Priority Communication

Biological Psychiatry: Celebrating 50 Years

Fighting Females: Neural and Behavioral Consequences of Social Defeat Stress in Female Mice

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ABSTRACT

BACKGROUND: Despite the twofold higher prevalence of major depressive and posttraumatic stress disorders in women compared with men, most clinical and preclinical studies have focused on male subjects. We used an ethological murine model to study several cardinal symptoms of affective disorders in the female targets of female aggression.

METHODS: Intact Swiss Webster (CFW) female resident mice were housed with castrated male mice and tested for aggression toward female intruders. For 10 days, aggressive CFW female residents defeated C57BL/6J (B6) female intruders during 5-minute encounters. Measures of corticosterone, c-Fos activation in hypothalamic and limbic structures, and species-typical behaviors were collected from defeated and control females. Ketamine (20 mg/kg) was tested for its potential to reverse stress-induced social deficits.

RESULTS: Housed with a castrated male mouse, most intact resident CFW females readily attacked unfamiliar B6 female intruders, inflicting $>$40 bites in a 5-minute encounter. Compared with controls, defeated B6 females exhibited elevated plasma corticosterone and increased c-Fos activation in the medial amygdala, ventral lateral septum, ventromedial hypothalamus, and hypothalamic paraventricular nucleus. Chronically defeated females also showed vigilance-like behavior and deficits in social interactions, novel object investigation, and nesting. The duration of social interactions increased 24 hours after chronically defeated female mice received a systemic dose of ketamine.

CONCLUSIONS: These findings demonstrate that CFW female mice living with male conspecifics can be used as aggressive residents in an ethological model of female social defeat stress. These novel behavioral methods will encourage further studies of sex-specific neural, physiological, and behavioral adaptations to chronic stress and the biological bases for interfemale aggression.

Keywords: Affective disorders, Aggression, Female, Ketamine, Social defeat stress, Social interaction

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The prevalence of affective disorders, including major depressive disorder and posttraumatic stress disorder, is twofold higher in women than in men, and sex can shape the trajectory of these disorders in terms of their onset, duration, and rate of recurrence (1–4). Despite substantial evidence for sex differences in the development, course, and biological underpinnings of affective disorders (5,6), most clinical and preclinical studies focus on male subjects. In preclinical research, the murine chronic social defeat stress protocol employs species-typical male aggression toward submissive conspecifics to induce several cardinal symptoms of stress-related affective disorders in a subset of defeated male mice (7–14). This protocol is highly reproducible between and within laboratories, suggesting that the translational effects of chronic social stress are both robust and reliable. In addition, long-term treatment with tricyclic antidepressants or serotonin reuptake inhibitors or a single ketamine administration can normalize defeat-associated social deficits and stress-induced molecular adaptations in male mice (9,13,15–18), making this protocol a valuable tool for investigating novel drugs and their mechanisms of action.

In developing a female model of social defeat stress that parallels the existing male protocol, substantial efforts have been made to foster female-directed male aggression by using male odorants transferred onto female mice (19) and through chemogenetic stimulation of the male ventromedial hypothalamus (VMH) (20). A vicarious social defeat stress model in which female mice witness intermale aggression (21) has also been employed. To provide an alternative method that closely mirrors the male chronic social defeat protocol and eliminates the need to generate atypical patterns of male aggression, we identify specific conditions that promote aggression in outbred female mice toward female opponents.

SEE COMMENTARY ON PAGE e31; SEE ALSO VIDEO CONTENT ONLINE

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Whereas male mice are aggressive under diverse experimental conditions (22), female aggression is most often studied either during pregnancy [(23–27), but see (28)] or during the first postpartum week while neonatal pups are suckling (29–31). As an adaptive defensive behavior, dams will engage in maternal aggression to prevent postpartum fertilization and to protect their offspring against unfamiliar male or female intruders (29,31–33). Outside of these brief gestational and postpartum windows, female mice are minimally aggressive when subjected to isolation housing (34), a technique often used to induce territorial aggression in outbred male mice (35,36), in female California mice (*Peromyscus californicus*) (37), and in female Syrian golden hamsters (*Mesocricetus auratus*) (38,39). In contrast, we report that most intact female mice (*Mus musculus*) housed with an intact or castrated male mouse will engage in intense aggression when confronted by a female opponent; although isolation-induced territorial competition may not promote interfemale agonistic behavior, a significant subset of female mice will readily fight a rival female intruder, possibly in competition for an available mate.

Upon identifying specific conditions to engender interfemale aggression, studies were conducted to 1) generate an ethological model of female chronic social defeat stress; 2) examine the effects of social defeat stress on plasma corticosterone concentrations; 3) determine if social defeat stress increases c-Fos activation in brain areas, including the medial amygdala (MeA), lateral septum, VMH, and paraventricular nucleus (PVN); 4) characterize defeat-associated deficits in species-typical social and nonsocial behaviors; and 5) increase the duration of social interactions initiated by chronically defeated female mice with acutely administered ketamine.

METHODS AND MATERIALS

See Supplement for additional details.

Animals

Twelve-week-old intact (n = 74) or ovariectomized (n = 27) Swiss Webster (CFW) female mice (Charles River Laboratories, Wilmington, MA) were housed in resident pairs with age-matched intact (n = 47) or castrated (n = 61) CFW male mice in clear polycarbonate cages (18.9 × 29.7 × 12.8 cm) lined with pine shavings. Twelve-week-old intact intruder C57BL/6J (B6) (n = 190) (Jackson Laboratories, Bar Harbor, ME) or CFW female mice (n = 40) were group-housed in cages (25.7 × 48.3 × 15.2 cm; n = 10/cage) with corncob bedding. Experimental 12-week-old B6 females were housed individually and assigned to control (n = 23), acute (n = 13), or chronic social defeat (n = 28) conditions. Animals were cared for according to the National Research Council *Guide for the Care and Use of Laboratory Animals*, and procedures were approved by the Tufts University Institutional Animal Care and Use Committee.

Aggression in Outbred Ovariectomized Female Mice

Ovariectomized CFW female mice, housed in resident pairs with intact CFW males, were evaluated for aggression in modified resident-intruder confrontations every other day starting 2 weeks after pair-housing (36). To test the effect of intruder strain and familiarity, male mice were removed and female intruders were introduced to resident home cages for 2-minute resident-intruder confrontations (Supplemental Figure S2). Attack latency and attack bite frequency were recorded. Following confrontations, female intruders were removed and male mice were returned to their resident home cages.

Aggression in Intact Outbred Female Mice

Intact female CFW residents, housed with intact male CFWs, were evaluated for aggression toward unfamiliar B6 female intruders. CFW litters were culled on postnatal day 1, at which time CFW female residents were housed singly. After the first postpartum week, there was a substantial decrease in interfemale aggression. To address whether this reduction was related to isolation housing, female residents were housed with castrated male mice, and aggressive confrontations continued 3 days later. After 5 days of living with castrated male mice, most female residents attacked unfamiliar female B6 intruders. Male cohabitation–induced interfemale aggression is referred to as rival aggression to distinguish it from maternal or gestational aggression. In subsequent groups, intact nulliparous CFW female mice were housed exclusively with castrated male CFWs in resident pairs. A significant subset of these female residents were highly aggressive toward unfamiliar female B6 intruders (n = 39 of 61; $>$15 bites/2-min confrontation); these residents were used as aggressors during the chronic social defeat protocol.

Sex-Specific Patterns of Aggression

Ten 5-minute resident-intruder confrontations between intact CFW female residents and unfamiliar B6 female intruders were videotaped in the resident home cage for detailed behavioral analyses (Supplemental Video S1). Similar archival videos of intermale confrontations were analyzed to compare the behavioral composition of aggressive encounters in male versus female mice (Supplemental Video S2).

Testing Aggressive Potential of Female Mice That Do Not Display Rival Aggression

To test whether pregnancy-induced aggression was distinct from rival aggression, consistently nonaggressive female residents that were pair-housed with castrated male mice (n = 7) for at least 2 months were assessed for gestational aggression. These female mice were housed with intact male CFWs for 3 days, then returned to their original castrated male partners. Aggression was evaluated every 2 or 4 days during 2-minute resident-intruder confrontations. Pups were culled on postnatal day 1, and female residents were tested for aggression 4 and 7 days postpartum.

10-Day Female Chronic Social Defeat Stress

Highly aggressive female CFWs were used as resident stimulus animals for the chronic social defeat stress protocol. Two days before the initial defeat episode, resident CFW pairs composed of intact female and castrated male mice were transferred to large polycarbonate cages (25.7 × 48.3 × 15.2 cm) divided in half by perforated, clear polycarbonate partitions [compare with (8,14)]. One day before defeats, female residents were tested for aggression to ensure behavioral reliability under the new housing conditions.

Daily 5-minute defeat episodes occurred in the large divided cages (Supplemental Video S3); male mice were temporarily removed, and experimental female B6 intruders were exposed to unfamiliar aggressive female residents. Following defeats, female B6 mice were housed opposite the female CFW residents that defeated them, and male mice were returned to be pair-housed with CFW female mice. During this 24-hour threat period, cage dividers permitted sensory contact between CFW resident pairs and experimental B6 mice but protected experimental mice from attack. For 10 consecutive days, B6 intruders were defeated by and rehoused adjacent to unfamiliar, aggressive CFW residents. Nondefeated, control B6 females were housed opposite unfamiliar resident CFW pairs daily, but were never physically attacked. Acutely defeated B6 experimental mice were treated as controls until the 10th day, when they were defeated once. Control and defeated mice were weighed every other day and singly housed after defeats concluded on the 10th day. During the 10-day protocol, resident CFW pairs received approximately 15 g of fresh pine shavings every other day, and cages were cleaned on the 5th day following the defeat. Reliably aggressive female CFW residents (i.e., >40 bites/5-min confrontation or >15 bites/2-min confrontation) were used in chronic social defeat stress experiments for 6 to 12 months.

Estrous Cycling

Vaginal cytology was monitored in experimental mice during the 10-day social defeat stress protocol using the lavage technique (40). Cyclicity was also evaluated in a subset of highly aggressive nulliparous resident CFWs (n = 23) housed with castrated male mice to determine if aggression varied according to estrous cycle phase [(41–43), but see (44,45)].

Corticosterone Measurements

At 2 time points (Supplemental Figure S1A), blood was collected from the submandibular vein of experimental female mice (n = 5 control; n = 5 acute defeat; n = 10 chronic defeat) using sterile 4-mm lancets (Goldenrod Animal Lancet; Medipoint, Inc., Mineola, NY) and centrifuged at 4°C at 3000 rpm for 10 minutes. Plasma (15 μL) was collected and stored at -80°C for corticosterone enzyme immunoassay (Arbor Assays, Ann Arbor, MI); standards (7.8125–1000 ng/mL) and samples were run in duplicate.

Tissue Collection and c-Fos Immunohistochemistry

On the 10th day of chronic social defeat stress, brains were collected from female B6 experimental animals after no defeat or a 5-minute social defeat stress episode followed by an hour-long threat period (Supplemental Figure S1B). Brain slices (50-μm) containing the anteromedial bed nucleus of the stria terminalis, ventral lateral septum (LSv), hypothalamic PVN (hPVN), periventricular nucleus, VMH, MeA, and dentate gyrus were selected for c-Fos immunohistochemistry.

Open Field Social Interaction

After a 2.5-minute habituation period in the social interaction apparatus (84 × 29 × 36 cm) containing an empty wire mesh stimulus cage (11-cm height, 10.5-cm diameter), control and defeated mice were briefly removed, and an unfamiliar, aggressive CFW female resident was placed in the stimulus cage. Experimental B6 mice were returned to the apparatus and evaluated for social interactions during a 2.5-minute test. Social interaction time was defined as the duration spent within a social interaction zone extending 2.25 cm past the radius of the stimulus cage. Social interaction videos (EthoVision XT version 14; Noldus Information Technology Inc., Leesburg, VA) were scored manually for vigilance-like behavior, defined as time spent oriented toward, but not interacting with, the stimulus animal [compare with (46)]. The open field and stimulus cage were cleaned and dried between mice.

Novel Object Investigation

Control and defeated female mice were briefly moved to clean holding cages while 4 rubber stoppers (Size No. 5; Thermo Fisher Scientific, Waltham, MA) were placed in their home cages. Mice were returned to their home cages for a 5-minute test. Rectal temperatures were collected immediately before and after tests using a temperature probe (2100 Tele-Thermometer; YSI, Inc., Yellow Springs, OH) lubricated with mineral oil.

Home Cage Social Interactions and Ketamine Administration

Social contact with a nonaggressive, group-housed B6 female stimulus animal was evaluated during 1.5-minute tests conducted in the home cage. Contact was defined as the duration of social investigation initiated by the experimental female mouse. Alternatively, home cage social interaction tests were conducted using anesthetized stimulus female mice (Supplemental Figure S1A). Rectal temperatures were collected from experimental female mice immediately before and after testing.

Chronically defeated and control female mice received intraperitoneal injections of 0.9% sodium chloride or the *N*-methyl-D-aspartate receptor antagonist ketamine hydrochloride diluted in 0.9% sodium chloride (20 mg/kg; Vedco Inc., St Joseph, MO) (17,21,47,48). Social interactions were evaluated 30 minutes, 24 hours, and 5 days after injection.

Nesting

Female mice received 2 g of nesting material (Nestlets; Ancare Corp., Bellmore, NY) at 1330 hours in the home cage. Five days later, nests were scored on a scale of 1 to 5 (49), nest heights and diameters were recorded, and nest images were evaluated for shape (i.e., circularity) in ImageJ (National Institute of Mental Health, Bethesda, MD).

RESULTS

Interfemale Rival Aggression

Most intact female CFW residents were aggressive when housed with a male mouse, but not when housed in isolation (Figure 1A) or following ovariectomy (Supplemental Figure S2). By the third aggressive confrontation, >90% of female residents that were housed with intact male CFW mice expressed gestational aggression (mean ± SEM attack bites = 26.62 ±

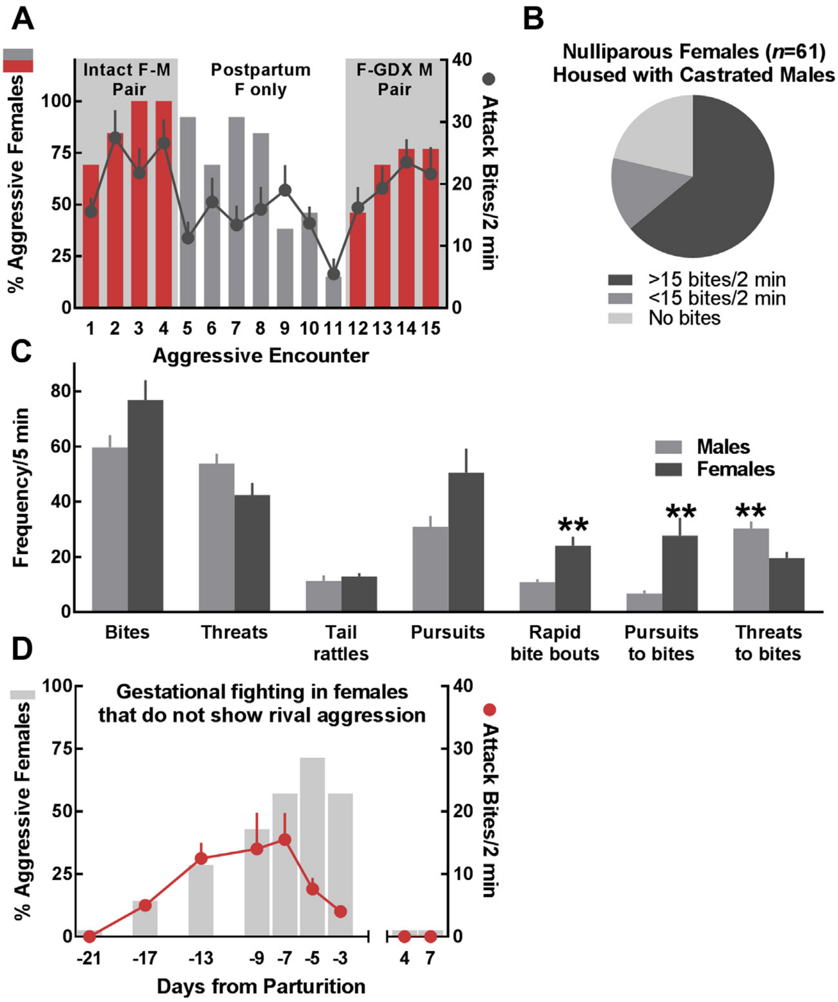

Figure 1. Most **(A)** multiparous and **(B)** nulliparous outbred female mice living with intact or gonadectomized (GDX), i.e., castrated, male mice developed aggression toward unfamiliar C57BL/6J female intruders. **(C)** Male and female attack bite frequencies were comparable, but resident female mice (n = 10) displayed more sequential bites (rapid bite bouts; t_{18} = 3.85), and their bites were more frequently preceded by pursuits (pursuits to bites; t_{18} = 3.20), whereas male mice (n = 10) exhibited more bites preceded by sideways threats (threats to bites; t_{18} = 3.14); data are shown as mean ± SEM; $^{**}p < .01$ male vs. female. **(D)** Most nonaggressive nulliparous female mice displayed pregnancy-induced aggression that was time-locked with the gestational period. **(A, D)** Left axes denote the percentage of animals that were aggressive, and right axes depict attack bite frequencies as mean ± SEM, calculated from female mice that fought. F, female; M, male.

3.83). However, by the 7th day after litters were culled and intact male mice were removed, <20% of female residents were aggressive, and mice that did express aggression showed substantially reduced attack bite frequencies (mean ± SEM attack bites = 5.5 ± 2.5). Five days after being rehoused with castrated CFWs, most (75%) female residents fought (mean ± SEM attack bites = 21.6 ± 4.34). Similarly, most intact nulliparous CFW residents housed exclusively with castrated male mice fought, and a significant subset (65%) emerged as highly aggressive toward unfamiliar female B6 intruders (Figure 1B).

Attack bite frequencies were similar between resident female and male aggressors (Figure 1C). During confrontations with an unfamiliar intruder, female residents exhibited more rearing behavior (Supplemental Figure S4) (50) and rapid bouts of consecutive bites that were often preceded by pursuits (Figure 1C and Supplemental Video S1). In contrast, male attacks were often preceded by sideways threats (Supplemental Video S2). Estrous cycle phase was determined in highly aggressive female residents (n = 23). Though an effect of phase on aggression was not apparent (Supplemental Figure S3), this could be explained by a ceiling effect and low variability in attack bite frequencies among aggressive female mice.

A subset of nulliparous residents housed with castrated male mice was consistently nonaggressive toward female intruders (n = 13 of 61). Half of these residents (n = 7) were tested for their sensitivity to the pro-aggressive effects of pregnancy. By late pregnancy, 75% of formerly nonaggressive female mice attacked an unfamiliar female intruder; however, after pups were culled on postnatal day 1, these residents returned to their nonaggressive, prepregnancy baselines (Figure 1D).

Neural and Physiological Effects of Chronic Female Social Defeat Stress

Acute or 10-day social defeat stress (Figure 2A and Supplemental Video S3) followed by a threat period increased circulating corticosterone more than the threat period alone (Figure 2B). Similar corticosterone concentrations in acutely and chronically defeated individuals suggest that B6 female intruders do not habituate to social defeat stress, much like acutely and repeatedly defeated outbred male intruders (51).

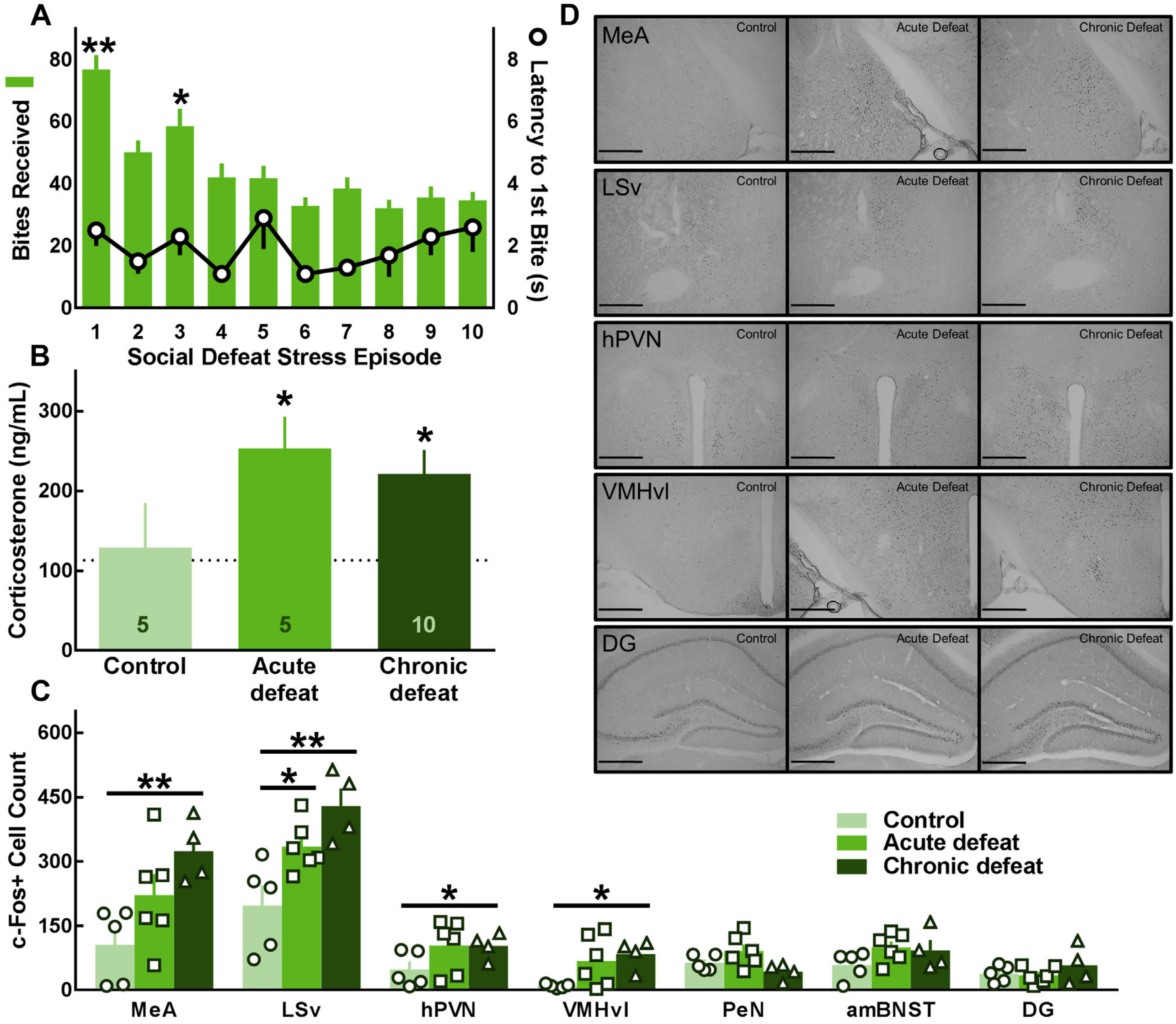

Figure 2. Female C57BL/6J mice were defeated by aggressive female CFW residents for 10 consecutive days. **(A)** Attack latencies were <5 seconds (right axis) and the greatest number of attacks were delivered early in the defeat protocol (left axis) ($F_{9,190} = 13.03$, $p < .0001$); *$p < .05$, **$p < .0001$ compared with day 5. **(B)** Elevated concentrations of plasma corticosterone were detected after acute or chronic social defeat stress (time: $F_{1,17} = 9.6$, $p = .007$; defeat: $F_{2,17} = 4.2$, $p = .033$); *$p < .05$ compared with control mice. **(C)** Social defeat stress increased c-Fos activation in the medial amygdala (MeA) ($F_{2,12} = 6.43$, $p = .013$), ventral lateral septum (LSv) ($F_{2,12} = 9.28$, $p = .004$), hypothalamic paraventricular nucleus (hPVN) ($F_{2,12} = 4.48$, $p = .035$), and ventrolateral division of the ventromedial hypothalamus (VMHvl) ($F_{2,12} = 4.17$, $p = .042$); *$p < .05$, **$p < .01$ compared with control mice. **(D)** Representative images of c-Fos in the MeA, LSv, hPVN, VMHvl, and dentate gyrus (DG). Scale bars = 200 μm. Data are shown as mean ± SEM. amBNST, anteromedial bed nucleus of the stria terminalis; PeN, periventricular nucleus.

However, neither estrous cycle nor body weight was significantly affected by chronic social defeat (Supplemental Figure S5A–E).

Compared with the control condition, acute or chronic social defeat stress significantly increased c-Fos activation in the LSv, whereas only chronic social defeat significantly increased the number of c-Fos$^+$ cells in the MeA, hPVN, and ventrolateral division of the VMH (VMHvl) (Figure 2C, D). Exploratory correlational analyses revealed patterns of interregional c-Fos activation. Specifically, there was a positive relationship between c-Fos$^+$ cell counts in the MeA and anteromedial bed nucleus of the stria terminalis in control mice, whereas an inverse correlation was observed in defeated mice (Supplemental Table S1). Female mice subjected to chronic social defeat also exhibited a unique pattern of inverse correlations in c-Fos in the periventricular nucleus and anteromedial bed nucleus of the stria terminalis or dentate gyrus.

Behavioral Effects of Chronic Social Defeat Stress in Female Mice

During social interactions in the home cage (Figure 3E) with a nonaggressive female B6 stimulus animal, chronically defeated mice displayed a greater number of defensive kicks and

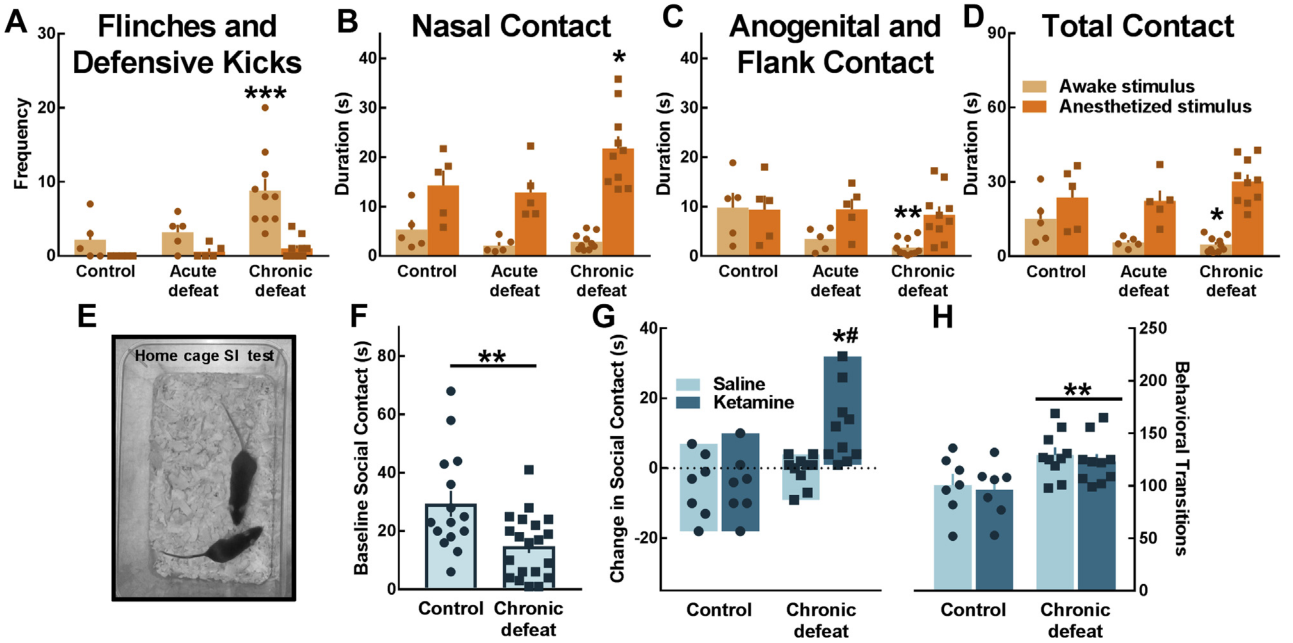

Figure 3. Chronically defeated female mice exhibited substantial social contact deficits, which were improved with ketamine, 24 hours after injection. **(A)** Defensive flinches and kicks were observed in chronically defeated female mice during social interactions with an awake conspecific (light bars) (stimulus × defeat interaction: $F_{2,17} = 3.67$, $p = .047$; stimulus: $F_{1,17} = 15.3$, $p = .001$; defeat: $F_{2,17} = 7.73$, $p = .004$). **(B–D)** Although chronically defeated mice engaged in more nasal contact with anesthetized stimulus animals (dark bars) (stimulus × defeat interaction: $F_{2,17} = 3.8$, $p = .043$; stimulus: $F_{1,17} = 54.53$, $p < .0001$), they displayed significantly less anogenital/flank contact (stimulus × defeat interaction: $F_{2,17} = 3.87$, $p = .041$; stimulus: $F_{1,17} = 13.67$, $p = .002$) and total contact (stimulus × defeat interaction: $F_{2,17} = 6.18$, $p = .0096$; stimulus: $F_{1,17} = 65.11$, $p < .0001$) with awake stimulus mice compared with control animals. **(E, F)** Baseline total social contact with an awake stimulus female mouse in the home cage was suppressed in chronically defeated mice ($t_{33} = 3.056$, $p = .0044$). **(G)** Ketamine (20 mg/kg) significantly increased social contact in chronically defeated female mice 24 hours after injection (drug × defeat interaction: $F_{1,30} = 4.26$, $p = .0478$; drug: $F_{1,30} = 4.26$, $p = .0478$; defeat: $F_{1,30} = 12.52$, $p = .0013$), **(H)** but did not reverse the high rate of behavioral transitions during social interaction tests (defeat: $F_{1,30} = 11.00$, $p = .0024$). **(A–D, F, H)** Data are shown as mean ± SEM. **(G)** Bars depict the maximum and minimum values. The dotted line marks no change in social contact between baseline and postinjection tests; values above the dotted line are increases from baseline, whereas values below the dotted line are decreases from baseline social contact time. Circles and squares represent individuals. $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$ **(A, C, D)** compared with control mice interacting with an awake social stimulus female mouse, **(B)** compared with control mice interacting with an anesthetized social stimulus female mouse, **(F, H)** compared with control mice, or **(G)** compared with ketamine-treated control mice; $^{\#}p < .05$, compared with saline-treated chronically defeated female mice. SI, social interaction.

flinches compared with control animals (Figure 3A) along with deficits in both anogenital/flank and total social contact (Figure 3C, D). When an anesthetized social stimulus mouse was placed in the home cage, chronically defeated female mice actually engaged in more nasal contact compared with animals in the control group (Figure 3B). Social investigation in the home cage also produced a substantial hyperthermic response, which was greater in chronically defeated versus control mice (Figure 4A); in contrast, there was no group difference in hyperthermia induced by novel object investigation (Figure 4B).

During pretreatment baseline home cage social interaction tests, social contact (i.e., nonaggressive social interaction initiated by the experimental female mouse) (Figure 3E) was significantly shorter among defeated animals (Figure 3F). Twenty-four hours after receiving a dose of ketamine (20 mg/kg), defeated female mice exhibited a significant increase from pretreatment social interactions compared with ketamine-treated control mice and saline-treated defeated mice (Figure 3G and Supplemental Videos S4–S7). This effect of ketamine was not evident 30 minutes or 5 days after injection (Supplemental Figure S6). Importantly, although ketamine increased social contact duration in animals subjected to chronic defeat, a defeat-associated increase in behavioral transitions during social interactions persisted (Figure 3H).

Experimental mice were also examined in an open field social interaction test (Figure 5C), which is often employed to identify depressive-like phenotypes in chronically defeated male mice (8–10). Female mice exposed to chronic defeat exhibited significantly more vigilance-like behavior compared with animals in the control condition (Figure 5A, C, D and Supplemental Video S8), though the duration of time spent in a predefined social interaction zone was comparable between groups (Figure 5B). Correlational analyses of chronically defeated individuals revealed a significant inverse relationship between time investigating a social partner in the open field and vigilance-like behavior (Supplemental Table S2).

In terms of nonsocial behaviors, chronically defeated animals constructed nests that were significantly less developed than control mice as illustrated by measures of nest peak diameter, height, circularity, and overall nest score (Figure 6A–E). Importantly, there was no group difference in baseline body temperature (Figure 4), suggesting that nest-building deficits in defeated mice were probably not due to stress effects on

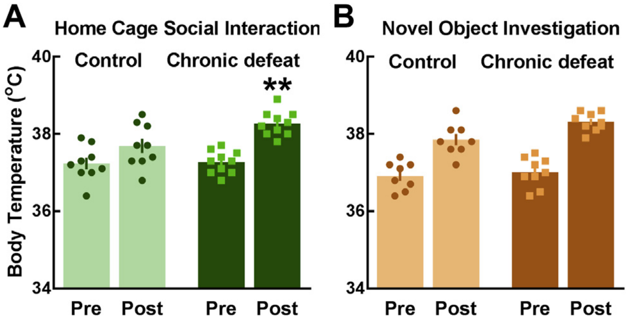

Figure 4. Hyperthermia was observed in response to 1.5-minute social interaction and 5-minute novel object investigation tests. Temperatures were measured immediately before (pre) and after (post) testing. **(A)** Chronically defeated female mice experienced a greater elevation in body temperature after social interactions compared with control animals (defeat × time interaction: $F_{1,17}$ = 5.91, p = .027; time: $F_{1,17}$ = 42.21, $p <$ .0001). **(B)** All mice showed a similar degree of hyperthermia in response to novel object investigation (time: $F_{1,15}$ = 113.6, $p <$ .0001). Data are shown as mean ± SEM; $^{**}p <$ .05, compared with control posttest temperature.

thermoregulation. A defeat phenotype was also evident when novel objects were placed in the home cage. Defeated female mice spent significantly less time investigating and exhibited a greater number of defensive startle-like behaviors (i.e., flinching and jumping) compared with control individuals (Figure 7B). In contrast, measures of general anxiety-like behavior collected during light/dark box testing were similar between control and chronically defeated mice (Supplemental Figure S8). Interestingly, a greater number of attack bites received during the 10-day chronic social defeat stress protocol predicted reduced home cage social interactions and time spent in the light chamber during light/dark box testing (Supplemental Figure S9).

DISCUSSION

We designed a novel and ethologically relevant model of chronic female social defeat stress that produces a distinct profile of neural and physiological effects along with pronounced depressive-like and anxiety-like behaviors in defeated female mice (Table 1). After 10 days of continuous social stress, female mice exhibited elevated levels of the stress hormone corticosterone and increased c-Fos activation in the MeA, LSv, VMHvl, and hPVN. In the days to weeks following social defeat, female mice engaged in atypical behaviors during novel object investigation, nest building, and social interactions. Active investigation of a nonaggressive social partner during social interactions increased in defeated ketamine-treated individuals, indicating that our model of female chronic social defeat stress produces a phenotype that is sensitive to some antidepressant compounds (52,53). Though ketamine increased social contact, defeated female mice that received drug treatment continued to exhibit atypically high rates of behavioral transitioning. Reduced behavioral stability in the presence of a nonaggressive individual may reflect a sensitized social threat response. The behavioral selectivity of ketamine raises the possibility that distinct mechanisms underlie chronic stress–induced deficits in social contact versus vigilance-related impairments. These observations should be considered and extended in future preclinical studies focusing on stress-related psychopathologies that occur at higher rates in women than in men.

Behavioral Effects of Chronic Female Social Defeat Stress

The pattern of aggressive behaviors recorded during interfemale agonistic encounters differed significantly from attack sequences during intermale fights. Considering the sophisticated exchange of multimodal sensory information between animals during ethological agonistic interactions, chronic social defeat stress procedures that rely on species-typical aggression may increase the translational potential of experimental findings. Similar to the pattern of aggression, significant features of the defeated female phenotype are distinct from the male phenotype, and in female mice, the severity of persistent

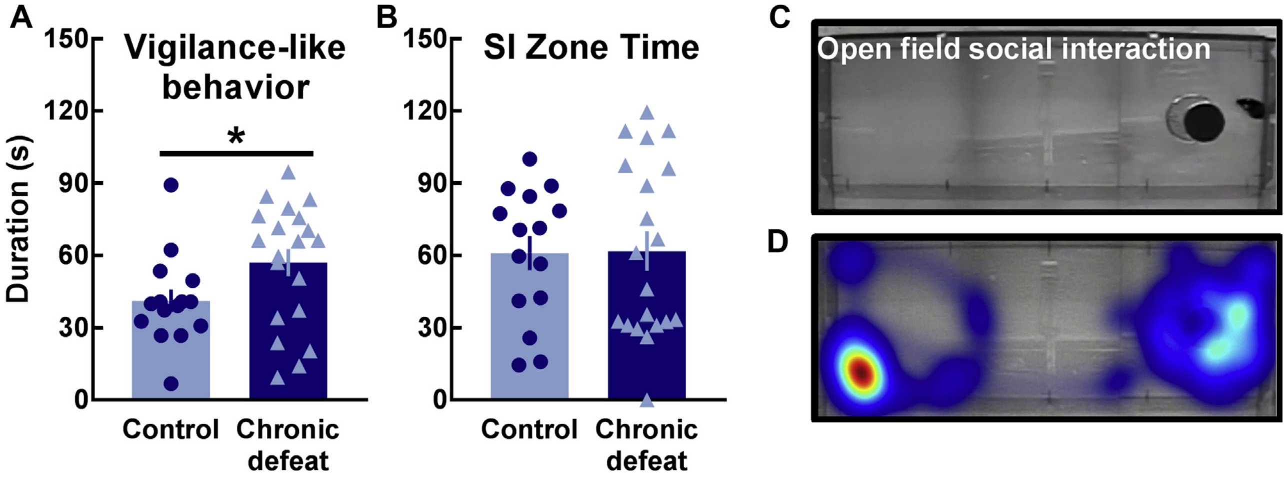

Figure 5. Chronically defeated female mice expressed more **(A)** vigilance-like behavior than control animals (t_{33} = 2.05, $^{*}p$ = .048) despite both groups spending **(B)** similar durations within the social interaction (SI) zone. **(C)** Representative image of vigilance-like behavior displayed by a chronically defeated female mouse and **(D)** the corresponding heat map of activity during the open field social interaction test.

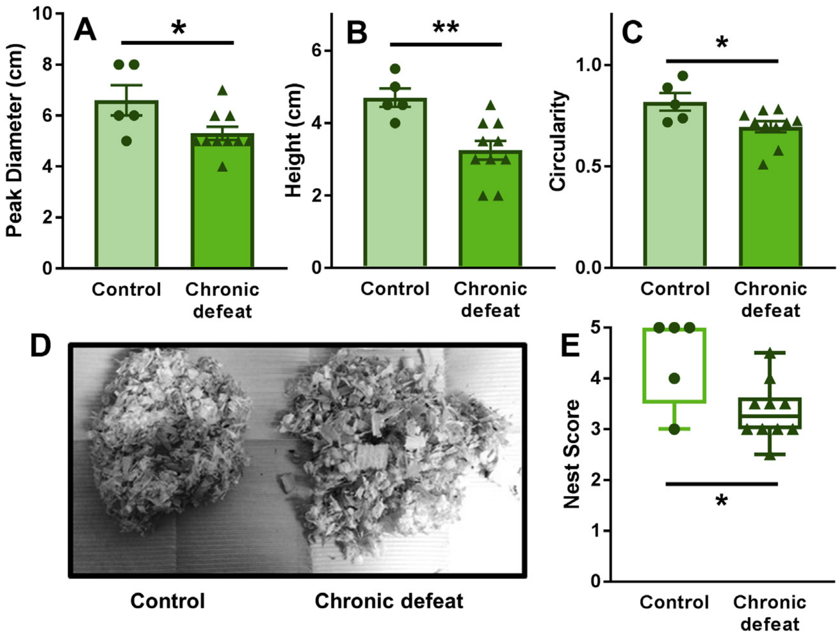

Figure 6. Nests constructed by chronically defeated female mice were underdeveloped compared with nests built by control animals, as measured by **(A)** nest peak diameter ($t_{13} = 2.347$), **(B)** nest maximal height ($t_{13} = 3.503$), **(C, D)** circularity (measured on a scale of 0–1, 1 = perfect circle) ($t_{13} = 2.515$), and **(E)** nest score (Mann-Whitney $U = 8.5$, $p = .039$). Data are shown as **(A–C)** mean ± SEM or as **(E)** maximum and minimum surrounding the median; $^*p < .05$, $^{**}p < .01$ compared with control mice.

stress-induced behavioral deficits may depend on the severity of the stress experience. These observations should encourage a sex-specific approach to evaluating the consequences of defeat in male and female mice and the development of strategies tailored to treat specific symptoms (54).

We observed a hypervigilant-like phenotype in female mice subjected to chronic stress (46,55), which may reflect an inability to distinguish threatening from nonthreatening stimuli. Defeated female animals showed exaggerated defensive behaviors, such as whole-body flinches, backwards jumps, and defensive kicks toward nonaggressive social partners. These tests occurred within a familiar, nonthreatening environment, further illustrating impairments in threat assessment. Importantly, social deficits were not readily detected when social interaction zone time, a putative indicator of depressive-like behavior in male mice (9), was used as the dependent measure. Some defeat-induced behavioral deficits manifest in a sex-specific fashion, highlighting the importance of evaluating novel potential pharmacotherapies with probes that can detect sexually dimorphic adaptations to chronic stress.

Nesting behavior was also impaired in chronically defeated female mice. Measures of nest construction can serve as an overall indicator of rodent health (56–58) and can be inhibited in male mice exposed to social stressors (59,60). As a goal-directed behavior, nesting requires a sequence of intricate actions to ultimately construct a protected, concave nest site (49,61). Among other possibilities, poor nesting may result from decreased concentration on task completion or impaired motivation to engage in potentially rewarding species-typical behaviors [nest material as a reinforcer (61–64)]. Defeated mice constructed incomplete nests, suggesting indecision or issues with concentration, both of which are cardinal symptoms of

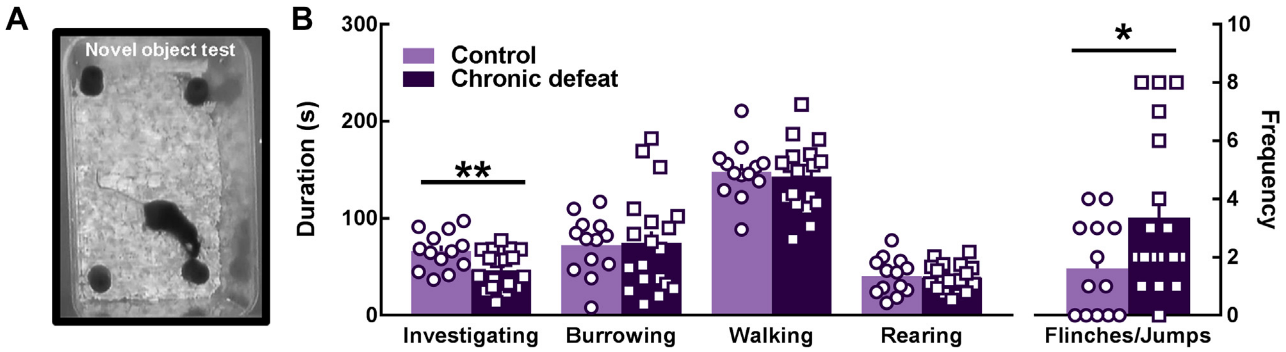

Figure 7. **(A, B)** Chronically defeated female mice spent less time investigating novel objects ($t_{30} = 2.849$, $p = .0078$) and exhibited more defensive flinches and jumps ($t_{30} = 2.126$, $p = .0419$) compared with control mice. All data are shown as mean ± SEM; $^*p < .05$, $^{**}p < .01$, control vs chronic defeat.

Table 1. Effects of Social Defeat Stress in Female Mice

	Acute Defeat	Chronic Defeat
Neural Activation (c-Fos)		
MeA	↔	↑
LSv	↑	↑
hPVN	↔	↑
VMHvl	↔	↑
PeN	↔	↔
amBNST	↔	↔
DG	↔	↔
Plasma Corticosterone	↑	↑
Social Interactions (HCSI)	↔	↓
Conspecific-Induced Defense (HCSI)	↔	↑
Social Hyperthermia (HCSI)	NA	↑
Social Vigilance (OFSI)	NA	↑
Social Interaction Zone Time (OFSI)	NA	↔
Novel Object Investigation (HCNO)	NA	↓
Novelty-Induced Defense (HCNO)	NA	↑
Novelty-Induced Hyperthermia (HCNO)	NA	↔
Nest Quality	NA	↓
Anxiety-like Behavior (LDB)	NA	↔

Consequences of acute or chronic social defeat stress in female C57BL/6J mice compared with control animals ($p < .05$).

↔, no difference from control mice; ↑, significantly greater than control mice; ↓, significantly less than control mice; amBNST, anteromedial bed nucleus of the stria terminalis; DG, dentate gyrus; HCNO, home cage novel object test; HCSI, home cage social interaction test; hPVN, hypothalamic paraventricular nucleus; LDB, light/dark box test; LSv, ventral lateral septum; MeA, medial amygdala; NA, not available; OFSI, open field social interaction test; PeN, periventricular nucleus; VMHvl, ventrolateral division of the ventromedial hypothalamus.

posttraumatic stress disorder and major depressive disorder (65). Preferential allocation of attentional resources for threat assessment may impede nest completion in animals that exhibit a hypervigilant-like phenotype. Future studies that evaluate action sequence planning as well as the anticipatory, motivational, and learning processes that drive nesting behaviors could reveal unique circuit-level mechanisms that contribute to the defeat phenotype observed in female mice (61,66).

Estrous cycling was similar between stressed and nonstressed female mice during chronic social defeat. Additional investigations need to determine if cycle phase and circulating hormone concentrations influence specific behavioral end points in female rodents defeated by aggressive conspecifics. Further work is also required to fully examine which stress-induced behavioral impairments are sensitive to one-time versus repeated ketamine administration in animals subjected to ethological female social defeat stress (67). In our sample of defeated female mice, we did not observe a distinct bimodal distribution of susceptible and resilient animals (Supplemental Figure S7A). Large-scale experiments paralleling the studies conducted by Krishnan *et al.* (10) in defeated male mice are necessary to definitively address the possibility of subgroups within the defeated female population. Such work may clarify the mechanisms that render some individuals more likely to develop affective psychopathologies and may guide the development of personalized treatment options.

Chronic Social Defeat Stress and Increased c-Fos Activation in Sexually Dimorphic Brain Regions

Chronically defeated female mice exhibited c-Fos activation within several sexually dimorphic brain regions that comprise overlapping social (68–70) and defensive behavioral and threat-processing networks (46,55,71–74), including the MeA, LSv, VMHvl, and hPVN. Whereas these areas are also activated in male rodents (75–80) exposed to repeated social defeat stress, regional sexual dimorphism can contribute to significant sex-dependent behavioral outcomes in response to cues (81–83) and experiences (84–86), illustrating the potential for sex-specific social defeat phenotypes despite similar patterns of c-Fos activation in chronically defeated male and female mice.

Interestingly, estrogen receptor α-expressing cells in the anterior portion of the VMHvl may control some aspects of active defense during acute social defeat (74). Our findings point to a similar cluster of cells that may be relevant to atypical social behaviors observed in chronically defeated female mice. Persistent stress-induced changes in estrogen signaling (5,87,88) within this cell population could contribute to exaggerated active defense and hypervigilance in female mice subjected to chronic social defeat stress.

Future Directions: Female Stress and Aggression

Aggression is most often studied in the context of the male behavioral repertoire; yet, male and female rodents, nonhuman primates, and humans will readily engage in aggressive acts under certain conditions (22,89–93). We show that consistent and intense interfemale aggression can be generated in intact but not ovariectomized female mice living with a male conspecific. In addition, distinct aggressive and nonaggressive female subtypes are present within the aggressive subpopulation; some female mice display exclusively gestational aggression, whereas others engage in gestational and rival aggression (Supplemental Figure S10). Comparable studies in rats have shown that intact, nulliparous animals housed with sterile male rats will become highly and persistently aggressive toward unfamiliar conspecifics (94), suggesting that the present murine model could be extended to study female rats under analogous defeat conditions. Additionally, similarities between female mice and rats (94–96) raise the possibility that there may be some adaptive and potentially conserved elements of interfemale rival aggression in rodents as well as other mammalian species.

Chronic social defeat stress produced a pattern of functional activation in brain areas also activated during mating or aggression in female mice, including the MeA (82,97) and VMHvl (98–101). It remains unclear whether these cell populations are functionally, molecularly, and spatially discrete or overlapping (86,102). Interactions between these networks could allow one social experience to modify later behaviors; for example, stress-associated activation of cells in aggression-related brain regions could affect aggressive performance or motivation to engage in future agonistic behaviors. To address this, further behavioral and molecular studies are necessary to test the potential for rival aggression to motivate operant responding in control and socially defeated female mice [compare with 103–108].

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