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ORIGINAL INVESTIGATION

Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: exploring a link between depression and drug abuse

Akiko Shimamoto · Joseph F. DeBold · Elizabeth N. Holly · Klaus A. Miczek

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Abstract
Rationale Women have twice the risk as men to develop depression. Approximately, 24% of major depression disorder cases have comorbid disorders with substance abuse. Several central systems, including dopaminergic and serotonergic pathways, are thought to be involved in such comorbidity.
Objectives The present study established a chronic social stress model in female rats, which produces some cardinal features of depressive-like symptoms. Further, we examined the effects of acute cocaine on dopamine (DA) and serotonin (5-HT) in the nucleus accumbens (NAc) using this model.
Methods Female Long-Evans rats confronted a nursing dam in its home cage for 30 min twice daily for 21 days. The non-stressed control group was handled daily throughout the experiment. During the 21 days of stress, behaviors during confrontations, weight, preference for saccharin, and estrous cycles were measured. Ten days after the last confrontation, the experimental rat was challenged with 10 mg/kg of cocaine, and levels of DA and 5-HT in the NAc were measured using in vivo microdialysis.
Results During the course of daily confrontations for 21 days, the experimental females significantly increased the duration of immobility, reduced weight gain and the preference for saccharin, and disrupted estrous cycles during the stress. Chronic social stress significantly attenuated cocaine-induced DA levels, and to some extent, attenuated a percent change of 5-HT compared to the non-stressed control group.
Conclusions Chronic social defeat stress for 21 days induced physiological and behavioral depression-relevant deficits and blunted response of dopaminergic and to some extent, serotonergic neurons to cocaine challenge in females.

Keywords Cocaine · Chronic social stress · Depression · Dopamine · Estrous cycles · Females · Nucleus accumbens · Serotonin

A. Shimamoto (✉) · J. F. DeBold · E. N. Holly · K. A. Miczek
Department of Psychology, Tufts University,
530 Boston Ave. (Bacon Hall),
Medford, MA 02155, USA
e-mail: akiko.shimamoto@tufts.edu

K. A. Miczek
Departments of Psychiatry, Pharmacology and Neuroscience,
Tufts University,
145 Harrison Avenue,
Boston, MA 02111, USA

Introduction

Women have twice the risk as men to develop depression (National Survey on Drug Use and Health 2008), and approximately 24% of major depression disorder cases have lifetime comorbidity with substance abuse (Kessler et al. 2003). Several central systems, including dopaminergic and serotonergic pathways, have been candidates for such comorbidity (Brady and Sinha 2005), and because behavioral and psychological effects of psychostimulants, such as cocaine, are more prominent in women than in men (Lukas et al. 1996; Kosten et al. 1996; Robbins et al. 1999; Elman et al. 2001), the mechanisms for such disorders in women need to be addressed.

In laboratory animals, foot-shock, tail-pinch, restraint, chronic mild stress (CMS), and social defeat stress procedures have been characterized as preclinical models for the study of symptoms of depression or chronic stress disorders, as assessed by hormonal, neuronal, and behav-

ioral responses (Deroche et al. 1992; Fuchs et al. 1992; Ortiz et al. 1996; Willner 1997; Rouge-Pont et al. 1998; Henn and Vollmayr 2005). Among these behavioral procedures, social defeat stress, as induced by a dominant animal, may have greater construct validity that is more analogous to what primates, including humans, experience outside of a laboratory setting, than other types of stressors (Fuchs and Flügge 2002; Rygula et al. 2005; Schmidt et al. 2008; Miczek et al. 2008). While social defeat stress can produce potent effects in males in terms of reduced weight gain and increased corticosterone (CORT) levels, its impact is much more subtle in females (Haller et al. 1999). Similarly, the CMS procedure, which was originally developed to model cardinal symptoms of depression, (Willner et al. 1987; Papp et al. 1991; Muscat et al. 1992; Gronli et al. 2004), either does not always produce anhedonia-like symptoms in females (Dalla et al. 2005), or is limited to strain-specific effects (Baker et al. 2006).

Social defeat stress, especially when experienced intermittently, induces behavioral sensitization as assessed by increased locomotor activity after a psychostimulant challenge (Miczek et al. 2008). Concurrently, cocaine-induced DA levels in the NAc increase after such intermittent social defeat stress (Tidey and Miczek 1996; Tidey and Miczek 1997). This is similar to what occurs with other types of intermittent stressors, such as foot-shock (Kalivas et al. 1988; Kalivas and Duffy 1989; Sorg and Kalivas 1991; Sorg and Steketee 1992; Steketee et al. 1992), food deprivation (Rouge-Pont et al. 1995), or restraint (Cuadra et al. 1999). On the other hand, prolonged stress suppresses basal and cocaine-induced DA and 5-HT levels in the NAc (Gambarana et al. 1999; Mangiavacchi et al. 2001), suggesting that duration and frequency of social stress could modify the aminergic response to acute cocaine. These hypotheses, however, are based on studies of male animals, and the effects of chronic social defeat stress in females have yet to be studied. Therefore, we have begun to study a reliable and replicable chronic social stress model in females comparable to social stress models for males (Miczek et al. 2008) and subsequently examined the effects of cocaine challenge on levels of DA and 5-HT in the NAc using this model.

Materials and methods

Subjects

Adult female Long-Evans rats (n=31) from Charles River Laboratories (Wilmington, MA, USA) weighing 200–225 g upon arrival in the laboratory were singly housed and adapted to our facility for at least a week. Stimulus male rats were housed next to the females' cages to help ensure regular estrous cycles. Resident multiparous female rats were housed in male–female pairs in large stainless steel cages (71×46×46 cm), as previously described (Miczek 1979; Haney et al. 1989). During the stress procedure, the experimental rats were housed in a protective wire mesh cage (20×30×20 cm) in the intervals between the confrontations as described below. In in vivo microdialysis experiments, the rats were housed after the surgery in custom-built polycarbonate cages (25×30×30 cm) with wire mesh screens as side walls, water bottle and food tray, and floors lined with Cellu-Dri pellet bedding. All cages were located in an environmentally controlled suite of vivaria and procedure rooms that were kept at 21±1°C, 35–40% humidity, and an inverted 12-h light–dark cycle (lights on 20:00 to 8:00 h). The experimental facilities and procedures were supervised and approved by the Tufts University Institutional Animal Care and Use Committee implementing the NIH Guide (National Research Council 2010).

Physiological and behavioral measurements

At least a week after the rats arrived, they were screened for saccharin preference. Saccharin was used instead of sucrose to avoid excess calorie intake during the testing across the experiment. A two-bottle choice method with 0.02% saccharin solution vs. unsweetened water was implemented for 1 h per day in the animals' home cages. The position of saccharin bottles was counterbalanced daily across rats. Water access was restricted for 3 h prior to the testing to ensure prompt fluid intake during the 1-h testing period. Intakes for both saccharin solution and unsweetened water were measured, and the volume of saccharin divided by total intake was calculated as preference for saccharin.

Vaginal smears were taken daily to track females' estrous cycles. The Giemsa staining method was used for cytological examinations (Staples and Geils 1965). Estrous cycle was determined in three phases: proestrus, estrus, and met/diestrus. Both estrous cycle and saccharin preference measurements were continued for at least 2 weeks in order to obtain stable baselines prior to the stress exposure. After collecting baseline measurements of the estrous cycling and saccharin testing, the experimental rats were randomly assigned to stressed or non-stressed control groups. Females that showed consistently lower intake of saccharin and irregular estrous cycles during the baseline periods (n=7) were excluded from the study.

Social stress procedure

A modification of the resident-intruder procedure developed for males was used for the social stress study in females (Miczek 1979; Haney et al. 1989). It consisted of

two daily confrontations followed by prolonged threat for the rest of the day for 21 days. Each female confronted a lactating female rat. These lactating females typically show the highest level of aggression during days 3–12 post-delivery in the presence of pups (Erskine et al. 1978). Confrontations took place for 30 min twice daily. Resident males were removed from the dams' home cages during confrontations. The confrontation was video recorded to measure the behavioral responses of the experimental females to the aggressors, including frequencies of being threatened, defensive upright postures, escape from the aggressors, being bitten or nipped, and duration of immobility, supine posture, walking, and sniffing. Behaviors were analyzed using The Observer XT software (Noldus Information Technology Inc., Leesburg, VA, USA) by a trained experimenter who is unfamiliar with the experimental status of the subjects. After the confrontation, the experimental female was placed in a wire mesh protective cage with unrestricted access to food and water. The protective cage was moved to the aggressor's home cage until the next unprotected confrontation. This component ensured that the experimental rat experienced continued threat from the aggressor without any physical harm. Each experimental female was confronted with a different resident female per session, producing social instability for the experimental rats. During the stress procedure, body weight and vaginal smears were collected daily and preference for saccharin was tested three times a week. The non-stressed control rats were handled daily during the entire experiment.

In vivo Microdialysis

Rats (n=10 for the stress group and n=14 for the non-stressed control group) were anesthetized with 100 mg/kg of ketamine and 6 mg/kg of xylazine. They underwent stereotaxic surgery with a unilateral guide cannula (BASi, West Lafayette, IN, USA) aimed at NAc with coordinates of AP; +2.1 mm from bregma, ML; +0.9 mm from midline, and DV; −5.8 mm from dura, according to stereotaxic atlas (Paxinos and Watson 1997). Three days prior to the test day, rats were injected with saline (I.P.) to ensure that the rats were accustomed to the injection and handling. The day before sample collection, the stylet in the cannula was replaced with a 2-mm active membrane probe (BASi, West Lafayette, IN, USA) that was connected to a syringe filled with artificial cerebrospinal fluid (aCSF) (CMA Microdialysis Inc., North Chelmsford, MA, USA). The infusion rate was 0.5 μl/min overnight. On the next day, the infusion rate was increased to 1.5 μl/min, and samples were collected every 10 min using a refrigerated fraction collector (CMA 142, CMA Microdialysis Inc., North Chelmsford, MA, USA). Each sample vial contained 5 μl of antioxidant which consisted of 20 mM phosphate buffer including 25 mM EDTA–2Na and 0.5 mM of ascorbic acid (pH 3.5). Five baseline samples were collected to measure tonic levels of DA and 5-HT. This was followed by saline injection and a cocaine challenge (10 mg/kg, I.P.). Twelve samples were collected thereafter in order to assess the time course of changes in DA and 5-HT levels after the cocaine injection. The average recovery of each probe for DA and 5-HT in this condition were 7.6% and 2.2%, respectively. Four rats (n=2 per group) were excluded from the study due to loss of head mount or unsuccessful microdialysate collection. Placements for each probe are shown in Fig. 1. At the end of the experiment, blood samples were collected for CORT assay using radioimmunoassay (Romero et al. 1993).

DA and 5-HT measurements

DA and 5-HT were analyzed using an LC10-AD pump (Shimadzu, Columbia, MD, USA), a manual injector (model 7,125; Rheodyne, Cotati, CA, USA) with a 0.1 ml sample loop, equipped with an electrochemical detection (ECD) system (DECADE II, Antec Leyden BV, Zoeterwoude, Netherland). Monoamines were separated using a cation-exchange column (CAPCELL PAK, 1.5 mm×250 mm, 5 μm I.D., Shiseido, Tokyo, Japan) with a column temperature set at 30°C. The mobile phase consisted of 150 mM ammonium acetate, 50 mM citric acid, 27 μM EDTA, 10% methanol, and 1% acetonitrile with pH adjusted to 4.6. The flow rate was set at 0.2 ml/min. The concentrations of DA and 5-HT were determined by using standard curves with known amounts of monoamines in a range of 1.875–18.75 pg. Under these conditions, the limit of detections (LODs) for DA and 5-HT

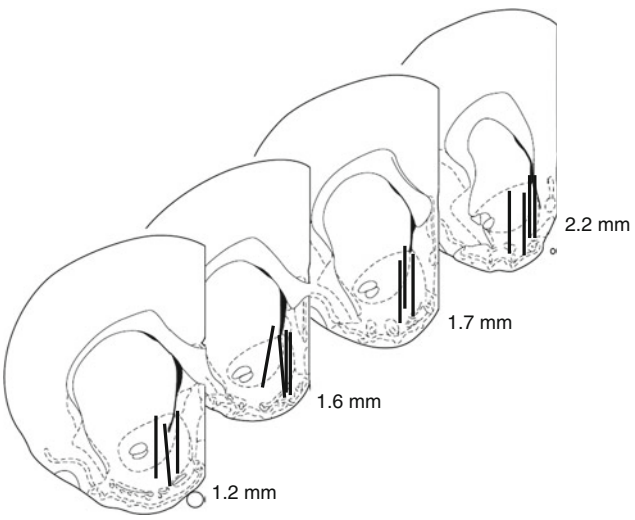

Fig. 1 Placement of microdialysis probe for animals with NAc implants for sample collection. Drawings are adapted from Paxinos and Watson (1997)

were 0.21 and 0.29 pg, respectively. Five rats (n=1 from the stressed and n=4 from the non-stressed control groups) were excluded due to lower levels of DA and 5-HT than the LODs.

After the sample collection, the experimental rats were deeply anesthetized with 100 mg/kg of pentobarbital to collect trunk blood for CORT assay. Once the blood was taken, the experimental rats were perfused with 4% paraformaldehyde solution prior to removing the brain for examination of probe placements. The fixed brains were sliced at 60 μm and mounted on gelatin-coated slides. Once the slices had dried, the brains were stained with cresyl violet. The precise placement of probes was determined using light microscopy (Fig. 1). One rat from the non-stressed control group was excluded from the study due to the misplacement of the probe.

Statistical analysis

Data analyses were performed using Sigma Stat 3.11 (Systat Software Inc., San Jose, CA, USA). One-way repeated measures ANOVAs were used to analyze repeated observations of experimental rats' behaviors during confrontations. Two-way repeated measures ANOVAs were used to analyze repeated observations of body weight and saccharin preference within the stressed and the non-stressed control groups; when indicated by a significant main effect, post hoc comparisons to the non-stressed control group or to the factor "Time" were performed using the Holm-Sidak method for pairwise multiple comparisons. For the microdialysis experiment, one-way repeated measures ANOVAs were used; when indicated by a significant main effect, post hoc comparisons to the five baseline levels of DA or 5-HT prior to the saline injections were performed using the Holm-Sidak method for pairwise multiple comparisons. When DA and 5-HT levels for stressed and non-stressed animals were compared, two-way repeated measures ANOVAs followed by Holm-Sidak method for pairwise multiple comparisons were performed. For CORT levels, unpaired t test was performed. P values of less than 0.05 were considered statistically significant.

Results

Changes of behaviors in experimental female rats during daily confrontations over the course of 21 days of social defeat

Table 1 shows the salient behaviors during confrontations for the experimental female rats (n=7) over 21 days of social defeat. The frequencies of being threatened, defensive upright posture, and escape from the aggressor were significantly reduced in week 3 compared to week 2 (P<0.05). On the other hand, the duration of immobility in the experimental female rats significantly increased during the course of social defeat stress (P=0.002). The CORT levels at the end of the experiment was 208.3± 19.43 ng/ml for the stressed group and 160.0±16.83 ng/ml for the non-stressed control group (n=7 per group, P<0.05).

Weight change

Figure 2 shows the weight change over the 21 days of chronic social stress, as calculated by a percent change from average weights for 3 days prior to the stress procedure. There was a significant main effect of chronic stress in weight change ($F_{1,\ 22}$=12.388, P=0.002) as well as "Time" ($F_{4,\ 71}$=39.622, P<0.001). Moreover, there was a significant interaction between chronic stress vs. weeks ($F_{4,\ 71}$=7.331, P<0.001). All pairwise multiple comparisons revealed a significantly lower weight gain in the stressed group compared to the non-stressed control group (P<0.05).

Reduction in preference for saccharin due to chronic social stress

During the chronic social stress protocol, a significant main effect of stress on intake for saccharin solution was observed ($F_{1,\ 17}$=5.429, P=0.031). Multiple comparisons revealed significant reduction of saccharin intake in the stressed group compared to the non-stressed control group in weeks 1 and 3 (P<0.05). Within the stress group, the saccharin intake was significantly reduced in week 1 compared to the intake in pre-stress period (P<0.05). There was a significant interaction between chronic stress vs. week ($F_{4,\ 59}$=4.583, P=0.003). Figure 3 shows preference for saccharin over the time course of stress. There was a significant main effect of stress on saccharin preference ($F_{1,\ 22}$=9.190, P=0.006). Multiple comparisons revealed significant reduction for the preference of saccharin in the stressed group compared to the non-stressed control group in weeks 1 and 3 and in the post-stress period (P<0.05). Within the stress group, the preference for saccharin was significantly decreased in the post-stress period compared to the pre-stress period (P<0.05).

Disruption in estrous cycle

In normal cycling rats, duration for one cycle was 4–5 days, which includes 2–3 days of met/diestrous phases (Fig. 4a). We combined metestrus and diestrus since these phases are considered to have fewer effects on behavioral and physiological outcomes (Quinones-Jenab et al. 1999; Zhang

Table 1 Time course changes in behaviors of socially defeated female rats (n=7 per week) during 30 min daily confrontations

		Week 1	Week 2	Week 3
Frequency	Being threatened	34.5±5.85	42.6±5.65	20.1±4.48*
	Defensive upright posture	12.5±2.55	15.5±3.04	7.0±1.01*
	Escape from the aggressor	20.6±5.38	22.9±4.96	7.2±1.82*
	Being bitten/nipped	47.2±8.64	70.6±9.69	52.1±11.29
Duration (s)	Immobile	175.3±61.47	415.2±70.74	828.3±159.80*,**
	Supine posture	27.6±8.22	38.3±10.49	21.5±7.83
	Walking	170.9±38.06	70.8±22.22	113.3±33.26
	Sniffing	40.7±12.59	42.0±13.03	61.3±18.53

All values are means ± SEM

**P<0.01 compared to week 2; *P<0.01 compared to week 1

et al. 2008; Keen-Rhinehart et al. 2009). When the rats were exposed to chronic social stress, the disruptions in the cycles were observed in the first week where met/diestrous phases were extended by 4–8 days (Fig. 4b). This disruption continued to be prominent during the second week of the stress procedure. On average, the percentage of regular cycles in the stressed group during the 21 days of social stress was 37%, whereas 81% of cycles were normal in the non-stressed control group (Fig. 4c).

Changes in the levels of NAc DA after cocaine challenge followed by chronic social stress

There was a significant main effect of cocaine on DA levels in the NAc for both the stressed ($F_{6,\ 12}$=4.502, P<0.001) and the non-stressed control groups ($F_{6,\ 12}$=4.156, P<0.001). All pairwise multiple comparisons revealed significant higher amounts of cocaine-induced DA from 20–40 min post-injection of cocaine in the stressed group and from 10–30 min post-injection in the non-stressed control group (P<0.05, Fig. 5a). When the two groups were compared using a two-way repeated measures ANOVAs, there was a significant interaction between chronic stress vs. "Time" ($F_{12,\ 136}$=2.944, P=0.001). All pairwise multiple comparisons revealed a significantly smaller amount of cocaine-induced DA in the NAc from 10–40 min post-injection of cocaine in the stressed group compared to the non-stressed control group (P<0.05, Fig. 5a). Chronic social stress did not affect the amount of tonic DA levels in the NAc. There were no statistical differences between the core vs. the shell placements; therefore, the data were collapsed.

When DA levels were expressed as a percentage of each rat's average baseline levels, a significant main effect of cocaine was observed for both the stressed ($F_{6,\ 12}$=5.472, P<0.001) and the non-stressed control groups ($F_{6,\ 12}$=12.650, P<0.001). The significant percentage changes were observed from 20–50 min post-cocaine in the stressed group and from 10–40 min in the non-stressed control group (Fig. 5b). There was a significant interaction between chronic stress vs. "Time" ($F_{12,\ 136}$=5.737, P<0.001), and a significant smaller change of cocaine-induced DA was observed from 10 to 30 min post-injection of cocaine in the stress group compared to the non-stressed control group (P<0.05, Fig. 5b).

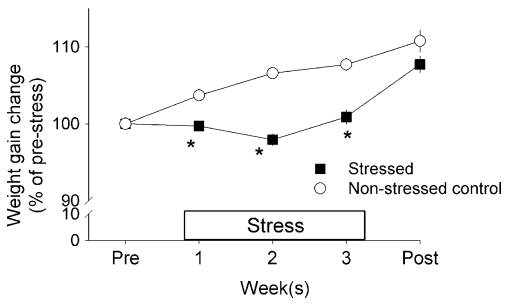

Fig. 2 The effect of 21 days of chronic social stress on weight gain change (mean±SEM). Data are expressed as percentage change from average of 3 days of values prior to the stress experiment. n=10 for the stressed group and n=14 for the non-stressed control group. *P<0.001 compared to the same week of the non-stressed control group

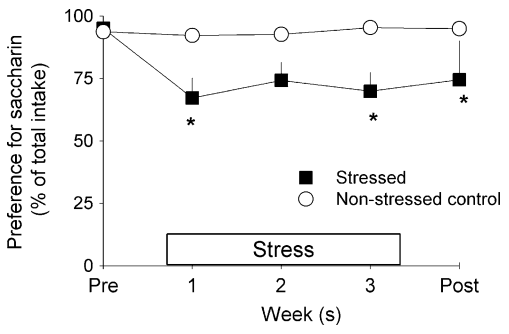

Fig. 3 The effect of 21 days of chronic social stress on preference for saccharin solution (mean±SEM). Data are expressed as percentage of saccharin intake over the total intake in 1-h testing. n=10 for the stressed group and n=14 for the non-stressed control group. *P< 0.001 compared to the same week of the non-stressed control group

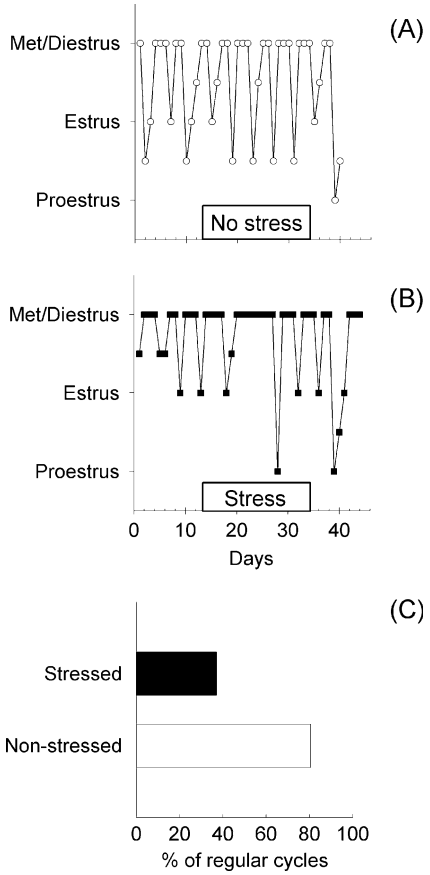

Fig. 4 Representative estrous cycles for (**a**) non-stressed control and (**b**) stressed rats over the time course of chronic social stress. Note that stressed rat showed lengthened met/diestrous period during the course of chronic stress. (**c**) Percentage of regular cycles during 21 days of chronic social stress in the stressed and the non-stressed control rats. n=10 for the stressed group and n=14 for the non-stressed control group

Changes in the levels of NAc 5-HT after cocaine challenge followed by chronic social stress

There was a significant main effect of cocaine on 5-HT levels in the NAc for both the stressed ($F_{6,\ 12}$=4.577, P<0.001) and the non-stressed control groups ($F_{6,\ 12}$= 7.231, P<0.001). All pairwise multiple comparisons revealed significantly higher amounts of cocaine-induced 5-HT from 30 to 60 min post-injection of cocaine in the stressed group and from 20 to 90 min post-injection in the non-stressed control group (P<0.05, Fig. 6a). When the two groups were compared using a two-way repeated measures ANOVAs, there was a significant "Time" effect ($F_{1,\ 12}$=10.839, P<0.001), but no stress effect was observed ($F_{1,\ 12}$=0.683, P=0.425).

When expressed as a percent change of five baseline samples, there was a main effect of cocaine in the non-stressed control group ($F_{6,\ 12}$=3.189, P=0.001), from 30 to 40 and 60 to 70 min post-cocaine (Fig. 6b). However, the main effect was abolished in the stressed group. Likewise, a two-way repeated measures ANOVAs revealed a significant "Time" effect ($F_{1,\ 12}$=4.246, P<0.001), but no stress effect ($F_{12,\ 140}$=1.069, P=0.391).

Discussion

The present study demonstrated for the first time in females that prior experiences with chronic social defeat stress resulted in significant reduction in preference for saccharin and in weight gain, disruption in estrous cycle, and significant suppression of cocaine-induced DA, and to some extent, 5-HT in the NAc.

We chose nursing dams as stressors, because lactating dams typically show aggression in defense of the young (Olivier et al. 1986; Rosenblatt et al. 1988; Haney et al. 1989; Miczek et al. 2004). In addition, threat periods maximize the defeat experiences without any physical damage to the defeated rats (Heinrichs et al. 1992; Tidey and Miczek 1996; Covington and Miczek 2001). For each confrontation, the experimental rat was exposed to a different dam in order to induce social instability and avoid habituation to the aggressors, which is more effective for female than for males, in terms of producing depressive-like symptoms, such as weight loss, increased adrenal weights, higher CORT levels, and loss of fluid intake

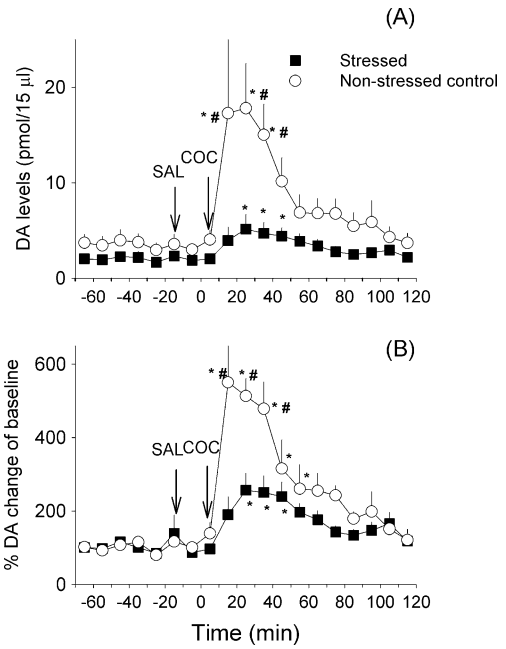

Fig. 5 The effect of chronic social stress on (**a**) DA levels (pmol/15 µl) and (**b**) percentage change of baseline after cocaine challenge in the NAc (n=7 per group, 10 mg/kg, I.P.) (mean±SEM). *SAL* saline, *COC* cocaine. *P<0.001, relative to the average of five baseline samples prior to the saline injections for each group. #P<0.05, relative time points to the stressed group

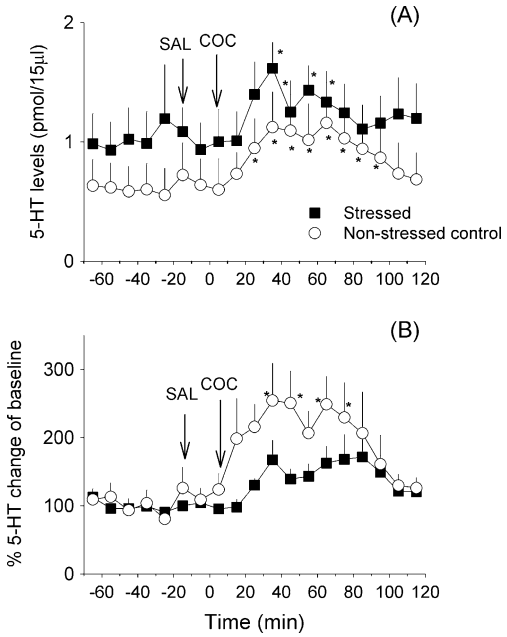

Fig. 6 The effect of chronic social stress on (**a**) 5-HT levels (pmol/15 µl) and (**b**) percentage change of 5-HT after cocaine challenge in the NAc (n=7 per group, 10 mg/kg, I.P.) (mean±SEM). *SAL* saline, *COC* cocaine. *P<0.001, relative to the average of five baseline samples prior to the saline injections for each group

(Haller et al. 1999; Herzog et al. 2009). The combination of social defeat stress and social instability in the current study significantly extended the duration of immobility during the confrontation over the course of stress, which resulted in fewer incidences of being attacked by the aggressors. These changes in behaviors were correlated with significant reductions in weight gain and preference for sweet solution and disruption of estrous cycles during the 21 days of continuous social stress. Although the weight gain was recovered after termination of the stress regimen, other characteristics such as preference for saccharin remained suppressed in the post-stress period. Besides, the CORT levels were significantly elevated in the stressed group at the end of the experiment, suggesting that the effect of chronic stress persists after the stress regimen is terminated. Future studies will have to validate whether the CORT response to stress correlate with the behaviors observed in the current study.

Estrogens directly increase striatal DA levels (Becker and Cha 1989; Becker 1990a, b; Xiao and Becker 1994) as well as 5-HT neural activity in females (Robichaud and Debonnel 2005). Because the estrous cycles in the stressed group were not entirely disrupted over the course of stress exposure, it is likely that estrogens do not play a significant role in suppression of cocaine-induced DA levels observed in the present study. Instead, chronic social stress induces behavioral, neurobiological, and neurochemical deficits (Miczek et al. 2008), which typically correspond to elevated plasma CORT levels (Pitman et al. 1988). CORT increases density of excitatory neurons, including glutamatergic neurons, in the prefrontal cortex (Seib and Wellman 2003). Since glutamate neurotransmission modulates stress-induced DA release also in the NAc (Kalivas et al. 1989; Morrow et al. 1993; Saulskaya and Marsden 1995; Feenstra et al. 1998), and the stress-induced NMDA/AMPA receptors ratio on DA cells is regulated by glucocorticoid receptors (Saal et al. 2003), the dampened cocaine-induced NAc DA levels seen in our present study could be modulated by glutamatergic neurotransmission from the prefrontal cortex, via glucocorticoids induced by chronic social stress. Moreover, glutamate neurotransmission has long been implicated in depression (Krystal et al. 2002; Paul and Skolnick 2003). For instance, the number of AMPA glutamate receptor 2 subtype in the NAc shell is reduced in mice which exhibit depressive-like behaviors after 10 days of severe social defeat stress (Vialou et al. 2010). In a human study, depressed patients had a significantly increased number of metabotropic glutamate receptor subtype 2/3 in the prefrontal cortex (Feyissa et al. 2010). Future work will have to determine specific glutamatergic/dopaminergic receptor subtypes that induce such deficits in cocaine-induced DA levels observed in the present study.

Although there was no significant interaction between stress and "Time" on 5-HT, the effect of cocaine was significant in the non-stressed control group but not in the stressed group, especially when percentage values were used for the analyses. This observation is possibly due to higher amount of tonic 5-HT observed in the stressed group. In fact, the Flinders sensitive line of rats, a genetic model of depression (Overstreet 1993), had higher amount of 5-HT in the NAc compared to controls (Zangen et al. 2001). On the other hand, the interval between the last social defeat and the sampling day could have restored 5-HT in the present study; the deficit in accumbal 5-HT release induced by 21 days of unpredictable stress is completely recovered at 14 days after the last defeat (Mangiavacchi et al. 2001). In addition, there is a sex difference in basal levels of accumbal 5-HT in naïve rats (Lucas and McMillen 2002; Festa et al. 2004), suggesting that chronic stress may affect the serotonergic response to stress differently between males and females. Because antidepressants, such as serotonin selective reuptake inhibitors, can alleviate some features of depressive-like symptoms (Willner et al. 1987; Moreau et al. 1992), future studies will have to examine the effect of antidepressants in these female rats.

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