-aminobutyric acidergic activities in prefrontal cortex of mice in social defeat model of depression. *Biol Psychiatry*. 76:231–238. [PubMed: 24239130]
37. Niv Y, Daw ND, Joel D, Dayan P (2007): Tonic dopamine: opportunity costs and the control of response vigor. *Psychopharmacology (Berl)*. 191:507–520. [PubMed: 17031711]

38. Cools R, Nakamura K, Daw ND (2011): Serotonin and dopamine: unifying affective, activational, and decision functions. *Neuropsychopharmacology*. 36:98–113. [PubMed: 20736991]
39. Barthas F, Kwan AC (2017): Secondary Motor Cortex: Where 'Sensory' Meets 'Motor' in the Rodent Frontal Cortex. *Trends Neurosci*. 40:181–193. [PubMed: 28012708]
40. Murakami M, Vicente MI, Costa GM, Mainen ZF (2014): Neural antecedents of self-initiated actions in secondary motor cortex. *Nat Neurosci*. 17:1574–1582. [PubMed: 25262496]
41. Murakami M, Shteingart H, Loewenstein Y, Mainen ZF (2017): Distinct Sources of Deterministic and Stochastic Components of Action Timing Decisions in Rodent Frontal Cortex. *Neuron*. 94:908–919 e907. [PubMed: 28521140]
42. Siniscalchi MJ, Phoumthipphavong V, Ali F, Lozano M, Kwan AC (2016): Fast and slow transitions in frontal ensemble activity during flexible sensorimotor behavior. *Nat Neurosci*. 19:1234–1242. [PubMed: 27399844]
43. Sul JH, Jo S, Lee D, Jung MW (2011): Role of rodent secondary motor cortex in value-based action selection. *Nat Neurosci*. 14:1202–1208. [PubMed: 21841777]
44. Gremel CM, Costa RM (2013): Premotor cortex is critical for goal-directed actions. *Front Comput Neurosci*. 7:110. [PubMed: 23964233]
45. Kim Y, Perova Z, Mirrione MM, Pradhan K, Henn FA, Shea S, et al. (2016): Whole-Brain Mapping of Neuronal Activity in the Learned Helplessness Model of Depression. *Front Neural Circuits*. 10:3. [PubMed: 26869888]
46. Miyamoto S, Mailman RB, Lieberman JA, Duncan GE (2001): Blunted brain metabolic response to ketamine in mice lacking D(1A) dopamine receptors. *Brain Res*. 894:167–180. [PubMed: 11251190]
47. Phoumthipphavong V, Barthas F, Hassett S, Kwan AC (2016): Longitudinal Effects of Ketamine on Dendritic Architecture In Vivo in the Mouse Medial Frontal Cortex. *eNeuro*. 3.
48. Ali F, Gerhard DM, Sweasy K, Pothula S, Pittenger C, Duman RS, et al. (2020): Ketamine disinhibits dendrites and enhances calcium signals in prefrontal dendritic spines. *Nat Commun*. 11:72. [PubMed: 31911591]
49. Ali F, Kwan AC (2020): Interpreting in vivo calcium signals from neuronal cell bodies, axons, and dendrites: a review. *Neurophotonics*. 7:011402. [PubMed: 31372367]
50. Rose T, Jaepel J, Hubener M, Bonhoeffer T (2016): Cell-specific restoration of stimulus preference after monocular deprivation in the visual cortex. *Science*. 352:1319–1322. [PubMed: 27284193]
51. Grewe BF, Langer D, Kasper H, Kampa BM, Helmchen F (2010): High-speed in vivo calcium imaging reveals neuronal network activity with near-millisecond precision. *Nat Methods*. 7:399–405. [PubMed: 20400966]
52. Lütcke H, Gerhard F, Zenke F, Gerstner W, Helmchen F (2013): Inference of neuronal network spike dynamics and topology from calcium imaging data. *Front Neural Circuits*. 7:201. [PubMed: 24399936]
53. Popoli M, Yan Z, McEwen BS, Sanacora G (2011): The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. *Nat Rev Neurosci*. 13:22–37. [PubMed: 22127301]
54. Moghaddam B (1993): Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. *J Neurochem*. 60:1650–1657. [PubMed: 8097232]
55. Yuen EY, Liu W, Karatsoreos IN, Ren Y, Feng J, McEwen BS, et al. (2011): Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. *Mol Psychiatry*. 16:156–170. [PubMed: 20458323]
56. McKlveen JM, Morano RL, Fitzgerald M, Zoubovsky S, Cassella SN, Scheimann JR, et al. (2016): Chronic Stress Increases Prefrontal Inhibition: A Mechanism for Stress-Induced Prefrontal Dysfunction. *Biol Psychiatry*. 80:754–764. [PubMed: 27241140]
57. Jackson ME, Moghaddam B (2006): Distinct patterns of plasticity in prefrontal cortex neurons that encode slow and fast responses to stress. *Eur J Neurosci*. 24:1702–1710. [PubMed: 17004934]
58. Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, et al. (2004): Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience*. 125:1–6. [PubMed: 15051139]

59. Liston C, Miller MM, Goldwater DS, Radley JJ, Rocher AB, Hof PR, et al. (2006): Stress- induced alterations in prefrontal cortical dendritic morphology predict selective impairments in perceptual attentional set-shifting. *J Neurosci.* 26:7870–7874. [PubMed: 16870732]
60. Liu RJ, Aghajanian GK (2008): Stress blunts serotonin- and hypocretin-evoked EPSCs in prefrontal cortex: role of corticosterone-mediated apical dendritic atrophy. *Proc Natl Acad Sci U S A.* 105:359–364. [PubMed: 18172209]
61. Martin KP, Wellman CL (2011): NMDA receptor blockade alters stress-induced dendritic remodeling in medial prefrontal cortex. *Cereb Cortex.* 21:2366–2373. [PubMed: 21383235]
62. Shansky RM, Hamo C, Hof PR, McEwen BS, Morrison JH (2009): Stress-induced dendritic remodeling in the prefrontal cortex is circuit specific. *Cereb Cortex.* 19:2479–2484. [PubMed: 19193712]
63. Bagot RC, Cates HM, Purushothaman I, Lorsch ZS, Walker DM, Wang J, et al. (2016): Circuit-wide Transcriptional Profiling Reveals Brain Region-Specific Gene Networks Regulating Depression Susceptibility. *Neuron.* 90:969–983. [PubMed: 27181059]
64. Hultman R, Ulrich K, Sachs BD, Blount C, Carlson DE, Ndubuizu N, et al. (2018): Brain-wide Electrical Spatiotemporal Dynamics Encode Depression Vulnerability. *Cell.* 173:166–180 e114. [PubMed: 29502969]
65. Teicher MH, Samson JA, Anderson CM, Ohashi K (2016): The effects of childhood maltreatment on brain structure, function and connectivity. *Nat Rev Neurosci.* 17:652–666. [PubMed: 27640984]
66. Fuchikami M, Thomas A, Liu R, Wohleb ES, Land BB, DiLeone RJ, et al. (2015): Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions. *Proc Natl Acad Sci U S A.* 112:8106–8111. [PubMed: 26056286]
67. Li N, Lee B, Liu RJ, Banasr M, Dwyer JM, Iwata M, et al. (2010): mTOR-dependent synapse formation underlies the rapid antidepressant effects of NMDA antagonists. *Science.* 329:959–964. [PubMed: 20724638]
68. Warden MR, Selimbeyoglu A, Mirzabekov JJ, Lo M, Thompson KR, Kim SY, et al. (2012): A prefrontal cortex-brainstem neuronal projection that controls response to behavioural challenge. *Nature.* 492:428–432. [PubMed: 23160494]
69. Hare BD, Shinohara R, Liu RJ, Pothula S, DiLeone RJ, Duman RS (2019): Optogenetic stimulation of medial prefrontal cortex Drd1 neurons produces rapid and long-lasting antidepressant effects. *Nat Commun.* 10:223. [PubMed: 30644390]

Figure 1: A self-paced, instrumental sucrose preference task for the head-fixed mouse

(A) Schematic of the experimental setup. The head-fixed mouse had access to a composite lick spout with three openings, each of which could deliver fluid independently. The mouse sat atop a skewered sphere or a treadmill and could run forwards or backwards.

(B) The task was based on a tandem fixed-ratio (FR), fixed-interval (FI) schedule. To illustrate the FR10-FI5 schedule, licks detected and reinforcement delivered from an actual behavioral session were plotted.

(C) The task had a block design, cycling through multiple types of reinforcement including 0%, 3%, and 10% sucrose solutions. The sequence was always 0%, 3%, 10%, 3%, and then 0%, 3%, 10%, 3% again, and so on.

(D) Lick rate for a typical session. Lick rate was calculated in 10-s bins. The colored patches indicated the reinforcement type and duration of the blocks. Note that there were time gaps between the blocks, because a new block would begin only after the completion of a FR10 action sequence. The animal responded for about 30 min before stopping due to satiation.

(E) Rewards earned for each block. This is the same session as (D).

(F) Rewards earned per session. Open circles, individual sessions; bar, mean $\pm$ s.e.m.

(G) Rewards earned for each reinforcement type, normalized by rewards earned in 0% blocks, per session. Open circles, individual sessions; bar, mean $\pm$ s.e.m.; Main effect of reinforcement type ($F_{3,114} = 47.8$; $P = 4 \times 10^{-20}$), comparisons: 0% vs. 3% pc, $P = 0.012$;

0% vs. 10%, $P = 4 \times 10^{-9}$; 3% nc vs. 3% pc, $P = 0.0011$; 3% nc vs. 10%, $P = 4 \times 10^{-9}$; 3% pc vs. 10%, $P = 1 \times 10^{-7}$, ANOVA with post hoc Tukey-Kramer test.

(H) Mean lick density relative to the time of reinforcement, plotted separately for each reinforcement type.

(I) Consummatory licks per reward for each reinforcement type, normalized by the number in 0% blocks. Open circles, individual sessions; bar, mean $\pm$ s.e.m; Main effect of reinforcement type ($F_{3,114} = 9.0$; $P = 6 \times 10^{-8}$), comparisons: 0% vs. 3% pc, $P = 0.02$; 0% vs. 10%, $P = 5 \times 10^{-7}$; 3% nc vs. 10%, $P = 2 \times 10^{-6}$; 3% pc vs. 10%, $P = 0.03$, ANOVA with post hoc Tukey-Kramer test.

(J) A separate cohort of mice was trained on a variant of the task with the same FR10-FI5 schedule, however reinforcement type was randomized for each reward. Open circles, individual sessions; bar, mean $\pm$ s.e.m; Main effect of reinforcement type ($F_{2,26} = 25.0$; $P = 9 \times 10^{-7}$), comparisons: 0% vs. 10%, $P = 8 \times 10^{-7}$; 3% vs. 10%, $P = 1 \times 10^{-4}$, ANOVA with post hoc Tukey-Kramer test.

(K) Another separate cohort of mice was trained on a variant of task in which the reinforcement types were 0, 0.23, and 1 mM sucralose.

(L) Similar to **(H)** except for sucralose task. Main effect of reward type ($F_{3,105} = 10.6$; $P = 4 \times 10^{-6}$), comparisons: 0 vs. 1.00, $P = 0.0006$; 0.23 nc vs. 0.23 pc, $P = 0.006$; 0.23 nc vs. 1.00, $P = 8 \times 10^{-6}$, ANOVA with post hoc Tukey-Kramer test.

(M) Similar to **(I)** except for sucralose task. Main effect of reward type ($F_{3,105} = 15.9$; $P = 1 \times 10^{-8}$), comparisons: 0 vs. 1.00, $P = 2 \times 10^{-8}$; 0.23 nc vs. 1.00, $P = 9 \times 10^{-6}$; 0.23 pc vs. 1.00, $P = 1 \times 10^{-4}$, ANOVA with post hoc Tukey-Kramer test.

Sample sizes were 39 sessions from 39 mice (**F – I**), 14 sessions from 2 mice (**J**), and 39 sessions from 9 mice (**K – M**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. In the list above, if a potential post hoc comparison was unnoted, $P = 0.05$.

Figure 2: Motivational anhedonia as a sustained phenotype in susceptible, but not resilient, mice.

(A) Timeline of the chronic social defeat stress and behavioral experiments.

(B) Based on the social interaction ratio, mice subjected to social defeat were divided into resilient and susceptible individuals. Controls were handled but did not experience social defeat. Each circle in the beeswarm plot represents an animal.

(C) Example movement trajectories for a susceptible and a resilient individual, when the CD1 aggressor mouse was present or absent in the interaction zone (red rectangle).

(D) Rewards earned for each reinforcement type, normalized by rewards earned in 0% blocks, per session, for control animals. For each animal, the value was an average across days according to three stages: “pre” for day –1 and 1, “stress” for day 5, 7, and 9, and “post” for day 11 and 13. Gray line, individual animal; bar, mean $\pm$ s.e.m. Main effect of reinforcement type ($F_{2,117} = 21.4$, $P = 1 \times 10^{-8}$), but not for stage ($F_{2,117} = 0.57$, $P = 0.6$) or interaction ($F_{4,117} = 0.16$, $P = 1$), ANOVA with reinforcement type and stage as fixed factors, subject as random factor.

(E) Similar to (D) for susceptible animals. Main effect of reinforcement type ($F_{2,90} = 20.0$, $P = 7 \times 10^{-8}$) and stage ($F_{2,90} = 6.16$, $P = 0.003$), but not interaction ($F_{4,90} = 1.28$, $P = 0.3$), with comparisons: pre vs. stress, $P = 0.005$; pre vs. post $P = 0.02$; stress vs. post, $P = 0.9$, ANOVA with reinforcement type and stage as fixed factors, subject as random factor, and post hoc Tukey-Kramer test.

(F) Similar to (D) for resilient animals. Main effect of reinforcement type ($F_{2,126} = 40.6$, $P = 3 \times 10^{-14}$), stage ($F_{2,126} = 5.05$, $P = 0.008$), but not interaction ($F_{4,126} = 1.70$, $P = 0.2$), with comparisons: pre vs. stress, $P = 0.013$; pre vs. post, $P = 1$; stress vs. post, $P = 0.02$, ANOVA with reinforcement type and stage as fixed factors, subject as random factor, and post hoc Tukey-Kramer test.

Sample sizes were 14 mice (**D**), 11 mice (**E**), and 15 mice (**F**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 3: A model of self-paced, instrumental sucrose preference based on motivational vigor
(A) Schematic of the model. Animal should select a response time that minimizes the total cost. Total cost is the sum of an energetic cost, incurred if actions have to be performed in quick succession, and an opportunity cost, incurred when potential rewards are delayed due to inaction. The opportunity cost depends on the expected reinforcement type, and a marginal utility term for diminishing returns due to satiation. From the optimal response time, we could estimate the number of completed actions per block.

(B) Task performance and model fit for an example session on day -1.

(C) Similar to **(B)** on day 9 for the same animal.

(D) The opportunity cost coefficients determined by fitting model to individual sessions for control animals.

Line, mean $\pm$ s.e.m. Shading, days with social defeat. Main effect of reinforcement type ($F_{2,305} = 14.0$, $P = 2 \times 10^{-6}$), but not for day ($F_{11,305} = 1.33$, $P = 0.2$) or interaction ($F_{22,305} = 0.541$, $P = 1$), ANOVA with reinforcement type and day as fixed factors, subject as random factor.

(E) Similar to **(D)** for susceptible animals. Main effect of reinforcement type ($F_{2,269} = 8.43$, $P = 3 \times 10^{-4}$) and day ($F_{10,269} = 4.82$, $P = 3 \times 10^{-6}$), but not for interaction ($F_{20,269} = 0.983$, $P = 0.48$), ANOVA with reinforcement type and day as fixed factors, subject as random

factor. Comparisons: for the c_{10} parameter, versus day -1 , $P = 1.0$ for day 1; $P = 1.0$ for day 3; $P = 0.06$ for day 5; $P = 0.04$ for day 7; $P = 0.02$ for day 9; $P = 0.04$ for day 11; $P = 0.7$ for day 13, from post hoc Tukey-Kramer test.

(F) Similar to **(D)** for resilient animals. Main effect of reinforcement type ($F_{2,344} = 13.8$, $P = 2 \times 10^{-6}$) and day ($F_{11,344} = 3.39$, $P = 2 \times 10^{-4}$), but not for interaction ($F_{22,344} = 0.544$, $P = 1.0$), ANOVA with reinforcement type and day as fixed factors, subject as random factor. Comparisons: for the c_{10} parameter, versus day -1 , $P = 1.0$ for day 1; $P = 1.0$ for day 3; $P = 0.1$ for day 5; $P = 0.4$ for day 7; $P = 0.5$ for day 9; $P = 1.0$ for day 11; $P = 1.0$ for day 13, from post hoc Tukey-Kramer test.

(G) The opportunity cost coefficient for the 10% sucrose reinforcement. On an animal-by-animal basis, the mean pre-stress values are plotted against the during-stress values. For susceptible animals, correlation coefficient = 0.77, $P = 0.006$. For resilient animals, correlation coefficient = 0.43, $P = 0.1$. Each circle in the scatter plot represents an animal. Black crosshair, mean $\pm$ s.e.m.

(H) The change-point parameter for the marginal utility, determined by fitting model to individual sessions for control animals. Line, mean $\pm$ s.e.m. Shading, days with social defeat. No effect of day ($F_{11,101} = 0.416$, $P = 1$), ANOVA with day as fixed factor, subject as random factor.

(I) Similar to **(H)** for susceptible animals. Main effect of day ($F_{10,89} = 3.89$, $P = 3 \times 10^{-4}$), ANOVA with day as fixed factor, subject as random factor. Comparisons: versus day -1 , $P = 1.0$ for day 1; $P = 0.07$ for day 3; $P = 0.07$ for day 5; $P = 0.008$ for day 7; $P = 0.004$ for day 9; $P = 0.2$ for day 11; $P = 1.0$ for day 13, from post hoc Tukey-Kramer test.

(J) Similar to **(H)** for resilient animals. Main effect of day ($F_{11,114} = 5.00$, $P = 3 \times 10^{-6}$), ANOVA with day as fixed factor, subject as random factor. Comparisons: versus day -1 , $P = 1.0$ for day 1; $P = 0.08$ for day 3; $P = 0.05$ for day 5; $P = 0.0006$ for day 7; $P = 0.0001$ for day 9; $P = 0.1$ for day 11; $P = 1.0$ for day 13, from post hoc Tukey-Kramer test.

Sample sizes were 14 mice (**D**, **H**), 11 mice (**E**, **I**), 15 mice (**F**, **J**). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 4: Chronic social defeat had only transient effect on consummatory actions

(A) Top, lick raster, chosen randomly from 10 trials of each reinforcement type from a session on day - 1. Bottom, mean lick density relative to the time of reinforcement, plotted separately for each reinforcement type, for the same session.

(B) Similar to (A) on day 9 for the same animal.

(C) Consummatory licks per reward for each reinforcement type, normalized by the number in 0% blocks, for control animals. For each animal, the value was an average across days according to three stages: “pre” for day -1 and 1, “stress” for day 5, 7, and 9, and “post” for day 11 and 13. Gray line, individual animal; bar, mean $\pm$ s.e.m. Main effect of reinforcement type ($F_{2,117} = 6.49$, $P = 0.002$), but not for stage ($F_{2,117} = 0.99$, $P = 0.4$) or interaction ($F_{4,117} = 0.28$, $P = 0.9$), ANOVA with reinforcement type and stage as fixed factors, subject as random factor.

(D) Similar to (C) for susceptible animals. Main effect of reinforcement type ($F_{2,90} = 3.35$, $P = 0.04$) and stage ($F_{2,90} = 3.30$, $P = 0.04$), but not interaction ($F_{4,90} = 0.22$, $P = 0.9$), with comparisons: pre vs. stress, $P = 0.048$; pre vs. post $P = 0.9$; stress vs. post, $P = 0.2$, ANOVA with reinforcement type and stage as fixed factors, subject as random factor, and post hoc Tukey-Kramer test.

(E) Similar to (C) for resilient animals. Main effect of reinforcement type ($F_{2,126} = 13.3$, $P = 5 \times 10^{-6}$), but not stage ($F_{2,126} = 1.27$, $P = 0.3$) or interaction ($F_{4,126} = 0.39$, $P = 0.8$), ANOVA with reinforcement type and stage as fixed factors, subject as random factor. Sample sizes were 14 mice (C, D), 11 mice (E, F), and 15 mice (G, H). * $P < 0.05$.

Figure 5: Longitudinal imaging of spontaneous activity of layer 2/3 pyramidal neurons in Cg1/M2

(A) Timeline of the experiments.

(B) A fixed coronal section showing the extent of virally mediated expression of GCaMP6s-mRuby2 in Cg1/M2.

(C) *In vivo* two-photon images from an awake, head-fixed mouse for GCaMP6s (green) and mRuby2 (red) in pyramidal neurons in Cg1/M2. The images were taken 6 days apart.

(D) Spontaneous fluorescence transients for an example cell from (C), recorded 2 days apart across 7 sessions spanning 13 days during a social defeat experiment. The insets show a still frame of the *in vivo* GCaMP6s and mRuby2 fluorescence on the corresponding days.

(E) Similar to (D) for two other cells.

Figure 6: Social defeat rapidly alters the prefrontal cortical activity

(A) The timeline for experiments to determine the impact of social defeat stress on prefrontal cortical activity. The shaded green area represents the time window used for analysis of the short-term effects.

(B) Based on the social interaction ratio, mice subjected to social defeat were divided into resilient and susceptible individuals. Controls were handled but did not experience social defeat. Each circle represents an animal. Note that the imaging animals were a new cohort and not part of the behavior-only study.

(C) Baseline rate of calcium events on day -1 for control, susceptible, and resilient animals. Filled circles, median; bar, 25th and 75th percentiles. Control versus susceptible: $P = 0.6$, control versus resilient: $P = 0.4$, susceptible versus resilient: $P = 0.4$, Wilcoxon rank-sum test. Control versus susceptible: $P = 0.2$, control versus resilient: $P = 0.01$, susceptible versus resilient: $P = 1 \times 10^{-4}$, Kolmogorov-Smirnov test.

(D) Change in neural activity from day -1 to 1 for control, susceptible, and resilient animals. The activity change was a normalized difference in the rate of events inferred from fluorescence transients, calculated separately for each cell. Filled circles, median; bar, 25th and 75th percentiles. Control versus susceptible: $P = 0.002$, control versus resilient: $P = 0.3$, susceptible versus resilient: $P = 0.0001$, Wilcoxon rank-sum test.

(E) Histogram of the neural activity change from day -1 to 1 for control, susceptible, and resilient animals. Control versus susceptible: $P = 2.1 \times 10^{-5}$, control versus resilient: $P = 0.005$, susceptible versus resilient: $P = 6.1 \times 10^{-6}$, Kolmogorov-Smirnov test. Sample sizes for (C - E) were 62 cells from 4 control animals, 86 cells from 4 susceptible animals, and 59 cells from 3 resilient animals.

** $P < 0.01$, *** $P < 0.001$.

Figure 7: Social defeat induces heterogeneous long-term activity changes in the prefrontal cortex

(A) The timeline for experiments to determine the impact of social defeat stress on prefrontal cortical activity. The shaded green area represents the time window used for analysis of the long-term effects.

(B) Heatmaps showing the change in spontaneous activity relative to the pre-stress baseline. Each row is a cell. The left and right heatmaps contain the 58 cells from 4 susceptible animals and 35 cells from 3 resilient animals respectively. Red indicates an increase in spontaneous activity relative to pre-stress day -1. Blue indicates a decrease.

(C) The dendrogram and pairwise similarity matrix for the hierarchical clustering with Euclidean distance as the distance metric on the entire data set of 93 cells.

(D) Cells were classified as Type 1 – 4 based on the cluster membership identified by the hierarchical procedure. For each Type, the change in spontaneous activity was averaged across cells. Solid line, median. Dotted lines, 20th and 80th percentiles.

(E) Proportion of cells in susceptible or resilient animal that was classified as Type 1 – 4. Comparing the proportions between susceptible and resilient animals, the difference was significant for Type 2 ($P = 0.002$, chi-square test), and did not reach significance for Type 1 ($P = 0.49$), Type 3 ($P = 0.05$), and 4 ($P = 0.20$).

(F) Baseline rate of calcium events on day –1 in susceptible or resilient animals for Type 1 – 4 cells. Filled circles, median; bar, 25th and 75th percentiles. Two-way ANOVA with factors of cell type and animal group. Main effect of cell type ($F_{3,85} = 14.0$; $P = 2 \times 10^{-7}$), but not for animal group ($F_{1,85} = 2.0$; $P = 0.2$) or interaction ($F_{3,85} = 2.0$; $P = 0.1$). Post hoc Tukey-Kramer comparisons: Type 1 vs. 2, $P = 0.0005$; Type 1 vs. 3, $P = 0.04$; Type 1 vs. 4, $P = 0.3$; Type 2 vs. 3, $P = 6 \times 10^{-7}$; Type 2 vs. 4, $P = 0.002$; Type 3 vs. 4, $P = 1$.

KEY RESOURCE TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Add additional rows as needed for each resource type	Include species and sex when applicable.	Include name of manufacturer, company, repository, individual, or research lab. Include PMID or DOI for references; use "this paper" if new.	Include catalog numbers, stock numbers, database IDs or accession numbers, and/or RRIDs. RRIDs are highly encouraged; search for RRIDs at https://scicrunch.org/resources .	Include any additional information or notes if necessary.
Antibody				
Bacterial or Viral Strain				
Biological Sample				
Cell Line				
Chemical Compound or Drug	Carprofen	Butler Animal Health		
Chemical Compound or Drug	Dexamethasone	Henry Schein Animal Health		
Chemical Compound or Drug				
Chemical Compound or Drug				
Commercial Assay Or Kit				
Deposited Data; Public Database	Imaging and behavioral data	Github		
Genetic Reagent				
Organism/Strain	C57BL/6J, male	Jackson Laboratory	#000664	
Organism/Strain	CaMKIIa-Cre, male	Jackson Laboratory	#005359	
Organism/Strain	CD-1 retired breeder	Charles River	#CrI:CD1(ICR), #022	
Peptide, Recombinant Protein				
Recombinant DNA	pAAV(AAV1)-hSyn1-Flex-mRuby2-GSG-P2A-GCaMP6s-W	Addgene	#68720-AAV1	
Recombinant DNA	pAAV(AAV1)-CaMKII-GCaMP6f-WPRE-SV40	Addgene	#100834-AAV1	
Sequence-Based Reagent				
Software; Algorithm	Presentation	Neurobehavioral Systems		
Software; Algorithm	ScanImage	Vidrio Technologies		
Software; Algorithm	MATLAB	MathWorks, Inc.		
Transfected Construct				
Other				