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AGE-DEPENDENT EFFECTS OF κ -OPIOID RECEPTOR STIMULATION ON COCAINE-INDUCED STEREOTYPED BEHAVIORS AND DOPAMINE OVERFLOW IN THE CAUDATE-PUTAMEN: AN *IN VIVO* MICRODIALYSIS STUDY

A. M. Cortez, S. Charntikov¹, T. Der-Ghazarian, L. R. Horn, C. A. Crawford, and S. A. McDougall*

Department of Psychology, California State University, San Bernardino, CA 92407, USA

Abstract

κ -Opioid receptor stimulation attenuates psychostimulant-induced increases in extracellular dopamine in the caudate-putamen (CPu) and nucleus accumbens of adult rats, while reducing cocaine-induced locomotor activity and stereotyped behaviors. Because κ -opioid receptor agonists (e.g., U50,488 or U69,593) often affect the behavior of preweanling rats in a paradoxical manner, the purpose of the present study was to determine whether κ -opioid receptor stimulation differentially affects dopaminergic functioning in the CPu depending on age. *In vivo* microdialysis was used to determine whether U50,488 (5 mg/kg) attenuates cocaine-induced dopamine overflow in the dorsal CPu on postnatal day (PD) 17 and PD 85. In the microinjection experiment, cocaine-induced stereotyped behaviors were assessed in adult and preweanling rats after bilateral infusions of vehicle or U50,488 (1.6 or 6.4 μ g per side) into the CPu. Results showed that U50,488 attenuated the cocaine-induced increases in CPu dopamine overflow on PD 85, while the same dose of U50,488 did not alter dopamine dialysate levels on PD 17. Cocaine also increased stereotyped behaviors (repetitive motor movements, behavioral intensity scores, and discrete behaviors) at both ages, but adult rats appeared to exhibit more intense stereotypic responses than the younger animals. Consistent with the microdialysis findings, bilateral infusions of U50,488 into the dorsal CPu decreased the cocaine-induced stereotypies of adult rats, while leaving the behaviors of preweanling rats unaffected. These results suggest that the neural mechanisms underlying κ -opioid/dopamine interactions in the CPu are not fully mature during the preweanling period. This lack of functional maturity may explain why κ -opioid receptor agonists frequently induce different behavioral effects in young and adult rats.

Keywords

Caudate-putamen; U50,488; Cocaine; Stereotyped behaviors; *In vivo* microdialysis

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*Corresponding author: Tel.: +1-909-537-5581; fax: +1-909-537-7003. smcdoug@csusb.edu (S. A. McDougall).

¹Present address: Department of Psychology, University of Nebraska-Lincoln, 238 Burnett Hall, Lincoln, NE 68588, USA

Full address: Sanders A. McDougall, Department of Psychology, California State University, San Bernardino, CA 92407, USA.

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INTRODUCTION

The basal ganglia consists of a set of interconnected subcortical nuclei, found in the telencephalon, diencephalon, and midbrain, that form a complex neuronal system capable of modulating a variety of behaviors, including reward, cognition, and motor movement (for reviews, see Alexander et al., 1986; Hauber, 1998; Middleton and Strick, 2000; Yin et al., 2008). Basal ganglia functioning varies across ontogeny, with age-dependent changes in behavior often being a consequence. Understanding the neural mechanisms underlying these age-dependent behavioral changes not only provides information about the developing organism, it serves as an additional tool for determining how neurotransmitter systems interact to mediate behavior (for a review, see Spear, 1979).

Some of the most prominent examples of age-dependent behavioral differences involve the dopamine and dynorphin neurotransmitter systems (i.e., dynorphin is the endogenous ligand at κ -opioid receptors). In general, κ -opioid receptor stimulation depresses the locomotor activity, rearing, and grooming of adult rats (Ukai and Kameyama, 1985; Di Chiara and Imperato, 1988; Jackson and Cooper, 1988; Leyton and Stewart, 1992). The same pattern of effects occurs in adult rats pretreated with psychostimulants, because κ -opioid agonists (e.g., U50,488 or U69,593) attenuate cocaine- and amphetamine-induced locomotor activity, rearing, hole poking, sniffing, and other stereotypies (Heidbreder et al., 1993; Duke et al., 1997; Gray et al. 1999; Vanderschuren et al., 2000; Tzaferis and McGinty, 2001; Smith et al., 2003; Puig-Ramos et al., 2008). The neural mechanisms underlying these κ -opioid/dopamine interactions involve striatal structures, since the nucleus accumbens is responsible for mediating locomotor activity and rearing (Staton and Solomon, 1984; Ikemoto, 2002; Ikemoto and Witkin, 2003; Sellings and Clarke, 2003; Sellings et al., 2006), while the caudate-putamen (CPu) is necessary for stereotyped motor movements and, to a lesser extent, locomotion (Staton and Solomon, 1984; Kelley et al., 1988; Dickson et al., 1994; Canales et al., 2000; Waszczak et al., 2002). Indeed, microdialysis studies show that U50,488 and U69,593 reduce basal dopamine dialysate levels and block the psychostimulant-induced increases in dopamine overflow in the nucleus accumbens and CPu of adult rats (Di Chiara and Imperato, 1988; Donzanti et al., 1992; Spanagel et al., 1992; Maisonneuve et al., 1994; Zaratini and Clarke, 1994; Gray et al., 1999). Apparently, κ -opioid receptors, located on the presynaptic terminals of dopamine-containing fibers, modulate cocaine-induced neurochemical and behavioral effects by decreasing dopamine release and, in some situations, by altering the functioning of the dopamine transporter (Di Chiara and Imperato, 1988; Spanagel et al., 1992; Ronken et al., 1993; Thompson et al., 2000).

Preweanling rats, like adults, show enhanced locomotor activity and stereotypy after acute treatment with a psychostimulant drug (Lal and Sourkes, 1973; Sobrian et al., 1975; McDougall et al., 1994, 1999, 2007). In sharp contrast to what is observed in adult rats, κ -opioid agonists do not attenuate cocaine- and amphetamine-induced locomotion during the preweanling period. Instead, U50,488, when given alone, causes a paradoxical increase in the locomotor activity and wall-climbing of preweanling rats (Jackson and Kitchen, 1989; McLaughlin et al., 1995; Duke et al., 1997; McDougall et al., 1997). Moreover, combined treatment with U50,488 and either cocaine or amphetamine produces a potentiated locomotor response (McDougall et al., 1999). A different pattern of results occurs when stereotyped behaviors are assessed, since κ -opioid agonists reduce cocaine- and amphetamine-induced stereotyped sniffing on postnatal day (PD) 17 (McDougall et al., 1999). The latter result is difficult to interpret, however, because the U50,488-induced increase in locomotor activity probably competes with the expression of stereotyped sniffing. Regardless, it is evident that the neural mechanisms mediating these U50,488-induced behavioral effects undergo substantial changes across postnatal development.

Whether these ontogenetic changes involve κ -opioid receptors in the CPu, nucleus accumbens, or some other brain region is uncertain.

The purpose of the present study was to determine whether κ -opioid receptors modulate cocaine-induced dopamine overflow and stereotyped behaviors in a similar manner during the preweanling period and in adulthood. *In vivo* microdialysis was used to examine whether U50,488 would attenuate cocaine-induced dopamine overflow in the CPu of adult and preweanling rats. In the microinjection experiment, cocaine-induced stereotypy was measured after bilateral infusions of U50,488 into the CPu. Cocaine-induced stereotypy, as well as *in vivo* microdialysis, were assessed on PD 85 and PD 17. At the latter age, κ -opioid receptors in the CPu have reached adult-like densities (Kornblum et al., 1987; Leslie and Loughlin, 1993), although no *in vivo* studies have determined whether these κ -opioid receptors modulate dopaminergic functioning similarly in young and adult rats.

EXPERIMENTAL PROCEDURES

Animals

Subjects were 179 rats of Sprague-Dawley descent (Charles River, Hollister, CA, USA) raised at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups at postnatal day (PD) 3 (day of parturition is PD 0). Mean body weights of the 40 male and 39 female rats tested on PD 17 were 39.9 g ($\pm$ 0.8, SEM) and 40.3 g ($\pm$ 0.8, SEM), respectively; whereas, the mean body weight of the 100 male rats tested on PD 85–89 was 395 g ($\pm$ 3.4, SEM). Rats tested at PD 17 were kept with the dam prior to testing, whereas rats tested in adulthood were maintained with same sex littermates after weaning. Both preweanling and postweanling rats were housed on racks in large polycarbonate maternity cages (56 $\times$ 34 $\times$ 22 cm) with wire lids. After surgery, adult rats were housed singly. Food and water were freely available. The colony room was maintained at 22°–24°C and kept under a 12-h light/dark cycle, with behavioral testing occurring during the light phase of the cycle. Subjects were cared for in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80–23) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Microdialysis testing was conducted in clear cylindrical chambers (Instech, Plymouth Meeting, MA, USA) equipped with dual-channel quartz-lined swivels (model 375/D/22QM, Instech) mounted on counterbalanced arms with tethers. Preweanling rats were tested in smaller chambers (14 cm in diameter) than adult rats (22 cm). Different microdialysis guide cannulae and probes were used at the two ages (PD 17, model CMA/7; PD 85–89, model CMA/11; CMA, Solna, Sweden), with the length of the permeable portion of the membrane being 3 mm for adult rats and 2 mm for preweanling rats. Behavioral testing was done in activity monitoring chambers that consisted of acrylic walls, a plastic floor, and an open top (Coulbourn Instruments, Allentown, PA, USA). To somewhat control for differences in body size, rats tested on PD 17 were placed in smaller sized chambers (26 $\times$ 26 $\times$ 41 cm) than rats tested on PD 85 (41 $\times$ 41 $\times$ 41 cm). Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine repetitive motor movements (a measure of stereotypy). Photobeam resolution for the large chambers was 1.27 cm and for the small chambers was 0.76 cm. The position of each rat was determined every 100 ms.

Drugs

(–)-Cocaine hydrochloride was dissolved in saline, while ($\pm$)-*trans*-U50,488 methanesulfonate salt (U50,488) was dissolved in distilled water. Systemically administered

drugs were injected intraperitoneally (IP) at a volume of 5 ml/kg (PD 17) or 1 ml/kg (PD 85–89); whereas, microinjected drugs were administered at a volume of 0.5 μ l per side (PD 17) or 1 μ l per side (PD 85). All drugs were purchased from Sigma (St. Louis, MO, USA).

Surgical procedures

For the microinjection experiments, surgeries were performed on PD 15 and PD 83 (i.e., two days prior to behavioral testing), whereas surgeries for the microdialysis experiments were conducted on PD 17 and PD 85. At both ages, anesthesia was induced by isoflurane (2.5–5%) mixed with oxygen. A topical lidocaine solution (1%) was applied to the scalp and ibuprofen (2 mg/kg, IP) was administered. A standard Kopf stereotaxic apparatus was used, with preweanling rats requiring a Cunningham Neonatal Rat Adapter for proper positioning. For the microdialysis experiments, a single craniotomy was performed and a dialysis guide cannula equipped with a dummy probe was surgically implanted in the left dorsal CPu of adult (M/L = +3.1, D/V = –3.7, A/P = +0.2 mm from bregma) and preweanling rats (M/L = +2.8, D/V = +5.8, A/P = +5.6 mm from the interaural line). For the microinjection experiments, two craniotomies were performed and either a stainless steel double guide cannula (preweanling rats) or two single guide cannulae (adult rats) were implanted in the dorsal CPu of adult (M/L + 3.1, D/V –4.2, A/P +0.2 mm from bregma) and preweanling rats (M/L +2.8, D/V +5.4, A/P +5.6 mm from the interaural line). The stereotaxic coordinates for adult rats are from the rat brain atlas of Paxinos and Watson (1998), whereas the developing rat brain atlas of Sherwood and Timiras (1970), which uses a different coordinate system, provided the stereotaxic coordinates for preweanling rats. Both sets of guide cannulae (22 gauge) were purchased from Plastics One (Roanoke, VA, USA). Stainless steel stylets (Plastics One) were used to seal guide cannulae until time of testing. For the microdialysis and microinjection experiments, guide cannulae were fixed in place using cyanoacrylate gel followed by dental cement (preweanling rats) or stainless steel anchor screws and dental cement (adult rats). After surgery, rats were allowed to recover away from other rats in temperature-controlled chambers (30°C).

In vivo microdialysis procedures: short surgical recovery

For both preweanling and adult rats, the microdialysis procedure began approximately 90 min after surgery. This short recovery period is typically used with preweanling rats (Kehoe et al., 1998; Kosten et al., 2003) because dams have a tendency to damage the microdialysis probe guides (Keller et al., 1996). To maintain consistency between age groups, adult rats were tested after the same recovery interval (both preweanling and adult rats were able to freely locomote 90 min after surgery).

At the start of testing, a microdialysis probe was inserted into the dialysate guide cannula and rats were placed into cylindrical testing chambers. Artificial cerebrospinal fluid (145 mM NaCl, 2.7 mM KCl, 1.4 CaCl₂, 1.0 MgCl₂, 2.0 Na₂HPO₄, pH = 7.4) was constantly perfused through the probe at a rate of 1.5 μ l/min by a microinfusion pump (model CMA/402, CMA). The equilibration period lasted for 120 min (see also De Souza Silva et al., 2008; Balla et al., 2009; Guyon et al., 2009; Serhsen et al., 2009), during which time all samples were discarded. An automated fraction collector (CMA/170; CMA) was then used to continuously collect basal dialysate samples for 100 min (divided into 20-min periods). Following collection of the last basal sample, preweanling and adult rats were injected with saline or U50,488 (5 mg/kg, IP) and returned to the cylindrical chambers for 20 min. Rats were then injected with saline or cocaine (25 mg/kg, IP) and returned to the cylindrical chambers for an additional 160 min. [These time parameters are similar to those described previously (Maisonneuve et al., 1994; Gray et al., 1999).] Throughout these procedures, dialysate samples were collected continuously in 250 μ l polyethylene vials containing 5 μ l of 0.2 N perchloric acid. Dialysate samples were frozen at –80°C until assay.

Dialysate samples were analyzed for dopamine using HPLC with electrochemical detection. Samples were tested using an autosampler (model 542; ESA, Chelmsford, MA, USA), an MD-150 column (ESA) and a Coulochem III electrochemical detector (triple detector electrode system, ESA). The mobile phase (75 mM NaH₂PO₄·H₂O, 1.4 mM OSA, 10 µM EDTA, 10% acetonitrile; pH 3.1 using H₃PO₄) was delivered via a model 584 HPLC pump (ESA) at a flow rate of 0.5 ml/min. The sensitivity of the assay was 0.5 pg/10 µl sample.

***In vivo* microdialysis procedures: short and long surgical recovery**

Separate groups of adult rats were allowed to recover either 90 min (short recovery) or 5 days (long recovery) prior to microdialysis testing. The microdialysis procedures were nearly identical to those just described. Specifically, there was a 120 min equilibration period followed by 100 min of baseline testing (divided into 20-min periods). Following collection of the last basal sample, adult rats from both the short and long recovery groups were injected with saline or U50,488 (5 mg/kg, IP) and returned to the cylindrical chambers for 20 min. Rats were then injected with saline (cocaine was not administered) and returned to the cylindrical chambers for an additional 160 min. Dialysate samples were collected and analyzed as described above.

Microinjection procedures and behavioral assessment

On PD 17 or PD 85 (48 h after surgery), rats were habituated to the activity chambers for 20 min. Rats were then removed from the activity chambers and the stainless steel stylets were replaced by infusion cannulae (Plastics One) which extended 1 mm below the guide cannulae. Hamilton microsyringes (10 µl) attached to dual infusion pumps (World Precision Instruments, Sarasota, FL, USA) were used to bilaterally microinject distilled water or U50,488 (1.6 or 6.4 µg) into the dorsal CPu over a 60 s period. After drug infusions, rats were returned to the activity chambers for 20 min. Rats were then injected (IP) with saline or 25 mg/kg cocaine and immediately returned to the chambers for 40 min. In summary, a three-phase testing procedure was used: rats were habituated to the apparatus for 20 min, distilled water or U50,488 was microinjected into the CPu and stereotypy was assessed for 20 min, then cocaine or saline were administered systemically and stereotypy was measured for an additional 40 min.

Time (s) engaged in repetitive motor movements (a measure of stereotypy) was assessed continuously across the testing session. Repetitive motor movements were defined as the total number of repetitive coordinate changes on the X-Y axes that occurred within 2 s (three back and forth movements were required before the behavior qualified as a repetitive motor movement). In addition, a subset of rats at both ages ($n=5-7$ per group) were videotaped and behavioral intensity scores (a measure of stereotypy) were later determined by an observer blind to treatment conditions. For this measure, rats were assessed every 5 min (for a 1-min period) using a behavioral intensity scale developed by Creese and Iversen (1973). This scale uses the following rating system: 0 = asleep or inactive, 1 = normal exploratory activity (no repetitive behaviors), 2 = discontinuous activity with prominent (repetitive) sniffing or rearing, 3 = continuous activity with repetitive sniffing and rearing in a fixed path, 4 = continuous activity with repetitive sniffing and rearing in the same location, 5 = continuous activity with repetitive chewing or gnawing in a fixed path, 6 = continuous activity with repetitive chewing or gnawing in the same location. Various stereotyped behaviors (e.g., head shaking and head-down sniffing) were measured by assessing whether a particular behavior was present (occurred over 50% of the time) at 20 s intervals during each 5-min time block.

Histology

After behavioral testing, rats were given an overdose of sodium pentobarbital and the brains were fixated in 4% paraformaldehyde. Brains were cryoprotected in a 20% sucrose solution, sectioned coronally (70 μ m) using a cryostat, and then stained with thionin. Histological assessment of cannula placements was done by observers blind to drug treatment conditions. Data from animals with inappropriate guide cannula placements were not included in the statistical analysis.

Data analysis

In the microdialysis experiments, data were analyzed using separate repeated measures analyses of variance (ANOVA) at each age. In the microinjection experiment, repeated measures (5-min time blocks) ANOVAs were used for statistical analysis of behavioral data. Because of ongoing experimental manipulations, separate ANOVAs were used to analyze time blocks 1–4 (habituation), 5–8 (U50,488 or vehicle), and 9–16 (cocaine or saline). When required, significant higher order interactions were further analyzed using one-way ANOVAs. Tukey tests were then used for making post hoc comparisons ($P < 0.05$).

Litter effects were minimized by assigning precisely one subject from each litter to a particular group (for a discussion of litter effects, see Zorrilla, 1997). In order to provide a sufficient number of subjects, an equal number of male and female preweanling rats were randomly assigned to groups. Unlike adults, prepubescent rats do not typically exhibit sex differences after treatment with a dopamine or κ -opioid agonist (see also Scalzo and Holson, 1992; Bowman et al., 1997; Duke et al., 1997; Snyder et al., 1998; McDougall et al., 2007). Preliminary statistical analyses showed that the behavioral and neurochemical data of our preweanling rats did not vary according to sex, so this variable was excluded from subsequent analyses. Male rats were exclusively used at PD 85.

RESULTS

Effects of U50,488 on cocaine-stimulated dopamine overflow using *in vivo* microdialysis in freely moving adult and preweanling rats: short surgical recovery

On PD 85, basal dopamine values in the dorsal CPu were 4.91 (± 0.35) pg/10 μ l dialysate. Overall, cocaine (25 mg/kg, IP) increased dopamine overflow in the dorsal CPu (Fig. 1) [Cocaine main effect, $F(1, 24) = 22.27$, $P < 0.001$], with this enhancement being statistically significant 20 to 140 min after cocaine treatment [Cocaine $\times$ Time interaction, $F(7, 168) = 12.59$, $P < 0.001$ and Tukey tests]. U50,488 attenuated the cocaine-induced increase in dopamine efflux, because adult rats in the U50,488-Cocaine group had dopamine dialysate levels no different than controls and significantly lower than the Saline-Cocaine group [Kappa $\times$ Cocaine interaction, $F(1, 24) = 5.77$, $P < 0.05$ and Tukey tests].

When measured on PD 17, basal dopamine values were 5.65 (± 0.15) pg/10 μ l dialysate. In general, cocaine affected preweanling and adult rats similarly, because cocaine also enhanced dopamine overflow in the dorsal CPu on PD 17 (Fig. 2) [Cocaine main effect, $F(1, 28) = 24.06$, $P < 0.001$]. At this age, cocaine-induced dopamine overflow was significantly greater than control values from 20 to 120 min post-injection [Cocaine $\times$ Time interaction, $F(7, 196) = 7.57$, $P < 0.001$ and Tukey tests]. Unlike adults, there was no evidence that U50,488 attenuated cocaine-induced dopamine efflux on PD 17 [Kappa $\times$ Cocaine interaction, $F(1, 28) = 0.01$, NS]. More specifically, U50,488 did not differentially affect dopamine overflow in saline- or cocaine-treated preweanling rats.

Effects of U50,488 on dopamine overflow using *in vivo* microdialysis in freely moving adult rats: short and long surgical recovery

Basal dopamine dialysate levels of adult rats were substantially greater after a long (5 day) surgical recovery than after a short (90 min) recovery (left panel, Fig. 3) [Recovery main effect, $F(1, 22)=144.60$, $P<0.001$]. Across the 160-min testing session, the long recovery groups continued to exhibit greater dopamine overflow than the short recovery groups (right panel, Fig. 3) [Recovery main effect, $F(1, 20)=59.64$, $P<0.001$]. In contrast to the previous experiment, U50,488 (5 mg/kg) caused a small, but statistically significant, reduction in dopamine overflow [Kappa main effect, $F(1, 20)=11.03$, $P<0.001$]. The Recovery $\times$ Kappa interaction was nonsignificant as was the three-way interaction involving the time variable.

Effects of bilateral infusions of U50,488 on cocaine-stimulated behaviors in adult and preweanling rats

Repetitive motor movements—Across the first four time blocks (i.e., the habituation phase), adult rats showed a significant decline in repetitive motor movements (a measure of stereotypy) (Fig. 4, left panels) [Time block effect, $F(3, 141)=10.04$, $P<0.001$]. This decline in repetitive motor movements continued across time blocks 5–8 (i.e., after vehicle or U50,488 infusions) [Time block main effect, $F(3, 135)=13.70$, $P<0.001$ and Tukey tests], although no treatment differences due to the κ -opioid agonist were apparent (Fig. 4, middle panels).

Systemically administering cocaine (25 mg/kg, IP) after time block 8 caused adult rats to exhibit a significant increase in repetitive motor movements when compared to saline controls (compare the upper and lower right panels of Fig. 4) [Cocaine main effect, $F(1, 42)=160.35$, $P<0.001$]. Further analysis of the cocaine groups showed that repetitive motor movements of adult rats increased progressively until time block 12 and then stabilized (Fig. 4, upper graph, right panel) [Time block main effect, $F(7, 147)=3.18$, $P<0.01$ and Tukey tests]. Bilateral infusions of U50,488 (1.6 or 6.4 μ g) significantly reduced the amount of time engaged in repetitive motor movements, but this effect was only apparent in cocaine-treated adult rats [Cocaine $\times$ Kappa interaction, $F(2, 42)=4.52$, $P<0.05$ and Tukey tests].

Prewanling rats responded in a manner similar to adults during the initial 8 time blocks (Fig. 5, left and middle panels). Specifically, repetitive motor movements declined during the habituation phase [Time block effect, $F(3, 138)=33.84$, $P<0.001$] and continued to show a slight decline after vehicle or U50,488 infusions [Time block main effect, $F(3, 132)=3.12$, $P<0.05$ and Tukey tests]. U50,488 alone did not significantly affect the repetitive motor movements of preweanling rats. On time blocks 9–16 (i.e., after cocaine or saline administration), preweanling rats given cocaine ($\bar{x} = 654.0$ s, SEM = 21.3) spent significantly greater time engaged in repetitive motor movements than rats given saline ($\bar{x} = 525.1$ s, SEM = 37.5) (Fig. 5, right panels) [Cocaine main effect, $F(1, 41)=8.44$, $P<0.01$]. Unlike what was observed in adult rats, bilateral infusions of U50,488 (1.6 or 6.4 μ g) did not impact the repetitive motor movements of preweanling rats [Cocaine $\times$ Kappa interaction, $F(2, 41)=0.44$, NS]. Although not assessed statistically, cocaine-induced repetitive motor movements appeared to be more pronounced at PD 85 than PD 17 (compare the right panels of Fig. 4 and Fig. 5).

Behavioral intensity—The effects of bilateral U50,488 infusions on the cocaine-induced stereotypy of adult and preweanling rats were also assessed using a behavioral intensity scale (Creese and Iversen, 1973). In adult rats, cocaine significantly enhanced behavioral intensity scores relative to saline controls (Fig. 6) [Cocaine main effect, $F(1, 25)=335.68$, $P<0.001$]. The behavioral intensity scores of saline-treated rats did not vary across the testing session, whereas the scores of cocaine-treated rats increased across the first 15 min

and then stabilized [Cocaine $\times$ Time block interaction, $F(7, 175)=13.10$, $P<0.001$ and Tukey tests]. U50,488 impacted behavioral intensity scores but only among the cocaine groups. Specifically, U50,488 (1.6 or 6.4 μg) significantly reduced the cocaine-induced increase in behavioral intensity scores, while not altering the scores of saline-treated rats [Cocaine $\times$ Kappa interaction, $F(2, 25)=5.06$, $P<0.05$]. Among the cocaine groups (Fig. 6, upper graph), a separate analysis of time blocks 13–16 showed that bilateral infusions of 6.4 μg U50,488 caused a significant reduction in the behavioral intensity scores of cocaine-treated adult rats [Kappa main effect, $F(2, 13)=4.18$, $P<0.05$ and Tukey tests], while the lower dose of U50,488 (1.6 μg) caused only a marginally significant decline ($P=0.10$) [Tukey tests].

Assessment of individual behaviors showed that intense head shaking was the predominant stereotypic behavior exhibited by cocaine-treated adult rats. Overall, head shaking was significantly more pronounced after systemic administration of cocaine than after saline [Cocaine main effect, $F(1, 25)=44.07$, $P<0.001$]. Among only the cocaine groups, U50,488 impacted head shaking on time blocks 13–16. Consistent with the other measures of stereotypy (i.e., repetitive motor movements and behavioral intensity scores), cocaine-treated adult rats given bilateral infusions of U50,488 (1.6 or 6.4 μg per side) exhibited significantly less head shaking than cocaine-vehicle controls (Table 1) [Kappa $\times$ Time block interaction, $F(6, 39)=2.51$, $P<0.05$ and Tukey tests].

In preweanling rats, cocaine significantly increased behavioral intensity scores relative to saline controls (Fig. 7) [Cocaine main effect, $F(1, 29)=233.12$, $P<0.001$]. The behavioral intensity scores of cocaine-treated rats increased in a progressive manner across time blocks 9–16, while the scores of saline-treated rats remained stable [Cocaine $\times$ Time block interaction, $F(7, 203)=2.36$, $P<0.05$]. Unlike what was observed in adult rats, bilateral infusions of U50,488 into the dorsal CPU did not significantly attenuate the behavioral intensity scores of cocaine-treated preweanling rats. Interestingly, saline-treated rats infused with 6.4 μg U50,488 exhibited heightened behavioral intensity scores on time block 16 (Fig 7, lower graph) [Cocaine $\times$ Kappa $\times$ Time block interaction, $F(14, 203)=2.57$, $P<0.01$ and Tukey tests]. The latter effect appeared to be due to a drug-induced increase in the grooming response. Separate statistical analyses, which included age as a factor in the ANOVA, showed that the cocaine-induced behavioral intensity scores of preweanling and adult rats did not differ [Age $\times$ Cocaine interaction, $F(1, 54)=0.36$, NS; Age $\times$ Cocaine $\times$ Kappa interaction, $F(2, 54)=0.61$, NS].

Head shaking was only infrequently observed at PD 17, whereas head-down sniffing was the predominant stereotyped behavior observed at this age. Specifically, the stereotyped sniffing of cocaine-treated preweanling rats was significantly greater than saline controls [Cocaine main effect, $F(1, 30)=185.92$, $P<0.001$], with this drug-induced effect becoming more pronounced as the session progressed [Cocaine $\times$ Time block interaction, $F(7, 210)=4.42$, $P<0.001$]. U50,488 did not modulate the actions of cocaine, because an analysis of stereotyped sniffing on time blocks 9–12 [Cocaine $\times$ Kappa interaction, $F(2, 30)=2.05$, NS] and 13–16 [Cocaine $\times$ Kappa interaction, $F(2, 30)=0.06$, NS] showed that preweanling rats treated with both cocaine and U50,488 (1.6 or 6.4 μg) did not differ from cocaine controls (i.e., the 0 μg U50,488-cocaine group) (Table 2).

DISCUSSION

When considered as a whole, the present results suggest that κ -opioid receptor stimulation differentially affects dopaminergic functioning in the dorsal CPU depending upon age of the animal. In adult rats, cocaine (25 mg/kg, IP) increased dopamine dialysate levels in the dorsal CPU by as much as 245%, which is consistent with typical lower-end literature values (e.g., Chen et al., 1996; Martin-Fardon et al., 1996; Dewey et al., 1997; Kreuter et al., 2004).

Importantly, this cocaine-induced increase in dopamine overflow was completely attenuated by a pretreatment injection of U50,488 (5 mg/kg, IP). Although this exact finding has not been reported before, it is well established that κ -opioid receptor agonists are capable of attenuating cocaine- and amphetamine-induced dopamine efflux in the ventral striatum of adult rats (Spanagel et al., 1990, 1992; Maisonneuve et al., 1994; Gray et al., 1999). In preweanling rats, cocaine increased dopamine dialysate levels in the CPu by as much as 174% (Fig. 1), but this effect was not modified by pretreatment with U50,488 (5 mg/kg, IP). Thus, κ -opioid receptor stimulation does not appear to cause an adult-like reduction in cocaine-induced dopamine overflow when measured on PD 17.

In the first microdialysis experiment κ -opioid receptor stimulation did not reduce basal dopamine dialysate levels in the dorsal CPu of preweanling or adult rats. In the subsequent experiment, adult rats showed a statistically significant 19.3% decline in dopamine overflow after U50,488 treatment (preweanling rats were not tested). The reason for the initial negative results is not known, although it is inconsistent with a number of studies showing that pretreatment with a κ -opioid receptor agonist produces either a modest (Maisonneuve et al., 1994) or substantial (Di Chiara and Imperato, 1988; Donzanti et al., 1992; Spanagel et al., 1992) reduction in accumbal or CPu dopamine overflow. Robust declines in extracellular dopamine are frequently observed when a high dose of U50,488 (10 mg/kg) is administered (e.g., Di Chiara and Imperato, 1988; Donzanti et al., 1992), so it is possible that the use of a lesser dose of U50,488 (5 mg/kg) was responsible for the more modest reduction in dopamine overflow reported here.

When assessed after a short recovery period, basal dopamine dialysate levels of preweanling and adult rats were 5.65 and 4.91 pg/10 μ l, respectively. The basal dialysate values of our PD 17 rats are very similar to literature values (Keller et al., 1994), however the basal values of our adult rats were lower than is typically reported (e.g., Keller et al., 1994; McDougall et al., 2008; Balla et al., 2009; Lindgren et al., 2010). To determine whether this effect was due to the short (90 min) recovery period, we did a separate experiment assessing how recovery duration affects basal dopamine overflow in the dorsal CPu. Results showed that basal dopamine dialysate levels were almost twice as great (9.38 vs. 4.80 pg/10 μ l) when *in vivo* microdialysis was conducted 5 days, instead of 90 min, after surgery. Although speculative, it is possible that tissue damage due to guide cannula placement caused a nearly immediate elevation in extracellular dopamine levels. This excess dopamine, in turn, may have triggered a compensatory reduction in dopamine dialysate levels via autoreceptor-mediated mechanisms. Alternatively, isoflurane has been reported to both potentiate dopamine release and inhibit dopamine synthesis (Adachi et al., 2005), so it is possible that anesthesia caused a short-term increase in extracellular dopamine that was followed by a decline lasting for at least a few hours (i.e., during microdialysis testing). Consistent with both of these explanations, the short recovery groups exhibited unusually high dihydroxyphenylacetic acid levels (data not shown), thus suggesting that large amounts of dopamine had been recently metabolized. Regardless of the explanation, the present results show that basal dopamine dialysate levels of adult rats were at literature values 5 days after surgery, but dopamine values were substantially lower when testing occurred 90 min after surgery.

Consistent with the microdialysis data, results from the microinjection experiment suggest that κ -opioid receptor stimulation differentially affects dopaminergic functioning depending upon age of the animal. In adult rats, bilateral infusions of U50,488 into the dorsal CPu decreased cocaine-induced stereotypies. This novel finding was apparent regardless of whether stereotypies were quantified as repetitive motor movements, individual stereotyped behaviors (i.e., head shaking), or through the use of a behavioral intensity scale. In preweanling rats, on the other hand, bilateral infusions of U50,488 into the dorsal CPu did not diminish cocaine-induced stereotypies. For example, U50,488 (1.6 and 6.4 μ g per side)

did not affect cocaine-induced repetitive motor movements or stereotyped sniffing on PD 17. In partial contrast to this finding, we have reported that systemic administration of U50,488 caused a dose-dependent reduction in the cocaine-, amphetamine-, and methylphenidate-induced stereotyped sniffing of preweanling rats (McDougall et al., 1999). Based on the present data, it appears that this previously reported U50,488 effect is either (a) not mediated by κ -opioid receptors in the dorsal CPu or (b) U50,488's locomotor activating effects may have competed with the ability to express stereotyped behaviors (e.g., Bordi et al., 1989).

In general, cocaine appeared to produce less robust neurochemical and behavioral effects in preweanling rats than adults. Although cross-age statistical comparisons were not conducted, cocaine did increase dopamine dialysate levels by 245% on PD 85 (when compared to basal levels), while only increasing dopamine concentrations by 174% on PD 17. In a similar vein, cocaine appeared to induce fewer repetitive motor movements in preweanling rats than adults, although statistical analysis of a more qualitative measure of stereotypy (i.e., behavioral intensity scores) suggested that the two age groups did not differ. Consistent with the latter results, Abrams and Bruno (1992) did not find "any obvious qualitative differences" in the ability of apomorphine to induce stereotypy at PD 21 and in adulthood. Even so, we have occasionally observed that preweanling rats seem to exhibit less intense drug-induced stereotypy than adults (e.g., Charntikov et al., 2008). Some of the difficulties associated with making this assessment are that the behavioral repertoire of the rat, as well as general motoric ability and body size, change across ontogeny (Moody and Spear, 1992). Differences in body size are relevant here, because repetitive motor movements were assessed using photobeam chambers. Even though photobeam resolution was adjusted for the different sized animals (see **Apparatus** section), it is uncertain whether the chambers were truly equated (i.e., age-dependent differences in repetitive motor movements may have been an artifact of the testing apparatus). Nonetheless, it is important to remember that cocaine significantly enhanced the repetitive motor movements, behavioral intensity scores, and discrete stereotypic behaviors of both preweanling and adult rats, but U50,488 only attenuated the cocaine-induced stereotyped behaviors of adults.

When the results of the present experiments are considered together, it appears that κ -opioid receptors in the CPu do not modulate dopaminergic functioning in a similar fashion during the preweanling period and in adulthood. The inability of U50,488 to block cocaine-induced dopamine overflow was not due to the late development of U50,488 binding sites, because κ -opioid receptor densities and mRNA levels are at approximately adult-like levels by PD 17 (Kornblum et al., 1987; Leslie and Loughlin, 1993; Georges et al., 1998). In adulthood, κ -opioid receptor stimulation activates voltage-dependent K^+ channels and inhibits both adenylyl cyclase activity and voltage-dependent Ca^{2+} channels via inhibitory G_i and G_o proteins (for reviews, see Childers, 1991; Minami and Satoh, 1995; Connor and Christie, 1999). No ontogenetic studies have established the age at which κ -opioid receptors are functionally coupled to these effector systems, so it is unclear whether the inability of U50,488 to modulate cocaine-induced dopamine overflow and stereotypy is due to an immaturity of κ -opioid receptor coupling. Importantly, an *in vitro* study showed that U50,488 is able to partially inhibit electrically evoked [3H]-dopamine release from striatal slices by late gestation (embryonic day 17) through the preweanling period (De Vries et al., 1990). Thus, it appears that the neural mechanisms underlying CPu κ -opioid/dopamine interactions emerge during early ontogeny, continue to develop across the preweanling period, and do not become fully mature until after PD 17. Whether κ -opioid receptors in the nucleus accumbens show a similar developmental pattern is uncertain, because Bolanos et al. (1996) reported that systemic administration of U50,488 blocked morphine-induced place preference conditioning on PD 10 and PD 17. This finding suggests that κ -opioid receptors may be capable of modulating accumbal dopamine release by the second postnatal week.

Although it is well established that κ -opioid receptors in the CPu and nucleus accumbens influence the unlearned behaviors of adult rats, κ -opioid receptors in other brain regions also impact motoric functioning. For example, microinjecting U50,488 or dynorphin (the endogenous ligand for κ -opioid receptors) into the substantia nigra pars reticulata (SNR) induces contralateral rotation in adult rats (in a unilateral preparation), while stimulating forward locomotion in preweanling rats (Herrera-Marschitz et al., 1984, 1986; Morelli and Di Chiara, 1985; Matsumoto et al., 1988; Thompson and Walker, 1992; Collins et al., 2000). For this reason it is surprising that systemic administration of a κ -opioid receptor agonist has nearly opposite effects on the motoric responsiveness of preweanling and adult rats. Specifically, systemically injected U50,488 increases motor activity in preweanling rats (Jackson and Kitchen, 1989; McLaughlin et al., 1995; Duke et al., 1997; McDougall et al., 1997), but reduces basal and psychostimulant-induced locomotor activity and stereotyped behaviors in adult rats (Heidbreder et al., 1993; Duke et al., 1997; Gray et al. 1999; Vanderschuren et al., 2000; Tzaferis and McGinty, 2001; Smith et al., 2003; Puig-Ramos et al., 2008). A likely explanation for this age-dependent difference is that the “balance” between κ -opioid systems (i.e., striatal and SNR) differs according to age. In adult rats, it is possible that the behavioral consequences of inhibiting the CPu and/or nucleus accumbens masks or overwhelms the motor activating effects of SNR stimulation. The opposite relationship may exist in preweanling rats, with the behavioral consequences of SNR activation overwhelming the motor inhibiting effects of κ -opioid receptor stimulation in the CPu and/or nucleus accumbens. The results of the present study provide support for this hypothesis, because κ -opioid receptors in the CPu did not modulate cocaine-induced dopamine efflux or behavior in a similar fashion during the preweanling period and in adulthood. Specifically, systemic administration of U50,488 (5 mg/kg) did not attenuate cocaine-induced dopamine overflow in preweanling rats, nor did bilateral infusions of U50,488 (1.6 or 6.4 μ g) into the CPu alter cocaine-induced stereotypies on PD 17. Thus, systemically administered U50,488 may increase the motoric activity of preweanling rats, because κ -opioid/dopamine interactions in the CPu (and potentially the nucleus accumbens) are not fully mature.

In summary, when examined during adulthood κ -opioid receptor stimulation had predictable effects on cocaine-induced dopamine overflow and stereotyped behaviors. Specifically, systemic administration of U50,488 fully attenuated cocaine’s ability to enhance dopamine overflow in the CPu of adult rats, while bilateral administration of U50,488 into the CPu of adult rats reduced cocaine-induced stereotypies. Quantitative ontogenetic differences were apparent in this study, because cocaine stimulated less dopamine overflow and less stereotypy in preweanling rats than adults. Of even more interest, systemically administered U50,488 did not alter cocaine-induced dopamine overflow in the CPu of preweanling rats, nor did bilateral infusions of U50,488 reduce dopamine-mediated stereotyped behaviors. These qualitative ontogenetic differences suggest that the κ -opioid receptor system does not function in an adult-like manner during the late preweanling period. A possible explanation is that the coupling between κ -opioid receptors and their second messengers are not fully mature by PD 17.

Abbreviations

ANOVA	Analysis of variance
CPu	caudate-putamen
IP	intraperitoneal
NS	nonsignificant

PD	postnatal day
SNR	substantia nigra pars reticulata

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Fig. 1.

Mean ($\pm$ SEM) dopamine dialysate levels in the dorsal CPu following administration of saline or cocaine (25 mg/kg, IP) on PD 85 ($n = 7$ per group). Adult rats were injected with saline or U50,488 (5 mg/kg, IP) 20 min prior to cocaine treatment. Dopamine overflow was measured using *in vivo* microdialysis in freely moving rats. Data are expressed as percent of baseline dopamine levels. †Significant difference between the cocaine- and saline-treated rats at the same time point (Cocaine $\times$ Time interaction, $P < 0.001$). *Significant difference between the Saline-Cocaine group and all other groups when collapsed across the 8 time points (Cocaine $\times$ Kappa interaction, $P < 0.05$).

Fig. 2.

Mean ($\pm$ SEM) dopamine dialysate levels in the dorsal CPu following administration of saline or cocaine (25 mg/kg, IP) on PD 17 ($n = 8$ per group). Prewanling rats were treated the same as described in Fig. 1. †Significant difference between the cocaine- and saline-treated rats at the same time point (Cocaine $\times$ Time interaction, $P < 0.001$).

Fig. 3.

Mean ($\pm$ SEM) dopamine dialysate levels in the dorsal CPu following administration of saline or U50,488 (5 mg/kg, IP) on PD 85 (i.e., after a 90-min recovery period) or on PD 89 (i.e., after a 5-day recovery period). Dopamine overflow was measured using *in vivo* microdialysis in freely moving adult rats ($n = 6$ per group). †Significant difference between the saline- and U-50,488-treated rats when collapsed across the 8 time points (Kappa main effect, $P < 0.01$). *Significant difference between the short and long recovery groups when collapsed across the 4 or 8 time points (Recovery main effects, $P < 0.001$).

Fig. 4.

Mean time (s) engaged in repetitive motor movements ($\pm$ SEM) during the 80-min behavioral testing session on PD 85 ($n = 8$ per group). Adult rats were habituated to the apparatus on time blocks 1–4. At the conclusion of time block 4 (indicated by the first dashed line), rats received bilateral infusions of vehicle or U50,488 (1.6 or 6.4 μ g) into the dorsal CPu. At the conclusion of time block 8 (indicated by the second dashed line), rats were injected with cocaine (25 mg/kg, IP). *Significantly different from the 0 μ g U50,488-cocaine group when collapsed across time blocks 9–16 (Cocaine $\times$ Kappa interaction, $P < 0.05$).

Fig. 5.

Mean (s) time engaged in repetitive motor movements ($\pm$ SEM) during the 80-min behavioral testing session on PD 17 ($n = 7-8$ per group). Preweanling rats were treated the same as described in Fig. 4. *Significantly different from the saline condition (lower graph) when collapsed across time blocks 9–16 (Cocaine main effect, $P < 0.01$).

Fig. 6. Mean behavioral intensity scores ($\pm$ SEM) of adult rats during the final 40 min of the behavioral testing session (i.e., only the eight time blocks occurring after cocaine administration are shown). Subjects were a subset of the adult rats ($n = 5-6$ per group) described in Fig. 4. *Significantly different from the 0 μ g U50,488-cocaine group when collapsed across time blocks 9–16 (Cocaine $\times$ Kappa interaction, $P < 0.05$).

Fig. 7.

Mean behavioral intensity scores ($\pm$ SEM) of preweanling rats during the final 40 min of the behavioral testing session (i.e., only the eight time blocks occurring after cocaine administration are shown). Subjects were a subset of the preweanling rats ($n = 5-7$ per group) described in Fig. 5. †Significantly different from the 0 μ g U50,488-saline group at the same time point (Cocaine $\times$ Kappa $\times$ Time block interaction, $P < 0.01$).

Table 1

Mean number of head shakes exhibited by cocaine- and saline-treated adult rats on time blocks 9–12 and time blocks 13–16

Drug Treatment	Time Blocks	
	9–12	13–16
0 µg U50,488-Saline	0.00 (±0.00)	0.05 (±0.05)
1.6 µg U50,488-Saline	0.00 (±0.00)	0.00 (±0.00)
6.4 µg U50,488-Saline	0.00 (±0.00)	0.00 (±0.00)
0 µg U50,488-Cocaine	5.29 (±1.45) [†]	7.54 (±2.14) [†]
1.6 µg U50,488-Cocaine	3.05 (±0.95) [†]	4.05 (±1.31) [†] *
6.4 µg U50,488-Cocaine	5.00 (±1.62) [†]	4.15 (±0.73) [†] *

Values represent mean (±SEM) number of head shakes per 5-min time block. The presence or absence of head shaking was assessed every 20 s using a time sampling procedure.

[†] Significantly different from saline-pretreated rats.

* Significantly different from the 0 µg U50,488-Cocaine group.

Table 2

Mean number of head-down sniffs exhibited by cocaine-and saline-treated preweanling rats on time blocks 9–12 and time blocks 13–16

Drug Treatment	Time Blocks	
	9–12	13–16
0 µg U50,488-Saline	0.78 (±0.28)	0.95 (±0.22)
1.6 µg U50,488-Saline	0.75 (±0.37)	0.07 (±0.05)
6.4 µg U50,488-Saline	0.42 (±0.26)	1.12 (±0.28)
0 µg U50,488-Cocaine	6.33 (±1.46) [†]	8.29 (±1.15) [†]
1.6 µg U50,488-Cocaine	8.58 (±0.54) [†]	8.71 (±1.29) [†]
6.4 µg U50,488-Cocaine	8.92 (±0.97) [†]	8.79 (±1.20) [†]

Values represent mean (±SEM) number of head shakes per 5-min time block. The presence or absence of head shaking was assessed every 20 s using a time sampling procedure.

[†]Significantly different from saline-pretreated rats.