Prenatal Exposure to Cocaine and Enriched Environment

Effects on Social Interactions

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ABSTRACT: Exposure to cocaine throughout gestation may produce several deleterious outcomes in the offspring that include effects on neurotransmitter systems and structure of the central nervous system. Such changes are most likely correlated with behavioral alterations. Environmental enrichment (EE) in early stages is a factor that affects structural and behavioral development. This article examines the effects, upon social interactions, of EE during the first month of life in rats prenatally exposed to cocaine. Wistar dams were subcutaneously exposed to 60 mg/kg of cocaine divided in two daily doses from gestational day (GD)8 to GD22. Pair-fed controls were given saline vehicle in the same protocol. Offspring were distributed to the different environments in four experimental groups. Group 1: offspring from dams prenatally exposed to cocaine as previously described and reared in EE from postnatal day (PND)1 to PND28; Group 2: pups from cocaine-exposed dams and reared in a standard environment (SE); Group 3: pups from pair-fed saline-exposed dams and reared in EE; Group 4: offspring from saline-exposed dams and reared in SE. On PND21, 24, and 28, rats were examined in several social behavioral categories (play fighting, social investigation, comfort behaviors, and solicitation to play) for 10 min. Animals reared in SE do not display any differences due to treatment in the behavioral categories analyzed. Control offspring reared in EE presented decreased play fighting, decreased solicitation to play, and decreased social investi-

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gation compared to the control SE group, while cocaine-exposed animals reared in EE did not present these variations. These results suggest that EE rearing may unmask hidden effects of prenatal cocaine exposure.

KEYWORDS: cocaine; development; enriched environment; social interactions

INTRODUCTION

Rats exhibit a complex and well-organized repertoire of social behavior. In juvenile rats, social activities are different from social play, which suggests that within the social repertoire of juvenile rats, it is possible to formulate a distinction between social behaviors related to play (pinning, boxing/wrestling, following/chasing) or unrelated to play (social exploration, contact behavior).[1–3] Social behaviors related to play occur mainly before sexual maturation, peaking at a midpoint of the periadolescent period, while other forms of social repertoire occur during the entire life span, such as social investigation and social contact behavior.[2,4,5] Play behavior seems to be an affiliate form of behavior, functioning to facilitate different aspects of social development, such as establishment of social organization in a group[6] and development of the ability to express and understand intraspecific-communicating signals,[4,7] which may serve to inhibit aggression[6,8] and increase group stability. Social play may be seen as facilitating the ability to cope with social conflicts.[9]

Behavioral studies have indicated that social play is a separate and relevant category of behavior and have reported that play and social investigation are influenced differentially by drugs' treatments.[10–12] Former studies suggest that behaviors related to play may be regulated through neuronal systems different from those that regulate social behaviors unrelated to play.[8,13] It was found that opioid systems have an important role in regulation and expression of social play. The motivational and rewarding aspects of play behavior are thought to be regulated by the endogenous opioid system.[13] The implication of the reward system in the regulation of social play suggested that long-term changes in this system probably would alter the expression of social play.

Cocaine reinforcing proprieties also utilize, in part, the same circuit implicated in the reinforcing properties of opiates.[14] Cocaine exposure throughout gestation results in an altered dopaminergic system[15,16] and this alteration produces some deficits in social behaviors.[17–21] Studies linking cocaine and social interaction showed that prenatal cocaine exposure results in diminished social play,[17,22] increased aggression,[19–21,23] and potentiation of responsiveness to acute or chronic stress.[24–27] Other groups reported that animals postnatally exposed to cocaine decreased aggression (acute treatment),[28] elicited less play solicitation from conspecifics, or did not differ from controls in their own play behavior.[22] These discrepancies in published data may be associated to the variety of test paradigms, animal models, and dosing regimens that have been

utilized. However, there is some convergence in basic research which suggests that exposure to drugs throughout development may lead to persistent abnormalities of in-brain systems and behavior. These permanent changes in the development of the brain circuits that process reward or reinforcement may result in a greater vulnerability to additive behaviors in adulthood.[29]

There are indications that both formal training on complex spatial tasks and living in spatially complex environments (environmental enrichment [EE]) alter neurochemistry, brain weight, and behavior in rats.[30] An environment that supplies a great variety of incentives provides a learning space to the animal, with increased possibilities of interaction, manipulation, and exploration of the environment.[31] Exploration presupposes the occurrence of learning, which probably alters the behavioral sequences (for review see Ref. 32). A complex environment increases the opportunity not only of social play but also of object interaction. Several studies found evidence of neurogenesis in response to EE (for review see Ref. 33), which might explain its beneficial effects on the course of neurodegeneratives diseases,[34] aging,[35] and recovery from brain damage.[36] The stimulation provided via EE, applied early in life, alters both brain and behavior and may be beneficial for the behavior development.[32]

The main objective of the present article was to analyze the effects of prenatal exposure to cocaine on development social behavior and to verify if a postnatal environmental condition (EE/standard) would affect the expression of social behaviors in rats exposed to cocaine during prenatal life.

MATERIAL AND METHODS

Subjects and Protocol of Drug Exposure

Rats used in this study were offspring born from Wistar females purchased from the Colony of the Gulbenkian Institute of Science, Oeiras, Portugal. They were bred at the Institute of Molecular and Cell Biology, University of Porto, Portugal. Animals were housed in a temperature- and humidity-controlled colony room, maintained on a 12/12-h light/dark cycle, with free access to food and water. At the onset of breeding, adult nulliparous females, weighing an average of 250–300 g, were mated with one male of the same strain. The morning a copulatory plug or sperm-positive vaginal cytology was observed was considered as gestational day 1 (GD1). Pregnant females were weighed, housed individually in standard cages containing wood bedding, and assigned to one of the following prenatal groups: a cocaine-exposed group (COC) and a control group, pair-fed to the COC group (PF). Dams in the COC group received subcutaneous injections (s.c.) of cocaine hydrochloride (Sigma Chemical Co., St. Louis, MO) at 60 mg/kg of body weight/day from GD8 to 22 and were given *ad libitum* food and water. The period of exposure comprises the major periods of organogenesis and of neurogenesis, migration and gliogenesis, covering also the onset of synaptogenesis.[37,38] Each daily

dose was divided into two equal parts, administered between 8:30–9:00 AM and 5:00–6:00 PM. Dams in the PF group received daily s.c. injections of vehicle (saline 0.9%), isovolumetric to cocaine and were submitted to food restriction (pair-fed to the cocaine females).

On the day after birth, postnatal day (PND)1, each litter was culled to eight pups, gender balanced, and pups weighed, marked with a felt-tip pen for identification, and housed in standard polycarbonate cages, living directly on wooden bedding. Pups were weaned on PND21. All procedures used were approved by the Portuguese Agency for Animal Welfare (General Board of Veterinary Medicine).

Housed Conditions

EE: large acrylic cage (100 × 70 × 70 cm) with wooden bedding and equipped with various objects (polyvinyl chloride [PVC] tubes, ping-pong and paper balls, wood objects, ladders, toys) that were replaced every 3 days. Standard environment (SE) standard acrylic cage type III, living directly on wooden bedding without objects.

Experimental Groups

Offspring were distributed into these two different environments completing 4 experimental groups: Group1 COC/EE: offspring from dams prenatally exposed to COC as previously described and reared in EE from PND 1 to PND28; Group 2 COC/SE: pups from COC exposed dam and reared in SE; Group 3 SAL/EE: pups from PF and saline (SAL)-exposed dam and reared in EE; Group 4 SAL/SE: offspring from SAL-exposed dams and reared in SE.

Behavioral Testing: Social Interactions

Social interactions were assessed in three periods of development: PND21—weaning and beginning of play-fighting behaviors,[39] PND24 and 28—prepubescent period. The social interaction test was performed in a sound-attenuated room, during the dark phase of the light cycle. The testing arena consisted in a (PVC) cage measuring 60 × 53 × 40 cm with wood shaving covered floor, containing the following objects: three ladders, a block and a siring, illuminated by an infrared light mounted 1 m above. The test was carried out between 4 PM and 5 PM, 6.5 h after the daily drug treatment. This period of time was chosen, to allow enough time to eliminate the influence of acute cocaine, considering a 2-h half-life after subcutaneous administration of cocaine (reviewed in Ref. 40). Behavior sessions were recorded by a video camera (SONY DCR-TRV9E; Sony Corp., Tokyo, Japan), for 10 min, after allowing a 5-min period for habituation to the test arena. Test was performed

in groups of four rats from the same experimental group but only one rat was observed by focal–animal method. The following behavioral categories were analyzed using the software Observer 4.1 (Noldus Information Technology, Wageningen Netherlands): frequency of play-fighting behaviors, divided into play-dominant (wrestling, boxing, pouncing, aggressive grooming, on-top-posture, chase, attack) and play-submissive (pining, escape, evade) behaviors; comfort behaviors (social grooming and pile-up behavior); play solicitation (crawl over/under, nosing); social investigation (sniffing the conspecific's body including the anogenital area); and total frequency social interaction.

Data Analyses

Behavioral data were analyzed by a Mann-Whitney *U* test. Since this Mann-Whitney *U* test did not indicate a significant effect of the gender factor, data were collapsed across gender and reanalyzed. Results were analyzed at a significance level of 5%, using the software Statistica version 5.5 (Statsoft Inc., 1999; Tulsa, OK).

RESULTS

Effects of Postnatal Exposure to Cocaine in SE

Prenatal exposure to cocaine had no significant effect on social behavior of developing rat pups reared in SE when compared to SAL/SE animals. No significant differences were found on frequency of play solicitation (see FIG. 1 A), play-fighting behaviors (dominant or submissive) (see FIG. 1 B,C), or social investigation (FIG. 1 D) comfort behaviors (FIG. 1 E) in rats COC/SE when compared to SAL/SE.

Effects of EE

For data concerning effects of EE, Mann-Whitney *U* test revealed a significant effect of housing conditions in several behavioral categories. Animals of SAL/EE group decreased play-dominant behaviors on PND21 ($Z = -2.92$; two-tailed $P < 0.01$) and on PND24 ($Z = -2.323$; two-tailed $P < 0.05$) (see FIG. 1 B), play-submissive behaviors on PND21 ($Z = -2.542$; two-tailed $P < 0.05$) and on PND24 ($Z = -3.192$; two-tailed $P < 0.001$) (see FIG. 1 C), social investigation on PND28 ($Z = -2{,}226$; two-tailed $P < 0.05$) (FIG. 1 D) and also decreased play solicitation on PND21 ($Z = -3.241$; two-tailed $P < 0.001$); and PND24 ($Z = -2.427$; two-tailed $P < 0.05$) (FIG. 1 A) when compared to SAL/SE rats.

Man-Whitney test showed a significant alteration in behavior of rats prenatally exposed to cocaine when they were reared in EE during the first month of life in three behavioral categories. COC/EE rats showed a decreased play-dominant behavior on PND21 ($Z = -2.353$; two-tailed $P < 0.05$) (see FIG. 1 B), of play-submissive behavior on PND21 ($Z = -2.672$; two-tailed $P < 0.01$) (see FIG. 1 C), and decreased social investigation on PND21 ($Z = -2.4$; $P < 0.05$) (FIG. 1 D). No other behavioral categories presented a significant effect for EE.

DISCUSSION

In the present study, effects of EE on prenatal cocaine-exposed rats were examined in several categories of social behavior. In the rat, the first month of postnatal life is a particularly sensitive developmental period in which environmental influences can have a significant effect on subsequent behavioral development.[11]

In an attempt to identify the effects of prenatal cocaine exposure on rat social behavior, we have examined the effects of chronic cocaine exposure on several behavioral categories of social interaction at three different points during the prepubescent period. However, control and prenatal cocaine-exposed rats reared in SE do not display any differences in the behavioral categories analyzed. Some studies report that prenatal cocaine exposure results in diminished social behavior,[17,22,41] decreased solicitation to play,[17,20,22] and increased aggression[18,20,21] in the offspring. These positive effects may be explained, in part, by methodological differences such as subject age at testing, dose of drug administered, and other differences in the protocol used.

Control animals reared in an EE (SAL/EE) displayed decreased play fight when compared to SE-reared rats (SAL/SE). This result is consistent with other studies, where a significant reduction of play-related behaviors on animals reared in EE was shown.[39,42] The reduction of social play in SAL/EE rats may reflect the fact that, in this study, rats were tested in an arena with novel objects. The novelty of objects' arrangements may have facilitated the exploration of environment.[42,43] Also, there is evidence that social interactions may increase object interaction.[43] Animals reared in an EE are more exploratory and seem to prefer to explore the novel environment instead of being engaged in social play,[42] which would reduce attention to the surroundings. The EE also decreased social investigation in SAL/EE when compared to SAL/SE-reared rats. This social behavior, unrelated to play, has the immediate goal of exploring cospecifics.[3] Social memory, the ability to form and retain information related to conspecifics,[44] seems to be improved in EE-housed animals. This result suggests that EE facilitated the retention of specific information, which is in accordance with the increased plasticity in the hippocampus described to be caused by EE.[33]

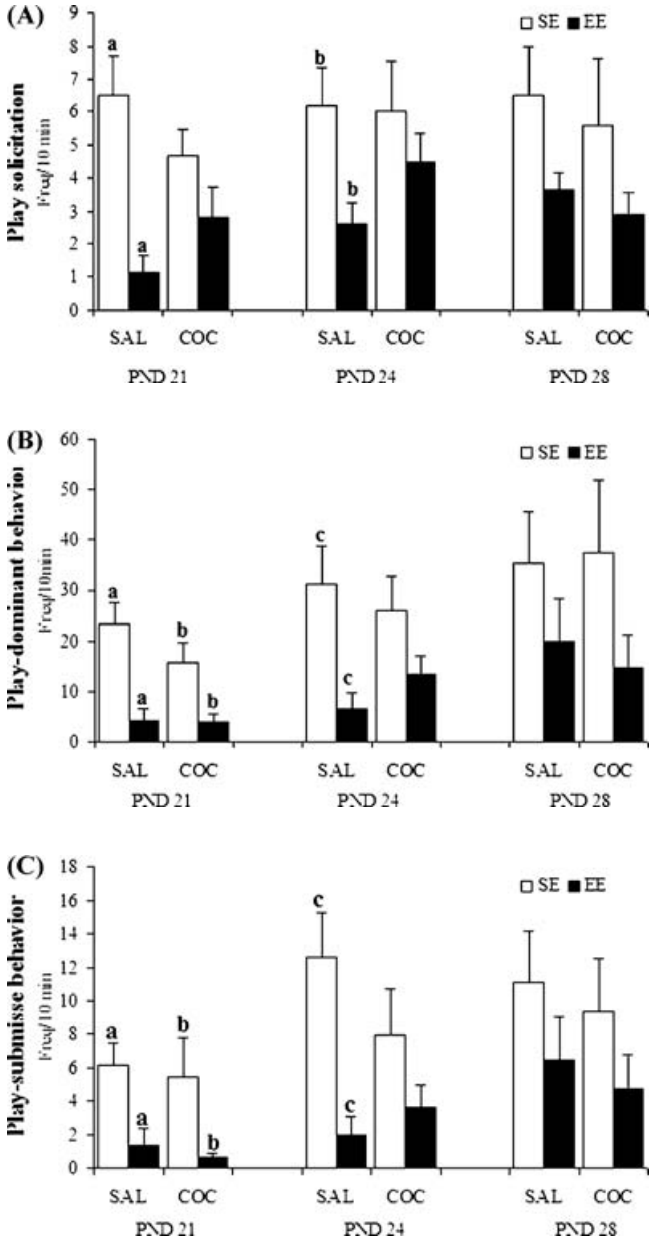

FIGURE 1. Effect of prenatal cocaine exposure and environmental enrichment on frequencies per 10 min of the follows behavioral categories: (**A**) play solicitation: (**a**) $P < 0.001$, (**b**) $P < 0.05$; (**B**) play-dominant behaviors: (**a**) $P < 0.01$, (**b**, **c**) $P < 0.05$; (**C**) play-submissive behaviors: (**a**) $P < 0.05$, (**b**) $P < 0.001$, (**c**) $P < 0.01$; (**D**) social investigation: (**a**, **b**) $P < 0.05$; and (**E**) comfort behaviors. Data are presented as means + SEM.

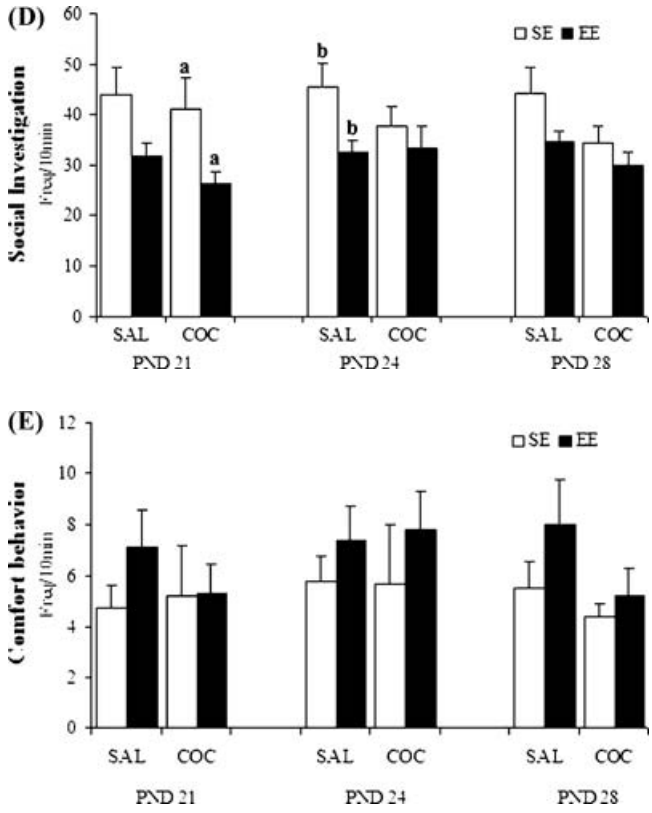

FIGURE 1. *Continued*

Play solicitation is less frequent in rats of SAL/EE group when compared to rats reared in SE. In the juvenile rat, specific behaviors classified as play solicitation include pounce, tail pulling, crawl over/under and darting, and precede bouts or sequences of play fighting.[4] Play solicitation has been described as a set of behaviors functionally inciting social play.[45] So, this decrease in solicitation to play can be related with the decreased play-fighting behavior.

Rats prenatally exposed to cocaine, when reared in EE, display decreased play-fighting behaviors (play-dominant and play-submissive behaviors) compared to COC/SE group. As already mentioned, reduction of play in animals reared in EE may be related with their preference to explore the novel environment instead of being engaged in social play.[42] Animals in EE present reduced levels of offensive and submissive behaviors such chasing, attacking, pining and fighting, suggesting that a complex environment most markedly affects play fighting. In fact, EE environment enhances the opportunities to spread out and to stay away, expanding the exploration of the behavioral repertoire.

Rats prenatally exposed to cocaine and reared in EE also decreased social investigation when compared to COC/SE animals. These results suggest that EE improves social memory in rats postnatally exposed to cocaine. Rats who were engaged in playful behavior with conspecifics in EE present increased dendritic arboration, have heavier cerebral cortices, and show improved learning abilities when compared to play-deprived rats in impoverished environment (for review see Ref. 46). Social exploration is not associated with play behavior. This social category, unrelated to play, seems to be regulated through different neural systems.[13] While social play seems to be primarily regulated through the opioid system,[47] cocaine is known to affect mainly the dopaminergic and serotonergic systems.[48,49] However, although these circuitries are not fully understood, there are strong evidences of direct and indirect (via GABAergic inhibition) influence of one system onto the other (for review see Ref. 10).

In the present work, no differences between cocaine-exposed and control rats could be determined when both groups were reared in a SE, nevertheless, when rats were given an extra developmental stimuli, such as those promoted by an EE, several differences were observed. Therefore, we propose that rearing in an EE may unmask hidden deficits of prenatal cocaine exposure that would not be evident under SE conditions.

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