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Individual Differences in Volitional Social Self-Administration and Motivation in Male and Female Mice Following Social Stress

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ABSTRACT

BACKGROUND: A key challenge in developing treatments for neuropsychiatric illness is the disconnect between preclinical models and the complexity of human social behavior. We integrate voluntary social self-administration into a rodent model of social stress as a platform for the identification of fundamental brain and behavior mechanisms underlying stress-induced individual differences in social motivation.

METHODS: Here, we introduced an operant social stress procedure in male and female mice composed of 3 phases: 1) social self-administration training, 2) social stress exposure concurrent with reinforced self-administration testing, and 3) poststress operant testing under nonreinforced and reinforced conditions. We used social-defeat and witness-defeat stress in male and female mice.

RESULTS: Social defeat attenuated social reward seeking in males but not females, whereas witness defeat had no effect in males but promoted seeking behavior in females. We resolved social stress-induced changes to social motivation by aggregating *z*-scored operant metrics into a cumulative social index score to describe the spectrum of individual differences exhibited during operant social stress. Clustering does not adequately describe the relative distributions of social motivation following stress and is better described as a nonbinary behavioral distribution defined by the social index score, capturing a dynamic range of stress-related alterations in social motivation inclusive of sex as a biological variable.

CONCLUSIONS: We demonstrated that operant social stress can detect stable individual differences in stress-induced changes to social motivation. The inclusion of volitional behavior in social procedures may enhance the understanding of behavioral adaptations that promote stress resiliency and their mechanisms under more naturalistic conditions.

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An ongoing focus of behavioral neuroscience is the identification of mechanisms that govern individual responses to social stress. Decades of work indicate that diverse neuronal and non-neuronal mechanisms drive this variability (1–3). Coping strategies for stress vary widely between individuals and play a significant role in subsequent vulnerability to neuropsychiatric disease (4,5), but little is known about how coping strategies for social stress are affected by social motivation. Furthermore, while many people (>50% of the general population) experience significant social and emotional stress in their lives, only a subset of these individuals (<10%) develop stress-related illness (6,7). Within this group, the rate of diagnoses of major depressive disorder and anxiety disorders is higher in women, and coping strategies differ between sexes (8,9–11). Such stress-related psychiatric disorders display sex-specific biases in attributes and symptomology, subsequently further modulating coping processes (12–14). Similarly, following stressful life events, women are more susceptible to hyperarousal whereas men are more susceptible to cognitive deficits (14). These changes are perceived as adaptive responses to acute or mild stressors and can become severely impaired with prolonged or chronic stress. Previous work has demonstrated the importance of social support as a strategy for improvement and prevention of neuropsychiatric disorders including anxiety and depression (15–19). Thus, because the selection of coping strategies varies widely but heavily influences an individual's outcome (20,21), expanding preclinical stress models to include volitional social motivation may better capture individual differences in the underlying neurobiology engaged by social stress.

A critical component of responding to stress is the decision to actively engage in, or avoid, socially rewarding interactions. The addition of operant social self-administration procedures to preclinical neuropsychiatric rodent models has revealed unexpected results, such as the existence of compulsive, addiction-like aggression seeking (22) and the role of voluntary

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social interaction in blunting incubation of drug craving (23). However, such voluntary procedures have not yet been incorporated into social stress models, which predominantly rely on involuntary social interaction tests. Here, we introduce operant social stress (OSS), a behavioral procedure used to evaluate voluntary social motivation in both male and female mice before, during, and after repeated social stress exposure. OSS is composed of 3 phases: 1) social self-administration training, 2) social stress exposure concurrent with daily reinforced self-administration to test social decision making, and 3) social reward seeking under both nonreinforced and reinforced conditions (Figure 1).

Currently, preclinical investigations primarily use variations of social-defeat or witness-defeat stress. In these procedures, mice either are subjected to physical repeated antagonistic social encounters (24–28) or witness them (29,30) and then are evaluated using involuntary social interaction tests measuring their exploratory sensory contact with a nonfamiliar target mouse (28). While clinical susceptibility and resilience exist on a spectrum (31–34), preclinical defeat procedures use binary classification for stress phenotypes based on minimal sensory contact duration. Although social- and witness-defeat procedures offer the benefits of high throughput and standardization, the inclusion of social self-administration procedures concurrent with social stress exposure will 1) allow assessment of voluntary social motivation, 2) provide expanded measures of individual variability in voluntary social behavior, and 3) provide a behavioral platform for resolving their underlying mechanisms.

In a series of experiments, we combined previously established models of social stress (28–30,35) with social self-administration procedures (22–38) and operant reward testing to investigate volitional social stress responses. Our results demonstrated sex-specific differences in stress-induced changes to volitional social self-administration and social motivation. Following social stress, males reduced social reward seeking but presented no changes following witness defeat. Females showed potential differences in coping mechanisms for social defeat versus witness defeat marked by no significant change in the former but increased reward seeking in the latter. Then, we integrated operant data into a social index that describes the spectrum of individual differences in social motivation following stress. Therefore, the OSS procedure provides a flexible behavioral platform for identifying fundamental mechanisms that drive individual responses to stress-induced changes in voluntary social motivation.

METHODS AND MATERIALS

Behavioral Procedures

Detailed descriptions of experimental rationales and procedures, experimental subjects, and apparatus are provided in Supplemental Methods. Below, we briefly describe 6 experiments; the experimental timeline is shown in Figure 1.

Experiment 1: Effect of Social Stress Exposure on Social Self-Administration and Social Reward Seeking. The goal of experiment 1 was to determine the consequence of social stress exposure on voluntary social self-administration and social reward seeking in male and female mice. In phase 1, we trained 89 male and 62 female mice for self-administration (Figure 2A–C; Figure S1A). Acquisition of social self-administration was defined as having an average of 2 or more social rewards across the last 3 days of training (days 8–10). All mice were tested for nonreinforced social reward seeking (1 hour) on the day following social self-administration training. During the social reward seeking tests, lever presses led to contingent delivery only of the discrete cue that had previously been paired with the delivery of the social partner. In phase 2, mice that acquired self-administration were placed into 2 groups per sex and stress type, for a total of 8 groups: male social-defeat stress and nonstressed controls, female social-defeat stress and nonstressed controls, male witness-defeat stress and nonstressed controls, and female witness-defeat stress and nonstressed controls (Figure 3). In phase 3, across sequential days, we tested all male and female social-defeat stress and witness-defeat mice for nonreinforced reward seeking (1 hour) and administered 2 consecutive once-daily reinforced progressive ratio (PR) tests (Figure 3).

Experiment 2: Effect of Active Lever Contingency on Social Self-Administration. The goal of experiment 2 was to determine the sensitivity of discriminative stimuli during social self-administration training, controlling for conditioned

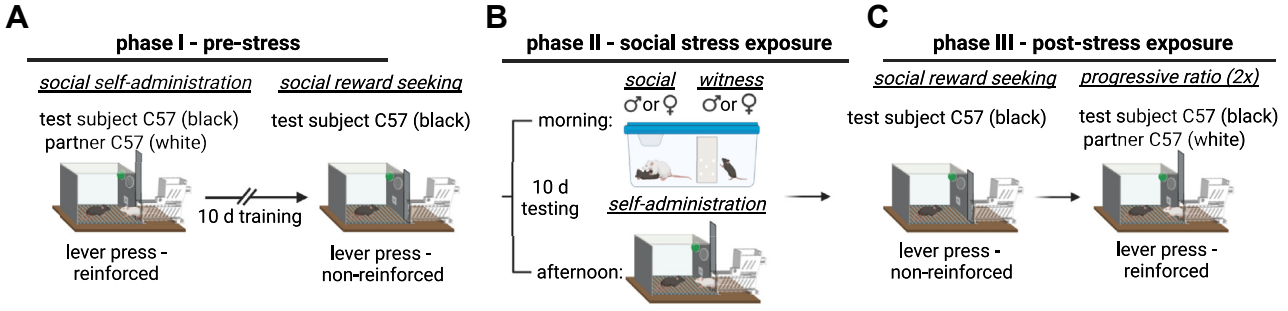

Figure 1. Operant social stress procedure schematic. **(A)** Phase 1 (prestress): male and female mice were trained for 10 days to lever press for freely moving access to a familiar social partner followed by a 1-hour nonreinforced reward-seeking test. **(B)** Phase 2 (social-defeat or witness-stress exposure): mice that acquired social self-administration were exposed to either social defeat or witness defeat for 10 days paired with daily self-administration test sessions with a familiar social partner. **(C)** Phase 3 (poststress exposure): following the final social stress exposure day, mice were tested for social reward seeking under extinction conditions (left) followed by 2 days of progressive ratio testing (right) to measure their social motivation breakpoint.

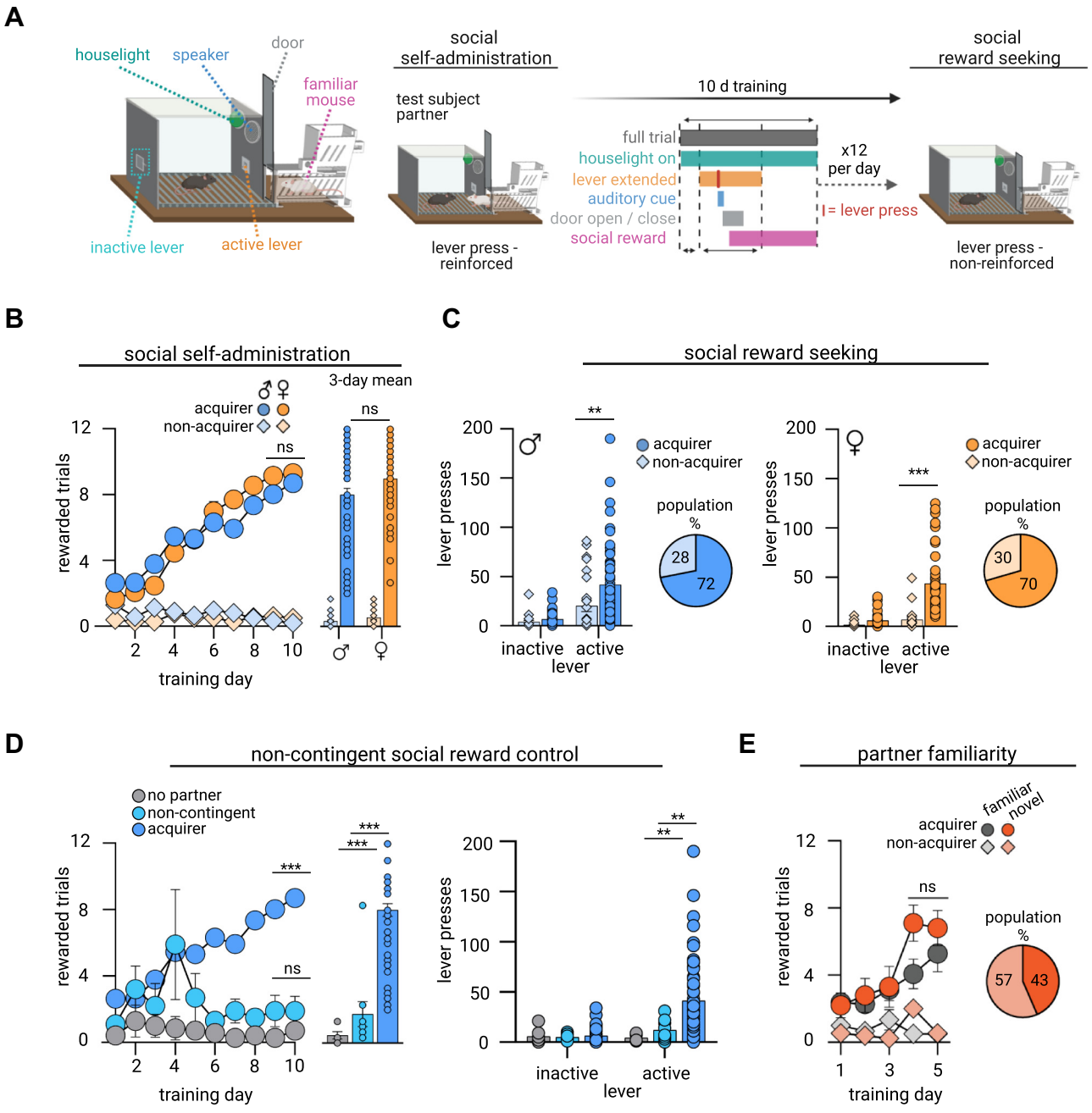

Figure 2. Male and female mice acquire social self-administration and exhibit social reward seeking. **(A)** Operant social stress chamber (left) and experimental timeline schematic (right) for social self-administration and social reward seeking. Test subject male and female C57BL/6J mice were trained to lever press for access to a familiar social partner. In the individual trial schematic inset, the vertical red line within the lever extended bin indicates an active lever press and the resulting sequence of events. **(B)** (Left) Number of rewarded trials over 10 days (48-minute session/day, 12 trials/day) of social self-administration under a trial-based fixed-ratio 1 reinforcement schedule with male (n = 89) and female (n = 61) mice. (Right) Mean rewarded trials from the final 3 days of social self-administration. **(C)** Number of nonreinforced male (left) and female (right) active and inactive lever presses during a 1-hour social reward-seeking test under extinction conditions following social self-administration. Pie charts show the proportion of each cohort that acquired social self-administration. **(D)** Noncontingent social reward controls. (Left) Number of rewarded trials over 10 days of social self-administration with either no partner (n = 7) or a noncontingent active lever (n = 10) compared with the acquirer (n = 64) male mice. (Middle) Mean rewarded trials from the final 3 days of social self-administration. (Right) Number of nonreinforced active and inactive lever presses during the social reward-seeking test. **(E)** Number of rewarded trials over 5 days of social self-administration with familiar (n = 20) and novel (n = 23) social partners; pie chart shows the percentage of mice that acquired social self-administration. Individual data denoted with symbols. $^{**}p < .001$, $^{***}p < .0001$. Data are mean ± SEM. ns, nonsignificant.

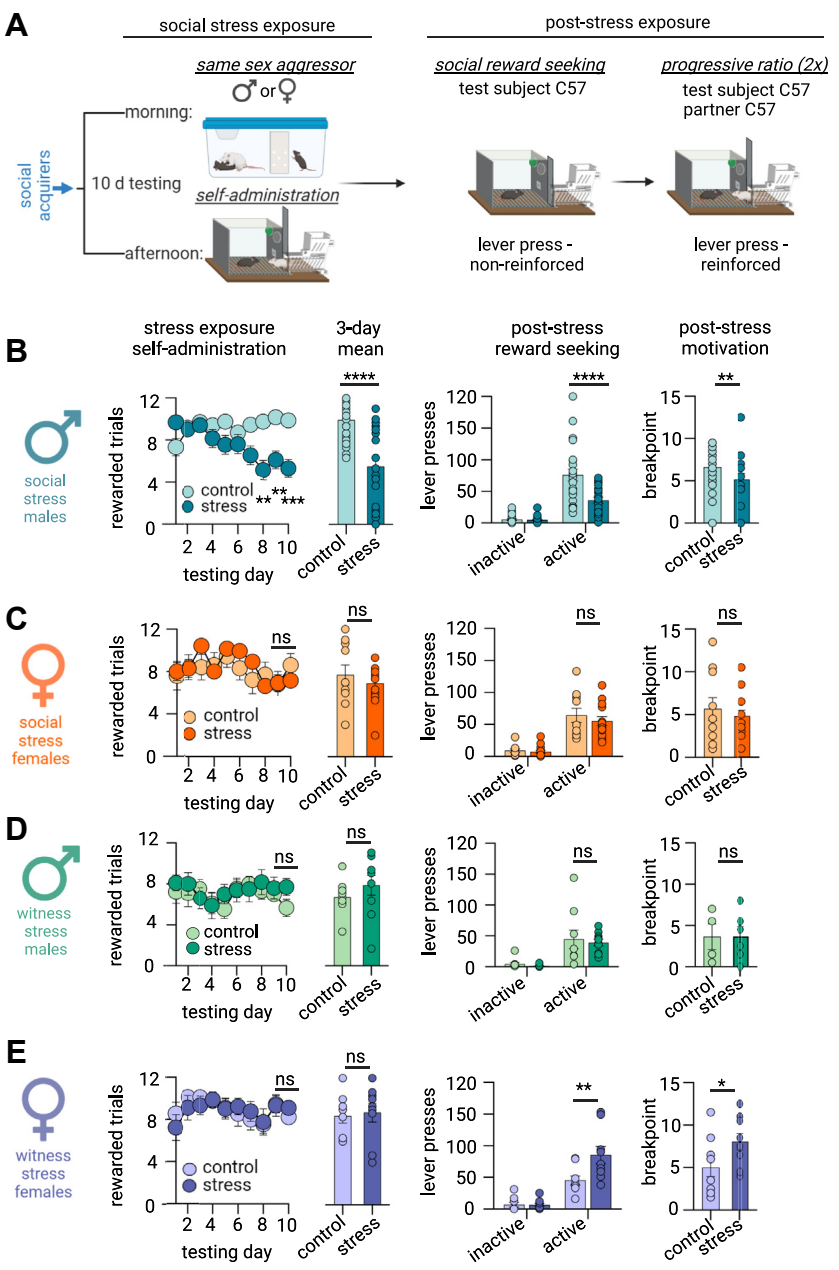

Figure 3. Social stress exposure affects social self-administration and social reward seeking. **(A)** Experimental schematic for stress and poststress phases of operant social stress. Male and female mice that acquired social self-administration were exposed to 10 days of once-daily social stress followed 4 hours later by social self-administration test sessions. After the last day of social stress exposure, mice were tested for nonreinforced social reward seeking and 2 days of reinforced progressive ratio testing. **(B–E)** For male social stress **(B)** (control, n = 24; stress exposed, n = 20), social stress female **(C)** (control, n = 10; stress exposed, n = 14), witness stress male **(D)** (control, n = 9: stress exposed, n = 10), and witness stress female **(E)** (control, n = 9; stress exposed, n = 9) mice, (left) number of rewarded trials over 10 days (12 trials/day) of social self-administration testing, with mean rewarded trials from the final 3 days of social self-administration testing; (middle) number of nonreinforced active and inactive lever presses during a 1-hour social reward-seeking test under extinction conditions following social stress exposure; (right) mean breakpoint earned during two 2-hour progressive-ratio tests for social reward. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. Data are mean ± SEM. ns, nonsignificant.

stimulus and unconditioned stimulus contingency (Figure 2D). We trained 2 cohorts of male black-coated C57BL/6J mice paired with white-coated familiar cagemates, where one cohort did not receive a social reward when responding to the active lever, and the second cohort received random social reward delivery unpaired from the contingent active lever. We compared these 2 controls to male acquirers from experiment 1 in our analysis. Following social self-administration training, we tested all mice for nonreinforced reward seeking (1 hour).

Experiment 3: Effect of Social Partner Coat Color on Social Self-Administration. The goal of experiment 3 was to determine the effect of social partner coat color on social self-administration (Figure S2), controlling for the use of a contrasting coat color that improves manual and automated behavioral annotation. We trained a cohort of male black-coated C57BL/6J mice paired with familiar, same coat color partners and a cohort of male black-coated C57BL/6J mice paired with familiar, different coat color partners. We included acquiring and nonacquiring mice in our analysis.

Experiment 4: Effect of Social Partner Familiarity and Prior Experience on Social Self-Administration. The goal of experiment 4 was to determine the consequence of

partner familiarity on the acquisition of social self-administration. We trained black-coated male C57BL/6J mice to self-administer nonfamiliar black-coated C57BL/6J social partners and compared their acquisition of self-administration with the black-coated males from experiment 2 that had familiar partners (Figure 2E). We included acquirer and non-acquirer mice in our analysis. To assess how prior social experience may modulate acquisition of social self-administration, we trained and tested male C57BL/6J mice (naïve) and then flipped (experienced, within subject) the roles of these mice with their familiar partners during subsequent self-administration and reward-seeking testing (Figure S3).

Experiment 5: Social Stress-Induced Clustering of Social Reward Classifications. The goal of Experiment 5 was to test whether the operant behavior we observed during OSS could be separated into discrete clusters. Specifically, we asked 1) how well data can be separated into 2 clusters (analogous to stress-susceptible and resilient phenotypes as defined in previous studies), and 2) whether there is an optimal number of clusters that can be defined. Using features derived from operant behavioral metrics during OSS, we tested the first question using a hierarchical clustering algorithm (Ward's method) constrained to 2 clusters. We tested the second question by performing a grid search on a set of Gaussian mixture models with a varying number of components and covariance matrix types to find the optimal number of clusters as determined by the lowest Bayesian information criterion score obtained during the search (22,39–44) (Figure 4). The set of 5 features for the clustering experiments were 1) the difference (Δ) in mean social rewards obtained over the last 3 days of training (phase 1) and stress exposure (phase 2), 2) active lever presses from the prestress social reward-seeking test, 3) mean social rewards obtained from the last 3 days of self-administration testing during stress exposure, 4) active lever presses from the poststress social reward-seeking test, and 5) mean breakpoint from PR tests. The detailed analytical pipeline is described in Supplemental Methods. The hierarchical clustering results were compared with the Calinski-Harabasz criterion (45) using the differences in *z*-scored OSS features for male and female clusters, the overlap coefficients between their fitted distributions, and Kolmogorov-Smirnov tests.

Experiment 6: Social Stress-Induced Indexing of Individual Variability in Social Reward Behavior. In experiment 6, we examined the spectrum of social motivation behavior following social stress (Figures 5 and 6; Figure S5) using the same 5 OSS features from experiment 5. The detailed analytical pipeline is described in Supplemental Methods and Supplemental Results. Briefly, the 5 features were *z* scored across mice of each sex and aggregated to compose a summarizing measurement for each mouse as their cumulative social index score. We distributed the social index of males and females based on their experimental condition (control, stress) to examine their similarity as described by their overlap coefficient. We determined which features were most impactful during the procedure using 3-component principal component analysis on the *z*-scored features that composed the cumulative score and labeled each animal by their group and cumulative score value, subsequently assessing the correlation of each feature with the resulting principal components (Pearson's *r*).

RESULTS

Male and Female Mice Exhibit Social Self-Administration and Reward Seeking

In phase 1 of OSS, we trained male and female mice to lever press for affiliative social interaction with a familiar partner (Figure 2A). A total of 64 male and 43 female mice acquired (acquirers) and 25 male and 18 female failed to acquire (nonacquirers) social self-administration (Figure 2B), with no difference found between male and female mice. Acquirers exhibited a higher average of rewarded trials ($F_{1,88} = 130.7$, $p < .0001$) across the last 3 days of training than nonacquirers, regardless of sex. In the social reward-seeking test, male ($F_{1,176} = 14.3$, $p = .001$) and female ($F_{1,176} = 14.3$, $p = .0003$) acquirers had greater active lever presses than nonacquirers (Figure 2C). Complete statistical results can be found in Table S1.

Social Self-Administration Is Contingent on Delivery of a Social Partner

To evaluate the social aspect of rewards on acquisition of social self-administration, we compared self-administration of male mice that were receiving pseudorandom social reward, unpaired from the active lever (noncontingent), or mice receiving no social reward when pressing the active lever (no-partner) to mice that acquired the task (acquirers) (Figure 2D). Compared with acquirers, there was a decrease in social self-administration across training days ($F_{1,490} = 102.3$, $p < .0001$) for no partner and for noncontingent mice ($F_{1,520} = 42.57$, $p < .0001$). In a reward-seeking test, both no partner ($F_{1,98} = 8.908$, $p = .0036$) and noncontingent ($F_{1,104} = 9.14$, $p = .0031$) mice decreased active lever responding compared with acquirers. Both no partner and noncontingent groups failed to acquire social self-administration.

Effect of Familiarity, Prior Experience, and Coat Color on Social Self-Administration and Reward Seeking

To test whether partner coat color affects acquisition of social self-administration, we compared the self-administration of male different coat color familiar partners to same coat color partners (Figure S2A). There was no effect of coat color across training days, but there was a significant difference in 3-day mean rewards ($F_{1,40} = 2.71$, $p = .023$) between same and different coat color acquirers (Figure S2B). In a reward-seeking test using same coat color partners, acquirers exhibited increased active lever responding ($F_{1,80} = 33.9$, $p = < .0001$) compared with nonacquirers. We also asked how familiarity with a social partner affected acquisition of social self-administration in male mice by using either familiar or novel social partners and observed no effect on social self-administration (Figure 2E). Last, we examined whether prior experience as a social partner would influence later acquisition of social self-administration (Figure S3). Both naïve and experienced male mice acquired social self-administration

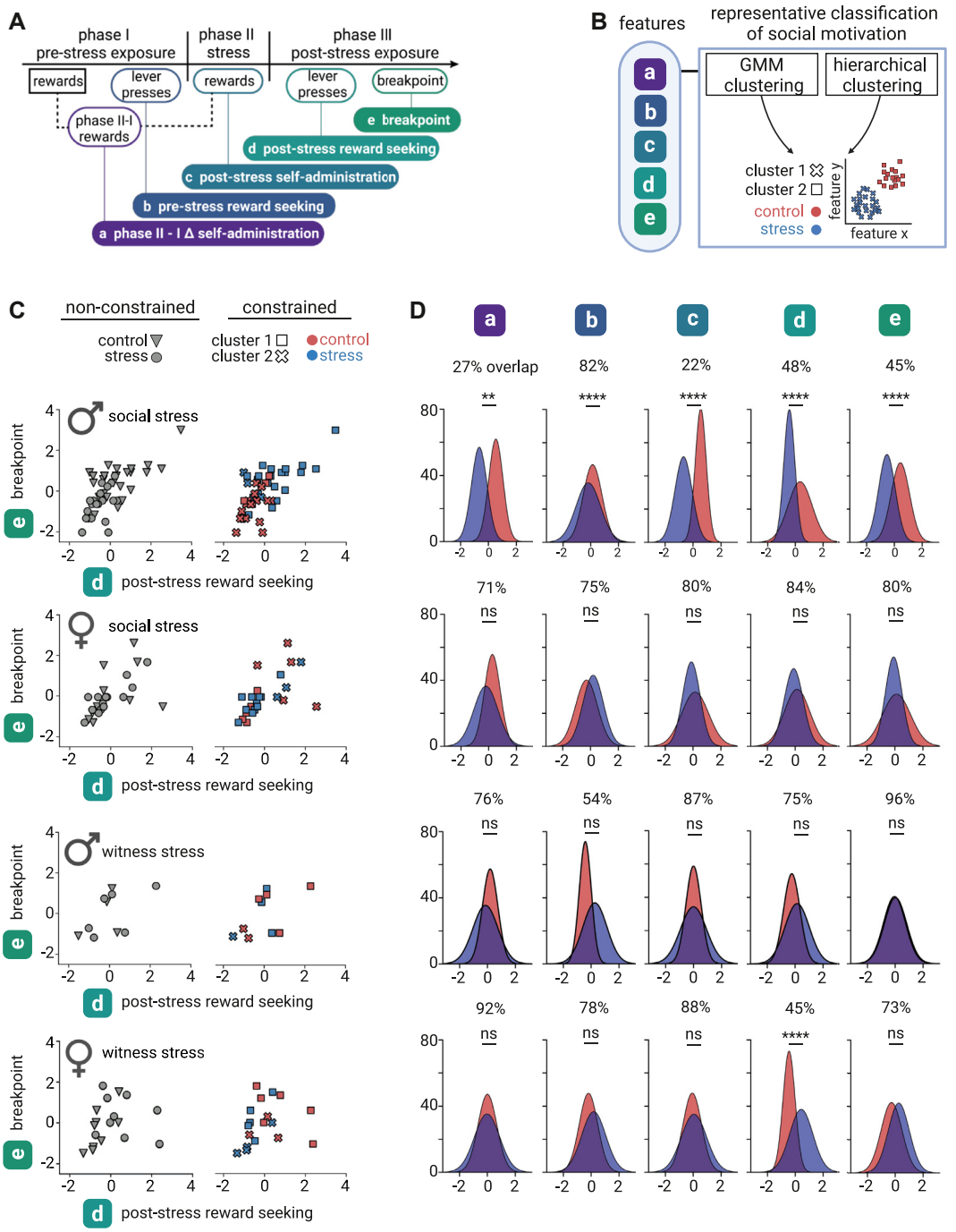

Figure 4. Social stress-induced clustering of social reward phenotypes. **(A)** Experimental schematic of social operant metrics as features for clustering analyses. Five operant metrics obtained during operant social stress: **(a)** the difference in 3-day mean of social self-administration before and during stress (phase 2 − 1 Δself-administration); **(b)** lever pressing on first lever test (prestress reward seeking); **(c)** the 3-day mean rewards obtained during social stress exposure (poststress self-administration); **(d)** lever pressing on the second lever test (poststress reward seeking); and **(e)** mean breakpoint across the 2 days of progressive ratio testing (breakpoint). The 5 metrics were *z* scored across experimental groups separately for male and female mice. **(B)** Schematic of hierarchical clustering classification approach. Inset, representative hypothetical results for classification of social behavior during operant social stress. Clearly defined populations among operant data from operant social stress metrics (cluster 1 and cluster 2) should yield 2 discrete clusters representing control (red) and stress (blue) populations of mice. **(C)** Male social stress (top) (n = 44), female social stress (middle) (n = 24), male witness stress (middle) (n = 10), and female witness stress (bottom) (n = 18) scatterplots of *z*-scored poststress metrics (reward seeking and breakpoint) labeled by stress condition (control, triangle; stress, circle) and cluster assignment from GMM (left) (single cluster: gray) and hierarchical clustering (right) (2 clusters, putative control cluster: red, putative stress cluster: blue) analyses. **(D)** Distribution of *z*-scored features **(a–e)** by hierarchical cluster assignment for male (top) (n = 18 resilient), stress female (middle) (n = 8), and witness female (bottom) (n = 9 resilient) mice. $^{**}p < .01$, $^{****}p < .0001$. GMM, Gaussian mixture model; ns, nonsignificant.

($F_{1,36}$ = 37.6, $p < .0001$), and naïve ($F_{1,72}$ = 25.5, p = .0008) and experienced ($F_{1,72}$ = 25.5, $p < .0001$) acquirers exhibited higher social reward seeking than nonacquirers, with a significant difference in active lever presses between naïve and experienced acquirers ($F_{1,36}$ = 1.12, p = .036).

Social Stress Exposure Alters Social Self-Administration and Reward Seeking in a Sex-Specific Manner

In phase 2, we assigned mice to either stress or no-stress control groups and exposed them to social-defeat or witness-defeat stress paired with social self-administration sessions (Figure 3A, left) to assess how social stress affects volitional self-administration of a familiar social partner. Stress males (Figure 3B) exhibited a reduction in rewarded trials across days ($F_{1,42}$ = 6.08, p = .018), whereas witness males (Figure 3D) exhibited no difference in social self-administration. Neither social-defeat stress (Figure 3C) nor witness-defeat stress (Figure 3E) females exhibited differences in social self-administration compared with controls. In phase 3 (Figure 3A, right), all mice were tested with a nonreinforced lever test followed by PR testing to gauge social reward seeking and motivation, respectively. Stress males (Figure 3B) lever pressed less often during nonreinforced ($F_{1,84}$ = 12.1, $p < .0001$) and reinforced ($F_{1,42}$ = 8.46, p = .006) reward seeking than controls. Stress females (Figure 3C) showed no difference in response to the active lever during both nonreinforced and reinforced reward-seeking tests. Witness males (Figure 3D) showed no difference in response to the active lever during both nonreinforced and reinforced reward-seeking tests. Witness females (Figure 3E) responded to the active lever significantly more often ($F_{1,32}$ = 6.43, $p < .0001$) during nonreinforced reward seeking and exhibited a higher breakpoint ($F_{1,17}$ = 4.46, p = .0499) than controls.

Cluster Analysis of Stress Susceptibility and Resilience Does Not Accurately Describe Stress Phenotypes

To determine whether individual differences in social self-administration and reward can be nonarbitrarily classified into 2 discrete clusters (typically analogous to susceptible and resilient), we evaluated social motivation following stress using key metrics (see Methods and Materials) as features for 2 different cluster analyses, Gaussian mixture model clustering and Ward's method for hierarchical clustering algorithm (Figure 4A, B). Social-defeat male clustering results were the most discrete, based on Calinski-Harabasz scores of 19.3, 10.7, 5.26, and 8.9 for male social, female social, male witness, and female witness defeat, respectively. The distribution of OSS features grouped by cluster assignment shows that for male social defeat (Figure 4D), the most discriminating operant features were Δself-administration, poststress self-administration, and breakpoint, whereas female social-defeat stress and male witness-stress clusters were not significantly separated by any feature distribution. Female witness-stress clusters were separated most by poststress reward seeking. These results suggest that the behavioral spectrum of social self-administration and reward in male and female mice following social stress is not clearly defined by 2 clusters, indicating that a different approach is required to better describe this relationship.

Individual Differences in Social Motivation Following Social Stress Exposure Are Better Described by a Spectrum

Our clustering results (Figure 4C, D) suggested that OSS individual variability is better modeled as a distribution along a spectrum. To test this, we aggregated the 5 OSS features into a cumulative score for each animal (social index) (Figure 5A) to summarize their social reward and motivation behavior. Consistent with previous behavioral results, stress males (Figure 5B) occupied the lower end of the social index distribution (29% overlap; Kolmogorov-Smirnov, $p < .0001$), while the stress female, witness male, and witness female distributions were not significantly different from their respective controls. Next, we explored how each OSS feature related to the social index distributions (Figure 5C) using principal component analysis to explain the feature-dependent variability across animals. These results demonstrate that OSS can be used to detect changes in social behavior due to stress that exists across multiple dimensions of operant behavior.

Stability of the Social Index

Next, we asked how well behavior from earlier OSS phases could predict final social index scores. We compared the ranked order of individual social index scores (Figure 6A) using 4 and 3 OSS features (without PR or both PR and poststress reward seeking, respectively) to those derived from all 5 features, across control and stress conditions for each group. Figure 6B–E (top) shows the distributions of social index scores for males and females. We tested the stability of individual social index rankings as a function of decreasing OSS feature space by ranking the social index scores for each feature set per group (Figure 6B–E, top and bottom, respectively). Rank similarity (Kendall's tau) tests found the rankings from reduced feature spaces to be similar to the ranking using all features for stress male, stress female, witness male, and witness female mice (Figure 6B–E, bottom). These results show that social index score distributions are stable even as individual differences are exhibited across OSS phases.

DISCUSSION

Inbred Male and Female Mice Acquire Social Self-Administration and Exhibit Social Reward Seeking

Both male and female inbred C57BL/6J mice acquired social self-administration using a trial-based procedure reinforced by freely moving physical social interaction, exhibiting social reward seeking under nonreinforced extinction conditions. Similar operant procedures have been used to study aggression reward for nearly 2 decades (46), and we have used a similar procedure in male CD-1 (22,47) and female CFW (48) outbred mice to examine behavioral, cell-type, and circuit-specific mechanisms of aggression reward seeking.

Recent work adapted operant aggression self-administration procedures to assess nonaggressive affiliative social reward, using barrier-based operant procedures that

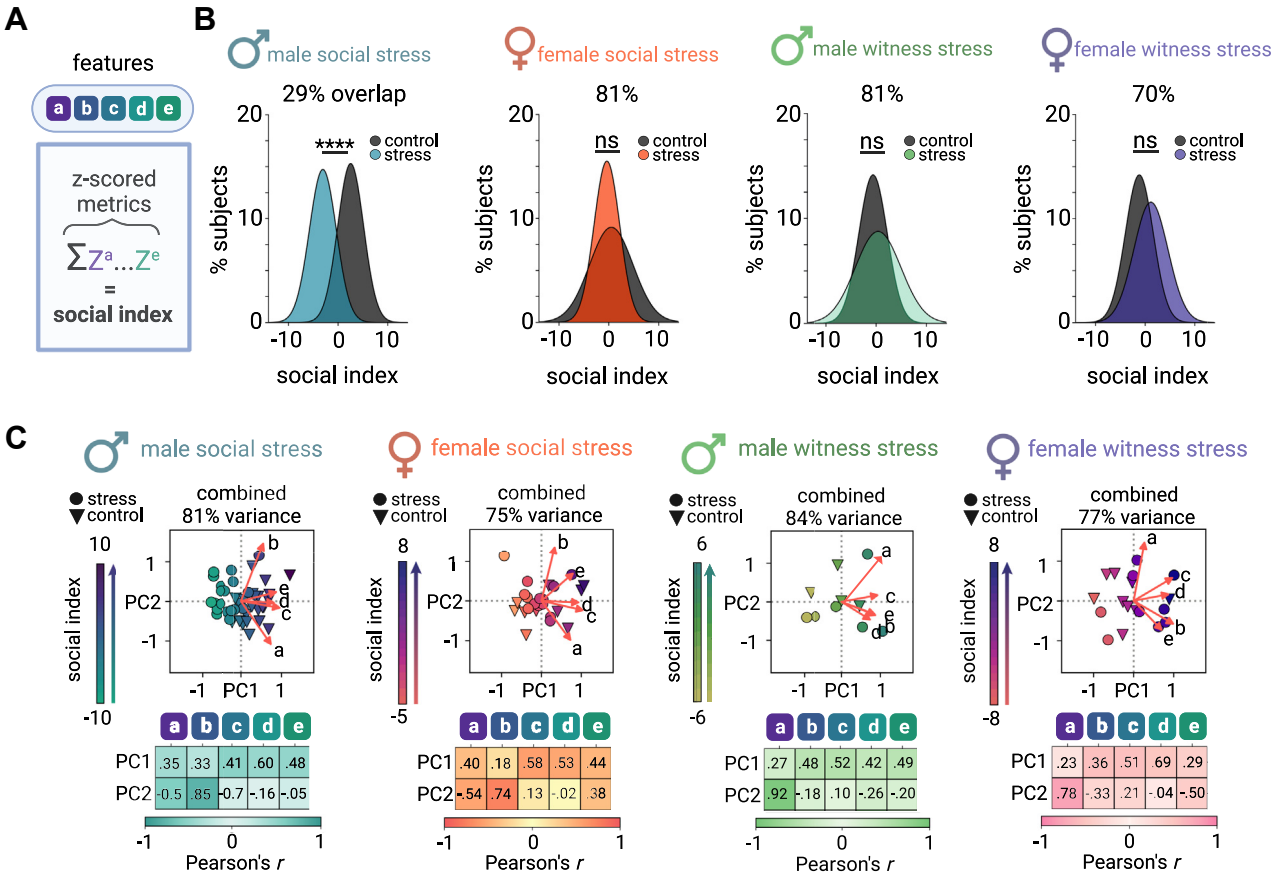

Figure 5. Social stress-modulated indexing of social reward behavior. **(A)** Social index score calculation. The 5 *z*-scored metrics used (Z^a–Z^e) for clustering analysis were aggregated per animal to obtain a cumulative sum of their features (social index). **(B)** Social index distributions for social stress males (left) (n = 44), social stress females (middle) (n = 24), witness stress males (middle) (n = 10), and witness stress females (right) (n = 18). **(C)** (Left) Biplot of PC analysis for features (top) and each feature's correlation with PC1 and PC2 (bottom) from social stress cohort males, social stress cohort females, witness stress cohort males, and witness stress cohort females. ****$p < .0001$. ns, nonsignificant; PC, principal component.

provide sensory access to a social partner. These studies have demonstrated that male C57BL/6J mice self-administer for sensory access to juvenile male social partners (36–38,49), and outbred female CD-1 but not inbred female C57BL/6J mice self-administer for sensory access to age-matched social partners (37). Our data show that both male and female inbred C57BL/6J mice self-administer familiar age-matched affiliative social partners when reinforced with freely moving social interaction. This may be because physical social interaction is more rewarding than sensory contact in mice (50). Similar mechanisms may modulate volitional social reward seeking, but comparisons with sensory contact have yet to be published and should be an area of future investigations.

Notably, the pragmatic tradeoffs when using sensory versus physical social self-administration create interpretive difficulties in understanding the motivation for performing the operant action. In the current work, several male mice were excluded during social self-administration training for displaying aggressive behaviors, without observation of these physical interactions, and their inclusion may have led to erroneous interpretation of affiliative social motivation.

Stress-Induced Modulation of Volitional Social Motivation in Male and Female Mice

Stress resilience and susceptibility in rodents are associated with neurophysiological changes in the mesolimbic dopaminergic reward system (51–56). However, this mechanistic understanding is constrained by pragmatic procedural considerations. First, social stress models typically use involuntary social interactions as the key metric of stress susceptibility or resilience, while downplaying the volitional aspect of social motivation in response to stress. Second, these procedures also stratify mice based on passive social exploration during standard open field–based social interaction tests, thus failing to capture the multidimensionality of individual variability. As an alternative, the OSS procedure provides a social self-administration procedure that directly evaluates changes in voluntary social reward-seeking behavior.

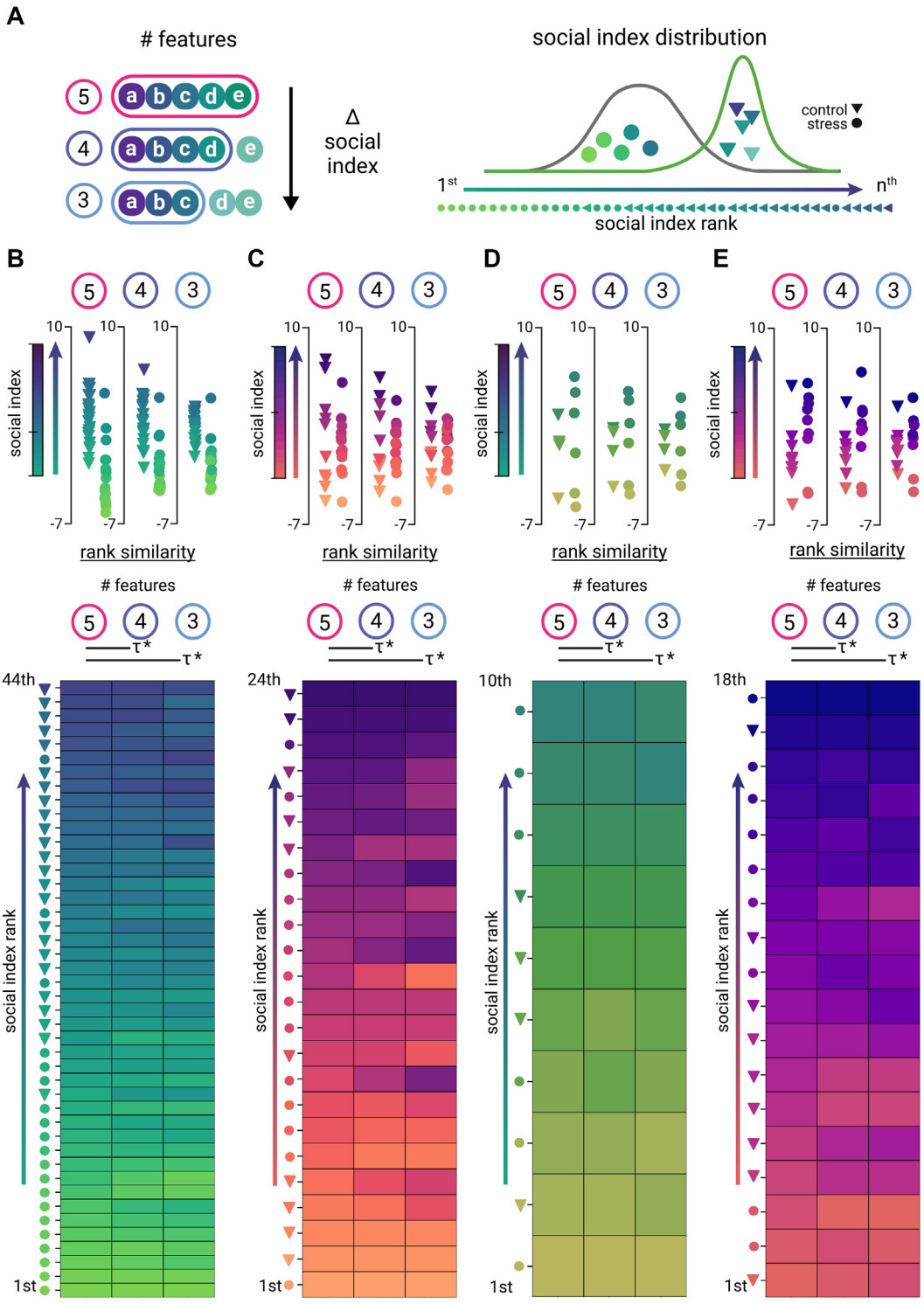

Figure 6. Stability of social index ranking. **(A)** (Left panel) Stability test schematic. To determine how well earlier features can predict the outcome of operant social stress, the social index for each animal was calculated without progressive ratio (4 features, purple) and without progressive ratio or poststress reward seeking (3 features, light blue) and compared against the social index obtained using all 5 features (pink). (Right panel) Social index rank distribution schematic. For rank comparisons, for each social index, calculated mice were ranked by their score from lowest (first) to highest (*n*th). **(B–E)** Distributions of social index scores (top panel) using 5, 4, or 3 operant social stress features and heatmaps (bottom panel) depicting the ordered ranking of social index scores (rows: controls, triangles; stress, circles) as a function of operant social stress features (columns), separately for social stress cohort males (left panel), social stress cohort females (middle panel), witness stress cohort males (middle panel), and witness stress cohort females (right panel). Kendall's τ tests were used to compare ranking similarity between 5 vs. 4 and 5 vs. 3 feature selection conditions. *$p < .0001$.

In this study, we found that socially defeated male mice exhibited volitional social avoidance as determined by attenuated social self-administration, which is consistent with general observations from other social-defeat stress models that have used standard open field social interaction testing (24,25,28,56). However, our witness males did not exhibit any changes in social reward seeking and motivation, which contradicts results of previous studies of witness-defeat stress using standard social interaction testing (29,30,35). Unlike male mice, our witness-defeat females exhibited elevated social reward seeking and motivation after stress, which is different from recent findings that females display decreased general sociability (57–59). These data parallel female witness-defeat results in which signs of heightened arousal (indicated by anxiogenic behavior, corticosterone levels in serum, proinflammatory markers) suggest a hypervigilance state (30,35). Such hypervigilance has also been reported in females following social-defeat stress during standard social interaction testing (60,61), which we did not observe in our social-defeat females using operant metrics.

Our results, particularly those of our witness males and both social-defeat and witness-defeat females, differ from previous reports of social avoidance (30,60), likely due to the involuntary versus voluntary nature of the social interaction tests and potential sex-specific stress-related adaptations such as social buffering (62,63). Specifically, the previously unreported differences between witness-defeat and social-defeat females may arise from impaired threat processing due to sensory perception of a threat but a lack of subsequent danger, which creates a continued anticipatory state of threat for which coping mechanisms may differ from that of chronic defeat (63,64). Moreover, females experiencing witness stress may also be compensating for perceived threats, the effects of which are further amplified by fear contagion or socially sensed threats coming from the defeated males (65,66), which results in increased social reward seeking and motivation. An attractive, but untested, alternative hypothesis is that control of volitional social interactions acts as a protective behavioral mechanism against stress in female, but not male, mice. These competing hypotheses should be the focus of future experiments.

In short, our OSS procedure allows comparisons of stressed mice to same-sex controls, revealing unexpected differences in social motivation and reward seeking between stress defeat types within sexes. We designed OSS to be modular; future iterations will allow for variations of social-defeat stress and witness-defeat stress (28,29,60,61,67–70) to be included to further allow for neurophysiological comparisons across sexes.

Individual Variability in Social Reward Seeking Following Stress

We demonstrated that operant features allowed for the identification of individual differences in volitional social motivation following stress, further incorporating volitional behavior as an end point for the widely used social-defeat and witness-defeat stress models in male and female mice, respectively. Using an unsupervised Gaussian mixture model selection approach on a subset of OSS features, we found that one cluster was the most optimal for grouping the data, contrary to our previous findings with aggression reward-seeking mice (22). Our hierarchical clustering analysis did not identify discrete subpopulations of social reward seeking among mice following stress. Rather, this analysis yielded clusters that discern distributions in some features but not others, thus failing to describe the breadth of behavioral differences that we observed in male and female mice. Instead, we used a method with minimal data transformation to summarize each animal's social motivation and reward-seeking behavior, while preserving the diversity of behavioral expression found across features (70,71). We also showed that the social index score relates meaningfully back to its individual OSS components and that its distribution across all mice accurately describes the spectrum of individual variability. The longitudinal tracking of operant responses before, during, and after social stress exposure also revealed the importance of social stress exposure in eliciting meaningful differences in social reward behavior.

Methodological and Experimental Considerations

We designed our OSS procedure to address the volitional aspects of social motivation and social reward, with an emphasis on tracking temporal changes in social motivation before, during, and after stress. Therefore, there are important methodological considerations. First, during the training phase, ~70% of mice acquire social self-administration, rendering the remaining 30% as nonacquirers (Figure 2B, C). While the neurobiological basis of nonacquisition may be of interest, especially for studies examining social deficits that are not attributed to stress-induced changes, it is beyond the scope of this study because our focus is on volitional social motivation following social stress. Another important facet of social motivation that we consider is that of social preference. We observed that fewer mice acquired social self-administration with a familiar partner than with a novel partner, replicating previous findings (72). Acquisition was also influenced by transference of social cues, wherein the (experienced) partners demonstrated higher social self-administration of their flipped-role (naïve) counterparts. This increase in social self-administration suggests a joint learning experience, via exchange of social information throughout repeated encounters, wherein experienced mice adopt social behavior exhibited by their naïve counterparts (73,74). The experienced social partners can be used in subsequent experiments as experimental animals given that they learn to acquire the social self-administration behavior more rapidly, thus supporting the 3 Rs (replacement, reduction, and refinement) of animal research.

Similarly, social transference plays an important role in behavioral responses attributed to stress exposure. OSS modifies standard social-defeat housing conditions where mice undergoing social or witness stress are now pair-housed with their same-sex conspecifics in home cages separate from their aggressors to avoid potential social contagion of fear, threat, or pain, which can affect mice witnessing agonistic interactions (65,66) and influence behavioral outcomes. Another potential housing caveat related to experimental differences in social-defeat procedures is the use of corncob bedding, which

has known estrogenic effects on social behavior and aggression in rodents (75,76). For this reason, all our mice were housed with woodchip bedding for life, including the entire duration of the experimental timeline.

Finally, further considerations for social-defeat and witness-defeat procedures, and others used to assess stress-related neuropsychiatric disorders, include pharmacological and neural manipulations to temper stress-related social behavior outcomes. Pharmacological agents used to attenuate depression-like and anxiety-like behaviors, such as fluoxetine and ketamine, reverse stress induced social avoidance and anhedonia (30,35,77). Future experiments that incorporate these manipulations will be critical to extending the utility of our OSS procedure as a preclinical animal model for stress-related neuropsychiatric disorders.

Conclusions

We have extended the use of social-defeat and witness-defeat stress to include operant metrics of social motivation and sex as a biological variable to introduce social index as an approach to describing individual differences. By shifting the focus to volitional behavior, the inclusion of females in social- and witness-defeat stress has raised new implications for research on stress-induced regulation of social behavior. Given the choice to socialize or not, female mice displayed different coping strategies than males, showing persistent or even higher motivation to pursue social interaction while the latter exhibited social avoidance consistent with previous findings. Our results demonstrate that including voluntary behavior engages a wider variety of innate and stress-mediated behaviors, which is crucial for understanding how phenotypes of stress susceptibility may develop, while also emphasizing the mitigating and protective effects of social buffering on stress. These data conceptually align with the social processes domain of the Research Domain Criteria system (78) that states that pathology presents as a spectrum, which is reflected in the social index distributions derived from the behavior we observed. In closing, we have provided a new procedure that may be used for the identification of neural mechanisms underlying individual differences and changes in social motivation following social stress exposure.

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JN, KNS, BMS, NLG, and SAG designed the experiments; JN, KNS, BMS, NLG, YYZ, ASS, YEG, PA, EG, and VST ran the behavioral experiments and collected data; JN, KNS, and BMS analyzed behavioral data; KNS and SAG established analysis pipelines; JN, KNS, and SAG wrote the paper. All authors reviewed and approved the final version of the manuscript prior to submission.

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