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Ontogeny of Striatal Dopamine Release in Rats After Acute Administration of Methamphetamine

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TSUCHIDA, K., K. AKIYAMA, K. SAKAI, H. UJIKE, X. LI AND S. KURODA. *Ontogeny of striatal dopamine release in rats after acute administration of methamphetamine.* PHARMACOL BIOCHEM BEHAV **53**(3) 575–580, 1996. – In the present study, we examined the effects of acute MAP administration on striatal extracellular levels of dopamine (DA) and its metabolites in groups of rats on postnatal days (PNDs) 14, 21, 28, and 56. A single injection of 4 mg/kg MAP (IP) induced increase in extracellular DA and decrease in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatal perfusates of rats on all PNDs examined. The magnitude of increase in DA concentrations at 20 min after the MAP injection was significantly smaller on PND 14 than PNDs 21, 28, and 56, whereas the magnitude of decrease in DOPAC concentrations after the MAP injection was significantly smaller on PND 14 than PNDs 21, 28, and 56. After the MAP injection, homovanillic acid levels decreased on PNDs 21, 28, and 56, but increased on PND 14. These results suggest that rats on PND 14 differ from those thereafter in MAP-induced DA release and changes in its metabolites, and that such developmental effect on MAP-induced DA release may be involved in the ontogeny of MAP-induced behavioral sensitization.

Dopamine DOPAC Ontogeny Methamphetamine Microdialysis Striatum

SEVERAL lines of study have revealed that psychostimulant-induced behavioral sensitization (1) occurs in rats only when repeated administration is initiated later than 3 weeks after birth (8,12,21). We previously investigated the effect of different postnatal periods for repeated methamphetamine (MAP) administration on formation of behavioral sensitization and MAP challenge-induced dopamine (DA) release, and found that a challenge dose of MAP (4 mg/kg) given 21 days after the last pretreatment MAP dose induced significantly enhanced stereotypy and significantly greater increase in striatal extracellular DA levels in MAP-pretreated than control rats only when repeated MAP pretreatment was initiated after postnatal day (PND) 21 or later (20).

Nigrostriatal dopaminergic neurons in the early PNDs exhibit in vivo electrophysiological characteristics that differ from those seen in adults (15,17–19). They respond to systemic administration of amphetamine (AMP) with anomalous electrophysiological prophiles that are not observed in adults (19). On the other hand, a study on ontogeny of AMP-induced striatal DA release using in vivo voltammetry indicated that a small dose of AMP increased striatal DA release in rats on PNDs 35–36 and in adult rats, but caused only a transient increase followed by decrease of DA in rats on PNDs 21–22 (10). These studies suggest that the manner by which dopaminergic neurons respond to AMP may be affected by postnatal development.

Because MAP-induced release of DA and changes in its metabolites depend upon maturity of dopaminergic neurons that allow ongoing synthesis of DA and the functional DA transporter, it is presumed that they may exhibit differential patterns over postnatal development, and that such postnatal effect on MAP-induced DA release and changes in its metabolites may be involved in the ontogeny of behavioral sensitization (8,12,20,21). However, only a few studies on DA release during the early PNDs have been carried out (2,9,10), and there are, to our knowledge, no reports on the ontogeny of

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psychostimulant-induced changes in extracellular DA and its metabolites determined using in vivo microdialysis. In the present study, we compared acute MAP administration-induced changes in DA and its metabolites in striatal perfusates of rats on different PNDs [14, 21, 28, and 56].

METHOD

Animals

Pregnant Sprague-Dawley rats were purchased from the Charles River Co. (Japan). Male pups were selected at random, divided into four groups and housed with their mothers (one family per cage) for 3 weeks in an environment of constant temperature (24°C), humidity, and lighting (12 L : 12 D cycle).

The experimental rats were treated with 4 mg/kg MAP hydrochloride (Dainippon Co., Japan) on PND 14 (group 1), 21 (group 2), 28 (group 3), and 56 (group 4). The pups in the groups 3 and 4 had been weaned on PND 21 and subsequently housed four to a cage.

In Vivo Microdialysis

A small-diameter concentric-style dialysis probe was constructed, as described by Robinson et al. (16). The dialysis membrane was made of ethylene vinyl alcohol plasmapheresis tubing with a diameter of 200 μm, length of 3.0 mm, and cutoff of 75,000 molecular weight (Evaflex A2; Kurare Co., Japan). In vitro experiments showed that recovery of DA from the external medium into the dialysis probe, at a flow rate of 2.0 μl/min, was approximately 5%. On the day before MAP administration, each rat was placed on a stereotaxic frame under pentobarbital sodium [50 mg/kg, intraperitoneally (IP)] anesthesia and a dialysis probe was implanted into the left anterior dorsal striatum according to the following respective coordinates (rostral from the bregma, lateral from the bregma, ventral from the dural surface, expressed in mm): rats on group 1 (PND 14): 2.0, 2.0, 5.0; group 2 (PND 21): 2.2, 2.2, 5.2; group 3 (PND 28): 2.3, 2.5, 5.5; group 4 (PND 56): 2.4, 3.0, 6.5. We confirmed histologically that the locations of the probe tips implanted into the striata did not differ among the groups. After surgery, preweanling rats in the groups 1 and 2 were not returned to the dam, and an individual rat was put into an experimental cage at constant temperature (24°C), humidity, and under lighting (12 L : 12 D cycle) overnight. During this overnight recovery, rats in all the groups were allowed to move freely, but without access to food or water. On the next day, rats, including the preweanling ones (groups 1 and 2), showed normal locomotor activity and subjected to in vivo microdialysis, during which the animals were conscious and moved freely with the aid of liquid swivel. The dialysis probe was perfused continuously at a flow rate of 2 μm/min with artificial cerebrospinal fluid (aCSF; Na, 140 mM; K, 3.35 mM; Ca, 1.26 mM; Mg, 1.15 mM; Cl, 151 mM, pH 6.5), and perfusates were collected every 20 min. After 60 min, when the initial three perfusate fractions used to determine the baseline levels had been collected, all the rats were injected (IP) with 4 mg/kg MAP and sample collection was continued for 3 h thereafter. The perfusates were injected directly into a reversed-phase high-performance liquid chromatography column connected to a coulometric electrochemical detection system (ESA Co., USA; guard electrode = +0.4 V, oxidation electrode = +0.05 V, reduction electrode = −0.35 V) to measure the DA, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations. DA, DOPAC, and HVA were separated at 27°C by ion-pair reversed-phase chromatography using Cosmosil ODS-C18 5-μm resin (Nacalai Co., Japan), with a mobile phase that comprised 0.05 M sodium dihydrogen phosphate buffer (pH 4.3) containing EDTA (0.05 mM), octanesulfonic acid (1 mM), 5% methanol, and 5% acetone. The mean concentrations of DA and its metabolites in the three fractions collected before MAP administration were regarded as baseline levels, and the levels of DA and its metabolites shown in the figures were expressed as percentages against respective baseline values.

Statistical Analysis

Differences in the absolute values of baseline levels of DA and its metabolites among the four groups were analyzed by Kruskal–Wallis test, which was followed by Mann–Whitney's *U*-test to compare differences between two groups in an arbitrary pair. Comparison of the extracellular levels of DA and its metabolites at different postinjection time points after MAP administration in each group was conducted using one-way ANOVA. Repeated measures of two-way ANOVA was used to evaluate whether changes in the extracellular levels of DA and its metabolites after MAP administration differ between two groups in an arbitrary pair. When a significant group vs. time interaction was found, repeated measures of two-way ANOVA was not considered to be eligible for further analysis, irrespective of whether between-group difference was significant or not. Subsequent analysis to determine whether significant differences occurred between two groups at each postinjection time point was conducted using one-way ANOVA and Fisher's protected least significant difference (PLSD) test as a posthoc test. *p*-Values less than 0.05 were considered as statistically significant.

RESULTS

Baseline Striatal Extracellular DA, DOPAC, and HVA Levels

The data are shown in Table 1. The absolute values of baseline levels of extracellular DA levels did not differ significantly among the four groups (Kruskal–Wallis test). There were significant differences in the absolute values of baseline extracellular DOPAC ($p = 0.0232$) and HVA ($p = 0.0065$) levels among the four groups (Kruskal–Wallis test). Mann–Whitney's *U*-test revealed significant differences between two groups: in DOPAC levels between group 1 and group 2 ($p = 0.0195$) and between group 1 and group 4 ($p = 0.0275$); in HVA levels between group 1 and group 2 ($p = 0.0109$), between group 1 and group 4 ($p = 0.0143$), between group 2 and group 4 ($p = 0.0446$), and between group 3 and group 4 ($p = 0.0283$).

Striatal DA Levels Following MAP Injection

The data are shown in Fig. 1. Acute MAP administration enhanced the extracellular DA levels in all the groups. Thus, there was a significant effect of postinjection time on the extracellular DA levels within individual groups, $F(9, 30) = 7.042$, $p < 0.0001$, for group 1; $F(9, 50) = 5.640$, $p < 0.0001$, for group 2; $F(9, 40) = 11.785$, $p < 0.0001$, for group 3; $F(9, 40) = 5.662$, $p < 0.0001$, for group 4 (one-way ANOVA). Repeated measures of two-way ANOVA conducted throughout all postinjection time points led to no significant difference in the extracellular DA levels between any two groups, but significant group vs. time interaction between group 1 and

TABLE 1

BASAL EXTRACELLULAR CONCENTRATIONS IN STRIATAL PERFUSATES

Group	N	DA	DOAPC	HVA
PND 14	4	2.73 ± 0.46	1700 ± 230	420 ± 136
PND 21	6	3.07 ± 0.42	3480 ± 346	1120 ± 95
PND 28	5	2.44 ± 0.34	4680 ± 1700	886 ± 143
PND 56	5	5.15 ± 1.18	9580 ± 3320	2680 ± 797

The absolute values (the mean ± SEM) of basal extracellular concentrations of DA, DOPAC, and HVA are expressed in fmol/μl. There was no significant difference in baseline extracellular DA levels among the four groups. There were significant differences in baseline extracellular DOPAC ($p = 0.0232$) and HVA ($p = 0.0065$) levels among the four groups (Kruskal-Wallis test). Significant differences between two groups were obtained by using Mann-Whitney's U-test: in DOPAC levels between PND 14 and PND 21 (*$p = 0.0195$) and between PND 14 and PND 56 (#$p = 0.0275$); in HVA levels between PND 14 and PND 21 (§$p = 0.0109$), between PND 14 and PND 56 (¶$p = 0.0143$), between PND 21 and PND 56 (‡$p = 0.0446$) and between PND 28 and PND 56 (†$p = 0.0283$).

group 2, $F(9, 72) = 3.669$, $p = 0.0007$, between group 1 and group 3, $F(9, 63) = 7.201$, $p < 0.0001$, between group 1 and group 4, $F(9, 63) = 4.417$, $p = 0.0002$, and between group 2 and group 4, $F(9, 81) = 3.082$, $p = 0.0031$, indicating ineligibility of using repeated measures of two-way ANOVA for analysis of between-group difference. One-way ANOVA and Fisher's PLSD test revealed significant differences in striatal DA levels between two groups at individual postinjection time points, as shown in the legend to Fig. 1.

Striatal DOPAC Levels Following MAP Injection

The data are shown in Fig. 1. Acute MAP administration significantly reduced the extracellular DOPAC levels in all the groups. Thus, there was a significant effect of postinjection time on the extracellular DOPAC levels within individual groups: $F(9, 30) = 37.186$, $p < 0.0001$, for group 1; $F(9, 50) = 75.574$, $p < 0.0001$, for group 2; $F(9, 40) = 23.235$, $p < 0.0001$, for group 3; $F(9, 40) = 17.228$, $p < 0.0001$, for group 4 (one-way ANOVA). Repeated measures of two-way ANOVA conducted throughout all postinjection time points revealed that there were significant differences in the extracellular DOPAC levels: $F(1, 8) = 35.36$, $p = 0.0003$, between group 1 and group 2; $F(1, 7) = 5.999$, $p = 0.0442$, between group 1 and group 3; $F(1, 7) = 15.687$, $p = 0.0055$, between group 1 and group 4. However, there were significant groups vs. time interactions between group 1 and group 2, $F(9, 72) = 11.183$, $p < 0.0001$, between group 1 and group 3, $F(9, 63) = 18.12$, $p < 0.0001$, between group 1 and group 4, $F(9, 63) = 5.647$, $p < 0.0001$, between group 2 and group 3, $F(9, 81) = 4.53$, $p < 0.0001$, between group 3 and group 4, $F(9, 72) = 2.747$, $p = 0.0081$, indicating ineligibility of using repeated measures of two-way ANOVA for analysis of between-group difference. One-way ANOVA and Fisher's PLSD test revealed significant differences in striatal DOPAC levels between two groups at individual postinjection time points, as shown in the legend to Fig. 1.

Striatal HVA Levels Following MAP Injection

The data are shown in Fig. 1. There was a significant effect of postinjection time on the extracellular HVA levels within individual groups: $F(9, 30) = 6.831$, $p < 0.0001$, for group 1; $F(9, 50) = 6.644$, $p < 0.0001$, for group 2; $F(9, 40) = 15.005$, $p < 0.0001$, for group 3; $F(9, 40) = 7.630$, $p < 0.0001$, for group 4 (one-way ANOVA). Thus, acute MAP administration reduced significantly the extracellular HVA levels in groups 2, 3, and 4, but significantly increased them in group 1. Repeated measures of two-way ANOVA conducted throughout all postinjection time points revealed that there were significant differences in the extracellular HVA levels: $F(1, 8) = 65.556$, $p < 0.0001$, between group 1 and group 2; $F(1, 7) = 91.671$, $p < 0.0001$, between group 1 and group 3; $F(1, 7) = 83.338$, $p < 0.0001$, between group 1 and group 4. However, there were significant groups vs. time interactions between group 1 and group 2, $F(9, 72) = 17.517$, $p < 0.0001$, between group 1 and group 3, $F(9, 63) = 24.056$, $p < 0.0001$, between group 1 and group 4, $F(9, 63) = 18.192$, $p < 0.0001$, between group 2 and group 3, $F(9, 81) = 5.404$, $p < 0.0001$, between group 3 and group 4, $F(9, 72) = 3.042$, $p = 0.0039$, indicating ineligibility of using repeated measures of two-way ANOVA for analysis of between-group difference. One-way ANOVA and Fisher's PLSD test revealed significant differences in striatal HVA levels between two groups at individual postinjection time points as shown in the legend to Fig. 1.

DISCUSSION

In the present study, we investigated acute MAP-induced striatal DA release and changes in its metabolites using in vivo microdialysis in rats on PNDs 14, 21, 28, and 56. The results shown in Table 1 are in agreement with those reported previously (2,9), wherein the absolute values of baseline DOPAC, which results from the metabolism of newly synthesized DA in the cytoplasmic pool by monoamine oxidase and thereby reflects ongoing DA synthesis (25), increased along with postnatal development, whereas the DA level per se was relatively stable from PND 10 until adulthood. The data on baseline DOPAC levels in the striatal perfusates in the previous (2,9) and present studies are consistent with ontogeny of tyrosine hydroxylase activity in the nigrostriatal neurons (6,13,14).

The major finding of the present study is that acute MAP administration altered striatal DOPAC and HVA levels differentially in rats on PND 14 and those on PNDs 21, 28, and 56 (Fig. 1). Thus, the magnitude of acute MAP-induced DOPAC level decrement was significantly attenuated in rats on PND 14 than those on PNDs 21, 28, and 56. Acute MAP administration significantly reduced HVA levels on PNDs 21, 28, and 56, but, conversely, significantly increased them on PND 14.

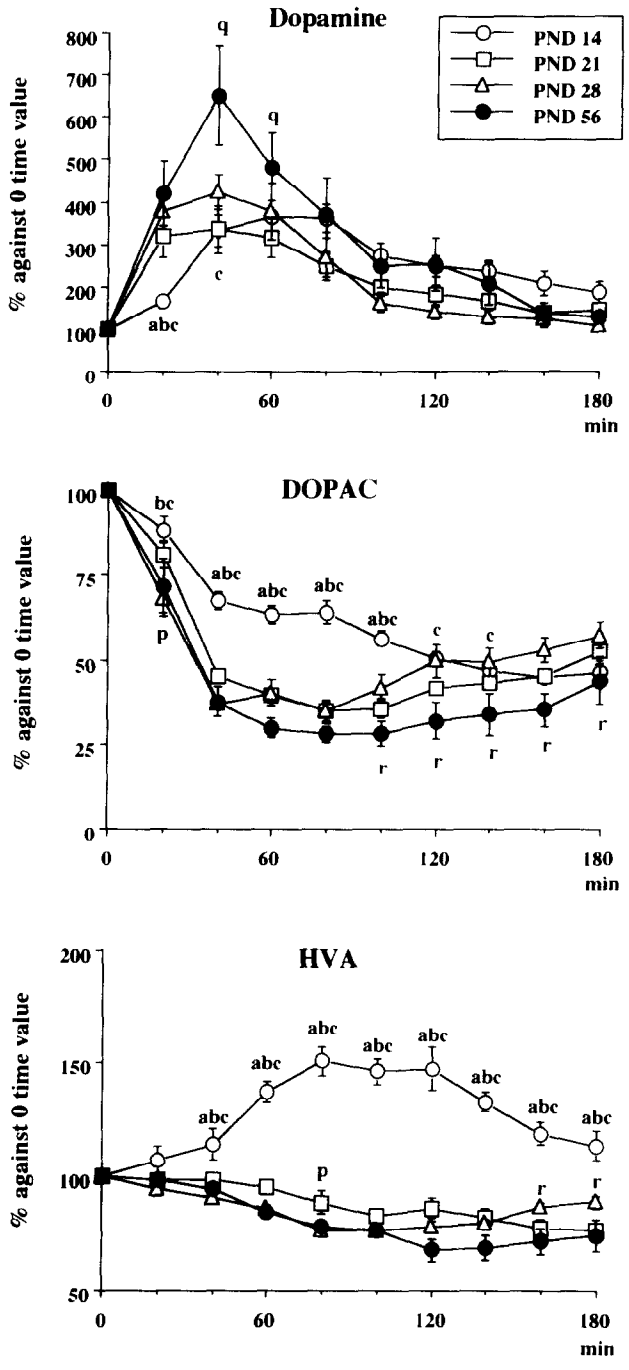

FIG. 1. Effect of methamphetamine (4 mg/kg) on DA, DOPAC, and HVA levels in the striatal perfusates of rats on PND 14 (○, n = 4), 21 (□, n = 6), 28 (△, n = 5), and 56 (●, n = 5). The data are expressed as percentage (mean ± SEM) of the zero time value. There was significant effect of time ($p < 0.0001$) on the extracellular DA, DOPAC, and HVA levels within individual groups (one-way ANOVA). Repeated measures of two-way ANOVA conducted throughout all postinjection time points revealed significant group vs. time interaction: in DA level, between PND 14 and PND 21, $F(9, 72) = 3.669$, $p = 0.0007$, between PND 14 and PND 28, $F(9, 63) = 7.201$, $p < 0.0001$, between PND 14 and PND 56, $F(9, 63) = 4.417$, $p = 0.0002$, and between PND 21 and PND 56, $F(9, 81) = 3.082$, $p = 0.0031$; in DOPAC level, between PND 14 and PND 21, $F(9, 72) = 11.183$, $p < 0.0001$, between PND 14 and PND 28, $F(9, 63) = 18.12$, $p < 0.0001$, between PND 14 and PND 56, $F(9, 63) = 5.647$, $p < 0.0001$, between PND 21 and PND 28, $F(9, 81) = 4.530$, $p < 0.0001$, between PND 28 and PND 56, $F(9, 72) = 2.747$, $p = 0.0081$; in HVA level, between PND 14 and PND 21, $F(9, 72) = 17.517$, $p < 0.0001$, between PND 14 and PND 28, $F(9, 63) = 24.056$, $p < 0.0001$, between PND 14 and PND 56, $F(9, 63) = 18.192$, $p < 0.0001$, between PND 21 and PND 28, $F(9, 81) = 5.404$, $p < 0.0001$, between PND 28 and PND 56, $F(9, 72) = 3.042$, $p = 0.0039$. Significant between-group differences (see the Results section) in the extracellular DOPAC and HVA levels found by two-way ANOVA were always accompanied by significant group vs. time interaction, indicating ineligibility of using repeated measures of two-way ANOVA for analysis of between-group difference. Accordingly, one-way ANOVA and Fisher's PLSD test were conducted at individual postinjection time points. Dopamine: $^{a}p < 0.01$, PND 14 < PND 21; $^{b}p < 0.01$, PND 14 < PND 28; $^{c}p < 0.01$, PND 14 < PND 56; $^{q}p < 0.01$ (40 min), $p = 0.017$ (60 min), PND 21 < PND 56. DOPAC: $^{a}p < 0.001$, PND 14 > PND 21; $^{b}p < 0.001$ (40–80 min), $p < 0.01$ (20 min), $p = 0.021$ (100 min), PND 14 > PND 28; $^{c}p < 0.001$ (40–100 min), $p < 0.01$ (20, 120 min), $p = 0.039$ (140 min), PND 14 > PND 56; $^{p}p < 0.01$, PND 21 > PND 28; $^{r}p = 0.003$ (120, 160 min), $p = 0.01$ (140 min), $p < 0.03$ (100, 180 min), PND 28 > PND 56. HVA: $^{a}p < 0.0001$ (60–180 min), $p = 0.014$ (40 min), PND 14 > PND 21; $^{b}p < 0.0001$ (60–160 min), $p < 0.001$ (40, 180 min), PND 14 > PND 28; $^{c}p < 0.0001$ (60–180 min), $p < 0.001$ (40 min), PND 14 > PND 56; $^{p}p = 0.049$, PND 21 > PND 28; $^{r}p < 0.03$, PND 28 > PND 56; (Fisher's PLSD test).

On the other hand, difference in acute MAP-induced DA release between rats on PND 14 and those on PNDs thereafter was observed only at 20 min after the injection. Using in vivo voltammetry, Gazzara et al. (10) reported that a small dose (1 mg/kg) of AMP consistently increased striatal DA release on PNDs 35–36 and in adults, but caused a transient increase that was followed immediately by decrease in rats on PNDs 21–22. Although repeated measures of two-way ANOVA was not be eligible for comparison between group 2 (PND 21) and group 4 (PND 56) due to significant group vs. time interaction, striatal extracellular DA levels were significantly lower in group 2 (PND 21) than group 4 (PND 56) at 40 and 60 min after the MAP injection in the present study.

Dopaminergic neuronal activity is regulated by several factors including ongoing DA synthesis, DA transporter-mediated reuptake of DA from the synaptic cleft into the nerve terminals, and feedback mechanisms such as somatodendritic autoreceptors and striatonigral long-loop feedback pathway. The data obtained herein should be discussed in conjunction with these regulatory factors.

Andersen and Gazzara (2) demonstrated that administration of a low dose of apomorphine (a mixed D_1/D_2 receptor agonist) attenuated extracellular DA and DOPAC levels in a similar magnitude in rats on PNDs 5, 10–11, 21–22, and 35–36 and in adult rats and concluded that synthesis-modulating DA autoreceptors on the nigrostriatal dopaminergic neurons are functional by PND 5. Electrophysiologically, the inhibitory effects of apomorphine (19) and quinpirole (15) on nigrostriatal dopaminergic neuronal activity were found to be similar in 2- and 4-week-old pups and adult rats. These results suggest that nigral dopaminergic neurons possess equivalent activity of inhibitory somatodendritic D_2 autoreceptor at all postnatal ages.

In the adult rat, it is well documented that striatonigral pathways function as long-loop afferent input to the nigral dopaminergic neurons and regulate their electrophysiological properties (5). It has been reported that development of stria-

tal neurons lags behind that of dopaminergic neurons, and that the adult-like electrophysiological properties of the neostriatal medium spiny neurons are observed only late in the fourth postnatal week (17,18). Given the immaturity of nigral afferents from the striatum at early PNDs, acute psychostimulant-induced dopamine release from dopaminergic terminals may not mediate long-loop feedback regulation of nigral dopaminergic neurons on such early PNDs. In fact, it has been reported that the nigral dopaminergic neurons in early PNDs exhibit paradoxical increases in neuronal firing on PNDs 1–6 and unresponsiveness on PNDs 7–15 after systemic administration of AMP, whereas it inhibits neuronal firing in the substantia nigra of adult rats (19). Because the electrophysiological properties such as spontaneous firing rate of mesencephalic DA-containing neurons are considered to affect DA release in the terminal fields (22), it remains to be investigated in a future study whether or not the immaturity of nigral afferents arising from the striatum may be associated with the unusual profile of striatal extracellular DOPAC and HVA after MAP administration on PND 14.

Not only DA per se but also AMP or MAP is known to be the substrate for DA transporter (4). Several lines of study have indicated that DA transporter is not fully developed before several postnatal weeks (3,7,13). It is likely that both the uptake of MAP into the dopaminergic nerve terminals and resultant DA release may be smaller in early PNDs, when DA transporter is not fully developed, than in adults. This hypothesis may be in line with the data of the present study. Given that MAP-induced DA release is reduced due to insufficient uptake of DA, MAP-induced decrease in DOPAC level, which represents DA release-induced reduction of DA metabolism by intracellular monoamine oxidase (25), would be attenuated. The inhibition by nomifensine, a DA uptake inhibitor, of both AMP-induced DA release and the decline in DOPAC levels (4) lends support to a hypothesis that underdeveloped DA transporter may be involved in the attenuation of decrease in striatal extracellular DOPAC after a MAP injection on PND 14. HVA level, which is considered to be derived from DOPAC and 3-methoxytyramine by catechol-*O*-methyltransferase (11) and by monoamine oxidase, respectively, in the extracellular compartment, may be affected by both increase of DA and concomitant decrease in DOPAC after a MAP injection. Although a reason for the unusual profile of increase in HVA levels after a MAP injection on PND 14 is unknown, it may represent a greater rate of DA turnover in the immature rats than in adult rats (9).

The 3-week postnatal development period has been demonstrated to be required for the formation of psychostimulant-induced behavioral sensitization (8,12,21). Furthermore, we recently reported that DA release induced by a MAP-challenge given 3 weeks after the last pretreatment MAP dose was significantly greater than in saline-pretreated controls only in animals that had been sensitized by MAP pretreatment starting on PND 21 or later (20). The present study suggests that MAP-induced striatal DA releasability does not reach maturity due to underdevelopment of DA transporter, and fails to contribute to the development of behavioral sensitization during the early postnatal period. With regard to factors related to DA releasability in early PNDs, however, a putative role of immaturity of striatonigral feedback pathways may be also involved.

Application of microdialysis technique to neonatal rats involves confounding problems due to insufficient reservoir for body fluid and unstable body temperature, which is prone to be influenced by environmental temperature. In this regard, neither the appropriate length of postsurgical recovery period nor the manner by which pups are handled while they are confronted with various restrictions for subsequent microdialysis is yet known. Whereas previous studies (2,9) conducted in vivo microdialysis in neonatal pups under anesthesia after a short recovery (1 h) from the implantation of probe, preweanling rats (groups 1 and 2) in the present study were not returned to the dam after the surgery and put in an experimental cage overnight without access to food or water. This method of recovery from the traumatic effect of surgery was used for all the groups, because both feeding (23) and drinking (24) are known to affect DA release. Although the body temperature was not monitored in rats of group 1 (PND 14), they were put in a constant environmental temperature at 24°C overnight and showed normal locomotor activity on the next day, suggesting the minimal effect of exposure to the temperature at 24°C on the core body temperature. Nevertheless, we are aware that the microdialysis in neonatal pups is hampered by the confounding factors (unstable body temperature and loss of body fluid), and note that further studies under strict control of these factors are necessary.

This is, we believe, the first neurochemical evidence that revealed differences in AMP-like psychostimulant-induced striatal DA release and changes in its metabolites between adult and early postnatal rats. Further studies are necessary to elucidate the putative role of dopaminergic neuronal maturity in the ontogeny of MAP-induced behavioral sensitization.

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REFERENCES

1. Akiyama, K.; Kanzaki, A.; Tsuchida, K.; Ujike, H. Methamphetamine-induced behavioral sensitization and its implications for relapse of schizophrenia. Schizophr. Res. 12:251–257; 1994.
2. Andersen, S. L.; Gazzara, R. A. The ontogeny of apomorphine-induced alterations of neostriatal dopamine release: Effects on spontaneous release. J. Neurochem. 61:2247–2255; 1993.
3. Bonnet, J.-J.; Costentin, J. Correlation between [^{3}H]dopamine specific uptake and [^{3}H]GBR 12783 specific binding during the maturation of rat striatum. Life Sci. 44:1759–1765; 1989.
4. Butcher, S. P.; Fairbrother, I. S.; Kelly, J. S.; Arbuthnott, G. W. Amphetamine-induced dopamine release in the rat striatum: An in vivo microdialysis study. J. Neurochem. 50:346–355; 1988.
5. Bunney, B. S.; Chiodo, L. A.; Grace, A. A. Midbrain dopamine system electrophysiological functioning: A review and new hypothesis. Synapse 9:79–94; 1991.
6. Coyle, J. T.; Campochiaro, P. Ontogenesis of dopaminergic-cholinergic interactions in the striatum: A neurochemical study. J. Neurochem. 27:673–678; 1976.
7. Fujita, M.; Shimada, S.; Nishimura, T.; Uhl, G. R.; Tohyama, M. Ontogeny of dopamine transporter mRNA expression in the rat brain. Mol. Brain Res. 19:222–226; 1993.
8. Fujiwara, Y.; Kazahaya, Y.; Nakashima, M.; Sato, M.; Otsuki, S. Behavioral sensitization to methamphetamine in the rat: An ontogenic study. Psychophamacology (Berlin) 91:316–319; 1987.
9. Gazzara, R. A.; Andersen, S. L. Calcium dependency and tetrodotoxin sensitivity of neostriatal dopamine release in 5-day-old

and adult rats as measured by in vivo microdialysis. J. Neurochem. 62:1741-1749; 1994.
10. Gazzara, R. A.; Fisher, R. S.; Howard, S. G. The ontogeny of amphetamine-induced dopamine release in the caudate-putamen of the rat. Dev. Brain Res. 28:213-220; 1986.
11. Kaakkola, S.; Wurtman, R. J. Effects of COMT inhibitors on striatal dopamine metabolism: A microdialysis study. Brain Res. 587:241-249; 1992.
12. Kolta, M. G.; Scalzo, F. M.; Ali, S. F.; Holson, R. R. Ontogeny of the enhanced behavioral response to amphetamine in amphetamine-pretreated rats. Psychopharmacology (Berlin) 100:377-382; 1990.
13. Le, W.-D.; Bostwick, R.; Appel, S. H. Use of [^{3}H]-GBR12935 to measure dopaminergic nerve terminal growth into the developing rat striatum. Dev. Brain Res. 67:375-377; 1992.
14. McGeer, E. G.; Fibiger, H. C.; Wickson, V. Differential development of caudate enzymes in the neonatal rat. Brain Res. 32:433-440; 1971.
15. Pitts, D. K.; Freeman, A. S.; Chiodo, L. A. Dopamine neuron ontogeny: Electrophysiological studies. Synapse 6:309-320; 1990.
16. Robinson, T. E.; Camp, D. M. The feasibility of repeated microdialysis for within-subjects design experiments: Studies on the mesostriatal dopamine system. In: Robinson, T. E.; Justice, J. B., Jr., eds. Microdialysis in the neurosciences. Amsterdam: Elsevier; 1991:189-234.
17. Tepper, J. M.; Damlama, M.; Trent, F. Postnatal changes in the distribution and morphology of rat substantia nigra dopaminergic neurons. Neuroscience 60:469-477; 1994.
18. Tepper, J. M.; Trent, F.; Nakamura, S. Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons. Dev. Brain Res. 54:21-33; 1990.
19. Trent, F., Nakamura, S.; Tepper, J. M. Amphetamine exerts anomalous effects on dopaminergic neurons in neonatal rats in vivo. Eur. J. Pharmacol. 204:265-272; 1991.
20. Tsuchida, K.; Ujike, H.; Kanzaki, A.; Fujiwara, Y.; Akiyama, K. Ontogeny of enhanced striatal dopamine release in rats with methamphetamine-induced behavioral sensitization. Pharmacol. Biochem. Behav. 47:161-169; 1994.
21. Ujike, H.; Tsuchida, K.; Akiyama, K.; Fujiwara, Y.; Kuroda, S. Ontogeny of behavioral sensitization to cocaine. Pharmacol. Biochem. Behav. 50:613-617; 1995.
22. Wang, L,; Pitts, D. K. Postnatal development of mesoaccumbens dopamine neurons in the rat: Electrophysiological studies. Dev. Brain Res. 79:19-28; 1994.
23. Westerink, B. H. C.; Teisman, A.; de Vries, J. B. Increase in dopamine release from the nucleus accumbens in response to feeding: A model to study interactions between drugs and naturally activated dopaminergic neurons in the rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 349:230-235; 1994.
24. Young, A. M. J.; Joseph, M. H.; Gray, J. A. Increased dopamine release in vivo in nucleus accumbens and caudate nucleus of the rat during drinking: A microdialysis study. Neuroscience 48:871-876; 1992.
25. Zetterström, T.; Sharp, T.; Ungerstedt, U. Further evaluation of the mechanism by which amphetamine reduces striatal dopamine metabolism: A brain dialysis study. Eur. J. Pharmacol. 132:1-9; 1986.