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Augmented D_1 dopamine receptor signaling and immediate-early gene induction in adult striatum following prenatal cocaine

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Abstract

Background—Prenatal exposure to cocaine can impede normal brain development triggering a range of neuroanatomical and behavioral anomalies that are evident throughout life. Mouse models have been especially helpful in delineating neuro-teratogenic consequences following prenatal exposure tococaine. The present study employed a mouse model to investigate alterations in D_1 dopamine receptor signaling and downstream immediate-early gene induction in the striatum of mice exposed to cocaine *in utero*.

Methods—Basal, forskolin- and D_1 receptor agonist-induced cAMP levels were measured *ex vivo* in the adult male striatum in mice exposed to cocaine *in utero*. Further studies assessed cocaine-induced *zif 268* and *homer 1* expression in the striatum of juvenile (P15), adolescent (P36), and adult (P60) male mice.

Results—The D_1 dopamine receptor agonist SKF82958 induced significantly higher levels of cAMP in adult male mice treated with cocaine *in utero* compared to saline controls. No effects of the prenatal treatment were found for cAMP formation induced by forskolin. Following an acute cocaine challenge (15 mg/kg, i.p.), these mice showed greater induction of *zif 268* and *homer 1*, an effect that was most robust in the medial part of the mid-level striatum and became more pronounced with increasing age.

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Conclusions—Together these findings indicate abnormally enhanced D_1 receptor signal transduction in adult mice following prenatal cocaine exposure. Such changes in dopamine receptor signaling may underlie aspects of long-lasting neuro-teratogenic effects evident in some humans following *in utero* exposure to cocaine, and identify the striatum as one target potentially vulnerable to gestational cocaine exposure.

Keywords

Immediate-Early Genes; Prenatal cocaine; Brain development; D_1; *zif 268*; *homer 1*

Introduction

Exposure to cocaine *in utero* continues to be a major epidemiological concern with estimates of 45,000 (1) to 375,000 (2) affected infants born each year. Roughly $352 million are spent annually in special education programs for these children in the US (3), and health care costs are estimated to be double that of a non-exposed infant (4). Despite the existing controversy over the definitive effects of prenatal cocaine (see (3)) accruing evidence identifying neuro-behavioral deficits in a subset of exposed offspring reinforce the need for further research aimed towards understanding mechanisms contributing to such long-term sequelae.

Numerous studies reporting findings from exposed offspring have linked prenatal cocaine exposure with microcephaly (5), low birth weight and preterm births (6), altered or delayed motor development (7), global hypertonia and coarse tremor (8), reduced cognitive development (9), and a subtle reduction in IQ (3) in a subset of exposed offspring. However, the data remains elusive as to the independent contribution of prenatal cocaine vs. confounding variables such as poly-drug use, poor prenatal; care, adverse living situations, and compromised maternal health in contributing to adverse postnatal neuro-behavioral outcomes. To control for some of these variables we created an animal model of prenatal cocaine exposure which has provided support for the role of cocaine independently contributing to some but not all of the clinical findings (reviewed in (10)). Of note,, rodents exposed to cocaine *in utero* display an altered response to drugs of abuse when tested as adults including impaired conditioned place preference (11,12), increased acquisition to (13,14) and enhanced reinforcing ability of cocaine (13,14) in self-administration tests, augmented brain stimulation reward following administration of cocaine and D_1 agonists (15), and enhanced stereotypy during cocaine induced behavioral sensitization (16).

The psychostimulant cocaine is a potent, indirect dopaminergic agonist, which inhibits reuptake by the dopamine transporter to increase synaptic dopamine levels (17). Dopamine acting on D_1 receptors activates the cAMP pathway and cAMP mediated gene expression. A significant amount of literature details the long-term effects of postnatal cocaine exposure on the dopamine system in rodents (18,19) reviewed in (20,21). However, much less is known about the long-term effects of prenatal exposure to cocaine. Evidence suggests decreased dopamine transporter activity (22,23), and enhanced D_1 (24,25) and D_2 (23) dopamine receptor levels/function in the striatum or midbrain of prenatally exposed animals. However, evidence that prenatal cocaine attenuates D_1 receptor signaling has also been found (26,27). Differences in the species studied, as well as the route, dose and gestational timing of cocaine utilized may contribute to such discrepant data. However, it is now well established that prenatal pharmacological manipulation of the embryonic dopamine system via cocaine seems to interrupt normal development possibly leading to an altered neuro-behavioral phenotype.

To date few studies have characterized alterations in gene expression in the brain of mice prenatally exposed to cocaine. Of special interest are genes encoding proteins that regulate neuronal plasticity including transcription factors (immediate-early genes, IEGs) such as c-

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fos and *zif 268* (28), and synaptic plasticity factors such as Homer proteins (29,30). The striatum is among the brain regions that show the most pronounced changes in gene expression following psychostimulant treatments (31,32). The striatum is part of anatomically distinct cortico-basal ganglia-cortical circuits that mediate planning and execution of motor functions and goal-directed behaviors (33–36). Striatal dysfunction is implicated in a variety of neuropsychiatric disorders (37–39), including drug addiction (40,41). Genes induced in striatal neurons by systemic psychostimulant administration include *c-fos* (42,43), *zif 268* (44) and *homer 1a* (45,46). Such gene induction is principally mediated by D_1-like receptors; however, D_2-like receptors additionally modulate such effects (47,48). Notably, psychostimulant-induced gene regulation is abolished by D_1 receptor blockade (42,44,49,50), or by targeted deletion of D_1 receptors (51–54).

The present study investigated the effects of prenatal cocaine exposure on dopamine receptor signaling. We assessed D_1 receptor signaling by first examining basal, forskolin-, and D1 receptor agonist-regulated cAMP levels in the striatum of adult mice exposed to cocaine *in utero*. This was followed by investigation of cocaine-induced expression of two IEGs, *zif 268* and *homer 1*, in the striatum of mice exposed to cocaine *in utero*, at three developmentally distinct ages, P15, P36 and P60, which correspond to juvenile, adolescent and adult age, respectively.

Methods and Materials

Prenatal Cocaine Treatment

Prenatal treatments were accomplished as previously described (55). Briefly, timed-pregnant Swiss Webster dams (Taconic Labs, New York) were assigned to one of two treatment groups to receive twice-daily subcutaneous (s.c.) injections (at 7:00 AM and 7:00 PM) from E8–E17, inclusive, of cocaine HCl (Sigma-Aldrich, St. Louis, MO, 20 mg/kg/injection, s.c., dissolved in saline, 2 mg/ml; PCOC40), or 0.9% saline (PSAL). In the adenylyl cyclase experiment two additional treatment groups were included; PCOC20 (2 daily injections of 10 mg/kg cocaine, s.c. in saline, 1mg/ml), and saline pair-fed (SPF), which were injected with 0.9% saline twice daily, but received a restricted diet to control for the anorectic effect of cocaine. Neither of these control groups was significantly different from the PSAL group regarding fetal growth (see supplemental data) or adenylyl cyclase activity, so offspring were not used in further studies.

All pups were surrogate fostered to control dams (Black Swiss Webster; Taconic Labs, New York), which had delivered within the previous 48 hours. To avoid the problem of 'oversampling' (56), no more than two offspring per litter were used.

Adenylyl Cyclase Measurement

Adult (P75-90) mice were exposed briefly to CO_2, decapitated and the dorsal striatum (caudate-putamen) was rapidly dissected on ice. Adenylyl cyclase activity was measured as previously described (19). Briefly, crude membranes were immediately prepared from the striatum of individual animals. Tissue homogenates (20–35 μg protein) were incubated in 10 mM imidizole (pH 7.4), 10 mM theophylline, 6 mM $MgSO_4$, 0.6 mM EGTA, 1.5 mM ATP, and 0.01 mM GTP in the absence or presence of 10 nM – 10 uM SKF82958 or 10 uM forskolin in triplicate for 5 minutes at 30°C. Adenylyl cyclase activity was terminated by placing the tubes into boiling H_2O for 2 minutes. The amount of cAMP formed was determined by a ^{3}H]cAMP binding protein assay (57). ^{3}H]cAMP (4 nM) in citrate-phosphate buffer (pH 5.0) followed by binding protein prepared from bovine adrenal glands was added to each sample. Standards for quantification were prepared with tissue containing known amounts of cAMP (0.5 – 20 pmol). A 90-minute competition reaction for the binding protein between formed cAMP and ^{3}H]

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cAMP was incubated at 4°C to reach equilibrium, and terminated with charcoal and centrifugation to separate the free cAMP from that bound to the binding protein. Aliquots of the supernatants were assayed for radioactivity by liquid scintillation spectrometry using CytoScint Scintillation Fluid (ICN Biomedicals, CA). Radioactivity counts were converted to pmol of cAMP protein by comparison to the standard curve. Protein concentrations were determined using a modification of the Lowry procedure (58).

Behavioral Testing

Horizontal locomotion was assessed in three different cohorts of male mice were tested at three distinct ages, postnatal day (P) 15, P36, and P60. On the test day, mice were placed individually in the test box (30 by 30 centimeter activity box with 16 photocells on each side connected to a computer, MedAssociates, Georgia, VT) for a 15-minute habituation period, removed from the box, injected with saline or cocaine (i.p., 15 mg/kg in saline,) and returned to their respective box for 30 minutes. Immediately following, mice were decapitated, their brains removed and rapidly frozen at −30°C in isopentane for in situ hybridization histochemistry.

In Situ Hybridization Histochemistry

Cryostat-cut coronal sections (12 μm) through the striatum were thaw-mounted onto glass slides (Superfrost/Plus, Daigger, Wheeling, IL), dried on a slide warmer and stored at −20°C. The sections were then fixed in 4% paraformaldehyde/0.9% saline for 10 minutes at room temperature, incubated in a fresh solution of 0.25% acetic anhydride in 0.1M triethanolamine/ 0.9% saline (pH 8.0) for 10 minutes, dehydrated, defatted for 2 × 5 minutes in chloroform, rehydrated, and air-dried. Slides were stored at −30°C until hybridization.

Oligonucleotide probes (48-mers; Life Technologies, Rockville, MD) were labeled with ^{35}[S]-dATP (59). The probes had the following sequence: *zif 268*, complementary to bases 1757–1804, GenBank accession number M28844; *homer 1*, bases 674–721, AF093257. The *homer 1* probe was a pan probe, targeting the beginning of the transcript, in order to produce a more robust signal (60).

Labeled probe (~3×10^6 cpm) in 100 μl of hybridization buffer was added to each slide (61). The sections were coverslipped and incubated at 37°C overnight. After incubation, the slides were first rinsed in 1X saline citrate (150mM sodium chloride, 15 mM sodium citrate), followed by three washes (20 minutes each) in 2X saline citrate/50% formamide at 40°C, and two washes (30 minutes each) in 1X saline citrate at room temperature. After a brief water rinse, the sections were air-dried and then apposed to X-ray film (BioMax MR-2, Kodak) for 4–10 days.

Analysis of Autoradiograms

Gene expression was assessed in sections from rostral (approximately at +1.5 mm rostral to bregma; (62,63), middle (+0.8), and caudal striatal levels (−0.2) (Fig. 1). Hybridization signals on film autoradiograms were measured by densitometry (NIH Image, Wayne Rasband, NIMH) across the total striatum on all 3 levels, as well as, on the rostral level, in medial and lateral striatal sectors and in the nucleus accumbens core, and medial and lateral shell, and on the middle level, in medial, central, lateral and ventral sectors (Fig. 1). The film autoradiograms were captured using a light table (Northern Light, Imaging Research, St. Catharines, Ontario, Canada) and a Sony CCD camera (Imaging Research). “Mean density” values of corresponding regions in the two hemispheres were averaged after correcting for background by subtracting mean density measured over white matter (corpus callosum). The illustrations of film autoradiograms (Fig. 4) are computer-generated images, and are contrast-enhanced when necessary.

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Statistical Analysis

Treatment effects were determined by one- or two-factor ANOVAs, followed by Fisher PLSD (gestational data), Tukey's, or Newman-Keuls post hoc tests (Statistica, StatSoft, Tulsa, OK).

All experimental protocols were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care and were in accordance with NIH and EEC directives for animal studies.

Results

Adenylyl cyclase activity

Adenylyl cyclase activity was measured under basal conditions and following the addition of forskolin or the selective D_1 receptor full agonist SKF82958 in the striatum of adult male PSAL, PCOC20, PCOC40 and SPF mice. Basal adenylyl cyclase activity was not significantly different between prenatal treatment groups including PSAL, PCOC20, PCOC40 and SPF ($p > 0.05$; Figure 2). Forskolin stimulated adenylyl cyclase activity by six- to eight-fold in all animals, and there were no significant differences between prenatal treatment groups ($p > 0.05$; Figure 2). SKF82958 produced an increase in cAMP formation at concentrations of 10 nM to 100 uM (Figure 2). Two-factor ANOVA revealed significant main effects of concentration ($p < 0.001$) and treatment factors ($p < 0.05$) as well as a concentration-treatment interaction ($p < 0.05$). Tukey post-hoc analysis revealed that adult male PCOC40 mice showed significantly greater stimulation of adenylyl cyclase activity by the D_1 receptor agonist as compared with PSAL mice ($p < 0.05$; Figure 2).

Behavioral effects

Three-way ANOVA revealed a significant main effect of postnatal treatment (coc vs. sal; $F(1,94)= 79.924$, $p < 0.0001$) on locomotion in all three age groups, which was more pronounced at P36 and P60 ($p < 0.0001$) than at P15 ($p < 0.01$), but no main effect of prenatal treatment at any age studied (Figure 3). For both PCOC and PSAL groups post hoc analyses revealed significantly higher locomotor activity in cocaine-treated mice compared to saline-treated controls at P36 and P60 ($p < 0.001$), but not at P15.

Cocaine-induced zif 268 expression in the striatum and nucleus accumbens

Basal *zif 268* expression (i.e., in PSAL mice) in the striatum was relatively low, but showed a medial-to-lateral gradient with higher levels medially, in all three age groups (Table 1). Cocaine challenge-induced *zif 268* expression displayed distinct regional patterns throughout the striatum. Induction of *zif 268* was most pronounced on the middle striatal level, slightly lower on the rostral level, but was minimal in the caudal striatum (Table 1). In both the rostral and middle striatum, *zif 268* mRNA levels were considerably higher medially than laterally after the cocaine challenge. However, due to the medial-lateral gradient in basal expression, the drug-induced increase tended to be greater laterally. On all levels, *zif 268* induction was less pronounced or absent in ventral striatal regions, including the nucleus accumbens (Table 1).

These expression patterns were in part age-dependent. Overall, younger animals tended to show a greater *zif 268* response than adults. For example, in the nucleus accumbens, at P15, there was a significant increase in *zif 268* expression after cocaine injection in the core ($p < 0.01$, both prenatal treatments), whereas at P36, the response just attained statistical significance ($p < 0.05$; core and shell), and at P60, no significant challenge effects were found in these subdivisions of the nucleus accumbens. The spatial pattern of *zif 268* expression shifted with increasing age in the dorsal striatum as well. While stable in the medial part at all ages studied, *zif 268* induction in the rostral lateral striatum gradually diminished from P15 to P60 (Table 1). Moreover, on the middle level, the *zif 268* response was statistically more robust in the

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central than in the lateral part of the striatum at P15, but gradually reversed to become more robust laterally at P60 (Figure 4).

Effects of prenatal cocaine treatment on *zif 268* expression were restricted to the middle striatum; these effects were maximal in the medial part and increased with age (Figure 5; Table 1). Overall, mice prenatally exposed to cocaine vs. saline, tended to show reduced *zif 268* mRNA levels following injection with saline (i.e., PCOC sal vs. PSAL sal), although this effect did not reach statistical significance. Because of this somewhat reduced basal expression, effects of the cocaine challenge were best revealed by the relative magnitude of the *zif 268* response (expressed as % of basal levels). No statistically significant effects of prenatal cocaine were seen at P15, with the one exception of a smaller challenge response ($p < 0.05$) in the total middle striatum in animals prenatally exposed to cocaine (Table 1; Figure 5). In contrast, at P36, there was a significantly increased relative *zif 268* response in the medial part of the middle striatum in animals prenatally exposed to cocaine (PCOC coc vs. PSAL coc, $p < 0.05$; Table 1). No other effects were seen at this age. At P60, a statistically more robust ($p < 0.01$) increase was found in the medial striatum. In addition, increased *zif 268* induction ($p < 0.05$) was also present in the lateral and ventral striatum, and the increased response thus reached statistical significance also for the total striatum on the middle level ($p < 0.01$; Table 1; Figure 5). In contrast, a decreased *zif 268* response ($p < 0.05$) was seen in the lateral shell of the nucleus accumbens (Table 1).

Cocaine-induced homer 1 expression in the striatum and nucleus accumbens

Basal *homer 1* expression in the striatum was also low, comparable to *zif 268* expression (Figure 4). After the cocaine challenge *homer 1* expression was increased, showing similar regional patterns as *zif 268* induction, but this increase was generally less robust than that for *zif 268*, especially in younger animals. Cocaine-induced *homer 1* expression was also more pronounced in the medial than the lateral striatum, on all striatal levels and at all ages examined. On the middle level, a similar, but less distinct, expression shift from more central than lateral at P15 to more lateral than central at P60 was apparent. In the ventral striatum, minimal or no effects of the cocaine challenge on *homer 1* expression were seen. In the core and in the lateral shell of the nucleus accumbens, there was increased expression, which just reached statistical significance ($p < 0.05$), in PCOC coc and PSAL coc mice, respectively, at P36 (Table 2).

Effects of prenatal cocaine treatment on *homer 1* expression were similar to those on *zif 268* expression, including the age-dependent expression pattern. No effects of the prenatal treatment were seen in P15 animals. In contrast, at P36, animals prenatally exposed to cocaine vs. saline displayed a significantly greater *homer 1* response to the cocaine challenge in the medial part of the middle striatum (PCOC coc vs. PSAL coc, % increase, $p < 0.01$; Table 2). Moreover, at P60, mice prenatally exposed to cocaine showed a significantly enhanced *homer 1* response in the rostral striatum (total, $p < 0.05$, absolute and relative increase; Table 2) and in the middle striatum (total, $p < 0.05$, relative; ventral, $p < 0.05$, absolute; Figure 5, Table 2).

Discussion

In this study we provide evidence for an enhanced D_1 dopamine receptor agonist-induced cAMP response in the striatum of adult male, but not female (see Supplemental Materials) mice prenatally exposed to cocaine compared to controls. Furthermore we show an age-dependent augmentation of prenatal cocaine treatment on cocaine-induced striatal IEG expression beginning at adolescence and becoming more robust into adulthood.

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Enhanced D_1 receptor signaling

The current data provide evidence for an enhanced cAMP response to the selective D_1 receptor full agonist SKF82958 in the striatum of adult male, mice exposed prenatally to cocaine vs. saline. We have previously shown that D_1 receptor-stimulated adenylyl cyclase activity in mice normally increases with age peaking at P20 (64). In that study mice exposed to cocaine *in utero* exhibited a decrease in D_1 receptor-stimulated adenylyl cyclase activity in the striatum at E18, 24 hours following the final exposure to cocaine, which was a result of a significant decrease in the expression of adenylyl under basal conditions. The present study, which demonstrated an increase in D_1 receptor-stimulated adenylyl cyclase activity in the striatum of adult mice, with no significant difference evident under basal conditions, suggests that the normal ontogeny of D_1 receptor signaling evident postnatally is exaggerated following prenatal cocaine exposure. P15, the earliest time at which we assessed cocaine-induced IEG induction coincides with the time by which basal and foskolin-induced adenyly cyclase activity have reached adult levels (64). The age-dependent increase in cocaine-induced gene expression we observed in mice prenatally exposed to cocaine vs. saline at P15, P36, and P60 thus may require a functionally mature signaling pathway to demonstrate the full effects of that exposure. The exaggerated and persistent increase in D_1 receptor-mediated intracellular signaling is a potential mechanism contributing to other behavioral and neuro-anatomical anomalies we and others have observed in adult mice following prenatal exposure to cocaine (e.g., 13,14). Despite such persistent neuro-adaptations it is clear that prenatal cocaine treatment does not affect the acute locomotor response to cocaine, similar to what has previously been seen at various postnatal ages (16,79,80).

Persistent deficits in striatal D_1 receptor signaling have been identified in a rabbit model of gestational cocaine exposure. Rabbits exposed to cocaine *in utero* displayed a functional uncoupling of D_1 receptors from G alpha s, without any changes in the concentration of G alpha s, D_2 coupling, or D_1 receptor antagonist binding (26,65). Furthermore, a recent report (66) demonstrated a decrease in D_1 receptor membrane expression in the striatum of preadolescent rabbits prenatally exposed to cocaine, a novel finding which may provide mechanistic insights into the reduction in G alpha s coupling seen in that model. At the level of D_1 receptor-activated signal transduction, Zhen et al. (67) have demonstrated an increase in phosphorylation of DARPP-32 at Thr 34, an inhibitor of protein phosphatase 1 (PP1), and an increase in D_1 receptor phosphorylation in the striatum of rabbits prenatally exposed to cocaine. They speculated that decreased PP1 activity leads to increased phosphorylation and increased internalization of D_1 receptors, which they hypothesized leads to decreased D_1 receptor signal transduction (26). Differences in the species studied, as well as the route, dose and gestational timing of cocaine utilized may contribute to the discrepancies between the present data obtained in mice and that published in the rabbit model.

Altered gene expression in the striatum

Our results show that male PCOC40 mice displayed greater induction of the IEGs *zif 268* and *homer 1* in the striatum after a cocaine challenge injection. This effect was age-dependent; it emerged during adolescence (P36) and was maximal in adults (P60). Regionally, this enhanced gene induction was most robust in the middle striatum and was maximal in the medial part. These findings are consistent with enhanced D_1 receptor signaling in the striatum of these mice.

The present study assessed changes in gene regulation in different functional domains of the striatum. We used a topographical mapping technique based on cortical input patterns (34, 68) that was developed for the rat (69,70) and simplified for the mouse. While limited anatomical studies in the mouse (e.g., 71) indicate that the overall topographical organization of the cortico-striatal projections is similar to that in the rat, the definitive association of the

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present gene regulation effects with particular functional domains in the mouse will need confirmation.

Our most important finding is the age-dependent effect of prenatal cocaine treatment on IEG induction in the striatum. Consistent with greater D_1 receptor-stimulated cAMP formation, the present study demonstrates that cocaine exposure *in utero* produces a long-term enhancement of IEG induction by cocaine. This effect was most pronounced at P60 and most robust in the middle striatum, and maximal in, but not limited to, the medial sector, which receives inputs from prelimbic and anterior cingulate cortex in rat (69) and mouse (73). This abnormal gene induction was best revealed when the challenge response was expressed relative to basal expression, especially for *zif 268*, as basal *zif 268* mRNA levels tended to be reduced in mice exposed prenatally to cocaine compared to saline-injected controls. A suppression of basal *zif 268* expression in the striatum by repeated cocaine treatment has been demonstrated before, although this suppression was found after repeated treatment in adults and recovered within 2–3 days of the treatment (74).

The enhanced induction of striatal *zif 268* and *homer 1* provides a potential mechanism for altered plasticity in striatal neurons. *Zif 268* encodes a zinc finger transcription factor that regulates a number of genes and has been implicated in a variety of neuroplastic processes (28). Homer 1 is a member of a family of scaffolding proteins (Homer/Vesl proteins) of the postsynaptic density that cluster and traffic glutamate receptors, link these to internal calcium stores, and play a role in various mechanisms of synapse structuring and plasticity (29,30, 75). The *homer 1* signal rapidly induced by psychostimulants (45,70,76) and other treatments mostly reflects the IEG isoform *homer 1a* (60). This truncated *homer 1* splice variant appears to act as a dominant negative regulator of glutamate synapse strength (77), by promoting disassembly and turnover of the signaling complex (29,30). This is seen as a necessary stabilizing process (78) during activity-dependent synaptic plasticity (77). Enhanced induction of these plasticity-associated molecules suggests that prenatal cocaine exposure renders these animals more susceptible to cocaine-induced neuroplasticity. There is the potential for an abnormal regulation in striatal synaptic growth and transmission related to altered induction of *homer 1* isoforms (77), and electrophysiological consequences related to altered *zif 268* induction (see 28).

Overall the current study provides evidence for abnormally increased striatal D_1 dopamine receptor activity following prenatal cocaine exposure as shown by an enhanced D_1 receptor agonist-induced cAMP response and increased striatal expression of the synaptic plasticity factor, Homer 1, and transcription factor, Zif 268. Further research is necessary to evaluate the mechanisms underlying the persistent augmentation of striatal D_1 receptor signaling, and its potential contribution to some of the neuro-behavioral deficits observed in preclinical models as well as in a subset of infants, children, adolescents and adults following prenatal exposure to cocaine, and identify the striatum as one target potentially vulnerable to gestational cocaine exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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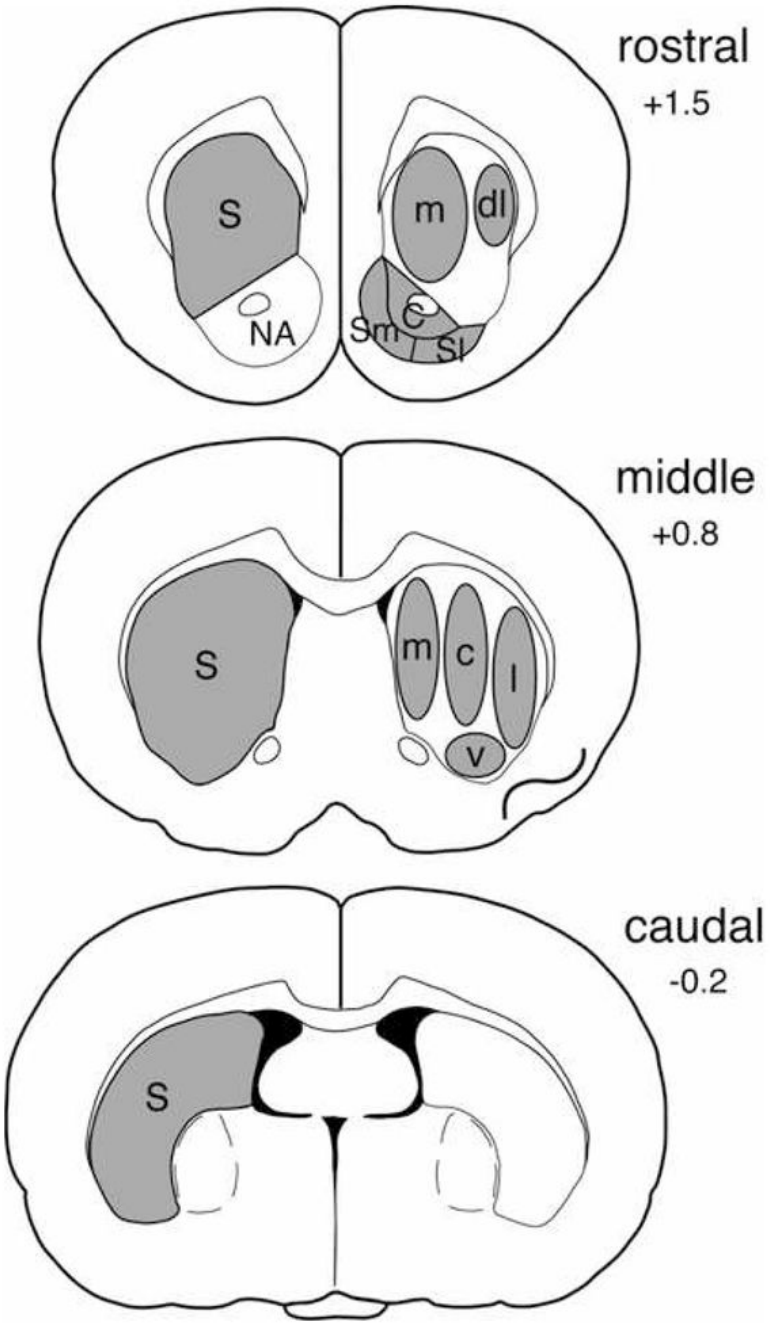

Figure 1.
Schematic illustration of sampling areas used to measure gene expression on rostral, middle and caudal striatal levels. Total striatal areas (S, left) and striatal and nucleus accumbens (NA) subregions (right) are shown. Striatum: m, medial; dl, dorsolateral; c, central; l, lateral; v, ventral; Nucleus accumbens: C, core; Sm, medial shell; Sl, lateral shell.

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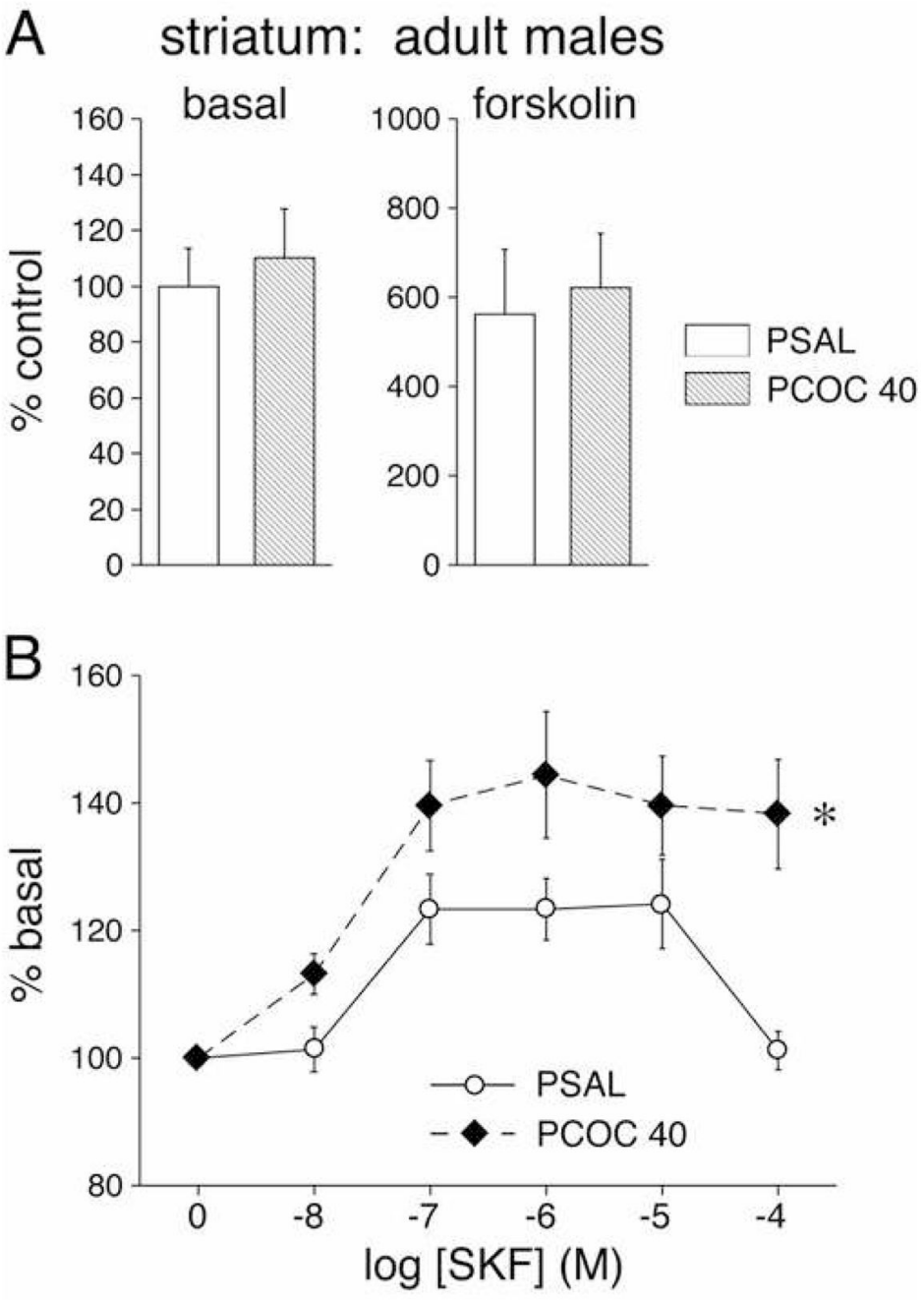

Figure 2.

Basal, forskolin- and D1 receptor agonist, SKF82958, stimulated adenylyl cyclase activity in the striatum of adult mice prenatally exposed to cocaine. Values (mean ± SEM) are given for males (N=8–9) that were exposed *in utero* to saline (PSAL) or cocaine (40 mg/kg/day, PCOC40). Data are expressed in percent of saline controls. A. Neither basal nor forskolin-stimulated adenylyl cyclase activity was significantly different between treatment groups. B. PCOC40 mice showed significantly higher D1 receptor-stimulated adenylyl cyclase activity than the PSAL control group (* $P<0.05$).

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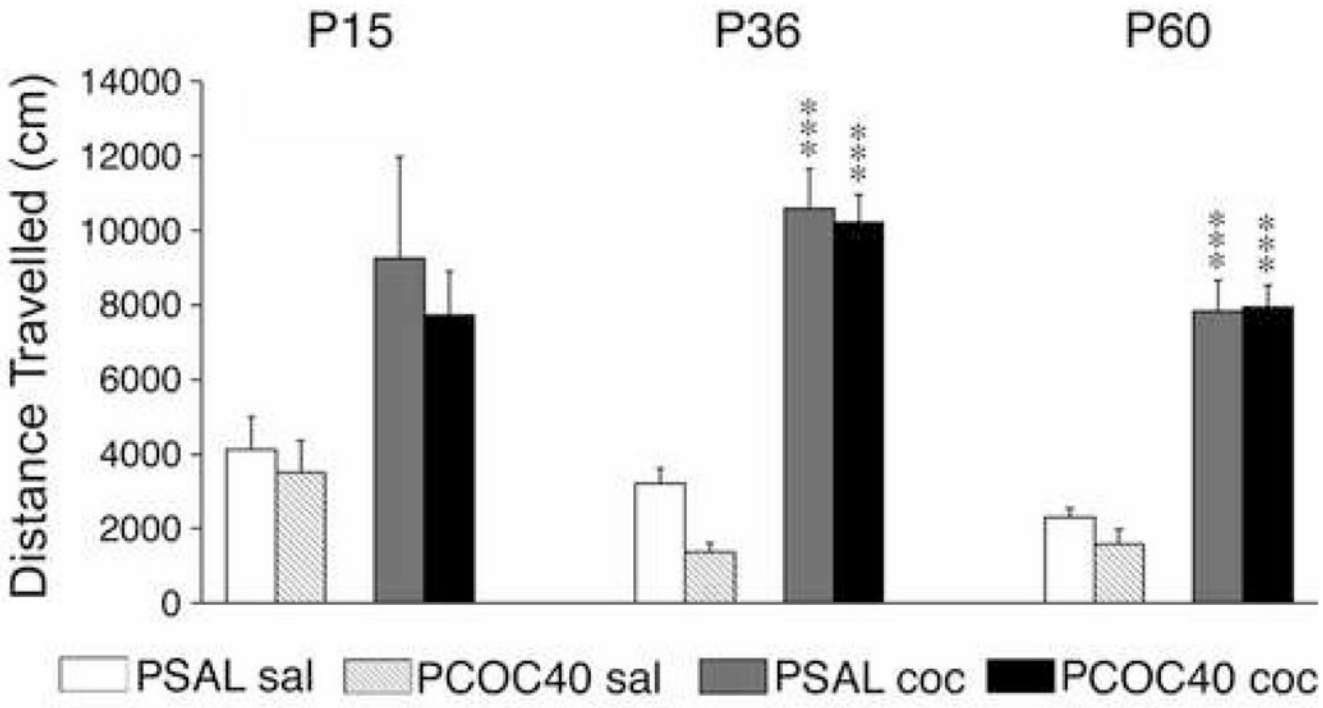

Figure 3.

Effects of prenatal cocaine exposure on basal and cocaine-induced locomotor activity at different ages. Distance traveled (mean±SEM) during a 30-min test is given for male mice that received an injection of saline (sal) or cocaine (15 mg/kg; coc) on postnatal (P) days 15, 36 or 60, following prenatal exposure to saline (PSAL) or cocaine (40 mg/kg/day; PCOC40). While the cocaine challenge increased locomotion at all 3 ages, no significant main effect of the prenatal treatment was observed. *** P<0.001, vs. respective saline controls.

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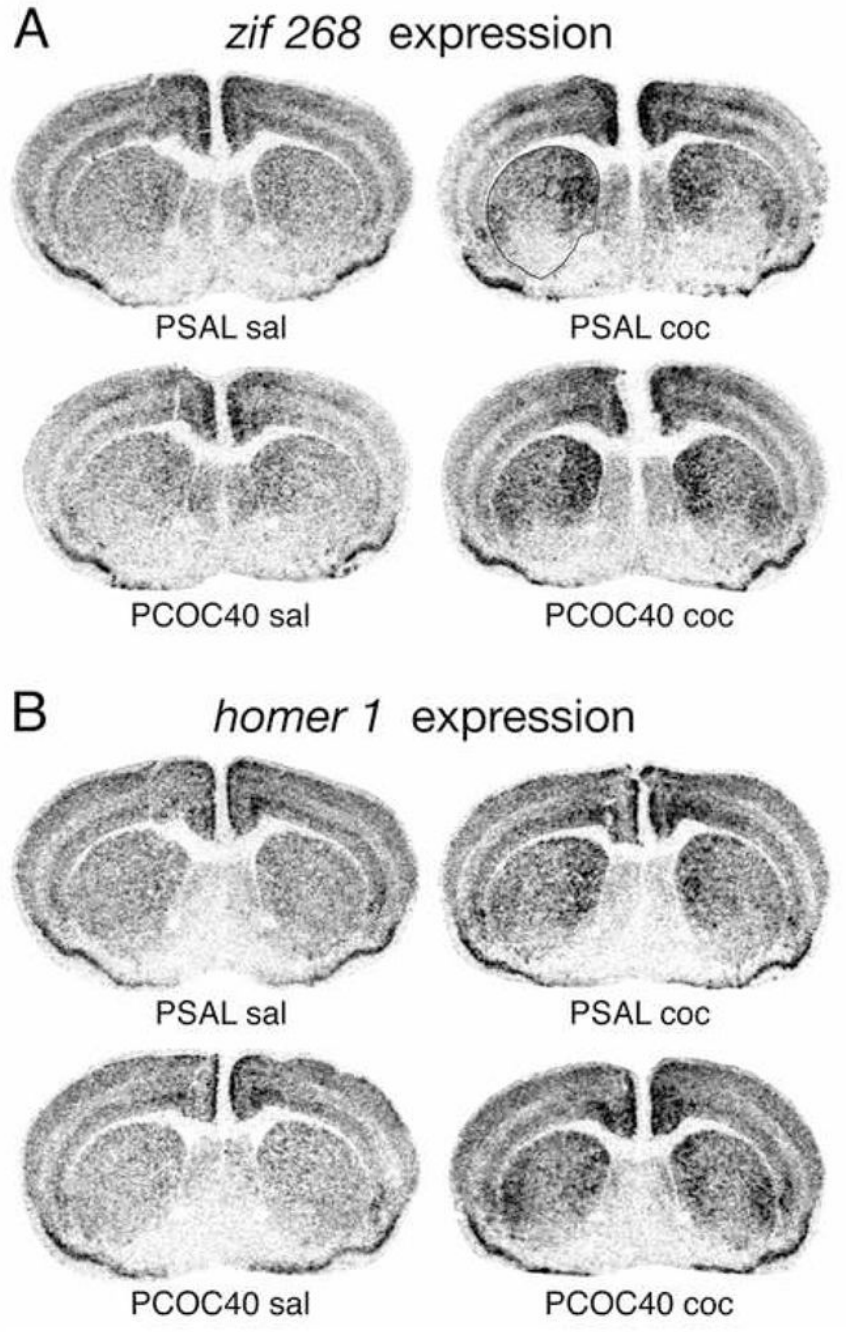

Figure 4.

Prenatal cocaine exposure enhanced cocaine-induced *zif 268* (A) and *homer 1* expression (B) in the striatum of adult mice. Illustrations of film autoradiograms depict gene expression in coronal sections from the middle striatal level in mice that received a saline (sal) or cocaine (15 mg/kg; coc) challenge injection at postnatal day (P) 60, following prenatal exposure to saline (PSAL) or cocaine (40 mg/kg/day; PCOC40). Maximal hybridization signal is black.

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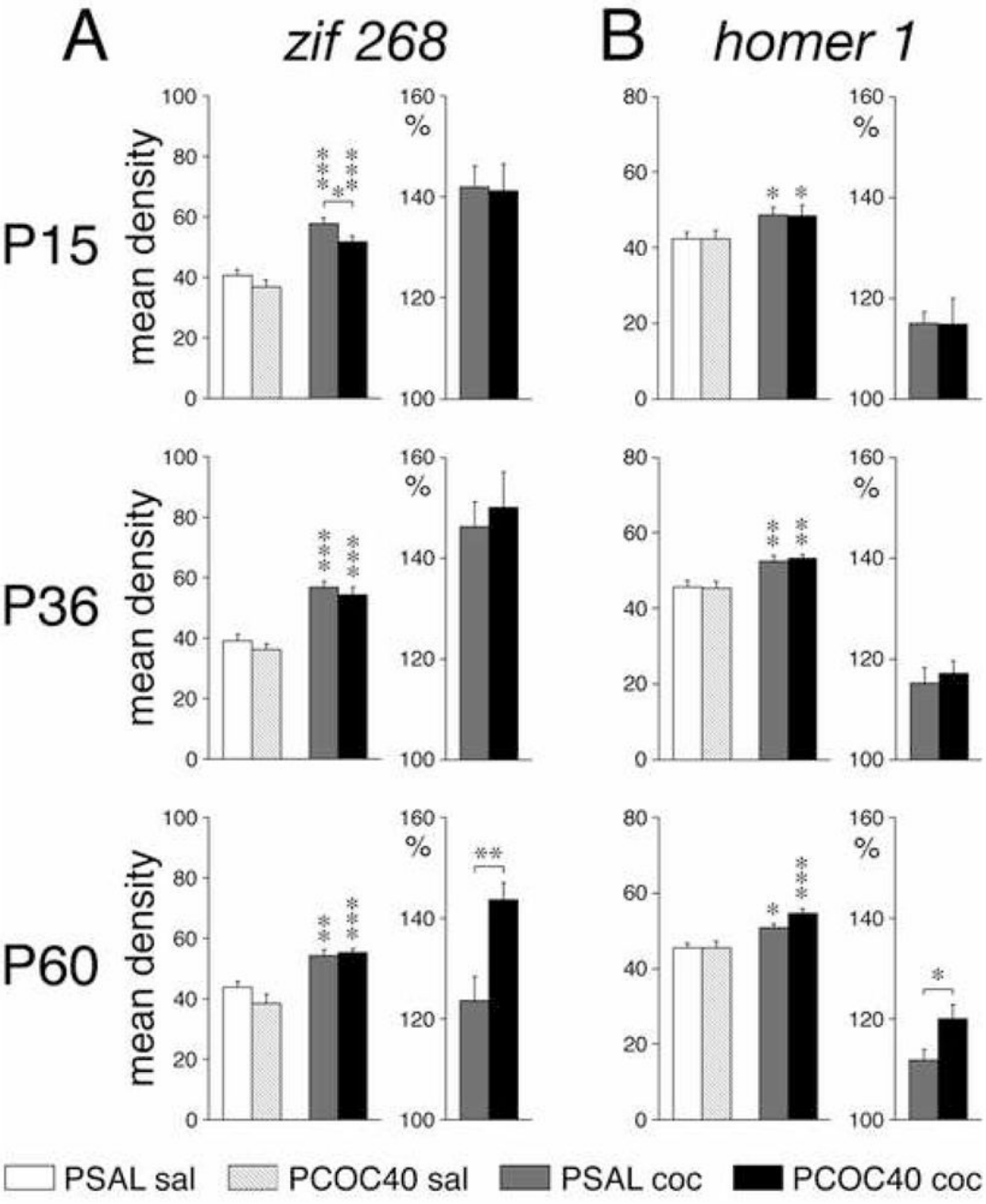

Figure 5.

Effects of prenatal cocaine exposure on *zif 268* (A) and *homer 1* expression (B) in the striatum at different ages. Mean density values (mean±SEM, arbitrary units) measured across the total striatum at the middle level are shown for mice that received a saline (sal) or cocaine (coc) challenge injection at postnatal day (P) 15, P36, or P60, following prenatal exposure to saline (PSAL) or cocaine (40 mg/kg/day; PCOC40). Cocaine challenge-induced gene expression is also given in percent (%) of expression in the respective saline control (right). PCOC40 mice showed enhanced induction of *zif 268* and *homer 1* by the cocaine challenge at P60. * P<0.05, ** P<0.01, *** P<0.001, vs. saline control or as indicated.

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Table 1

Effects of prenatal cocaine treatment on the postnatal expression of *zif 268*.

Mean density values (mean±SEM) measured in different striatal regions on rostral, middle and caudal levels are given for mice that were prenatally exposed to saline (PSAL) or cocaine (PCOC) and tested at postnatal days (P) 15, P36 or P60 after an injection of saline (sal) or 15 mg/kg cocaine (coc). Responses to the cocaine challenge are also shown in percentage of values in respective saline controls (%). Striatum (S): m, medial; dl, dorsolateral; c, central; l, lateral; v, ventral; tot, total. Nucleus accumbens (NA): C, core; Sm, medial shell; Sl, lateral shell.

age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
P15	rostral						
	S m	54.5±2.5	53.4±2.3	78.7±2.8***	79.0±3.5***	144.2±5.1	147.8±6.5
	S dl	47.3±1.7	44.8±1.3	60.9±1.8***	58.7±2.6***	128.9±3.7	131.3±5.8
	S tot	48.1±1.8	47.1±1.4	68.0±1.7***	67.2±3.0***	141.1±3.6	142.6±6.3
	NA C	38.0±2.6	40.6±2.8	57.6±4.1**	58.2±4.5**	151.3±10.7	143.3±11.2
	NA Sm	35.1±1.2	36.3±1.0	40.5±3.3	37.0±2.6	115.4±9.4	101.8±7.1
	NA Sl	27.0±1.0	27.3±1.3	36.0±3.8	31.6±3.2	133.4±14.2	115.6±11.7
	middle						
	S m	56.7±3.6	52.6±2.6	80.2±3.5***	78.9±3.0***	141.4±6.2	150.1±5.8
	S c	42.8±1.9	39.8±1.6	64.8±3.0***	63.7±4.8***	151.2±7.1	160.1±12.1
	S l	40.1±1.7	40.5±2.9	56.4±3.7**	52.0±4.1*	140.4±9.1	128.4±10.0
	S v	20.8±1.7	24.3±2.4	27.7±2.8	25.4±3.5	132.9±13.4	104.8±14.5
	S tot	40.7±1.9	36.8±2.3	57.8±1.7***	51.9±1.9*** #	142.0±4.1	141.3±5.3
	caudal						
	S tot	31.3±1.8	32.4±2.1	39.1±2.5	33.9±2.6	124.7±7.9	104.7±7.9
P36	rostral						
	S m	55.8±3.1	52.8±1.9	79.7±2.4***	77.9±2.3***	142.8±4.2	147.3±4.3
	S dl	43.5±2.7	42.6±2.7	53.8±1.2**	52.6±2.0**	123.6±2.8	123.5±4.7
	S tot	48.1±2.7	45.9±2.0	65.0±2.0***	63.4±2.2***	135.2±4.2	138.1±4.9

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age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
	NA C	26.5±3.3	21.6±2.0	36.7±3.2*	34.3±2.3*	138.8±12.0	158.4±10.6
	NA Sm	31.3±2.3	25.6±1.9	35.9±2.7	34.5±2.1*	114.7±8.6	134.4±8.2
	NA Sl	19.1±1.6	18.0±0.9	25.7±1.9*	26.4±2.4*	134.7±9.8	146.5±13.6
	middle						
	S m	57.6±3.3	51.3±2.0	83.0±1.8***	81.7±3.0***	143.9±3.1	159.1±5.9#
	S c	41.0±2.9	38.2±2.4	57.5±2.1***	54.8±2.8***	140.4±5.2	143.5±7.2
	S l	32.9±1.9	33.7±2.8	56.9±1.9***	51.7±3.4***	172.9±5.9	153.4±10.2
	S v	19.3±2.3	15.3±1.5	22.2±2.7	17.3±2.3	115.0±14.1	113.0±15.1
	S tot	39.0±2.3	36.3±2.0	57.0±1.9***	54.4±2.6***	146.2±4.9	150.0±7.1
	caudal						
	S tot	27.0±2.3	24.5±2.0	32.1±2.2	30.4±1.4	118.8±8.1	123.9±5.9
P60	rostral						
	S m	59.8±3.2	56.1±3.2	76.6±2.4***	73.5±2.2***	128.0±3.9	131.0±3.9
	S dl	49.5±3.6	46.9±2.2	56.4±2.2	51.6±1.4	114.0±4.4	110.0±3.0
	S tot	51.3±2.7	48.8±3.0	64.3±1.4***	59.8±1.6**	125.3±2.7	122.5±3.3
	NA C	39.9±3.8	33.3±3.1	47.6±2.5	42.5±2.0	119.2±6.3	127.6±6.1
	NA Sm	39.4±2.2	37.6±2.4	43.0±2.1	36.1±1.6	109.1±5.2	96.1±4.4
	NA Sl	30.8±3.1	32.1±1.5	32.9±2.9	26.6±1.9	106.9±9.3	82.8±5.9#
	middle						
	S m	61.8±2.2	55.7±4.1	79.9±1.7***	79.7±1.7***	129.2±2.8	143.1±3.0##
	S c	43.9±2.8	39.8±3.5	53.2±3.0	53.0±2.0*	121.2±6.8	133.2±5.1
	S l	38.3±2.8	33.1±2.8	54.2±5.0**	59.6±2.4***	141.6±12.9	180.0±7.1#
	S v	23.5±2.4	16.9±3.0	22.5±1.7	23.4±2.4	95.7±7.1	138.2±14.3#
	S tot	43.9±2.0	38.6±3.0	54.3±2.1**	55.4±1.3***	123.7±4.7	143.6±3.5##

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age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
	caudal						
	S tot	32.5±0.9	29.7±1.4	37.8±2.0	35.7±2.2	116.3±6.2	120.3±7.4

*** $p < 0.001$,

** $p < 0.01$,

* $p < 0.05$, vs. respective saline control;

$p < 0.01$,

# $p < 0.05$, vs. PSALcoc.

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Table 2

Effects of prenatal cocaine treatment on the postnatal expression of *homer 1*.

Mean density values (mean±SEM) measured in different striatal regions on rostral, middle and caudal levels are given for mice that were prenatally exposed to saline (PSAL) or cocaine (PCOC) and tested at postnatal days (P) 15, P36 or P60 after an injection of saline (sal) or 15 mg/kg cocaine (coc). Responses to the cocaine challenge are also shown in percentage of values in respective saline controls (%). Striatum (S): m, medial; dl, dorsolateral; c, central; l, lateral; v, ventral; tot, total. Nucleus accumbens (NA): C, core; Sm, medial shell; Sl, lateral shell.

age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
P15	rostral						
	S m	46.1±0.7	44.2±1.5	53.1±1.4*	54.9±2.8**	115.1±3.1	124.2±6.3
	S dl	46.1±1.4	45.9±1.2	49.1±1.5	51.0±2.2	106.5±3.2	111.0±4.8
	S tot	44.5±0.8	42.7±1.0	48.7±1.2	51.9±2.5**	109.4±2.8	121.7±5.9
	NA C	35.7±1.8	32.7±1.8	36.4±2.4	39.6±2.4	101.8±6.8	120.9±7.4
	NA Sm	33.6±2.1	32.5±2.2	29.2±2.3	29.7±1.8	86.8±7.0	91.2±5.4
	NA Sl	35.0±2.1	37.7±2.5	35.3±2.1	32.8±2.5	100.8±6.1	86.9±6.5
	middle						
	S m	43.1±0.9	40.6±1.1	53.1±1.8***	53.8±2.3***	123.0±4.3	132.5±5.8
	S c	45.4±0.6	44.8±1.2	52.7±1.6*	53.1±2.6**	116.1±3.5	118.5±5.7
	S l	50.7±0.9	50.8±1.7	55.7±1.6	52.7±2.2	109.7±3.2	103.8±4.2
	S v	27.3±1.2	29.7±1.9	30.5±1.8	30.4±1.3	111.7±6.6	102.4±4.3
	S tot	42.3±0.7	42.2±1.4	48.7±1.0*	48.5±2.1*	115.0±2.4	114.9±5.0
	caudal						
	S tot	33.8±1.4	33.5±1.0	36.0±2.2	34.6±2.1	106.4±6.4	103.4±6.4
P36	rostral						
	S m	47.7±1.3	47.9±1.3	56.9±1.2***	59.5±1.1***	119.2±2.4	124.3±2.3
	S dl	48.9±1.5	51.1±1.6	53.2±1.3	54.7±1.5	108.7±2.8	107.0±2.9
	S tot	47.4±1.2	47.0±1.2	53.0±1.0**	55.4±1.2***	111.9±2.1	117.8±2.5
	NA C	33.4±1.3	30.2±1.8	37.9±1.9	36.2±1.6*	113.5±5.8	120.0±5.3

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age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
	NA Sm	33.8±1.5	31.4±1.7	35.4±2.2	33.0±1.8	104.7±6.4	105.0±5.7
	NA Sl	30.8±3.3	35.7±1.8	38.9±2.1*	40.1±1.4	126.2±6.7	112.1±3.8
	middle						
	S m	49.6±1.7	47.2±1.4	59.7±1.1***	63.0±1.8***	120.2±2.2	133.3±3.8##
	S c	46.4±1.9	45.9±1.6	51.0±1.7	52.5±1.3*	110.0±3.7	114.3±2.9
	S l	50.2±2.2	51.9±2.0	58.9±2.2*	58.2±1.9*	117.3±4.3	112.0±3.7
	S v	29.9±2.3	31.5±2.0	31.2±0.7	30.9±0.6	104.3±2.3	98.1±1.9
	S tot	45.6±1.8	45.4±1.7	52.5±1.5**	53.1±1.2**	115.1±3.2	117.0±2.6
	caudal						
	S tot	35.9±1.8	34.7±1.6	38.0±1.5	40.3±1.1	105.8±4.3	115.9±3.3
P60	rostral						
	S m	51.4±0.9	51.8±1.3	59.8±0.8***	62.3±0.5***	116.3±1.5	120.2±1.1
	S dl	50.9±2.6	51.5±2.4	54.4±1.9	57.3±1.7	106.9±3.7	111.2±3.3
	S tot	50.5±1.1	50.5±1.1	54.6±0.7**	57.4±0.6*** #	108.2±1.5	113.6±1.3#
	NA C	41.2±2.6	43.3±3.6	41.4±1.4	43.3±1.8	100.4±3.4	100.0±4.3
	NA Sm	41.2±1.9	41.5±2.3	40.1±1.3	39.6±2.0	97.3±3.0	95.4±4.9
	NA Sl	39.9±2.5	43.5±1.8	43.1±1.8	43.3±2.4	107.9±4.5	99.5±5.6
	middle						
	S m	52.3±2.0	50.4±2.7	64.6±1.8***	66.6±1.8***	123.6±3.4	132.2±3.5
	S c	44.8±1.5	44.4±1.9	49.2±1.3	51.1±1.8*	109.9±2.8	115.2±4.0
	S l	49.7±2.1	48.3±1.8	57.1±2.2*	62.9±2.8**	114.8±4.5	130.1±5.9
	S v	29.9±2.4	35.3±1.6	30.9±1.9	37.5±1.3#	103.2±6.4	106.2±3.7
	S tot	45.5±1.4	45.6±1.7	50.9±1.0*	54.7±1.3***	111.8±2.2	120.0±2.9#

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age	area	PSALsal	PCOCsal	PSALcoc	PCOCcoc	PSALcoc(%)	PCOCcoc(%)
	caudal						
	S tot	36.8±1.5	37.1±1.6	41.3±0.5	42.3±1.6*	112.3±1.4	114.0±4.2

*** P < 0.001,

** p < 0.01,

* p < 0.05, vs. respective saline control;

p < 0.01,

# p < 0.05, vs. PSALcoc.