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Fighting females: Neural and behavioral consequences of social defeat stress in female mice

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Abstract

Background: Despite the two-fold higher prevalence of major depressive disorder and posttraumatic stress disorder in females compared to males, most clinical and preclinical studies focus on male subjects. We introduce 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 mice were housed with castrated males and tested for aggression toward female intruders. For 10 days, aggressive CFW females defeated C57BL/6J (B6) females during 5-min encounters. Measures of corticosterone, c-Fos activation in hypothalamic and limbic structures, and species-typical behaviors were collected from defeated and non-defeated females. Ketamine (20 mg/kg) was tested for its potential to reverse stress-induced social deficits.

Results: Housed with a castrated male, most intact CFW females readily attacked unfamiliar B6 females, inflicting >40 bites in a 5-min encounter. Compared to 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 hrs after chronically defeated females received a systemic dose of ketamine.

Conclusions: These findings demonstrate that CFW females 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 on the biological bases for interfemale aggression.

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Keywords

Aggression; Female; Social defeat stress; Affective disorders; Social interaction; Ketamine

Introduction

The prevalence of affective disorders including major depressive disorder (MDD) and posttraumatic stress disorder (PTSD) is two-fold 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 males. In preclinical research, the murine chronic social defeat stress protocol employs species-typical male aggression toward a submissive conspecific 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, chronic treatment with tricyclic antidepressants or serotonin reuptake inhibitors or acute 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 mechanisms.

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 females (19) and through chemogenetic stimulation of the male ventromedial hypothalamus (20). A vicarious social defeat stress model in which females 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.

While male mice are aggressive under diverse experimental conditions (22), female aggression is most often studied either during pregnancy (23–27; but 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 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 females will readily fight a rival female, 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, lateral septum, ventromedial hypothalamus, and paraventricular nucleus, 4.) characterize defeat-associated deficits in species-typical social and non-social behaviors, and to 5.) increase the duration of social interactions initiated by chronically defeated females with acutely administered ketamine.

Methods and Materials

See Supplement for additional details.

Animals

Twelve-week-old intact (n=74) or ovariectomized (OVX; n=27) Swiss Webster (CFW) female mice (Charles River Laboratories, Wilmington, MA, USA) were housed in resident pairs with age-matched intact (n=47) or castrated (n=61) CFW males 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, USA) or CFW females (n=40) were group-housed in cages (25.7×48.3×15.2 cm; n=10/cage) with corn cob bedding. Experimental twelve-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's *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 OVX females

OVX CFW females, housed in resident pairs with intact CFW males, were evaluated for aggression in modified resident-intruder confrontations every other day starting two weeks after pairhousing (36). To test the effect of intruder strain and familiarity, males were removed and intruder females were introduced to resident home cages for 2-min confrontations (Fig. S2). The attack latency and attack bite frequency were recorded. Following confrontations, female intruders were removed and males were returned to their resident home cages.

Aggression in intact outbred female mice

Intact CFW females, housed with intact CFW males, were evaluated for aggression toward unfamiliar B6 females. CFW litters were culled on postnatal day one (PND1) at which time CFW females 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, CFW females were housed with castrated males and three days later, aggressive confrontations continued. After five days of living with castrated males, most resident females attacked unfamiliar B6 females. Here, 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 females were housed exclusively with castrated males in resident pairs. A significant subset of these females were

highly aggressive toward unfamiliar B6 intruders (n=39/61; >15 bites/2-min); these residents were used as aggressors during the chronic social defeat protocol.

Sex-specific patterns of aggression

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

Testing the aggressive potential of females that do not display rival aggression

To test whether pregnancy-induced aggression was distinct from rival aggression, consistently *non-aggressive* females that were pair-housed with castrated males (n=7) for at least two months were assessed for gestational aggression. These females were housed with intact CFW males for three days, then returned to their original castrated male partners. Aggression was evaluated every two or four days during 2-min resident-intruder confrontations. Pups were culled on PND1 and resident females were tested for aggression four and seven days postpartum.

Ten-day female chronic social defeat stress

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

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

Estrous cycling

Vaginal cytology was monitored in experimental B6 females 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 females (n=23) housed with castrated males to determine if aggression varied according to estrous cycle phase (41–43; but 44, 45).

Corticosterone measurements

At two time points (Fig. S1A), blood was collected from the submandibular vein of B6 females (n=5 control; n=5 acute defeat; n=10 chronic defeat) using sterile 4 mm lancets (Goldenrod Animal Lancet, Medipoint, Inc., Mineola, NY, USA), centrifuged at 4°C at 3000 rpm for 10 min. Fifteen microliters of plasma were collected and stored at −80°C for corticosterone enzyme immunoassay (Arbor Assays, Ann Arbor, MI, USA); standards (7.8125–1,000 ng/mL) and samples were run in duplicate.

Tissue collection and c-Fos immunohistochemistry

On the tenth day of chronic social defeat stress, brains were collected from B6 females after no defeat or a 5-min social defeat stress episode followed by an hour-long threat period (Fig. S1B). Fifty micron brain slices containing the anteromedial bed nucleus of the stria terminalis (amBNST), ventral lateral septum (LSv), hypothalamic paraventricular nucleus (hPVN), periventricular nucleus (PeN), ventromedial hypothalamus (VMH), medial amygdala (MeA), and dentate gyrus (DG) were selected for c-Fos immunohistochemistry.

Open field social interaction

After a 2.5-min 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; Fig. 5C), control and defeated females were briefly removed while an unfamiliar, aggressive CFW female was placed in the stimulus cage. Experimental mice were returned to the apparatus and evaluated for social interactions during a 2.5-min 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 (Ethovision XT v. 14). Social interaction videos were scored manually for vigilance-like behavior, defined here as time spent oriented toward but not interacting with the stimulus animal (cf., 46). The open field and stimulus cage were cleaned and dried between mice.

Novel object investigation

Experimental females were briefly moved to clean holding cages while four rubber stoppers (14-135G/14-130G; Fisher Scientific, Agawam, MA) were placed in the home cage (Fig. 7A). Females were returned to the home cage for a 5-min test. Rectal temperatures were collected from experimental females immediately prior to and following tests using a thermo-probe (2100 Tele-thermometer, YSI, Inc., Yellow Springs, OH, USA) lubricated with mineral oil.

Home cage social interactions and ketamine administration

Experimental female-initiated social contact with a non-aggressive, group-housed B6 stimulus female was evaluated during 1.5-min tests in the experimental female's home cage. Alternatively, home cage social interaction tests were conducted using anesthetized stimulus females (Fig. S1A). Rectal temperatures were collected from experimental females immediately prior to and following testing.

Chronically defeated and control females received intraperitoneal injections of 0.9% NaCl or the *N*-methyl-D-aspartate (NMDA) receptor antagonist, ketamine hydrochloride diluted in 0.9% NaCl (20 mg/kg; VedCo Inc., Saint Joseph, MO, USA;17, 21, 47, 48). Social interactions were evaluated 30 min, 24 hrs, and 5 days post-injection.

Nesting

Females received two grams of nesting material (Nestlets, Ancare Corp., Bellmore, NY, USA) at 1330hr in the home cage. Five days later, nests were scored on a scale of 1–5 (49), nest heights and diameters were recorded, and nest images were evaluated for shape (i.e., circularity) in ImageJ.

Results

Interfemale rival aggression

Most intact resident CFW females were aggressive when housed with a male, but not when housed in isolation (Fig. 1A) or following ovariectomy (Fig. S2). By the third aggressive confrontation, >90% of resident CFW females that were housed with an intact male expressed gestational aggression (attack bites: $M\pm SEM$=26.62±3.83). However, by the seventh day after litters were culled and intact males were removed, <20% of females were aggressive and females that did express aggression showed substantially reduced attack bite frequencies ($M\pm SEM$=5.5±2.5). Five days after being rehoused with castrated males, most resident females fought (75%; attack bites: $M\pm SEM$=21.6±4.34). Similarly, most intact nulliparous CFW females housed exclusively with castrated males fought and a significant subset (65%) emerged as highly aggressive toward unfamiliar B6 intruders (Fig. 1B).

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

A subset of nulliparous resident females housed with castrated males were consistently nonaggressive toward intruder females (n=13/61). Half of these mice (n=7) were tested for their sensitivity to the pro-aggressive effects of pregnancy. By late-pregnancy, 75% of formerly non-aggressive females attacked an unfamiliar female intruder; however, after pups

were culled on PND1, these residents returned to their non-aggressive, pre-pregnancy baselines (Fig. 1D).

Neural and physiological effects of chronic female social defeat stress

Acute or ten-day social defeat stress (Fig. 2A; Video S3) followed by a threat period increased circulating corticosterone more than the threat period alone (Fig. 2B). Similar corticosterone concentrations in acutely and chronically defeated mice suggest that females do not habituate to social defeat stress, much like acutely and repeatedly defeated outbred males (51). However, neither estrous cycle nor body weight was significantly affected by chronic social defeat (Fig. S5A–E).

Compared to 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 VMHvl (Fig. 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 amBNST in control whereas an inverse correlation was observed in defeated mice (Table S1). Females subjected to chronic social defeat also exhibited a unique pattern of inverse correlations in c-Fos in the PeN and amBNST or DG.

Behavioral effects of chronic social defeat stress in female mice

During social interactions in the home cage (Fig. 3E) with a non-aggressive B6 stimulus female, chronically defeated individuals displayed a greater number of defensive kicks and flinches compared to controls (Fig. 3A) along with deficits in both anogenital/flank and total social contact (Fig. 3C, D). When an anesthetized social stimulus mouse was placed into their home cage, chronically defeated females actually engaged in more nasal contact compared to controls (Fig. 3B). Social investigation in the home cage also produced a substantial hyperthermic response which was greater in chronically defeated females compared to controls (Fig. 4A); in contrast, there was no group difference in hyperthermia induced by novel object investigation (Fig. 4B).

The total duration of experimental female-initiated social contact (i.e., non-aggressive social interaction) during baseline home cage testing (Fig. 3E) in mice that later received ketamine or saline was significantly lower among defeated animals (Fig. 3F). Twenty-four hours after receiving a dose of ketamine (20 mg/kg), defeated females exhibited a significant increase from pre-treatment social interactions compared to ketamine-treated controls and compared to defeated mice that received saline (Fig. 3G; Videos S4–7). This effect of ketamine was not evident 30 min or 5 days post-injection (Fig. S6). Importantly, although ketamine increased social contact duration in chronically defeated females, a defeat-associated increase in behavioral transitions during social interactions persisted (Fig. 3H).

Control and defeated females were also examined in an open field social interaction test (Fig. 5C) which is often employed to identify depressive-like phenotypes in chronically defeated male mice (8–10). Chronically defeated females exhibited significantly more vigilance-like behavior compared to controls (Fig. 5A, C, D; Video S8) though the duration of time spent in a predefined social interaction zone was comparable between groups (Fig.

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 (Table S2).

In terms of non-social behaviors, chronically defeated females constructed nests that were significantly less developed than controls as illustrated by measures of nest peak diameter, height, circularity, and overall nest score (Fig. 6A–E). Importantly, there was no group difference in baseline body temperature (Fig. 4), suggesting that nest-building deficits in defeated females were probably not due to stress effects on thermoregulation. A defeat phenotype was also evident when novel objects were placed in the home cage. Defeated females spent significantly less time investigating and exhibited a greater number of defensive startle-like behaviors (i.e. flinching and jumping) compared to controls (Fig. 7B). In contrast, measures of general anxiety-like behavior collected during light-dark box testing were similar between control and chronically defeated mice (Fig. 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 (Fig. 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- and anxiety-like behaviors in defeated female mice (Table 1). After ten days of continuous social stress, females 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, females engaged in atypical behaviors during novel object investigation, nest-building, and social interactions. Active investigation of a non-aggressive social partner during social interactions increased in defeated ketamine-treated females, 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 females that received drug treatment continued to exhibit atypically high rates of behavioral transitioning. Reduced behavioral stability in the presence of a non-aggressive 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 vs. 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. Like the pattern of aggression, significant features of the defeated female phenotype are

distinct from males, and in female mice, the severity of persistent 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 females subjected to chronic stress (46, 55), which may reflect an inability to distinguish threatening from non-threatening stimuli. Defeated females showed exaggerated defensive behaviors such as whole-body flinches, backwards jumps and defensive kicks toward non-aggressive 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 males (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 females. Measures of nest construction can serve as an overall indicator of rodent health (56–58) and can be inhibited in males 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 PTSD and MDD (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 non-stressed females during chronic social defeat. Additional investigations need to determine if cycle phase and circulating hormone concentrations influence specific behavioral endpoints in females defeated by aggressive conspecifics. Further work is also required to fully examine which stress-induced behavioral impairments are sensitive to acute vs. repeated ketamine in female mice subjected to ethological stress conditions (67). We also did not observe a distinct bimodal distribution of “susceptible” and “resilient” chronically defeated females (Fig. S7A). Large-scale studies paralleling those conducted by Krishnan, Han et al. (10) in defeated males 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 compared to others 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 females 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. While 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 alpha-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 females. Persistent stress-induced changes in estrogen signaling (5, 87, 88) within this cell population could contribute to exaggerated active defense and hypervigilance in defeated females.

Future directions: Female stress and aggression

Aggression is most often studied in the context of the male behavioral repertoire; yet, male *and female* rodents, non-human primates, and humans will readily engage in aggressive acts under certain conditions (22, 89–93). Here, 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 non-aggressive female subtypes are present within the aggressive subpopulation; some females display exclusively gestational aggression while others engage in gestational and rival aggression (Fig. S10). Comparable studies in rats have shown that intact, nulliparous females housed with sterile males become highly and persistently aggressive toward unfamiliar females (94), suggesting that the present murine model could be extended to study female rats under analogous defeat conditions. Additional 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, and perhaps in 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 females (cf., 103–108).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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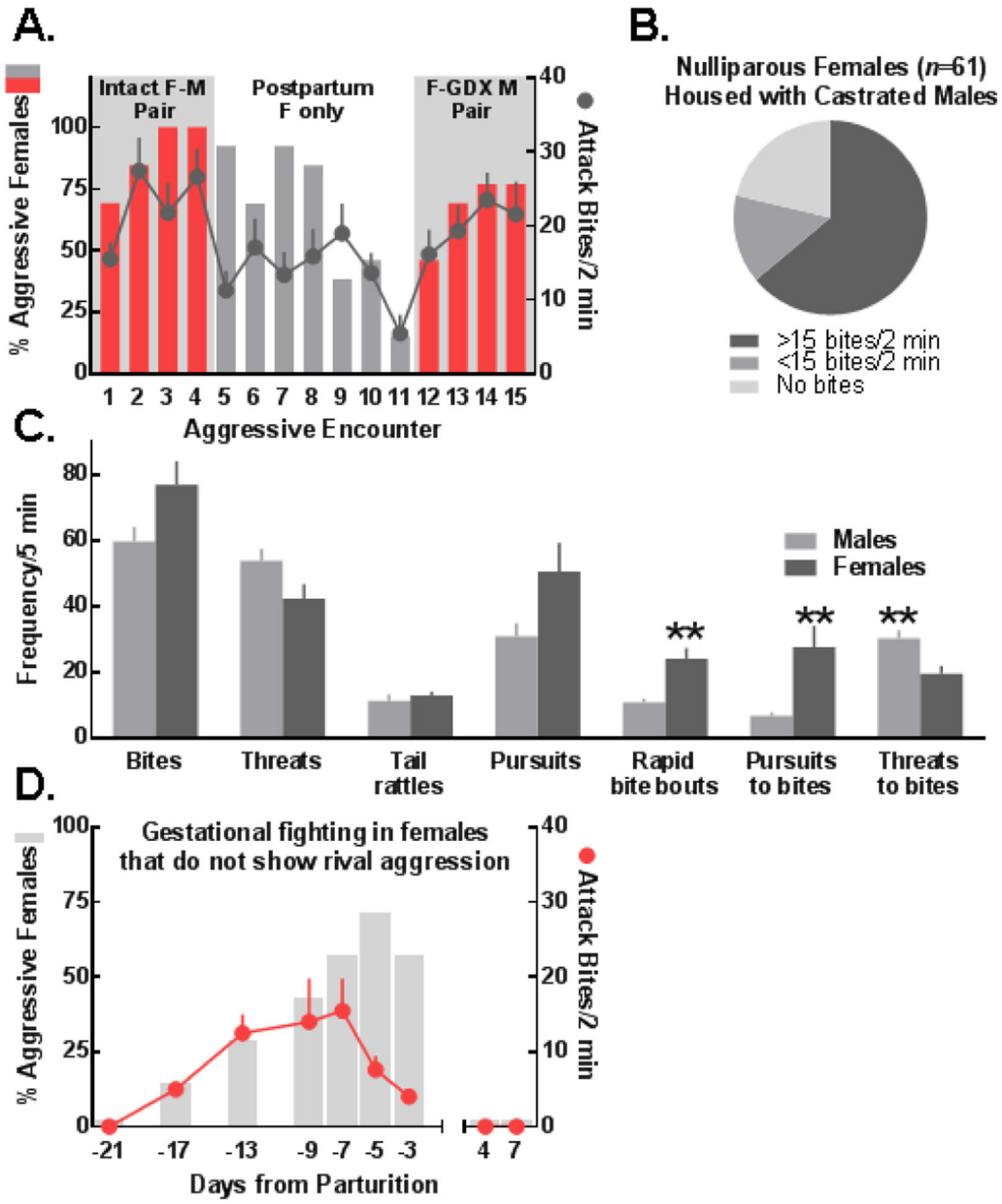

Figure 1.

(**A**) Most multiparous and (**B**) nulliparous outbred females living with intact or gonadectomized (GDX; i.e., castrated) males developed aggression toward unfamiliar C57BL/6J female intruders. (**C**) Male and female attack bite frequencies were comparable, but females (*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 males (*n*=10) exhibited more bites preceded by sideways threats (threats to bites; $t(18)=3.14$); data shown as Mean ± SEM; $^{**}p<0.01$ male vs. female. (**D**) Most non-aggressive nulliparous females 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 females that fought.

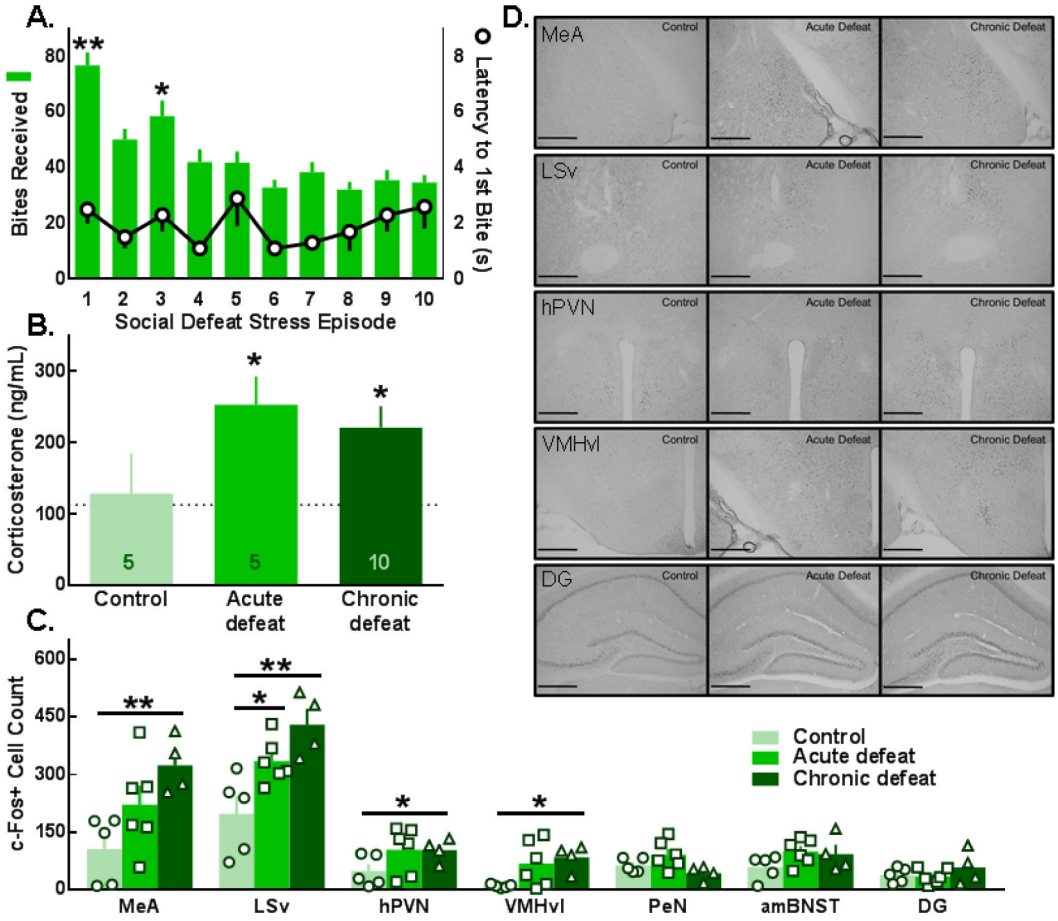

Figure 2.

Female C57BL/6J mice were defeated by aggressive resident CFW females for 10 consecutive days. (**A**; right axis). Attack latencies were <5 sec and (**A**; left axis) the greatest number of attacks were delivered early in the defeat protocol (left axis; $F(9,190)=13.03$, $p<0.0001$; *$p<0.05$, **$p<0.0001$ compared to day 5). (**B**) Elevated concentrations of plasma corticosterone were detected after acute or chronic social defeat stress (time: $F(1,17)=9.6$, $p=0.007$; defeat: $F(2,17)=4.2$, $p=0.033$; *$p<0.05$ compared to control). (**C**) Social defeat stress increased c-Fos activation in the medial amygdala (MeA; $F(2,12)=6.43$, $p=0.013$), the ventral lateral septum (LSv; $F(2,12)=9.28$, $p=0.004$), the hypothalamic paraventricular nucleus (hPVN; $F(2,12)=4.48$, $p=0.035$), and the ventrolateral division of the ventromedial hypothalamus (VMHvl; $F(2,12)=4.17$, $p=0.042$); *$p<0.05$, **$p<0.01$, compared to control. (**D**) Representative images of c-Fos in the MeA, LSv, hPVN, VMHvl, and dentate gyrus (DG); PeN, periventricular nucleus; amBNST, anteromedial bed nucleus of the stria terminalis; scale bars are 200 μm. Data are shown as Mean ± SEM.

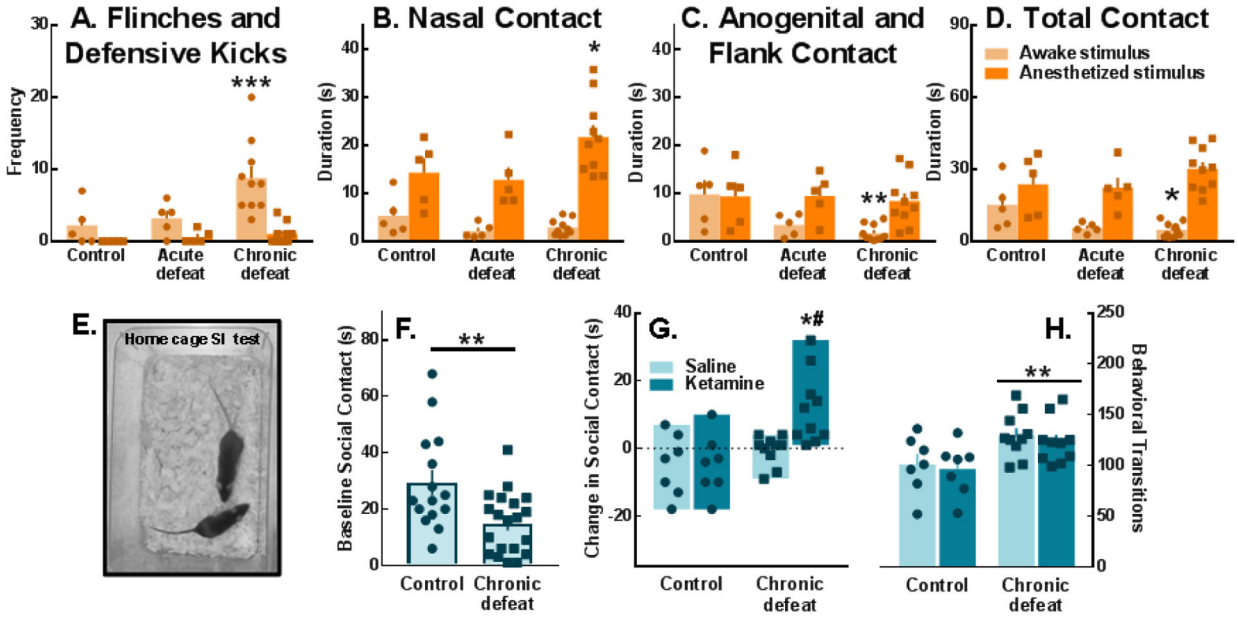

Figure 3.

Chronically defeated females exhibited substantial social contact deficits which were improved with ketamine, 24 hrs post-injection. (**A**) Defensive flinches and kicks were observed in chronically defeated females during social interactions with an awake conspecific (light bars; stimulus × defeat interaction: $F(2,17)=3.67$, $p=0.047$; stimulus: $F(1,17)=15.3$, $p=0.001$; defeat: $F(2,17)=7.73$, $p=0.004$). (**B-D**) Though chronically defeated females engaged in more nasal contact with anesthetized stimulus animals (dark bars; stimulus × defeat interaction: $F(2,17)=3.8$, $p=0.043$; stimulus: $F(1,17)=54.53$, $p<0.0001$), they displayed significantly less anogenital/flank contact (stimulus × defeat interaction: $F(2,17)=3.87$, $p=0.041$; stimulus: $F(1,17)=13.67$, $p=0.002$) and total contact (stimulus × defeat interaction: $F(2,17)=6.18$, $p=0.0096$; stimulus: $F(1,17)=65.11$, $p<0.0001$) with awake stimulus mice as compared to non-defeated controls. (**E**, **F**) Baseline total social contact with an awake stimulus female in the home cage was suppressed in chronically defeated mice ($t(33)=3.056$, $p=0.0044$). (**G**) Twenty-four hours post-injection, ketamine (20 mg/kg) significantly increased social contact in chronically defeated females (drug × defeat interaction: $F(1,30)=4.26$, $p=0.0478$; drug: $F(1,30)= 4.26$, $p=0.0478$; defeat: $F(1,30)= 12.52$, $p=0.0013$), (**H**) but did not reverse the high rate of behavioral transitions during social interaction tests (defeat: $F(1,30)=11.00$, $p=0.0024$). (**A-D, F, H**) Data are portrayed as the Mean ± SEM. (**G**) Bars depict the max and min values. The dotted line marks no change in social contact between baseline and post-injection tests; values above the dotted line are increases from baseline while values below are decreases from baseline social contact time. Circles and squares represent individuals. *$p<0.05$, **$p<0.01$, ***$p<0.001$ (**A**, **C**, **D**) compared to controls interacting with an awake social stimulus female, (**B**) compared to controls interacting with an anesthetized social stimulus female, (**F**, **H**) compared to controls, or (**G**) compared to ketamine-treated controls; #$p<0.05$, compared to saline-treated chronically defeated females.

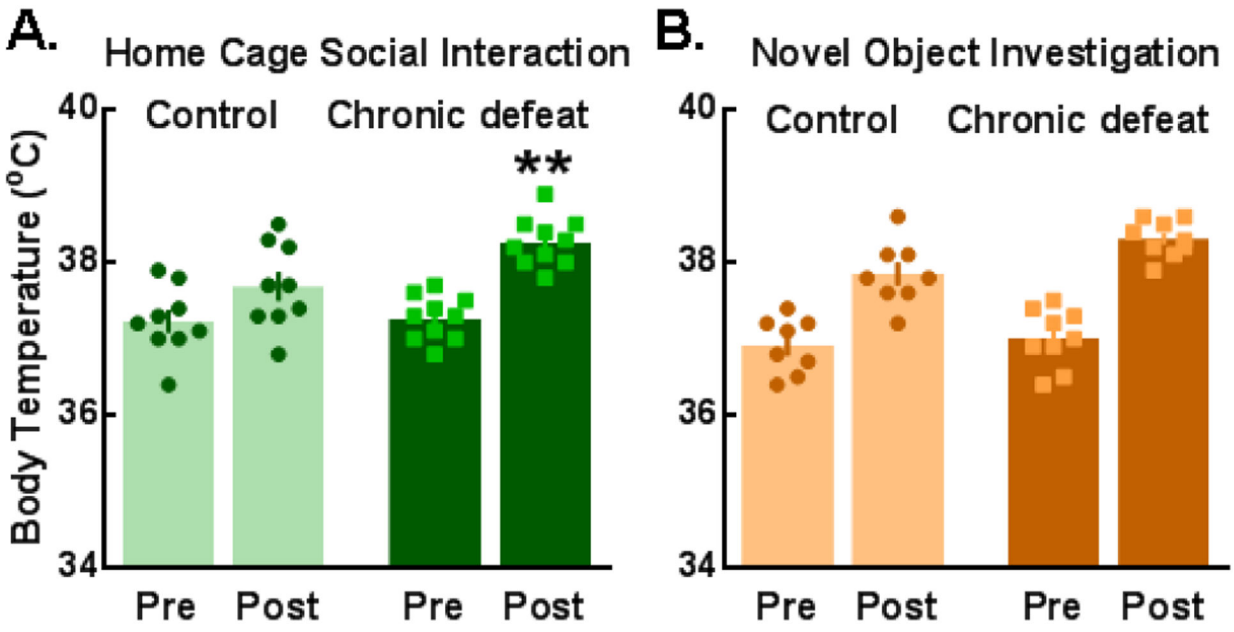

Figure 4.

Hyperthermia was observed in response to 1.5-min social interaction and 5-min novel object investigation tests. Temperatures were measured immediately prior to (pre) and following (post) testing. (**A**) Chronically defeated females experienced a greater elevation in body temperature after social interactions compared to non-defeated controls (defeat × time interaction: $F(1,17)=5.91$, $p=0.027$; time: $F(1,17)=42.21$, $p<0.0001$). (**B**) All mice showed a similar degree of hyperthermia in response to novel object investigation (time: $F(1,15)=113.6$, $p<0.0001$). Data are depicted as the Mean ± SEM; $^{**}p<0.05$, compared to control post-test temperature.

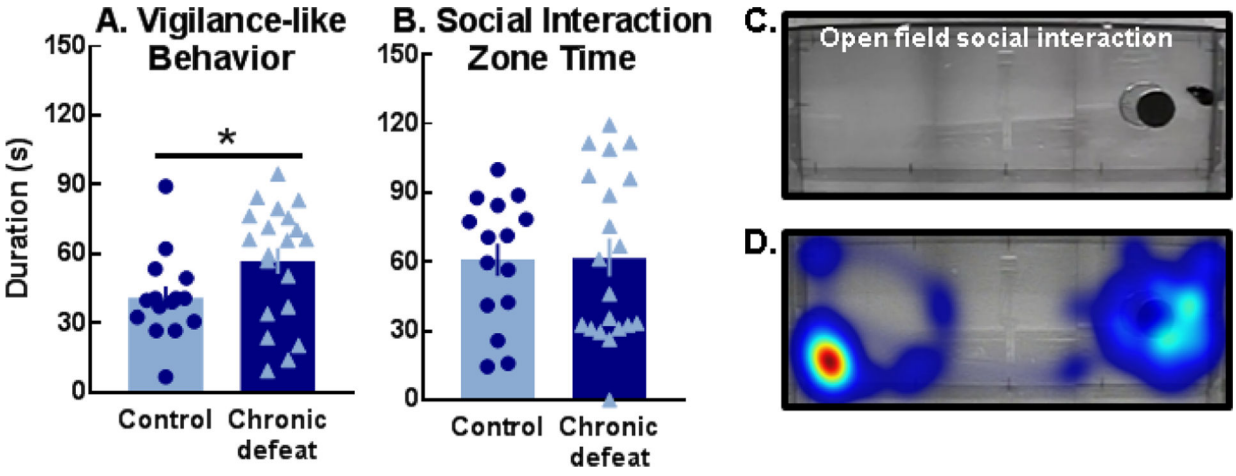

Figure 5.

Chronically defeated females expressed more (**A**) vigilance-like behavior than controls ($t(33)=2.05$, $*p=0.048$) despite both groups spending (**B**) similar durations within the social interaction zone. (**C**) Representative image of vigilance-like behavior displayed by a chronically defeated female and (**D**) the corresponding heat map of activity during the open field social interaction test.

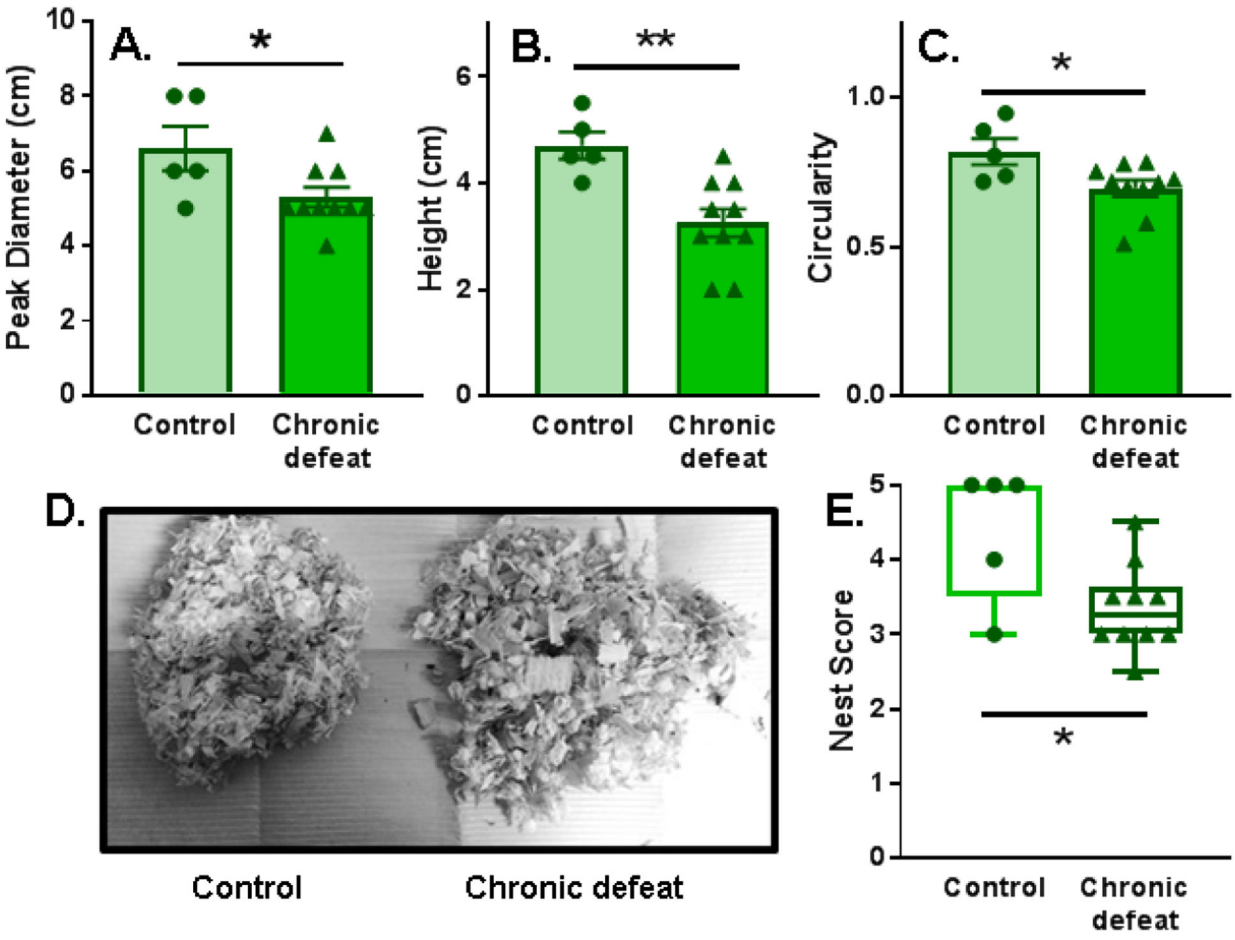

Figure 6.

Nests constructed by chronically defeated females were underdeveloped compared to nests built by non-defeated 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 $U=8.5$, $p=0.039$). (**A**-**C**) Data are portrayed as the Mean ± SEM (**D**) or as the max and min surrounding the median; *$p<0.05$, **$p<0.01$ compared to control.

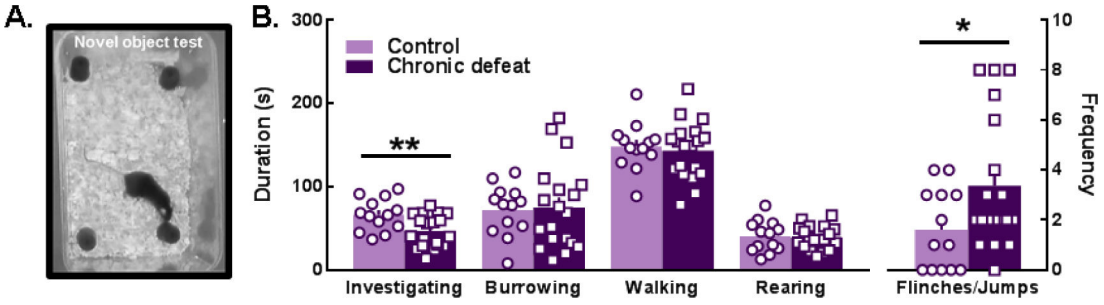

Figure 7.

(**A**, **B**) Chronically defeated females spent less time investigating novel objects ($t(30)=2.849$, $p=0.0078$) and exhibited more defensive flinches and jumps ($t(30)=2.126$, $p=0.0419$) compared to nondefeated females. All data are shown as the Mean ± SEM; *$p<0.05$, **$p<0.01$, control vs. chronic defeat.

Table 1.

Effects of social defeat stress in female mice

Neural activation (c-Fos)	Acute defeat	Chronic defeat
MeA	↔	↑
LSv	↑	↑
hPVN	↔	↑
VMHvl	↔	↑
PeN	↔	↔
amBNST	↔	↔
DG	↔	↔
Plasma corticosterone	↑	↑
Social interactions (HCSI)	↔	↓
Conspecific-induced defense (HCSI)	↔	↑
Social hyperthermia (HCSI)	N/A	↑
Social vigilance (OFSI)	N/A	↑
Social interaction zone time (OFSI)	N/A	↔
Novel object investigation (HCNO)	N/A	↓
Novelty-induced defense (HCNO)	N/A	↑
Novelty-induced hyperthermia (HCNO)	N/A	↔
Nest quality	N/A	↓
Anxiety-like behavior (LDB)	N/A	↔

Consequences of acute or chronic social defeat stress in female C57BL/6J mice compared to non-defeated controls (p <0.05); no difference from controls, ↔; significantly greater than controls, ↑; significantly less than controls, ↓; not available, N/A; medial amygdala, MeA; ventral lateral septum, LSv; hypothalamic paraventricular nucleus, hPVN; ventrolateral division of the ventromedial hypothalamus, VMHvl; periventricular nucleus, PeN; anteromedial bed nucleus of the stria terminalis, amBNST; dentate gyrus, DG; home cage social interaction test, HCSI; open field social interaction test, OFSI; home cage novel object test, HCNO; light dark box test, LDB

KEY RESOURCES TABLE

Resource Type	Specific Reagent or Resource	Source or Reference	Identifiers	Additional Information
Antibody	Rabbit polyclonal anti-c-Fos	Proteintech Group, Inc.	Cat# 26192–1-AP	
Antibody	Biotinylated goat anti-rabbit	Vector Laboratories	Cat# BA-1000	
Chemical Compound or Drug	Ketamine HCl	VedCo Inc.	Cat# VINV-KETA-0VED	
Commercial Assay or Kit	Vectastain avidin-biotin complex (ABC) kit	Vector Laboratories	Cat#PK-4000	
Commercial Assay or Kit	3,3'-diaminobenzidine (DAB) kit	Sigma-Aldrich	Cat# D4418	
Organism/Strain	Mouse (*Mus musculus*): CFW, male and female	Charles River Laboratories	RRID: IMSR_CRL:24	
Organism/Strain	Mouse *(Mus musculus)*: C57BL/6J, female	The Jackson Laboratory	RRID: IMSR_JAX: 000664	
Software; Algorithm	Ethovision XT	Noldus Information Technology	RRID: SCR_000441	
Software; Algorithm	The Observer XT	Noldus Information Technology	RRID: SCR_004074	
Software; Algorithm	ImageJ	National Institutes of Health	RRID: SCR_003070	
Software; Algorithm	Prism	GraphPad	RRID: SCR_002798	