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# Neonatal Cocaine Alters Behavioural Responsiveness to Scopolamine and Cholinergic Development in Mice

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CALAMANDREI, G., L. RICCERI AND A. VALANZANO. *Neonatal cocaine alters behavioural responsiveness to scopolamine and cholinergic development in mice.* PHARMACOL BIOCHEM BEHAV **56**(4) 557–563, 1997.—CD-1 mice received daily subcutaneous injections of either cocaine (20 mg/kg or 40 mg/kg) or saline solution (0.9% NaCl) from postnatal days 2 to 15. Pups were tested on days 16–17 for learning and 24-h retention of a passive avoidance task, where entering a dark compartment was punished with a mild foot shock. Locomotor activity and general behaviour in an open field arena were assessed on day 21, following administration of either the muscarinic blocker scopolamine (0.8 mg/kg) or saline solution. In addition, immunostaining for the enzyme choline acetyltransferase (ChAT) was measured in different basal forebrain areas (medial septum, striatum, and nucleus basalis) on day 30. Cocaine treatment failed to affect either learning or retention capabilities. Nonetheless, neophobic behaviour during the learning session was enhanced in control nonpunished mice exposed to the 20-mg/kg dose. In the open field test, although baseline activity levels were unaffected by cocaine exposure, the 40-mg/kg cocaine-treated pups showed decreased sensitivity to the hyperkinetic effects of scopolamine. ChAT immunocytochemistry revealed a significant reduction of the number of ChAT-immunopositive neurons in the nucleus basalis but not in the other cholinergic basal forebrain regions. © 1997 Elsevier Science Inc.

Cocaine Neonatal development Passive avoidance learning Forebrain cholinergic neurons Choline acetyltransferase Scopolamine Mice

A NUMBER of human studies suggest that in utero exposure to cocaine has teratogenic effects on the developing brain. Asymmetrical brain growth retardation, abnormal EEG pattern, and sudden infant death syndrome appear to be related to maternal cocaine addiction (18,32). Moreover, infants born to cocaine-abusing mothers have been found to exhibit depression of interactive behaviour, overreactivity to environmental stimuli, and increased irritability (10,15,33).

Several attempts have been made to investigate neurobehavioural deficits following prenatal exposure to cocaine in animal models. In rats, administration of cocaine during pregnancy appears to be associated with a variety of behavioural alterations in the offspring, including hyper- or hypoactivity (12,25,26,43,45), deficits in spontaneous alternation and spatial learning (28,43), and poor performance in odor associative learning (22,23,44,45) and in place preference (23). In addition, prenatal exposure to cocaine results in altered functioning of the dopaminergic system as well as other neural systems. Dow-Edwards et al. (16) reported lasting changes of functional activity in several brain regions in rats following prenatal cocaine exposure, and Anderson-Brown et al. (3) found that cocaine acutely inhibits DNA synthesis in specific regions of the developing rat brain. Late-prenatal cocaine exposure resulted in accelerated behavioural maturation and in decreased locomotor response to both *d*-amphetamine and cocaine challenge on postnatal day 15 (43). Finally, although increased striatal dopamine D2 receptor binding has been found in rats prenatally exposed to cocaine (40), Akbari et al. (1) reported a delay in the maturation of the serotonergic system.

More recently, neonatal treatment schedules have been used in rodents to model third trimester human fetal exposure; during the third trimester of pregnancy, the developing brain is in fact highly susceptible to teratogenic influences, as intensive neuronal growth and proliferation occur (the so-called brain growth spurt) (9,14). In rodents, this period of brain growth takes place during the first weeks after birth (14). Although

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only a few studies have examined neonatal cocaine effects in rodents, alterations in central nervous system (CNS) development as well as in behavioural maturation have been reported. Specifically, Barron and Irvine (4,5) found that postnatal cocaine administration affected behavioural performance, such as locomotor activity, spontaneous alternation, balance, and coordination, but not associative learning. Ricceri et al. (37) reported impairment of passive avoidance learning in 12- to 13-day-old rats exposed to cocaine during the first 10 days of life. They also found a reduction in the activity of the enzyme choline acetyltransferase (ChAT; the acetylcholine synthesizing enzyme, a very specific marker of cholinergic neurons) in the septal area, a finding that is suggestive of an alteration in cholinergic system development. As both in vitro and in vivo evidence in the adult rat seems to indicate a direct interaction of cocaine with the muscarinic cholinergic receptor (30,48,49), it is worth investigating whether exposure to cocaine during a period of intensive brain growth may affect cholinergic behavioural regulations.

The aim of the present study was to assess in developing mice the effects of postnatal cocaine exposure on behavioural end points that are highly sensitive to experimental manipulation of cholinergic transmission. Mouse pups were treated daily with cocaine at two different doses (20 and 40 mg/kg) from postnatal day (pnd) 2 to pnd 15. Learning and 24-h retention of a passive avoidance response were assessed on pnd 16 and 17. On pnd 21, the same animals were tested in an open field apparatus after IP injection of either saline or scopolamine (0.8 mg/kg). Regarding the age of testing and the type of tests chosen, it is well known that central cholinergic systems in mice undergo a crucial maturational transition from the end of the second postnatal week onward (2,21). In immature rodents, acquisition of a passive avoidance task (in which the pup has to withhold an escape response in order to avoid a negative reward) is already possible at the end of the first postnatal week or a few days thereafter (35,46), and is facilitated by cholinomimetics and impaired by centrally administered muscarinic and nicotinic blockers (8,17). However, 24-h retention of a passive avoidance response is not generally seen until the end of the second postnatal week, and this appears to be related to the functional maturation of septohippocampal connections (19,36). Moreover, rodents start responding to administration of the cholinergic blocking agent scopolamine in an adultlike fashion (hyperactivity and impaired habituation) by pnd 21. The appearance of the scopolamine hyperactivity syndrome around weaning time in altricial rodents is indeed considered an index of normal cholinergic development (2). Thus, if cocaine exerts a detrimental effect on the ontogenesis of central cholinergic systems, we expected that this would affect pups' performance in either learning or activity tests or both. Finally, to evaluate potential long-lasting alterations in the development of specific cholinergic areas, brain samples were taken on pnd 30 and processed for ChAT immunocytochemistry. The number of ChAT-immunopositive neurons was quantified in the medial septum, striatum, and nucleus basalis by means of a computerized image analysis system.

## METHOD

### *Animals*

Mice of an outbred Swiss-derived strain (CD-1) were purchased from Charles River Italia (Calco, Italy). The animals were kept in an air-conditioned room at 21 ± 1°C and 60 ± 10% relative humidity, with a red/white light cycle (white light from 2130 to 0930 h). Males and multiparous females were housed separately in groups of five in 42 × 27 × 14-cm Plexiglas boxes with a metal top and sawdust as bedding. Pellet food (enriched standard diet purchased from Mucedola, Settimo Milanese, Italy) and tap water were continuously available. Two weeks after their arrival, 14 breeding pairs were formed and housed in 33 × 13 × 14-cm boxes; the females were inspected daily for vaginal plug and for delivery (pnd 1). The stud was removed 10 days after the discovery of the plug. All litters were culled at birth to six males and two females to maintain an appropriate litter size and gender composition (27); the six males were randomly assigned to either saline (two pups), cocaine 20 mg/kg (two pups), or cocaine 40 mg/kg (two pups) treatment.

### *Cocaine Treatment*

Pups received an SC injection of either saline, cocaine 20 mg/kg (coc 20), or cocaine 40 mg/kg (coc 40) in the nape of the neck from pnd 2 to pnd 15. Cocaine hydrochloride (Merck, Darmstadt, Germany) was dissolved in physiological saline. Injections (needle size: 30G 1/2) were performed between 1000 and 1200 h. Pups appeared to tolerate the injections well, and no lesions were observed on their bodies. Body weight was recorded daily from pnd 1 to pnd 15.

### *Passive Avoidance Test*

The avoidance apparatus for immature mice (Ugo Basile, 21025-Comerio, Varese, Italy) consisted of a tilting-floor Plexiglas cage divided into two compartments (the Start and the Escape compartments, 18 × 9.5 × 16 cm each) by a sliding door. The Start compartment was white and was illuminated by a white light suspended 5 cm above it, while the Escape compartment was black and was covered by a lid that kept it completely dark. The metallic grid floor (bars 0.3 cm in diameter spaced 5 mm apart) was connected to a scrambling shocker (model E13-08; Colbourn Instruments, Leigh Valley, PA, USA) set at 0.4 mA. Avoidance tests were performed between 0930 and 1230 h (i.e., during the initial portion of the dark period). The procedure consisted of two phases, Acquisition and Retention, which took place on two subsequent days.

*Acquisition phase (pnd 16).* The two saline, two coc 20, and two coc 40 pups of a litter were randomly assigned to one of the two testing conditions (Punished and Nonpunished). Mice underwent a multitrial session. Punished pups were individually placed into the Start compartment facing away from the doorway. The sliding door between the compartments was raised and the pup was allowed to cross into the dark chamber. When the pup crossed (four-paw criterion), lowering the tilting floor, the door shut and a 3-s 0.4-mA foot shock was delivered to the grid floor. The trial ended when the mouse gave the step-through response or remained in the Start compartment for 120 s, whichever event occurred first. At the end of each trial, pups were removed from the apparatus and left undisturbed for a 60-s intertrial interval. The Acquisition phase ended when either the subject had remained in the Start compartment for two consecutive trials (learning criterion), or after 10 trials ended by stepping through. If the learning criterion was achieved before the 10th trial, then all remaining trials (to a total of 10) were considered as 120-s latencies. Mice of the Nonpunished control group were subjected to a similar multitrial session in the same apparatus, but step-through responses were not punished by foot shock. The mean

latency to enter the dark compartment was used as an index of learning.

*Retention phase (pnd 17).* The procedure was identical for Punished and Nonpunished pups and consisted of one trial not punished by foot shock. The Retention trial ended when the pup either gave the step-through response (head and forepaws in the dark compartment) or remained in the Start compartment for 120 s. Latency to enter the dark compartment was used to evaluate the effect of previous training on Retention performance.

### *Open Field Test*

On pnd 21, saline, coc 20, and coc 40 mice of each litter were assigned to either scopolamine or saline treatment. Punished and Nonpunished mice were evenly distributed in the scopolamine or saline treatment. They were weighed and injected IP with either 0.8 mg/kg of scopolamine hydrobromide (Sigma Chemical Co.) or an equal volume of 0.9% saline solution. This particular scopolamine dose was chosen on the basis of pilot experiments that showed it is effective in inducing hyperactivity and impaired habituation in 21-day-old mice tested either in an open field or in a Varimex automated apparatus.

Fifteen minutes after treatment, individual pups were transferred from the home cage to an open field arena (35 × 35 cm) made of black Plexiglas with a light grey bottom subdivided by black lines into 7 × 7-cm squares. The test started by placing the animal at the center of the arena. The behaviour of the animal was then observed and video recorded for 20 min using a Sony VO-5360 apparatus equipped with CH-video cameras for red lights. Immediately after each test, the apparatus was thoroughly cleaned with cotton pads wetted with 70% ethanol. Recordings were then scored by an observer blind to the treatments received by the animals. No recourse was made to simultaneous scoring by two experimenters, because no experimenter biases were found in previous control experiments in the case of the response considered.

The 20-min session was divided in four blocks of 5 min each, and the following behavioural categories were scored during the first 3 min of each block: number of crossings (crossing the square limits with both forepaws), and number and duration of wall rearing, open rearing, and grooming behaviours.

### *Immunocytochemistry and Analysis of Staining*

On pnd 30, one saline and one coc 40 pup from five different litters were anesthetised with Nembutal before being perfused transcardially with 15 ml cold 0.01 M phosphate-buffered saline (PBS), followed by 15 ml cold 4% paraformaldehyde dissolved in 0.1 M phosphate buffer. The brains were removed and placed in fresh 4% paraformaldehyde for 24 h at 4°C. The brains were then transferred to a 30% sucrose solution in PBS and left overnight at 4°C. Coronal sections (30 μm) of the forebrain including the septum and nucleus basalis were cut on a freezing microtome and processed for ChAT immunocytochemistry. The free-floating sections were rinsed in PBS plus 0.1% Triton X-100 (PBS-T), treated with 5% normal rabbit serum, and then incubated at 4°C overnight in anti-ChAT monoclonal antibody (Boehringer Mannheim, 1:3 dilution in PBS-T). After washings with PBS, sections were incubated for 1 h in rabbit anti-rat IgG and then in avidin–biotin–horseradish peroxidase (Vectastain Elite Kit, Vector Laboratories, Burlingame, CA, USA) followed by 0.06% diaminobenzidine + $H_2O_2$ (Sigma Chemical Co.). After additional rinsing, sections were wet mounted on gelatin-coated slides, dried, dehydrated in ascending concentrations of ethanol, cleared in xylene, and coverslipped.

The number of ChAT-positive neurons was measured in three forebrain cholinergic regions (medial septum, striatum, and nucleus basalis) using a computerized image analysis system (Image 1.41, National Institutes of Health, Bethesda, MD, USA) coupled to a Nikon Optiphot 2 microscope (10× magnification). The total number of stained cells in three consecutive sections, separated by 30 μm, was divided by the total area considered, to obtain the total number of ChAT-positive cells per $mm^2$.

### *Statistical Analysis*

Mixed-model analysis of variance (ANOVA) for repeated measures was used for processing body weight, open field, immunocytochemical, and passive avoidance latency data, always considering the litter random variable. Post hoc comparisons were performed by Tukey's HSD test with Bonferroni's correction.

## RESULTS

### *Body Weight*

ANOVA yielded a significant effect of cocaine treatment on body weight [$F(2, 22) = 17.57$, $p < 0.01$]. Post hoc comparisons revealed that only the 40-mg/kg dose was effective in reducing weight gain (saline vs. coc 40, and coc 20 vs. coc 40, $p < 0.01$).

### *Passive Avoidance*

Learning and retention performances in the passive avoidance test are shown in Fig. 1. In the Acquisition session (pnd 16), ANOVA yielded a significant interaction between cocaine treatment and training condition [$F(2, 18) = 3.7$, $p < 0.05$]. Mean latency to step-through was significantly higher in Punished than in Nonpunished mice pretreated with either saline or coc 40 (post hoc comparisons, $p < 0.01$), whereas mean latency of Nonpunished mice in the coc 20 group did not differ from that of coc 20/Punished pups. In addition, coc 20/Nonpunished mice had significant higher latency to step-through than both saline and coc 40 Nonpunished mice (post hoc comparisons, $p < 0.05$). In the Retest session (Fig. 1, right panel), the avoidance performances of Punished pups were significantly improved in comparison with those of Nonpunished pups, irrespective of the saline or cocaine treatment received [$F(1, 9) = 9.93$, $p < 0.05$].

### *Open Field*

Figure 2 shows locomotor activity levels in the open field. Baseline activity levels were not affected by cocaine pretreatment. Scopolamine significantly increased the number of crossings in both saline and cocaine animals [$F(1, 8) = 101.93$, $p < 0.001$]. However, mice exposed to the 40-mg/kg cocaine dose showed a decreased sensitivity to the hyperkinetic effects of scopolamine as compared with either saline or coc 20 animals [interaction of cocaine treatment × scopolamine challenge, $F(2, 16) = 3.90$, $p < 0.05$; $p < 0.05$ after post hoc comparisons].

Table 1 shows the frequency of the behavioural items recorded during the open field test. No significant main effect of cocaine treatment or interaction between cocaine treatment

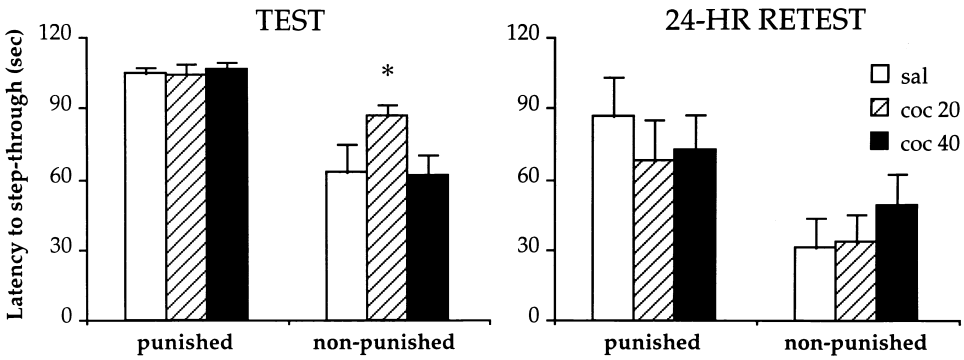


FIG. 1. Acquisition and 24-h retention of a passive avoidance step-through task measured in 16–17-day-old mice injected SC with either saline (sal), 20 mg/kg cocaine (coc 20), or 40 mg/kg cocaine (coc 40) from pnd 2 to 15. Data are mean ± SEM. $n = 10$ in each final group. *Significant effect of cocaine treatment ($p < 0.05$).

and scopolamine challenge was found. Scopolamine increased the frequency of wall rearing [$F(1, 8) = 16.97, p < 0.01$] while decreasing open rearing [$F(1, 8) = 30.88, p < 0.001$] and grooming [$F(1, 8) = 4.91, p = 0.05$]. The effect of scopolamine on grooming frequency seemed more marked in cocaine-treated than in control animals: however, the interaction between cocaine and scopolamine challenge was not statistically significant.

*Immunocytochemistry*

Analysis of ChAT immunostaining (Figs. 3 and 4) in the three forebrain areas considered revealed that coc 40 animals had considerably fewer ChAT-positive neurons in the nucleus basalis, but not in the septum and striatum, than saline-treated mice [interaction between cocaine treatment and brain region, $F(2, 14) = 8.32, p < 0.01; p < 0.01$ after post hoc comparisons].

## DISCUSSION

The results of the present study indicate that neonatal administration of cocaine to mice from pnd 2 to 15 exerts very limited effects on learning and retention of a passive avoidance task, while altering in a dose-dependent fashion behavioural sensitivity to scopolamine in the open field test. The decrease in the number of ChAT-positive neurons in the nucleus basalis suggests that neonatal cocaine exposure influenced the development of at least a subset of forebrain cholinergic neurons.

Significant dose-related reduction of body weight gain from pnd 1 to 15 was observed in cocaine-treated pups. On pnd 15, coc 20 and coc 40 pups decreased to 92% and 82%, respectively, compared with the saline group. A similar finding has also been reported in neonatally treated rats, although no effect on total brain weight was found as a consequence of cocaine treatment (11). It has been suggested that growth retardation in cocaine-exposed pups might result from cocaine's anorexic effect, and in particular from the interference of the drug with suckling behaviour (6,11). The absence of body growth retardation in artificially reared pups neonatally treated with cocaine would support such a hypothesis (5). In this respect, the effects of cocaine on general somatic growth would not be related to direct neurotoxic effects.

As regards the effect of cocaine on early learning and retention capabilities in rats, prenatal exposure only evidenced limited effects in Pavlovian conditioning and sensory precon-

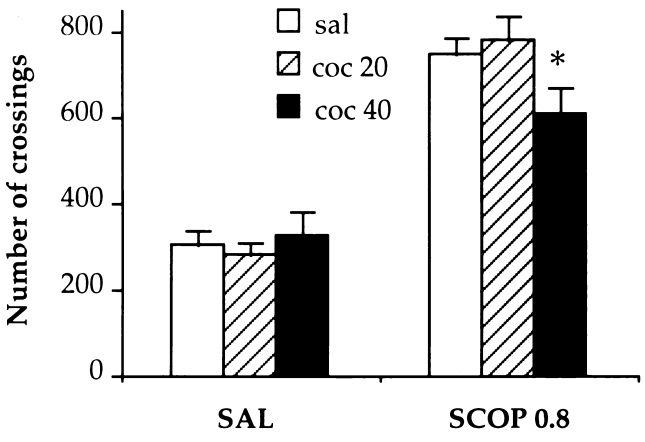


FIG. 2. Locomotor activity of 21-day-old mice recorded during a 20-min open field test. Animals were injected SC with either saline (sal), 20 mg/kg cocaine (coc 20), or 40 mg/kg cocaine (coc 40) from pnd 2 to 15, and received an IP administration of saline or scopolamine (0.8 mg/kg) 15 min before the test. Data are mean ± SEM. $n = 9$ for sal and coc 20 animals; $n = 7$ for coc 40 animals. *Significant effect of cocaine treatment ($p < 0.05$).

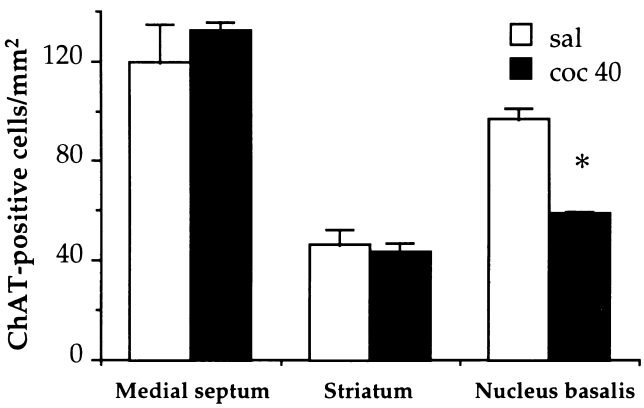


FIG. 3. Mean number (± SEM) of ChAT-immunopositive cells per mm² in the medial septum, striatum, and nucleus basalis of 30-day-old mice treated SC with saline (sal) or 40 mg/kg cocaine (coc 40) from pnd 2 to 15. $n = 4$ for sal animals; $n = 5$ for coc 40 animals. *Significant effect of cocaine treatment ($p < 0.01$).

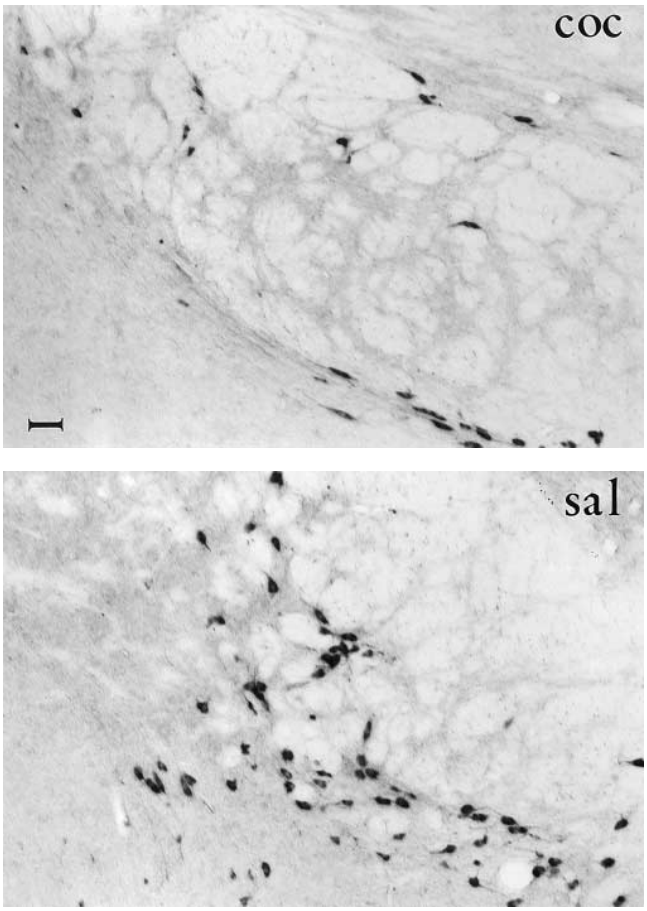


FIG. 4. Photomicrographs of coronal sections through the brain of 30-day-old mice previously treated with either saline (sal) or cocaine (coc) showing ChAT-immunoreactive neurons in the nucleus basalis. Scale bar = 100 μm.

ditioning (22) and deficits in learning of an odor/milk association (44), whereas no effects on learning and retention of a passive avoidance task were found (12,26). Among the few studies on the neurobehavioural effects of neonatal exposure to cocaine, that of Barron and Irvine (5) failed to evidence impairment in passive avoidance learning on pnd 23–24 in rats receiving cocaine from pnd 4 to 10, while Ricceri et al. (37) found a deficit in passive avoidance learning, mainly limited to the 24-h retention of this same response. The results of the present study do not support an effect of neonatal cocaine treatment on learning and retention capacities of preweaning mice. Several factors may account for the discrepancy of the present findings from those of Ricceri et al.: a) species-specific differences between mice and rats, b) different length of exposure to cocaine and different age at testing, and c) the demands of the two learning tasks (step-down vs. step-through avoidance), calling for different ecological dispositions.

The only significant change observed in the acquisition phase concerned the behaviour of Nonpunished coc 20 mice, which were more hesitant to explore the dark compartment than saline and coc 40 animals. Increased neophobia has also been observed in rats exposed prenatally to cocaine, which were reluctant to leave the start box to explore an open field when tested as adolescent and adults (26). Thus, our findings extend a cocaine-related effect on neophobia behaviour to developing mice postnatally exposed to cocaine limited to the lower cocaine dose. This is not surprising, as several studies from the prenatal cocaine literature suggest that exposure to low or high doses of cocaine may have diverse repercussions on brain neurochemistry as well as on behaviour (34). Some behavioural responses might be more sensitive to low cocaine doses than to high ones. For example, Barron and Irvine (5) reported an effect of neonatal cocaine on spontaneous alternation on pnd 19 at a 20-mg/kg dose but not at a higher dose (60 mg/kg).

The results of the open field test on pnd 21 showed that neither baseline activity levels nor general behaviour were affected by cocaine treatment. On the basis of the somewhat conflicting results concerning the effect of cocaine on general activity, it appears that alterations in locomotor activity in animals prenatally or postnatally exposed to cocaine depend on both type of test and cocaine dose (12,15,26,43,44). Similarly, when neonatal treatment schedules were used, cocaine was found to have no effect on running wheel activity in rats exposed to either 20 or 40 mg/kg cocaine (6), whereas moderate hyperactivity was observed in the open field only in rats exposed to a 60-mg/kg dose of cocaine (5). It must be noted that, notwithstanding the increased neophobia displayed by coc 20 animals during the passive avoidance task, their locomotor and exploratory behaviour in the open field was not affected. However, because many psychotropic drugs have been found to reduce exploration without exerting any effect on motor activity or vice versa [for an overview, see (31)], we cannot exclude the possibility that the use of a test designed specifically to analyse exploratory behaviour in a totally novel environment (i.e., hole board test, measurement of the latency to approach a novel object) might reveal increased neophobia in coc 20-treated animals. This issue certainly deserves further investigation.

As for the altered response to scopolamine, the reduced behavioural sensitivity to this drug in the coc 40 animals is the first evidence of a change in sensitivity to a cholinergic challenge in developing rodents upon cocaine pretreatment, and is in accordance with previous biochemical and behavioural data for adult rats showing lowered binding to muscarinic receptors and a long-lasting decrease in behavioural responsiveness to scopolamine after cocaine exposure (29,30,41, 49). As mentioned in the introduction, both in vivo and in vitro studies support the direct action of cocaine on the cholinergic system (39,47), in addition to probable indirect effects resulting from the imbalance of other neurochemical systems (1,24,29,38). Specifically, cocaine appears to act as a direct antagonist of muscarinic receptors (41) and was found to induce downregulation of these same receptors at the hippocampal level (29). In this respect, quantitative and/or qualitative changes in muscarinic receptor populations might be induced by the administration of cocaine in a period of critical development for the central cholinergic systems and could in turn account for the decreased response to scopolamine challenge.

Immunocytochemical data showing decreased ChAT immunoreactivity in the nucleus basalis on pnd 30 further support a long-lasting alteration in the cholinergic function associated with neonatal administration of cocaine. Indeed, a lower number of ChAT-positive neurons was also recorded in the septum and nucleus basalis of 12-day-old rats treated with 25 mg/kg cocaine from birth to pnd 11 (37). Nucleus basalis neurons represent the major extrinsic cholinergic input to the neocortex, and their functional and neurochemical maturation is influenced by neurotrophic agents released by cortical target

neurons (21). Thus, altered functionality of cholinergic transmission at the cortical level in cocaine-exposed animals might have affected the normal development of the basalo-cortical pathway and resulted in biochemical (decreased ChAT synthesis) and/or morphological (cell shrinkage, atrophy) changes in nucleus basalis neurons. We cannot exclude the possibility that immunocytochemical analysis performed shortly after interruption of cocaine treatment in the present study would have revealed alterations in ChAT immunostaining in forebrain cholinergic areas other than the nucleus basalis, such as the septum. Moreover, due to the lack of immunocytochemical data in coc 20 animals, it is difficult to draw definite conclusions on the correlation between behavioural changes and alteration of cholinergic development. Nonetheless, some of the behavioural alterations reported in the neonatal cocaine literature and in the present study are reminiscent of those induced by nucleus basalis lesions. Indeed, the nucleus basalis is involved in the regulation of a number of behavioural responses, including passive avoidance learning, spatial alternation. and exploration/habituation [see (13) for a comprehensive review]. A specific role of the nucleus basalis in activity is supported by the finding that injections of scopolamine in this area lead to hyperactivity (7). Therefore, alteration in this subset of cholinergic neurons would be in agreement with the reduced sensitivity to scopolamine. However, as Dekker and coauthors pointed out, the nucleus basalis is a diffuse structure, and variations in the size and location of the lesion can result in different behavioural deficits in different studies (13). This could account for the lack of effects on passive avoidance learning and retention observed in the present study.

The present data suggest that neonatal cocaine has some teratogenic effects on neurobehavioural maturation in mice. In agreement with data on rats, these effects are more subtle than those induced by other teratogenic agents such as alcohol (9). However, the alteration in behavioural sensitivity to scopolamine and the reduction in the number of ChAT-positive neurons in the nucleus basalis support previous evidence indicating an influence of cocaine treatment on cholinergic neurobehavioural regulation. Inasmuch as the mechanisms involved in the neurochemical and behavioural effects of cocaine are far from being fully understood (20), these findings point to the need for investigating the responses to cocaine administration of systems other than the dopaminergic one.

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## REFERENCES

1. Akbari, H. M.; Kramer, H. K.; Whitaker-Azmitia, P. M.; Spear, L. P.; Azmitia, E. C.: Prenatal cocaine exposure disrupts the development of the serotonergic system. Brain Res. 572:57–63; 1992.
2. Alleva, E.; Bignami, G.: Development of mouse activity, stimulus reactivity, habituation, and response to amphetamine and scopolamine. Physiol. Behav. 34:519–523; 1985.
3. Anderson-Brown, T.; Slotkin, T. A.; Seidler, F. J.: Cocaine acutely inhibits DNA synthesis in developing rat brain regions—Evidence for direct actions. Brain Res. 537:197–202; 1990.
4. Barron, S.; Irvine, J.: The effects of neonatal cocaine exposure on two measures of balance and coordination. Neurotoxicol. Teratol. 16:89–94; 1994.
5. Barron, S.; Irvine, J.: Behavioural effects of neonatal cocaine exposure using a rodent model. Pharmacol. Biochem. Behav. 50:107–114; 1995.
6. Barron, S.; Kaiser, D. H.. Hansen, L. S.: Neonatal cocaine exposure, activity and responsivity to cocaine in a rodent model. Neurotoxicol. Teratol. 16:401–409; 1994.
7. Baud, P.; Mayo, W.; le Moal, M.; Simon, H.: Locomotor hyperactivity in the rat after infusion of muscimol and [D-Ala$^2$]Met-enkephalin into the nucleus basalis magnocellularis. Possible interaction with cortical cholinergic projections. Brain Res. 452:203–211; 1988.
8. Blozovski, D.; Hennocq, N.: Effect of antimuscarinic cholinergic drugs injected systemically or into the hippocampo-entorhinal area upon passive avoidance learning in young rats. Psychopharmacology 76:351–358; 1982.
9. Bonthius, D. J.; West, J. R.: Permanent neuronal deficits in rats exposed to alcohol during the brain growth spurt. Teratology 44:147–163; 1991.
10. Chasnoff, I. J.; Griffith, D. R.; MacGregor, S.; Dirkes, K.; Burns, K. A.: Temporal patterns of cocaine use in pregnancy. JAMA 257:957–961; 1987.
11. Chen, W. A.; Andersen, K. H.; West, J. R.: Cocaine exposure during the brain growth spurt: Studies of neonatal survival, somatic growth and brain development. Neurotoxicol. Teratol. 15:267–273; 1993.
12. Church, M. W.; Overbeck, G. W.: Prenatal cocaine exposure during the brain growth spurt: Studies of neonatal survival, somatic growth, and brain development. Neurotoxicol. Teratol. 15:267–273; 1993.
13. Dekker, A. J.; Connor, D. J.; Thal, L. J.: The role of cholinergic projections from the nucleus basalis in memory. Neurosci. Biobehav. Rev. 15:299–317; 1991.
14. Dobbing, J.; Sands, J.: Comparative aspects of the brain growth spurt. Early Hum. Dev. 3:79–83; 1979.
15. Dow-Edwards, D. L.: Functional effects of cocaine given during critical developmental periods. Pediatr. Res. 23:241A; 1988.
16. Dow-Edwards, D. L.; Freed, L. A.; Fico, T. A.: Structural and functional effects of prenatal cocaine exposure in adult rat brain. Dev. Brain Res. 57:263–268; 1990.
17. Dumery, V.; Derer, P.; Blozovski, D.: Enhancement of passive avoidance learning through doses of intra-amygdaloid physostigmine in the young rat. Its relation to the development of acetylcholinesterase. Dev. Psychobiol. 21:553–556; 1988.
18. Durand, D. J.; Espinoza, A. M.; Nickerson, B. G.: Association between prenatal cocaine exposure and sudden infant death syndrome. J. Pediatr. 117:909–910; 1990.
19. Feigley, D. A.; Spear, N. E.: Effect of age and punishment condition on long-term retention by the rat of active and passive avoidance learning. J. Comp. Physiol. Psychol. 73:515–526; 1970.
20. Gawin, F. H.: Cocaine addiction: Psychology and neurophysiology. Science 251:1580–1585; 1991.
21. Hefti, F.; Hartikka, J.; Knusel, B.; LaPlume, M. O.; Mash, D. C.: Nerve growth factor and cholinergic neurons of the mammalian brain. In: Steriade, M.; Biesold, D., eds. Brain cholinergic systems. New York: Oxford University Press; 1990:173–201.
22. Heyser, C. J.; Chen, W. J.; Miller, J.; Spear, N. E.; Spear, L. P.: Prenatal cocaine exposure induces deficits in Pavlovian conditioning and sensory preconditioning among infant rat pups. Behav. Neurosci. 104:955–963; 1990.
23. Heyser, C. J.; Goodwin, G. A.; Moody, C. A.; Spear, L. P.: Prenatal cocaine exposure attenuates cocaine-induced odor preferences in infant rats. Pharmacol. Biochem. Behav. 42:169–173; 1992.
24. Hurd, Y. L.; Weiss, F.; Koob, G.; Ungerstedt, U.: The influence of cocaine self-administration on in vivo dopamine and acetylcholine neurotransmission in rat caudate-putamen. Neurosci. Lett. 109: 227–233; 1990.

25. Hutchings, D. E.; Fico, T. A.; Dow-Edwards, D. L.: Prenatal cocaine: Maternal toxicity, fetal effects and locomotor activity in rat offspring. Neurotoxicol. Teratol. 11:65–69; 1989.
26. Johns, J. M.; Means, L. L.; Means, M. J.; McMillen, B. A.: Prenatal exposure to cocaine II: Effects on open-field activity and cognitive behaviour in Sprague–Dawley rats. Neurotoxicol. Teratol. 14:343–349; 1992.
27. Laviola, G.; Loggi, G.: Sexual segregation in infancy and bi-directional benzodiazepine effects on hot-plate response and neophobia in adult mice. Pharmacol. Biochem. Behav. 42:865–870; 1992.
28. Levin, E. D.; Seidler, F. J.: Sex-related spatial learning differences after prenatal cocaine exposure in the young adult rat. Neurotoxicology 14:23–28; 1993.
29. Lipton, J. W.; Ellison, G. D.: Continuous cocaine induces persisting changes in behavioural responsivity to both scopolamine and diazepam. Neuropsychopharmacology 7:143–148; 1992.
30. Lipton, J. W.; Olsen, R. W.; Ellison, G. D.: Length of continuous cocaine exposure determines the persistence of muscarinic and benzodiazepine receptor alterations. Brain Res. 676:378–385; 1995.
31. Lister, R. G.: Ethologically-based animal models of anxiety disorders. Pharmacol. Ther. 46:321–340; 1990.
32. Little, B. B.; Snell, L. M.: Brain growth among fetuses exposed to cocaine in utero—Asymmetrical growth retardation. Obstet. Gynecol. 77:361–364; 1991.
33. Neuspiel, D. R.; Hamel, S. C.; Hochberg, E.; Greene, J.; Campbell, D.: Maternal cocaine use and infant behaviour. Neurotoxicol. Teratol. 13:229–233; 1991.
34. Raum, W. J.; McGivern, R. F.; Peterson, M. A.; Shryne, J. H.; Gorski, R. A.: Prenatal inhibition of hypothalamic sex steroid uptake by cocaine: Effects on neurobehavioural sexual differentiation in male rats. Dev. Brain Res. 53:230–236; 1990.
35. Ray, D.; Nagy, Z. M.: Emerging cholinergic mechanisms and ontogeny of response inhibition in the mouse. J. Comp. Physiol. Psychol. 92:335–349; 1978.
36. Ricceri, L.; Calamandrei, G.; Chiarotti, E.; Alleva, E.: Nerve growth factor affects passive avoidance learning and retention in developing mice. Brain Res. Bull. 39:219–226; 1996.
37. Ricceri, L.; Tirassa, P.; Aloe, L.; Alleva, E.: Postnatal cocaine exposure affects neonatal passive avoidance performance and cholinergic development in rats. Pharmacol. Biochem. Behav. 45:283–289; 1993.
38. Robertson, M. W.; Leslie, C. A.; Bennet, J. P., Jr.: Apparent synaptic dopamine deficiency induced by withdrawal from chronic cocaine treatment. Brain Res. 538:337–339; 1991.
39. Robinson, S. E.; Hambrecht, K. L.: The effect of cocaine on hippocampal cholinergic and noradrenergic metabolism. Brain Res. 457:383–385; 1988.
40. Scalzo, F. M.; Ali, S. F.; Frambes, N. A.; Spear, L. P.: Weanling rats exposed prenatally to cocaine exhibit an increase in striatal D2 dopamine binding associated with an increase in ligand affinity. Pharmacol. Biochem. Behav. 37:371–373; 1990.
41. Smith, R. F.; Mattran, K. M.; Kurkjian, M. F.; Kurtz, S. L.: Alterations in offspring behaviour induced by chronic prenatal cocaine dosing. Neurotoxicol. Teratol. 11:35–38; 1989.
42. Sharkey, J.; Ritz, M. C.; Schenden, J. A.; Hanson, R. C.; Kuhar, M. J.: Cocaine inhibits muscarinic cholinergic receptors in heart and brain. J. Pharmacol. Exp. Ther. 246:1048–1052; 1988.
43. Sobrian, S. K.; Burton, L. E.; Robinson, N. L.; Ashe, W. K.; James, H.; Stokes, D. L.; Turneer, L. M.: Neurobehavioural and immunological effects of prenatal cocaine exposure in rats. Pharmacol. Biochem. Behav. 35:617–629; 1990.
44. Spear, L. P.; Kirstein, C. L.; Bell, J.; Yootanasumpum, V.; Greenbaum, R.; O'Shea, J.; Hoffman, H.; Spear, N. E.: Effects of prenatal cocaine exposure on behaviour during the early postnatal period. Neurotoxicol. Teratol. 11:57–63; 1989.
45. Spear, L. P.; Kirstein, C. L.; Frambes, N. A.: Cocaine effects on the developing central nervous system: Behavioural, psychopharmacological, and neurochemical studies. Ann. N.Y. Acad. Sci. 562:290–307; 1989.
46. Stehower, D. J.; Campbell, B. A.: Ontogeny of passive avoidance: Role of task demands and development of species-typical behaviors. Dev. Psychobiol. 13:385–390; 1980.
47. Tan, X.; Costa, L. G.: Inhibition of muscarinic receptor-stimulated phosphoinositide metabolism by cocaine, norcocaine and cocaethylene in rat brain. Dev. Brain Res. 79:132–135; 1994.
48. Witkin, J. M.; Goldberg, S. R.; Katz, J. L.; Kuhar, M. J.: Modulation of the lethal effects of cocaine by cholinomimetics. Life Sci. 45:2295–2301; 1989.
49. Zeigler, S.; Lipton, J.; Toga, A.; Ellison, G.: Continuous cocaine administration produces persisting changes in brain neurochemistry and behaviour. Brain Res. 552:27–35; 1991.