Cocaine enhances resistance to extinction of responding for brain-stimulation reward in adult prenatally stressed rats

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Competing Interest Statement

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Abstract

The present experiment assessed whether prenatal stress (PS) can alter the ability of acute and chronic cocaine administration to increase and decrease the rewarding effectiveness of the medial forebrain bundle (MFB) using intracranial self-stimulation (ICSS), and also whether PS can affect the extinction of the MFB stimulation response. Adult male offspring of female rats that received PS or no PS (nPS) were implanted with MFB stimulating electrodes, and were then tested in ICSS paradigms. In both nPS and PS offspring, acute cocaine injection decreased ICSS thresholds dose-dependently. However, the threshold-lowering effects at any dose were not significantly different between groups. There was also no group-difference in the threshold-elevating effects of chronic cocaine administration. Nevertheless, chronically drug-administered PS rats exhibited a resistance to the extinguishing of the response for brain-stimulation reward when acutely treated with cocaine, as compared to extinction without cocaine treatment. The results suggest that PS may weaken the ability for response inhibition under cocaine loading in male adult offspring.

Keywords: cocaine, intracranial self-stimulation, medial forebrain bundle, prenatal stress, reward

Abbreviations

ANOVA: analysis of variance; HPA: hypothalamic-pituitary-adrenal, ICSS: intracranial self-stimulation, i.p.: intraperitoneally, MFB: medial forebrain bundle, nPS: no PS, PS: prenatal stress,

Exposure to maternal stress during critical periods of brain development can have permanent and often profound influences on the physiology and behavior of offspring. Clinical research suggests links between maternal stress during pregnancy and many behavioral aberrations in later life, including attention/deficit hyperactivity disorder [9, 15, 25, 29, 38], poor social interaction [30], cognitive dysfunction [46, 47], increased anxiety [46, 47] and substance abuse disorders [47]. In animals, especially rats, repeated maternal exposure to stress during the last week of gestation has been used to model prenatal stress (PS) and validated in a number of laboratories: This procedure results in behavioral modifications, strikingly similar to those in humans, ranging from stronger locomotor responses to a novel environment [10, 41], reduced propensity for social interaction [43], impaired sexual behavior [42, 45], and learning impairments in a reversal task on a Morris water-maze task [16, 39] to increased anxiety-like behaviors such as decreased visits to the "open-arms" on an elevated-plus maze [37, 41]. However, preclinical investigations of alterations in brain reward systems after gestational stress underlying substance use disorders are extremely limited.

Drug self-administration and intracranial self-stimulation (ICSS) are useful operant methods for studying the reinforcing effects of addictive drugs in animal models. Only a recent study by Kippin et al. [23] reported a modulatory effect of PS on cocaine self-administration. Specifically, offspring of female rats that were exposed to repeated maternal restraint stress during the last week of gestation exhibited, when allowed to press a lever for cocaine, elevated responding both during extinction and cocaine-primed reinstatement, but not during self-administration. This suggests that PS induces altered

responsivity of brain reward systems to cocaine. However, no studies have used ICSS measures to investigate the role of PS in cocaine vulnerability. As ICSS directly activates brain reward systems, its thresholds are believed to give an operational measure of brain reward function. Thus, the lowering of ICSS thresholds observed in the period immediately after cocaine administration [11, 22, 28] reflects an increase in brain reward function that may be responsible for the euphoric symptoms associated with cocaine addiction in humans [11]. Conversely, the elevation of ICSS thresholds seen in the period after acute effects of cocaine injection disappeared [1, 20, 28] reflects a decrease in brain reward function that may underlie postcocaine anhedonic/dysphoric symptoms in humans [11]. These findings suggest ICSS paradigms may be used to assess both the euphorigenic and dysphorigenic responses induced by cocaine [11].

To test whether PS affects both responses, therefore, we first examined the dose-response effects of acute cocaine administration on ICSS, and then assessed changes in ICSS thresholds during the withdrawal periods between daily repeated cocaine injections. This was accomplished by determining the effects of cocaine on ICSS rate-frequency functions immediately after and 24 hrs after drug injection. Finally, since it has been reported that prenatally stressed animals can slow the extinguishing of the response during extinction with cocaine self-administration [23], we compared the responses in chronically drug-injected rats during extinction after ICSS training when treated with or without cocaine.

All experiments were approved by the Hamamatsu University School of Medicine Animal Care and Use Committee, and were carried out with the National Institute of Health

Guide for the Care and Use of Laboratory animals (NIH Publications No.80-23). All efforts were made to minimize animal suffering and reduce the number of animals used.

Pregnant Sprague-Dawley (Japan SLC Inc., Shizuoka, Japan) rats were individually kept under a12:12hr light/dark cycle (lights on at 0700h), with food and water *ad libitum*. PS treatment was performed daily from gestational days 13 to 19 (G13 - G19). The dams were put into a narrow animal holder and exposed to bright light for 45 min three times a day (started from 1000, 1300, and 1600h). Control dams were left undisturbed. The litters were nursed by their mothers, weaned at postnatal day 21, and housed three or four to a cage with their own littermates, maintaining the main treatment group segregation.

Male offspring of females that received PS and no PS (nPS) at the age of 10 to 12 weeks were used. The rats were pretreated intraperitoneally (i.p.) with atropine sulfate (0.05 mg, i.p.), anesthetized for surgery with pentobarbital (50 mg/kg, i.p.) and positioned in a stereotaxic frame. A monopolar electrode (stainless steel wire that served as cathode, 0.2 mm in diameter) was inserted into the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (coordinates: anteroposterior -4.0 mm; mediolateral 1.6 mm; and dorsoventral 8.0 mm) [33]. A cortical screw served as anode. Electrodes were secured to the skull with anchor screws and dental cement. Rats were allowed 7-10 days to recover from surgery.

In Experiment I, PS (n=9) and no PS (nPS) (n=8) rats were first trained to poke their nose into a hole in a wall of a transparent acrylic chamber (30.0 × 30.0 × 35.0 cm) for self-stimulation. The rewarding effect of ICSS was measured as previously described [8]. A fixed-interval (1 s) reinforcement schedule was used for this experiment. Each nose-poke

delivered a 0.3-s train of monophasic cathodal rectangular pulses with 0.1-ms duration. During the preliminary session the frequency was held constant at 65 pulses per second (pps) and the current intensity was progressively increased until the subjects showed vigorous self-stimulation. Rats were then tested using two alternating series of ascending and descending current intensities varied in steps of 50 μA. The current-threshold of each rat was defined as the value of stimulus that evoked 50% of the maximal rate of self-stimulation. These intensity values (120-240 μA) were then held constant for the subsequent testing of the frequency-threshold. The rats were again tested using two alternating series of ascending and descending pulse frequencies. The frequencies increased by 0.1 log unit steps (e.g., 26, 33, 41, 52, 65, 82, 103, 130, 163, 206 pps). Each frequency was tested within a 120-s trial. During each testing trial, rats received 10 priming stimulations at the beginning and nose pokes were recorded only in the last 60 sec. A 120-s warm-up trial (65 pps) followed by a 120-s extinction trial was given before the testing trials. Drug tests began when the rate–frequency curves were stable for at least three consecutive days (once daily). A baseline rate-frequency curve was measured (for 40 min) at 3 hr before the drug administration. Cocaine hydrochloride (cocaine: Dai-Nippon Ltd., Osaka, Japan) was dissolved in 0.9% saline and administered in a volume of 1.0 ml/kg of body weight. Cocaine doses (2.5, 5, 10, 20, or 30 mg/kg i.p.) were based on the salt form. The rate-frequency curve was measured again immediately after drug injection. In each group, vehicle and all drugs doses were tested in an ascending order for each rat, and a 3-day interval was allowed between injections.

In Experiment II, another cohort of PS (n=7) and nPS (n=5) rats had ICSS threshold

tests 24 hr after daily drug administration. First, once the rate–frequency curves were stable for at least three consecutive days, the rats were intraperitoneally injected with vehicle for five consecutive days to obtain a baseline. Next, rats were injected with 20 mg/kg of cocaine for five consecutive days and then with 40 mg/kg of cocaine for five additional days. The rats were removed from the test chamber immediately after the ICSS session, and injected with vehicle or cocaine in their home cage. Because previous studies demonstrated that 40 mg/kg was the cocaine dose that induces a generalized motor seizures after repeated daily drug exposure and electrical brain stimulation [24], a combination of 20 mg/kg, a lower dose, and then 40 mg/kg of the drug, a higher dose, was used for repeated cocaine administration to minimize this possibility. All subjects twice underwent a training trial of 65 pps and extinction trial of 1 pps, at the end of chronic cocaine exposure. Each session consisted of three series of 10-min training and 10-min extinction. Half the animals were injected first with vehicle and then with 10 mg/kg of cocaine, while the remaining rats received drugs in the reversed order. The effect of PS on the extinction of nose-poking for ICSS was examined with and without cocaine treatment.

Group differences in the stimulation currents required for maintaining reliable responding between PS and nPS animals were evaluated using a Student's t-test. ICSS thresholds were obtained by fitting the Gompertz growth model to the data [32]: $y=\alpha e^{-e^{b(Xi-X)}}$ (α, *b and Xi* represent *the maximum rate, an index of the slope,* and *threshold* respectively). The percentage changes in mean threshold value and maximum rate of responding produced by vehicle or cocaine for PS and nPS rats were analyzed using mixed design two-way analysis of variance (ANOVA) (prenatal treatment x cocaine dose). The numbers

of responses during training and during extinction for PS and nPS rats were also analyzed using mixed design two-way ANOVA (prenatal treatment x cocaine dose). Significant effects were further analyzed using post hoc Bonferroni tests.

At the end of each experiment, ICSS rats were overdosed with pentobarbital (100 mg/kg, i.p.), perfused intracardially with a 10% formalin-saline solution, and brains were removed. Brains were subsequently sectioned at 30 μm using the frozen technique, and sections were stained with hematoxylin eosin to estimate locations of the electrode tips.

The electrode tips in nPS and PS rats that received the rewarding stimulation were confirmed to be located within or near the MFB. Distribution of electrode placements was essentially identical in both groups of animals (data not shown).

In Experiment I, nPS rats maintained reliable responding at 183.8±10.9 μA (mean±SEM), whereas PS rats maintained it at 185.6±13.3 μA; these currents did not differ significantly between groups ($t=0.10$, $df=15$, ns). Cocaine caused acutely dose-dependent leftward shifts of the rate-frequency functions regardless of prenatal treatment (Fig. 1A). These shifts in the mean ICSS thresholds, expressed as percentages of the pre-drug baseline, revealed a significant main effect of cocaine dose ($F(5,75)=19.82$, $p<0.001$) but not of prenatal treatment ($F(1,15)=0.29$, ns) and also no interaction effect of cocaine dose and prenatal treatment ($F(5,75)=1.72$, ns)(Fig. 1B). That is, in both nPS and PS rats, acute cocaine injection did not have significant effects at 2.5, 5 and 10 mg/kg, but it significantly decreased ICSS thresholds at 20 ($p<0.001$) and 30 mg/kg ($p<0.001$) when compared with threshold values after vehicle (cocaine 0 mg/kg). However, the threshold-lowering effects at any dose were not significantly different between groups. On the other hand, the mean of

maximal response rates did not reveal any significant main effects of cocaine dose ($F(5,75)=0.55$, ns) as well as prenatal treatment ($F(1,15)=2.83$, ns), and no interaction between cocaine dose and prenatal treatment ($F(5,75)=0.80$, ns) (data not shown).

In Experiment II, nPS and PS rats required 172.0±9.7 μA and 165.7±13.4 μA, respectively, to sustain reliable responding; these currents did not differ significantly between treatments ($t=0.35$, $df=10$, ns). The rate-frequency function was slightly shifted to the right in both nPS and PS animals, as determined 24 hrs after daily cocaine administration (Fig. 2A). The data were averaged over the last three consecutive days for baseline and each cocaine treatment. However, the mean ICSS thresholds revealed no significant main effects of cocaine dose ($F(2,20)=1.42$, ns) and prenatal treatment ($F(1,10)=0.99$, ns), and no interaction effect of cocaine dose and prenatal treatment ($F(2,20)=0.43$, ns). After the chronic cocaine exposure, all animals were given extinction trials of responding for ICSS with or without cocaine treatment. Nose-poking for ICSS was allowed to stabilize during 10-min training trials before each rat was exposed to 10 min of extinction trials (responding without ICSS reward). Cocaine (10 mg/kg) did not increase the response rate during training trials in PS as well as nPS rats. The total number of nose-pokes over three training trials did not show any statistical difference between vehicle and cocaine ($F(1,10)=2.38$, ns), between prenatal treatments ($F(1,10)=0.10$, ns), and also interaction between drug and prenatal treatments ($F(1, 10)=0.21$, ns) (Table 1). On the other hand, the drug clearly increased the response rate during extinction trials in PS but not nPS rats (Fig. 2B). The total number of nose-pokes over three extinction trials revealed a significant interaction between cocaine dose and prenatal treatment ($F(1,10)=7.28$, $p=0.02$)

and marginal main effect in cocaine dose ($F(1,10)=4.49$, $p=0.06$), but no main effect in prenatal treatment ($F(1,10)=1.86$, ns) was found. Following cocaine treatment, the total number of nose-pokes during extinction trials was significantly high only in PS rats, compared with vehicle-treated trials ($p<0.01$), as shown in Fig. 2B.

The results of this study demonstrated for the first time that prenatal stress augments responding during extinction of ICSS with but not without cocaine treatment in male adult offspring. However, our results also revealed that prenatal stress affected neither the stimulant effects of the rewarding impact of the MFB stimulation induced acutely by exposure to cocaine, nor its depressant effects during the withdrawal periods in repeated drug exposures.

PS is well known to alter the hypothalamic-pituitary-adrenal (HPA) axis in ways that weaken its negative feedback in later life [48]. That is, repeated stress to pregnant rats by activating the HPA axis causes excessive secretion of glucocorticoid hormones in the mother and fetus [44]. High levels of corticosterone cross the placenta and blood-brain barriers to reach the fetal brain [2, 3] and results in dysregulation of the fetal HPA axis [48]. Consequently, the offspring of mothers that were prenatally exposed to stress evidently exhibit a prolonged elevation of plasma corticosterone following an acute restraint stress in adulthood.

Several findings have shown that the HPA axis is crucial for cocaine reinforcement [35, 36]. Cocaine self-administration cannot be acquired at low doses (0.125 mg/kg/infusion or lower) unless the level of corticosterone is increased above a threshold critical for cocaine reward [12, 13, 14]. Conversely, adrenalectomy effectively prevents the increase in cocaine

self-administration by footshock-induced corticosterone [14]. Like stress, ICSS activates the HPA axis leading to elevated levels of corticosterone in circulating blood [7, 40]. Based on these combined results, one can speculate that PS and the resulting heightened corticosterone secretion might increase the sensitivity of brain-stimulation reward to cocaine in offspring.

We first examined acute dose-response effects of cocaine administration on ICSS in male adult offspring by determining the effects of cocaine on ICSS rate-frequency functions. However, PS failed to alter the lowest dose of cocaine (20 mg/kg, i.p.) for inducing a significant decline of ICSS thresholds, and no significant difference in ICSS thresholds was found between PS and nPS rats at any dose of cocaine (Fig.1). Results also showed that cocaine did not alter the maximal rates of responding at any dose examined, suggesting that ICSS thresholds observed here were largely unaffected by performance-altering effects of drug manipulations such as increased locomotion and/or stereotypy evident at a high dose. It is worth noting that other behavioral models, such as self-administration and conditioned place preference paradigms also provided results consistent with the present finding: When both nPS and PS rats were trained to self-administer cocaine intravenously at doses of 0.25, 0.5 and 1.0 mg/kg/infusion, there was no difference in the number of sessions required to reach the self-administration criterion between groups [23]. In addition, nPS and PS rats developed a dose-dependent conditioned place preference, and no significant difference was observed between groups at cocaine doses of 2.5, 5 and 10 mg/kg, i.p. (our unpublished data). In parallel with these results, the present findings indicate that PS and the resulting impaired activity of HPA axis

might not affect the stimulant effect of the rewarding effectiveness of the MFB stimulation induced acutely by cocaine in male adult offspring.

Next, we assessed daily changes in ICSS thresholds during the withdrawal periods after repeated exposures to cocaine administration. According to the literature, adrenalectomy blocks acquisition of cocaine self-administration [14, 26], whereas repeated treatment with corticosterone decreases ICSS thresholds in adult rats [4], suggesting that PS and the resulting elevated levels of corticosterone may enhance the rewarding value of stimuli such as drugs and stimulation. Results showed that both PS and nPS rats tended to develop elevations of ICSS thresholds daily determined at 24 hrs post-cocaine administration. Moreover, no significant difference in ICSS thresholds was found between PS and nPS rats after either 20 or 40 mg/kg of cocaine was repeatedly administrated (Fig. 2A). This finding suggests that PS and the resulting weakened negative feedback of the HPA axis might not affect the cocaine-induced depressant effect of brain-stimulation reward in male adult offspring. It is unexpected, however, that the present study failed to duplicate a previous result in the nPS rats showing that daily repeated administration of cocaine at a high dose of 40 mg/kg over seven consecutive days dampened the sensitivity of the brain reward systems by post-drug elevation of ICSS thresholds [24]. The reason for this difference in response is not clear, but may be due to differences in drug regimen. Although rats in the present study were treated with 20 mg/kg once-daily for 5 days followed by further 5 days of treatment with 40 mg/kg once-daily, the treatment period of 40 mg/kg cocaine (5 days exposure) was shorter than that in the previous study (7 days exposure) which showed that only the post-cocaine increase in reward thresholds for brain stimulation seen on Day 7 was

significantly higher from baseline [24]. In addition, it is also important to note that brain reward systems may respond differentially to self vs imposed drug injections. Indeed, ICSS thresholds are elevated after chronically self-administered cocaine injections more reliably than by chronically experimenter-administered cocaine injections [1, 22, 28].

As mentioned above, PS and the resulting dysregulation of the HPA axis most likely does not affect the potentiation as well as depression of brain stimulation reward appearing shortly and long after cocaine administration, respectively. Nonetheless, we found noticeable evidence that chronically drug-administered PS rats exhibited high levels of responding during extinction trials than did nPS rats when acutely treated with cocaine, whereas responding during extinction was unchanged in both groups when treated with vehicle. Thus, this evidence is partially in accordance with a recent finding showing that PS rats increased lever-pressing during extinction of cocaine self-administration relative to nPS rats [23]. The mechanisms by which PS enhances non-reinforced responding during extinction are unclear but may involve alterations of the mesolimbocortical dopamine system as well as the HPA axis. Indeed, PS decreases corticosteroid receptors in hippocampus [3, 17] and frontal cortex [27] of male offspring, areas that are strongly involved in the regulation of the negative feedback of the HPA axis [18, 19]. On the other hand, PS increases dopamine receptors in these brain areas [5, 6] and also enhances basal [23] as well as cocaine-stimulated dopamine transmission in the prefrontal cortex of cocaine-experienced male rats [23]. Moreover, PS males show a reduction in spine density and dendritic complexity in hippocampus [20] and medial prefrontal cortex [31]. Damage to the hippocampus [21, 49] or the medial prefrontal cortex [34] could display a high

resistance to extinction in some appetitive learning situations. Thus, altered responsiveness of the HPA axis and the dopamine system in these brain regions may contribute to the behavioral deficit observed here in PS rats.

In summary, the present findings showed that gestational stress potentially increases resistance to extinction of responding for a brain-stimulation reward under cocaine loading in male adult offspring. It is thus suggested that PS might decrease the ability to inhibit inappropriate responding, which becomes obvious under cocaine administration.

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References

[1] Ahmed SH, Kenny PJ, Koob GF, Markou A. Neurobiological evidence for hedonic allostasis associated with escalating cocaine use. Nature Neurosci 2002;5:625-626.

[2] Arishima K, Nakama S, Morikawa Y, Hashimoto Y, Eguchi Y. Maternal-foetal interrelations of plasma corticosterone concentrations at the end of gestation in the rat. J Endocrinol 1977;72:239-40.

[3] Barbazanges A, Piazza PV, Le Moal M, Maccari S. Maternal glucocorticoid secretion mediates long-term effects of prenatal stress. J Neurosci 1996;16:3943-9.

[4] Barr AL, Brotto LA, Phillips AG. Chronic corticosterone enhances the rewarding effect of hypothalamic self-stimulation in rats. Brain Res 2000;875:196-201.

[5] Barros VG, Berger MA, Martijena ID, Sarchi MI, Perez AA, Molina VA, Tarazi FI, Antonelli MC. Early adoption modifies the effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. J Neurosci Res 2004;76:488-496.

[6] Berger MA, Barros VG, Sarchi MI, Tarazi FI, Antonelli MC. Long-term effects of prenatal stress on dopamine and glutamate receptors in adult rat brain. Neurochem Res 2002;27:1525-1533.

[7] Burgess ML, Davis JM, Borg TK, Wilson SP, Burgess WA, Buggy J. Exercise training alters cardiovascular and hormonal responses to intracranial self-stimulation. J Appl Physiol 1993;75:863-9.

[8] Chen J, Nakamura M, Kawamura T, Takahashi T, Nakahara D. Roles of pedunculopontine tegmental cholinergic receptors in brain stimulation reward in the rat. Psychopharmacology 2006;184:514-22.

[9] Clements AD. The incidence of attention deficit-hyperactivity disorder in children whose mothers experienced extreme psychological stress. Ga Edu Res 1992;91:1-14.

[10] Deminiére JM, Piazza PV, Guegan G, Abrous N, Maccari S, Le Moal M, Simon H. Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. Brain Res 1992;586:135-9.

[11] Frank RA, Manderscheid PZ, Panicker S, Williams HP, Kokoris D. Cocaine euphoria, dysphoria, and tolerance assessed using drug-induced changes in brain-stimulation reward. Pharmacol Biochem Behav 1992;42:771-9.

[12] Goeders NE. The HPA axis and cocaine reinforcement. Psychoneuroendocrinology 2002;27:13-33.

[13] Goeders NE. Stress and cocaine addiction. J Pharmacol Exp Ther 2002;301:785-9.

[14] Goeders NE, Guerin GF. Effects of surgical and pharmacological adrenalectomy on the initiation and maintenance of intravenous cocaine self-administration in rats. Brain Res 1996;722:145-52.

[15] Grizenko N, Shayan YR, Polotskaia A, Ter-Stepanian M, Joober R. Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. J Psychiatry Neurosci 2008;33:10-6.

[16] Hayashi A, Nagaoka M, Yamada K, Ichitani Y, Miake Y, Okado N. Maternal stress induces synaptic loss and developmental disabilities of offspring. Int J Neurosci 1998;16:209-16.

[17] Henry C, Kabbaj M, Simon H, Le Moal M, Maccari S. Prenatal stress increases the hypothalamo-pituitary–adrenal axis response in young and adult rats. J Neuroendocrinol 1994;6:341–345.

[18] Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog Neuro-Psychoph 2005;29:1201-13.

[19] Holmes A, Wellman CL. Stress-induced prefrontal reorganization and executive dysfunction in rodents. Neurosci Biobehav Rev 2009;33:773-83.

[20] Hosseini-Sharifabad M, Hadinedoushan H. Prenatal stress induces learning deficits and is associated with a decrease in granules and CA3 cell dendritic tree size in rat hippocampus. Anat Sci Int 2007;82:211-7.

[21] Kelley SP, Mittleman G. Effects of hippocampal damage on reward threshold and response rate during self-stimulation of the ventral tegmental area in the rat. Behav Brain Res 1999;99:133-41.

[22] Kenny PJ, Polis I, Koob GF, Markou A. Low dose cocaine self-administration transiently increases but high dose cocaine persistently decreases brain reward function in rats. Eur J Neurosci 2003;17:191-195.

[23] Kippin TE, Szumlinski KK, Kapasova Z, Rezner B, See RE. Prenatal stress enhances responsiveness to cocaine. Neuropsychopharmacology 2008;33:769-82.

[24] Kokkindis L, McCarter BD. Postcocaine depression and sensitization of brain-stimulation reward: analysis of reinforcement and performance effects. Pharmac Biochem Behav 1990;36:463-71.

[25] Linnet KM, Dalsgaard S, Obel C, Wisborg K, Henriksen TB, Rodriguez A, Kotimaa A, Molianen I, Thomsen PH, Olsen J, Jarvelin MR. Maternal lifestyle factors in pregnancy risk of attention deficit hyperactivity disorder and associated behavior: review of the current evidence. Am J Psychiatry 2003;160:1028-40.

[26] Mantsch JR, Saphier D, Goeders NE. Corticosterone facilitates the acquisition of cocaine self-administration in rats: opposite effects of the type II glucocorticoid receptoragonist dexamethasone. J Pharmacol Exp Ther 1998;287:72-80.

[27] McCormick CM, Smythe JW, Sharma S, Meaney MJ. Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal response to stress and brain glucocorticoid receptor density in adult rats. Dev Brain Res 1995;84:55-61.

[28] Markou A, Koob GF. Postcocaine anhedonia an animal model of cocaine withdrawal. Neuropsychopharmacology 1991;4:17-26.

[29] McIntosh DE, Mulkins RS, Dean RS. Utilization of maternal perinatal risk indicators in the differential diagnosis of ADHA and UADD children. Int J Neurosci 1995;81:35-46.

[30] Meijer A. Child psychiatric sequelae of maternal war stress. Act Psychiatr Scand 1985;72:505-11.

[31] Murmu MS, Salomon S, Biala Y, Weinstock M, Braun K, Bock J. Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. Eur J Neurosci 2006;24:1477-87.

[32] Panagis G, Kastellakis A, Spyraki C, Nomikos G. Effects of methyllycacotinine (MLA), an alpha 7 nicotinic receptor antagonist, on nicotine- and cocaine-induced potentiation of brai stimulation reward. Psychopharmacology Berl 2000;149:388-96.

[33] Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 4th ed. New York: Academic Press; 1998.

[34] Peters J, LaLumiere RT, Kalivas PW. Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J Neurosci 2008;28:6046-53.

[35] Piazza PV, Le Moal M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Res Rev 1997;25:359-72.

[36] Piazza PV, Le Moal M. The role of stress in drug self-administration. Trends Pharmacol Sci 1998;19:67-74.

[37] Poltyrev T, Keshet GI, Kay G, Weinstock M. Role of experimental conditions in determining differences in exploratory behavior of prenatally stressed rats. Dev Psychobiol 1996;29:453-62.

[38] Rodriguez A, Bohlin G. Are maternal smoking and stress during pregnancy related to ADHA symptoms in children? J Child Psychol Psychiatry 2005;46:246-54.

[39] Szuran T, Zimmermann E, Welzl H. Water maze performance and hippocampal weight of prenatally stressed rats. Behav Brain Res 1994;65:153-5.

[40] Terry LC, Martin JB. Hypothalamic-pituitary responses to intracranial self-stimulation in the rat. Brain Res 1978;157:89-104.

[41] Vallée M, Mayo W, Dellu F, Le Moal M, Simon H, Maccari S. Prenatal stress induces high anxiety and postnatal handling induces low anxiety in adult offspring: correlation with stress-induced corticosterone secretion. J Neurosci 1997;17:2626-36.

[42] Ward IL. Prenatal stress feminizes and demasculinizes the behavior of males. Science 1972;175:82-4.

[43] Ward IL, Stehm KE. Prenatal stress feminizes juvenile play patterns in male rats. Physiol Behav 1991;50:601-5.

[44] Ward IL, Weisz J. Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. Endocrinology 1984;114:1635-44.

[45] Ward OB, Monaghan EP, Ward IL. Naltrexone blocks the effects of prenatal stress on sexual behavior differentiation in male rats. Pharmacol Biochem Behav 1986;25:573-6.

[46] Weinstock M. Alterations induced by gestational stress in brain morphology and behaviour of the offspring. Prog Neurobiol 2001;65:427-51.

[47] Weinstock M. The potential influence of maternal stress hormones on development and mental health of the offspring. Brain Behav Immunity 2005;19:296-308.

[48] Welberg LAM, Seckl JR. Prenatal stress, glucocorticoids and the programming of the brain. J Neuroendocrinol 2001;13:113-28.

[49] Zimmermann PK, Wagner U, Krauth J, Huston JP. Unilateral lesion of dorsal hippocampus enhances reinforcing lateral hypothalamic stimulation in the contralateral hemisphere. Brain Res Bull 1997;44:265-71.

Figure legends

Fig. 1. Effects of acute cocaine administration to potentiate rewarding effects of ICSS (Experiment I). (A) Rate of nose-poking as a function of stimulation frequency during pre-drug baseline and after vehicle or cocaine (30 mg/kg, i.p.) for PS (n=9) and nPS (n=8). Each data point denotes the mean±SEM of reinforced responses. (B) Effects of cocaine on ICSS thresholds in PS (n=9) and nPS (n=8) rats. Cocaine caused dose-dependent decreases in ICSS thresholds (mean±SEM) in all animals. However, the threshold-lowering effects of cocaine were not different between groups. ***$p<0.001$

Fig. 2. Effects of repeated cocaine administration to dampen rewarding effects of ICSS and responding in ICSS extinction trials (Experiment II). (A) Effects of chronic cocaine administration on the ICSS thresholds in nPS (n=5) and PS (n=7). Immediately after the ICSS session rats were daily injected with cocaine at 20 mg/kg (i.p.) for successive 5 days and then at 40 mg/kg (i.p.) for another successive 5 days. ICSS threshold was measured at 24-hr post-cocaine. Data were presented as the mean±SEM of values averaged over the last 3 successive days for each injection. (B) Effects of 10 mg/kg cocaine on non-reinforced nose-pokes during extinction trials of ICSS responding. Each bar denotes the mean±SEM of total nose-poke responses over 3 extinction trials. **$p<0.01$

Table legend

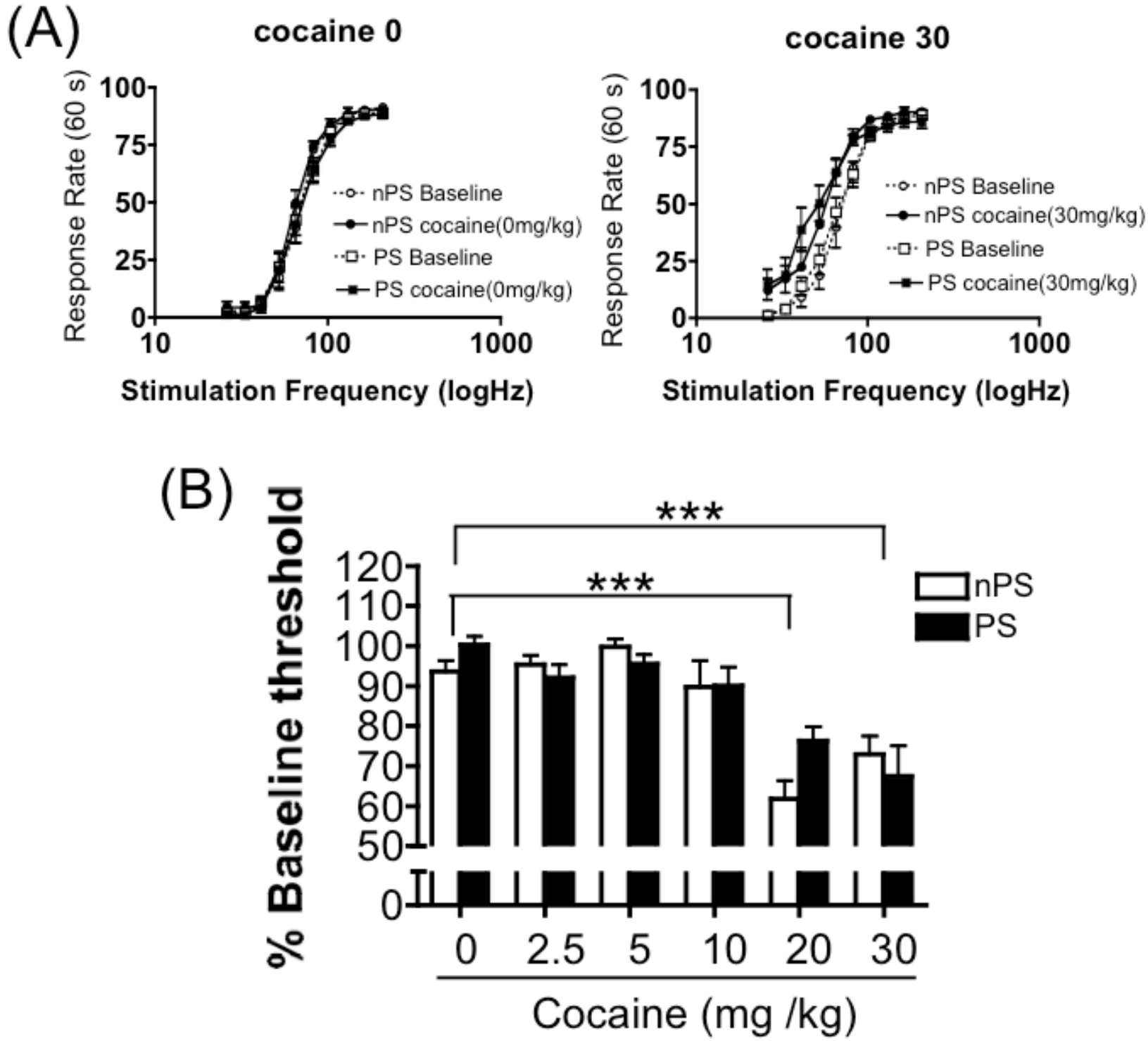
Table 1. Effects of 10 mg/kg cocaine on reinforced nose-pokes during training trials of ICSS

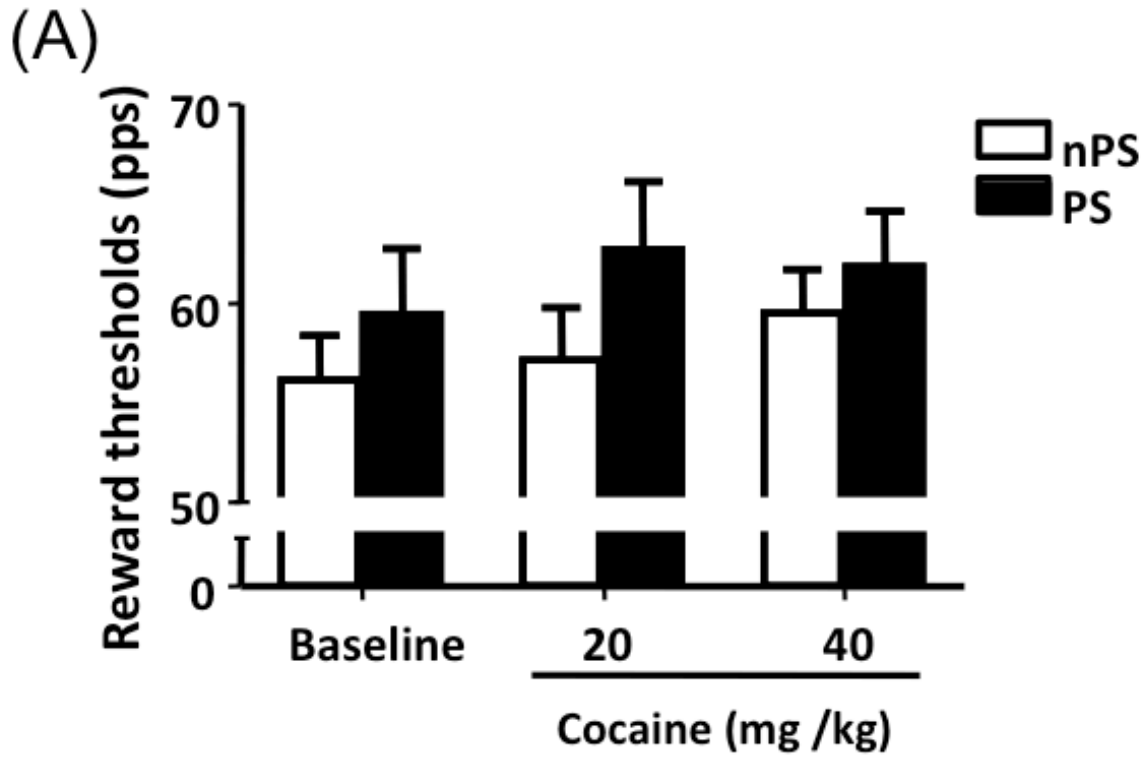
Values given are mean±SEM number of total nose-pokes over 3 training trials with or without cocaine. Although cocaine increased ICSS rates, there was no significant difference in responding between treatments in both groups.

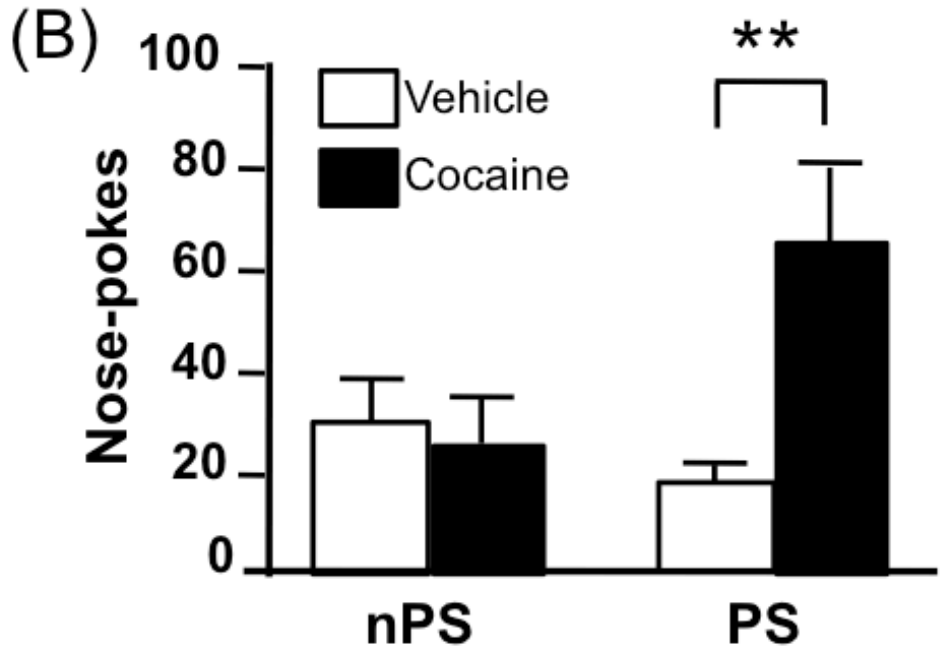
Group	Drug treatment	Training (10 min/trial)			Total
		1	2	3	
nPS (n=5)	Vehicle	289.0±32.5	271.2±45.7	266.2±31.4	826.4±83.7
	Cocaine	313.2±32.1	322.8±28.7	293.8±43.3	929.8±96.8
PS (n=7)	Vehicle	318.9±23.0	321.4±23.2	276.3±41.4	877.1±69.6
	Cocaine	339.0±27.3	376.4±23.1	326.6±42.2	948.7±84.1

Table 1. Effects of cocaine on the rate of ICSS

Values given are mean±SEM number of nose-pokes per 10 min with or without cocaine (10 mg/kg, i.p.). Although cocaine increased ICSS rates, there was no significant difference in responding between treatments in both groups.


(A)
cocaine 0
Response Rate (60 s)
100
75
50
25
0
10
100
1000
Stimulation Frequency (logHz)
nPS Baseline
nPS cocaine(0mg/kg)
PS Baseline
PS cocaine(0mg/kg)
cocaine 30
Response Rate (60 s)
100
75
50
25
0
10
100
1000
Stimulation Frequency (logHz)
nPS Baseline
nPS cocaine(30mg/kg)
PS Baseline
PS cocaine(30mg/kg)
(B)
***
***
% Baseline threshold
120
110
100
90
80
70
60
50
0
nPS
PS
0
2.5
5
10
20
30
Cocaine (mg /kg)


(A)
Reward thresholds (pps)
70
60
50
0
nPS
PS
Baseline
20
40
Cocaine (mg /kg)


(B)
Nose-pokes
100
80
60
40
20
0
Vehicle
Cocaine
**
nPS
PS