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Amphetamine exerts anomalous effects on dopaminergic neurons in neonatal rats in vivo

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The effects of amphetamine, apomorphine and haloperidol on the spontaneous activity of electrophysiologically identified nigral dopaminergic neurons were examined with extracellular recordings in vivo in neonatal rats ranging in age from postnatal day 1 to postnatal day 28, and in adult rats. In postnatal day 1–6 pups amphetamine (5 mg/kg i.p.) produced a paradoxical increase in neuronal firing in 45% and had no effect on 30% of the 20 neurons examined. During the second week half of the neurons recorded were unresponsive to amphetamine. Typical amphetamine-induced inhibition was observed in only 25% of the neurons from postnatal day 1–6 and 50% of those from postnatal day 7–15 rats compared to 81.8% in postnatal day 16–28 pups and 100% in adults. Apomorphine (50–200 μg/kg i.p.; 5–20 μg/kg i.v.), significantly inhibited the spontaneous activity of dopaminergic neurons, including cells that previously failed to be inhibited by amphetamine, independent of age. The apomorphine-induced inhibition was consistently reversed by administration of haloperidol (0.5–2.0 mg/kg, i.p.; 50–200 μg/kg i.v.). The anomalous responses to amphetamine in early neonatal rats may be related to its paradoxical behavioral effects in human children afflicted with attention deficit disorder.

Amphetamine; Substantia nigra; Dopaminergic neurons; Attention deficit disorder; Neonate; (Stimulants)

1. Introduction

Dopamine and other biogenic amines make a precocious debut during the prenatal period and therefore have been implicated as important trophic and/or survival factors in early brain development (Lauder and Bloom, 1974, 1975; Noisin and Thomas, 1988; Seiger and Olson, 1973; Olson and Seiger, 1972). Despite the early appearance of these amines and their corresponding biosynthetic enzymes, the bulk of the synaptic and neurophysiological development of the basal ganglia is temporally delayed and occurs postnatally (Loizou and Salt, 1970; Kalsbeek et al., 1988; Herregodts et al., 1990). We recently reported that significant changes occur in the in vivo electrophysiological properties of nigral dopaminergic neurons in neonatal rat pups over the first postnatal month (Tepper et al., 1990a), and that the time course of the postnatal development of the cytoarchitecture of the substantia nigra is temporally correlated with these neurophysiological changes (Tepper et al., 1990b).

During the last decade there has been a growing interest in studying the functional development of the basal ganglia brought about in part by the advent of grafting fetal dopaminergic neurons into the neostriatum as a possible treatment for Parkinson's disease. Fisher et al. (1991) examined the in vivo electrophysiological properties of fetal dopaminergic neurons implanted into the adult dopamine-depleted neostriatum and reported that many of the electrophysiological properties of grafted dopaminergic neurons were very similar to those observed among dopaminergic neurons from intact neonates. These findings imply that dopaminergic neurons in neonatal rats may be a valuable model for grafted neurons and may help to predict the functional properties of dopaminergic neurons in neostriatal grafts.

In addition to serving as a model for neostriatal transplants, neonatal dopaminergic neurons may prove to exhibit significant changes in their pharmacological responsiveness over postnatal development that ultimately may give insight into similar age-related changes in drug responsivity observed clinically in human chil-

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dren. For example, it has been known for several decades that administration of psychomotor stimulants (e.g. d-amphetamine or methylphenidate) to children having attention deficit disorder (hyperkinesis) exerts a paradoxical calming effect on behavior and is clinically effective in alleviating hyperactivity, improving impulse control and increasing attention span (e.g. Gittelman, 1983; Wender, 1987). In support of a neonatal rodent model for attention deficit disorder, behavioral studies have reported that rat pups are spontaneously hyperactive during the first month postpartum (Hartley and Seeman, 1983) and like attention deficit disorder-afflicted children, are distractable (Feigley et al., 1972) and have learning difficulties (Campbell and Spear, 1972). The physiological mechanisms underlying the paradoxical effects of amphetamine in children are unknown, although it has been found that in adults, psychomotor stimulants like amphetamine or methylphenidate interact with brain monoamine systems and suppress the spontaneous activity of nigrostriatal dopaminergic neurons, at least in part by causing local dopamine release resulting in self-inhibition of dopaminergic neuron firing (Groves et al., 1975; Groves and Tepper, 1983).

The present study examined the effects of systemic amphetamine on the neuronal activity of neonatal nigral dopaminergic neurons in vivo and aimed to chart changes in their pharmacological responsiveness from the day of birth (postnatal day 1) through postnatal day 28. Part of this work has appeared previously in abstract form (Trent et al., 1990).

2. Materials and methods

2.1. Subjects

Subjects consisted of 77 Sprague–Dawley rat pups (Institute of Animal Behavior, Rutgers) ranging in age from the day of birth (postnatal day 1) to postnatal day 28 and weighing between 6.3 and 81.3 g. Pups were anesthetized with urethane (1.3 g/kg i.p.) and supplemented by inhalation of metofane (methoxyfluorane) if necessary. In some cases the femoral vein was cannulated or an i.p. catheter was inserted. The procedure for stereotaxic installation of neonatal rats has been described elsewhere (Nakamura et al., 1987). Body temperature was maintained at 37 ± 1°C for the duration of the experiment. All animals were treated in strict accordance with guidelines set forth in the PHS manual 'Guide for the Use and Care of Laboratory Animals'.

In addition, 17 adult male Sprague–Dawley rats (Institute of Animal Behavior, Rutgers) ranging in weight from 262 to 376 g were anesthetized with urethane (1.3 g/kg i.p.), intubated with either femoral or i.p. catheters and installed into a stereotaxic frame according to standard procedures.

2.2. Electrical stimulation

After removal of the scalp, a small burr hole was drilled overlying the anterior-lateral neostriatum for insertion of a stimulating electrode (coordinates 0.5–0.7 mm anterior to bregma, 2.5–3.3 mm lateral to the midline, depending on the age of the pup and 1.0 mm anterior to bregma and 3.7 mm lateral to the midline for adults). After puncturing the atlanto-occipital membrane to release cerebrospinal fluid and reduce brain pulsation, a bipolar stimulating electrode constructed from fine enamel-coated stainless steel wires (California Fine Wire), having a diameter of 100 μm, a tip separation of approximately 150 μm, and in vitro impedance of about 10 KΩ was lowered to depths ranging from 2.2 to 3.4 mm below the cortical surface in pups and 4.0 mm in adults. Constant current stimuli consisting of monophasic square wave pulses of 500 μs duration were generated with a Winston A-65 timer/stimulator and SC-100 constant current stimulus isolators, delivered at a rate of 0.67 Hz and ranged in intensity from 0.2 to 5 mA.

2.3. Recording

For recording, a burr hole approximately 1.5–2.5 mm in diameter was drilled overlying the substantia nigra at coordinates 0.5–1.5 mm anterior to lambda and 1.1–2.0 mm lateral to the midline in neonates while one of 3.0 mm diameter was drilled in adults at coordinates 2.0 mm anterior to lambda and 2.0 mm lateral to the midline in adults. Single unit extracellular recordings were obtained using glass micropipettes filled with 2 M NaCl containing 2% pontamine sky blue and possessing in vitro impedances of 5–15 MΩ. Recordings were amplified by a Neurodata IR183 preamplifier, displayed on a Tektronix 5113 storage oscilloscope and stored on magnetic tape for off-line analysis.

2.4. Drugs

All drugs were dissolved in 0.9% saline or 0.9% saline containing 0.1 mg/ml ascorbate for apomorphine, and were administered through either a femoral or i.p. catheter. The drugs used in this study and their concentrations were as follows: d-amphetamine sulfate (1–5 mg/kg i.p.; 0.25–2.0 mg/kg, i.v., Sigma); apomorphine hydrochloride (50–200 μg/kg i.p.; 5–20 μg/kg i.v., Sigma); and haloperidol lactate (0.5–2.0 mg/kg i.p.; 50–200 μg/kg i.v., McNeil Pharmaceuticals). All doses are expressed in terms of the salt.

2.5. Data analysis

Spontaneous activity of identified nigral dopaminergic neurons was monitored on-line using a Macintosh II computer equipped with a National Instruments MIO16L multifunction board as well as taped for off-line analysis. At least 4–5 min of stable spontaneous pre-drug activity was obtained before drugs were administered either i.p. or i.v. At the end of each experiment, data were played back from tape off-line and spike trains were analyzed to determine the effects of drugs on firing rate and pattern and were used to construct rate meters, first order interval histograms and autocorrelograms. Pre-drug firing rates were determined by averaging the last 2–5 min preceding drug administration. The effects of drugs administered i.p. on neuronal activity were computed by dividing the firing rate measured for 1 min beginning 3 min post-injection by the baseline firing rate. The effects of i.v. drug administration were similarly computed at 30 s intervals following drug administration. Maximal effects were reached by 3 min after i.p. injections and 30 s after i.v. injections. In addition, spike trains were analyzed by computer for the occurrence of burst discharges. Bursts were defined as beginning with the first interspike interval of 80 ms or less and terminating with the first interspike interval greater than 160 ms (Grace and Bunney, 1984; see Tepper et al., 1990a).

Data were pooled by assigning animals to one of the following age groups: postnatal day 1–6, postnatal day 7–15, postnatal day 16–28 and adult. Drug-induced effects on cell firing at appropriate post-injection time points were analyzed using a one-way analysis of variance by age group and differences between groups were tested with Scheffé's F-test at the $P < 0.1$ level.

2.6. Histology

At the end of each experiment, pontamine sky blue was ejected iontophoretically (-25 μA, 20 min) to mark the last recording site, and the neostriatal stimulating site was marked by making a small electrolytic lesion by passing 0.25-0.50 mA DC for 1 s through the stimulating electrode. Animals were perfused intracardially with 10–20 ml of saline followed by 50–100 ml of 10% formalin or 4% paraformaldehyde containing 0.2% glutaraldehyde in 0.15 M sodium phosphate buffer at pH 7.4. After removal from the skull, brains were post-fixed for 1–7 days, and 80 μm sections were cut on a freezing microtome or a vibratome. Sections were stained with neutral red, dehydrated in alcohol, cleared, and coverslipped. The location of stimulating and recording sites was verified and in some cases drawn at $1\times$ using a Nikon Optiphot microscope equipped with a drawing tube.

3. Results

Dopaminergic neurons were identified either by antidromic activation from the ipsilateral neostriatum, as illustrated in fig. 1, or by careful examination of neurophysiological parameters including spike waveform and duration, spontaneous firing rate and pattern, and depth below the cortical surface (Grace and Bunney, 1983; Tepper et al., 1990a). The antidromic nature of neostriatal-evoked responses was determined by collision extinction with spontaneously occurring spikes. Seventy-three percent of the neurons in this study were antidromically identified. After independent analyses of antidromically-identified and non-identified neurons showed no difference in response to either amphetamine or apomorphine, these data were pooled.

Amphetamine (5 mg/kg i.p.) was administered to 39 neonates and six adult male rats. A single i.p. injection of amphetamine (5 mg/kg) evoked a range of responses from dopaminergic neurons in the postnatal day 1–6 group. In these early neonates, amphetamine induced a paradoxical increase in firing (i.e. 249.4 ±

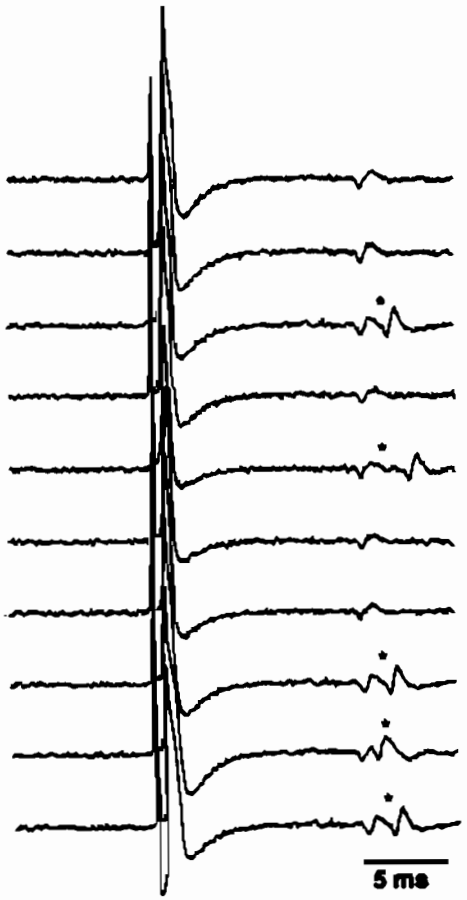

Fig. 1. Ten consecutive oscilloscope sweeps illustrating neostriatal-evoked antidromic responses of a postnatal day 1 nigrostriatal neuron. Note the high proportion of full antidromic spikes consisting of both initial segment and somadendritic components (asterisks).

TABLE 1

Developmental changes in the proportion of dopaminergic neurons inhibited by systemic amphetamine (5 mg/kg i.p.). Percentages for each type of amphetamine-induced response represent the proportion of total cells examined for each age group. Changes in firing rate of less than ±10% were considered to represent instances of 'no effect'. Percentages enclosed in parentheses indicate the percent control firing rate after amphetamine administration.

Age group	n	Facilitation	Inhibition	No effect
Postnatal day 1–6	20	45% (249.4 ± 53.8%)	25% (82.3 ± 3.1%)	30% (98.3 ± 1.8%)
Postnatal day 7–15	10	–	50% (61.9 ± 7.8%)	50% (101 ± 2.1%)
Postnatal day 16–28	11	–	82% (59.9 ± 9.1%)	18% (100 ± 0.6%)
Adult	6	–	100% (38.5 ± 13.2%)	–

53.8% of control) in nine cells, produced a very slight inhibition in five cells (i.e. 82.3 ± 3.1% of control) and had no effect (i.e. 98.3 ± 1.8% of control) on the firing rate in the remaining six dopaminergic neurons of this group, as shown in table 1. After systemic amphetamine administration, the overall mean percent change in control firing rate in this age group (+62.4 ± 29.6%; n = 20) significantly differed from that observed in postnatal day 16–28 pups and adults ($F(3,46) = 4.6$, $P < 0.01$), as shown in fig. 2. Ratemeters illustrating the time courses of responses to amphetamine from typical examples from each age group are shown in fig. 3. During the second postnatal week, five of the 10 dopaminergic neurons were pharmacologically unresponsive to systemic amphetamine administration, even at a high dose (5 mg/kg i.p.), as illustrated for a typical case in fig. 3B. A clear and consistent inhibition to amphetamine in a majority of neurons was first observed in the postnatal day 16–28 group, which did not differ from adults. In postnatal day 1–15 pups the amphetamine-induced percent increase in firing rate was inversely correlated with the pre-drug spontaneous firing rate of the neuron (5 mg/kg i.p., n = 30, F = 6.7, r = 0.44, $P < 0.05$) while an analogous relationship was not found in either the postnatal day 16–28 or adult groups.

In an effort to dissociate the anomalous effects of amphetamine on nigral dopaminergic neurons from the route of drug administration, amphetamine (0.25, 0.5, 1.0 and 2.0 mg/kg) was given i.v. as sequential injections administered at 1 min intervals to 12 pups and four adult male rats. In agreement with the i.p. data, amphetamine administered i.v. at 2 mg/kg to rats within the first two weeks postpartum also induced atypical responses in dopaminergic neurons which significantly differed from adult values ($F = 5.9$, $df = 12$, $P < 0.05$) as illustrated in fig. 3E. In 11 cases, after allowing sufficient time to elapse for attainment of the maximal amphetamine-exerted effect, the dopamine autoreceptor agonist, apomorphine (50–200 μg/kg, i.p.; 5–20 μg/kg i.v.), was systemically administered to determine whether altered somadendritic autoreceptor function was responsible for the anomalous effects of

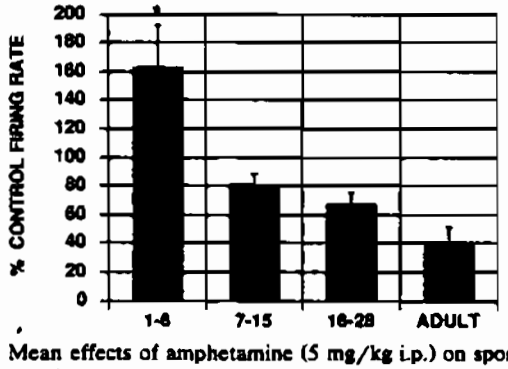

Fig. 2. Mean effects of amphetamine (5 mg/kg i.p.) on spontaneous firing rate of nigral dopaminergic neurons measured at 3 min post-injection. Numbers within bars represent numbers of cells. Error bars represent S.E.M. Asterisk indicates significant difference from postnatal day 16–28 and adult groups ($P < 0.05$).

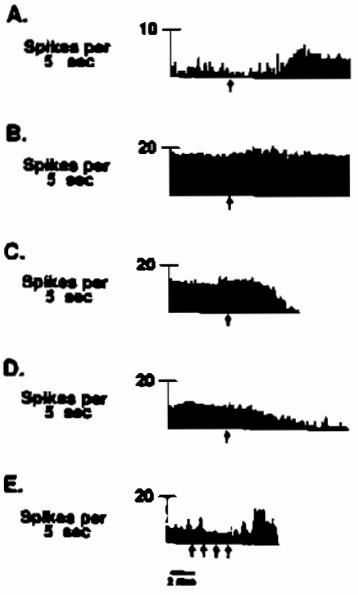

Fig. 3. Ratemeters illustrating typical responses of nigral dopaminergic neurons after a single injection of amphetamine (5 mg/kg i.p.). (A) Amphetamine induces a paradoxical increase in firing rate (+340.1%) in a postnatal day 1 rat. (B) No change (+6.3%) in firing rate is seen after amphetamine administration to a postnatal day 11 pup. (C) Amphetamine causes complete inhibition of firing in a postnatal day 23 rat that is similar to that seen in adults (D; −66.4%). (E) Sequential i.v. injections of amphetamine (0.25, 0.50, 1.0, 2.0 mg/kg) to a postnatal day 8 rat evokes a significant increase in firing (+43.2%). Arrows indicate the time(s) of amphetamine injection.

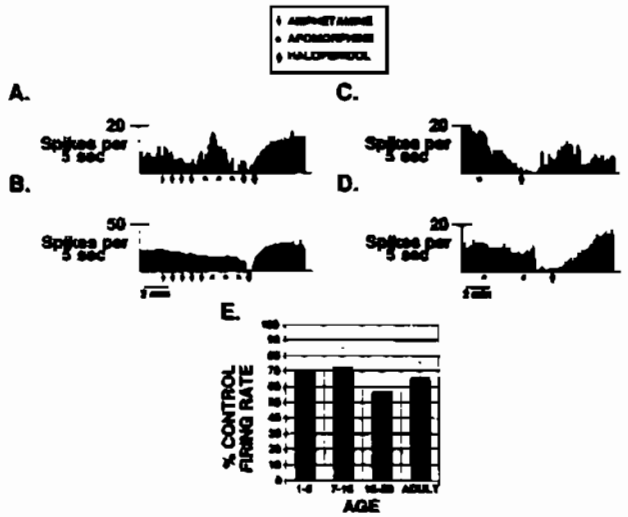

Fig. 4. Ratemeters illustrating the effects of amphetamine, apomorphine and haloperidol on the firing of nigral dopaminergic neurons. (A) Sequential injections of amphetamine (↑; 0.25, 0.5, 1.0, 2.0 mg/kg i.v.) significantly increase the spontaneous activity of a dopaminergic neuron in a postnatal day 8 pup while subsequent administration of apomorphine (*; 10, 20, 50 μg/kg i.v.) causes a complete cessation of neuronal firing that is reversed by haloperidol (↑↑; 50, 100 μg/kg i.v.). (B) Repeated injections of amphetamine (0.125, 0.50, 1.0, 2.0 mg/kg i.v.) do not significantly alter firing of a dopaminergic neuron from a postnatal day 18 rat although subsequent apomorphine (5, 10, 20 μg/kg i.v.) causes complete inhibition of spontaneous activity. (C) Apomorphine (200 μg/kg i.p.) results in complete inhibition of neuronal firing in a postnatal day 7 pup as it does in adults (D; 200, 400 μg/kg i.p.). Haloperidol reversed the apomorphine-induced inhibition in all cells shown ((A) 50, 100 μg/kg, i.v.; (B) 50 μg/kg, i.v.; (C, D) 500 μg/kg i.p.). Symbols indicate the times at which each drug was administered. (E) Mean effects of apomorphine (0.2 mg/kg, i.p.) on baseline firing rate measured at 3 min post-injection. Response to apomorphine does not vary as a function of age. Numbers within bars represent numbers of cells. Error bars represent S.E.M.

amphetamine seen in early neonates. Apomorphine administered by either route produced approximately a 50% reduction in the pre-drug spontaneous activity of dopaminergic neurons that had previously failed to be inhibited by systemic amphetamine indicating that dopamine autoreceptors are functional at least by the time of birth as illustrated in fig. 4.

Apomorphine (200 μg/kg i.p.; 10 and 20 μg/kg i.v.) was also administered to 21 amphetamine-naive pups and six adult rats and consistently decreased the pre-drug firing rate of dopaminergic cells by approximately the same amount in all ages (i.e. 30.8 ± 2.4% after 200 μg/kg i.p.; 40.8 ± 3.2% after 10 μg/kg i.v.; 55 ± 7.1% after 20 μg/kg, i.v.) as shown in fig. 4E. In three additional cases in postnatal day 1–6 pups, higher doses of apomorphine (1 mg/kg i.p.) caused almost complete inhibition of spontaneous firing (−99.0 ± 1.0%, $P < 0.001$). The apomorphine-induced inhibition of dopaminergic cell activity was reversed in animals of all ages by systemic administration of the D_1/D_2 receptor antagonist, haloperidol (0.5–2.0 mg/kg i.p.; 50–200 μg/kg, iv.) as illustrated in fig. 4. Haloperidol-induced reversal of apomorphine inhibition consistently produced firing rates in excess of the original pre-amphetamine or apomorphine levels.

4. Discussion

Nigral dopaminergic neurons respond to systemically administered amphetamine in an age-dependent manner. Over the first postnatal month the effects of amphetamine on neuronal activity progress from a paradoxical increase in firing rate in a significant proportion of early neonates (postnatal day 1–6), to an anomalous lack of effect during the second week postpartum and finally, by the third postnatal week, to the normal inhibitory effect seen in adults. These age-dependent effects of amphetamine do not appear to be due to some age-related differential action of the anesthetic agents employed since, as we have previously described (Tepper et al., 1990a), on the occasions in which it was necessary to administer supplemental anesthetic (metofane or urethane), no changes in rate or pattern of spontaneous activity were noted.

Behavioral studies examining the effects of systemic amphetamine on locomotor activity show that an age-dependent shift in dose–response for amphetamine occurs between postnatal day 15 pups and adults (Lanier and Isaacson, 1977), consistent with what we have observed on a cellular level. In addition, studies employing a pretreatment–challenge dose paradigm have found that amphetamine-induced behavioral sensitization does not develop until after the third postnatal week possibly due to the immaturity of both dopamine carrier and uptake systems (Kolta et al., 1990).

One explanation for the anomalous effects of amphetamine in the substantia nigra of neonates may be that neonates possess reduced D_2 autoreceptor function. However, our results indicate that the sensitivity of D_2 somadendritic autoreceptors in neonates is similar to that of adults since (1) systemically administered apomorphine suppressed the neuronal activity of dopaminergic neurons in all age groups with equal potency and (2) this apomorphine-induced inhibition was consistently reversed beyond the original pre-drug baseline by the dopamine receptor antagonist, haloperidol. This latter phenomenon suggests that, as in adults, nigral neurons in neonates exhibit some tonic level of activation of inhibitory somadendritic autoreceptors by endogenous dopamine. These observations are in agreement with previous in vivo studies in which systemic administration of direct-acting dopamine agonists (i.e. apomorphine) to adult rats was shown to inhibit the firing of nigral dopaminergic neurons by

direct activation of somadendritic D_2 autoreceptors (e.g. Bunney et al., 1973). In contrast to the age-independent response to apomorphine reported here, others have suggested that during the second and fourth postnatal weeks, nigrostriatal dopaminergic neurons are less sensitive to the inhibitory effects of i.v. administered apomorphine, but not quinpirole, than those of adults (Pitts et al., 1990). In that study, the maximum cumulative intravenous doses of apomorphine required to completely suppress the spontaneous firing of dopaminergic neurons in neonates and adults were significantly higher than those that we administered (i.e. 256 μg/kg for neonates, 64 μg/kg for adults vs. 20 μg/kg for all ages in the present report) and, therefore, may account in part for the observed discrepancy.

Our observations suggest that the anomalous amphetamine-evoked responses of dopaminergic neurons in early neonates may not be due to altered mechanisms intrinsic to the substantia nigra itself, but may be the result of atypical responses of nigral afferents to amphetamine-induced dopamine release from dopaminergic terminal fields. It has been suggested that in adult rats, the inhibitory effects of amphetamine on dopaminergic cell activity results from both local dopaminergic self-inhibition and nigral afferent feedback (Bunney and Aghajanian, 1978; Groves et al., 1975). Severing the reciprocal connections between the substantia nigra and the neostriatum by diencephalic transection increases the basal firing rate of dopaminergic neurons and attenuates systemic amphetamine-induced inhibition of dopaminergic cell firing without affecting responsiveness to apomorphine (Bunney and Aghajanian, 1978). Similar results have been obtained from studies examining the effect of habenular and entopeduncular lesions on inhibition induced by the related compound, methamphetamine (Sasaki et al., 1990). These data indicate that the mechanism of amphetamine-mediated inhibition of nigral dopaminergic neurons involves not only local self-inhibition within the substantia nigra but also the participation of synaptic input originating from regions receiving dopaminergic afferents.

During the early postnatal period in rat, the neurophysiological development of striatal neurons lags behind that of dopaminergic neurons as indicated by the delayed onset of spontaneous activity which is not detected until the second postnatal week in neostriatum and which remains at minimal levels until after postnatal day 18, whereas nigral dopaminergic neurons are spontaneously active at least by the day of birth (Napier et al., 1985; Tepper et al., 1990a).

In intact adults, many neostriatal neurons are spontaneously active, and stimulation of the cerebral cortex evokes an initial excitatory response followed by a period of prolonged (100–350 ms) inhibition due to the disfacilitation of excitatory cortical afferents (Wilson et al., 1983). In neonates, spontaneous activity is extremely rare, and cortical stimulation evokes the initial excitatory phase, but the succeeding cortically evoked long lasting inhibition is absent until the end of the third postnatal week, near the time that the neurons in the present study began to exhibit adult-like responsiveness to amphetamine (Trent and Tepper, 1991). This lack of disfacilitation closely resembles the synaptic responses of neostriatal neurons observed in in vivo intracellular recordings obtained from adults following either cortical lesions or in recordings of fetal neostriatal cells grafted into the kainic acid-lesioned neostriatum (Wilson et al., 1983; Xu et al., 1991). In agreement with these findings, in vivo intracellular recordings from the striatal and entopeduncular-pallidal neurons in kittens indicate that during the early postnatal period, afferent stimulation evokes atypical excitatory responses that gradually develop into the excitation–inhibition sequence seen in adults (Levine et al., 1974, 1979; Morris et al., 1979). The apparent similarity in the responses of neostriatal neurons to cortical stimulation among neonates, decorticate adults and fetal neostriatal graft neurons may arise, in part, from decreased afferent input to the neostriatum resulting in the reduction or removal of the tonic excitatory tone normally exerted by the cerebral cortex in intact adults (Xu et al., 1991). In addition to its neurophysiological immaturity, the anatomy of the neostriatum is underdeveloped with respect to adults until the end of the first postnatal week (Fishell and Van der Kooy, 1987). By virtue of its anatomical and physiological immaturity, the neostriatum and possibly other nigral afferents (e.g., those arising from the entopeduncular nucleus or habenular nuclei) may not respond to their afferent inputs as in adults and as a result, may contribute to the anomalous effects of amphetamine during the first two postnatal weeks.

In contrast to the consistent amphetamine-induced inhibition of nigral cell firing observed in adults, the effects of amphetamine on neostriatal neuronal activity vary as a function of location and cell type (e.g. Groves and Tepper, 1983; Rebec, 1987). Behavioral evidence suggests that systemic amphetamine may be acting through neostriatal D_1 and D_2 receptors to produce stereotypy (Braun and Chase, 1986). In addition, electrophysiological and behavioral reports have found that neostriatal D_1 and D_2 receptors interact synergistically and presumably subserve a modulatory role in the action of systemically administered dopamine agonists (Braun and Chase, 1986; Hu and Wang, 1988). In neonates, neostriatal D_1 and D_2 receptors have not reached adult values until the end of the first postnatal month (Pardo et al., 1977; Murrin and Zeng, 1986; Noisin and Thomas, 1988; Zeng et al., 1988). This decreased receptor number coupled to the previously

discussed reduced afferent input as well as the anatomical and neurophysiological immaturity of the neostriatum as a whole may alter the responsiveness of neostriatal neurons to systemic amphetamine administration thereby contributing to the observed anomalous effects in substantia nigra in early neonates.

Another factor accounting for the anomalous effects of amphetamine in early neonates may be the functional immaturity of the dopamine uptake system. In adults, amphetamine causes dopamine release from nigral dendrites and terminals and interferes with its re-uptake resulting in self-inhibition by an autoreceptor-mediated mechanism (Groves et al., 1975; Groves and Tepper, 1983). In neonates, amphetamine-induced release of dopamine from nigral dendrites may be insufficient to stimulate dopaminergic autoreceptors and may therefore fail to induce the characteristic self-inhibition seen in adults even though these neurons are capable of synthesizing and storing dopamine before birth (Voorn et al., 1988). In vivo microdialysis studies examining extracellular dopamine levels in the neostriatum and nucleus accumbens of adult rats report that amphetamine-induced release is significantly reduced by nomifensine, a selective dopamine re-uptake inhibitor, indicating that the dopamine carrier system must be operative for amphetamine-induced release to occur (Zetterström et al., 1988; Arbuthnott et al., 1990). Since the dopamine uptake system is not fully developed before the fourth postnatal week (Kolta et al., 1990), the acquisition of adult-like responsiveness to amphetamine should occur concomitantly with the maturation of the uptake system by the end of the first postnatal month, as our results indicate.

The circumscribed time window during which neonatal dopaminergic neurons are pharmacologically unresponsive to amphetamine may represent an important point in the postnatal development of the substantia nigra. The transition from anomalous 'immature' responses to amphetamine characteristic of neonates to the normal 'adultlike' inhibitory response by the third postnatal week temporally coincides with that of the most marked postnatal changes in cytoarchitectural and morphological organization of the substantia nigra (Tepper et al., 1990b) as well as that of a transient shift in the firing pattern of nigrostriatal dopaminergic neurons to a pacemaker-like mode and a transient decrease in the duration of post-stimulus inhibition evoked from stimulation of the neostriatum (Tepper et al., 1990a). These structural and physiological reorganizations culminate in the mature anatomy and seem to signal the onset of the acquisition of 'adultlike' responsiveness to systemic amphetamine.

The paradoxical effects of amphetamine on nigrostriatal dopaminergic neurons in early postnatal rats may be related to its paradoxical behavioral effects in human children with attention deficit disorder. One model proposed to explain the underlying physiological basis of hyperactivity suggests that attention deficit disorder-afflicted children suffer from delayed cognitive development that manifests itself as behavioral immaturity persisting throughout childhood until the onset of puberty (Kinsbourne, 1973). Since in the present study the paradoxical effects of amphetamine disappeared in a time-dependent fashion, perhaps attention deficit disorder is due in part to a functional immaturity of striatal efferent systems and/or the substantia nigra itself.

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References

Arbuthnott, G.W., I.S. Fairbrother and S.P. Butcher, 1990, Brain microdialysis studies on the control of dopamine release and metabolism in vivo, J. Neurosci. Meth. 34, 73.

Braun, A.R. and T.N. Chase, 1986, obligatory D1-D2 receptor coactivation and the generation of dopamine agonist related behaviors, Eur. J. Pharmacol. 131, 301.

Bunney, B.S. and G.K. Aghajanian, 1978, d-Amphetamine-induced depression of central dopamine neurons: Evidence for mediation by both autoreceptors and a striato-nigral feedback pathway, Naunyn-Schmiedeb. Arch. Pharmacol. 307, 255.

Bunney, B.S., G.K. Aghajanian and R.H. Roth, 1973, Comparison of effects of L-Dopa, amphetamine and apomorphine on firing rate of rat dopaminergic neurones, Nature New Biol. 245, 123.

Campbell, B.A. and N.E. Spear, 1972, Ontogeny of behavioral arousal: The role of environmental stimuli, Psychol. Rev. 79, 215.

Feigley, D.A., P.J. Parsons, L.W. Hamilton and N.E. Spear, 1972, Development of habituation to novel environments in the rat, J. Comp. Physiol. Psychol. 79, 443.

Fishell, G. and D. Van der Kooy, 1987, Pattern formation in the striatum: Developmental changes in the distribution of striatonigral neurons, J. Neurosci. 7, 1969.

Fisher, L.J., S.J. Young, J.M. Tepper, P.M. Groves and F.H. Gage, 1991, Neostriatal grafts of fetal mesencephalic cell suspensions: Electrophysiological and morphological characterization, Neuroscience 40, 109.

Gittelman, R., 1983, Experimental and clinical studies of stimulant use in hyperactive children and children with other behavioral disorders, in: Stimulants: Neurochemical, Behavioral and Clinical Perspectives, ed. I. Creese (Raven, New York) p. 205.

Grace, A.A. and B.S. Bunney, 1983, Intracellular and extracellular electrophysiology of nigral dopaminergic neurons – 1. Identification and characterization, Neuroscience 10, 301.

Grace, A.A. and B.S. Bunney, 1984, The control of firing pattern in nigral dopamine neurons: Burst firing, J. Neurosci. 4, 2877.

Groves, P.M. and J.M. Tepper, 1983, Neuronal mechanisms of action of amphetamine, in: Stimulants: Neurochemical, Behavioral and Clinical Perspectives, ed. I. Creese (Raven, New York) p. 81.

Groves, P.M., C.J. Wilson, S.J. Young and G.V. Rebec, 1975, Self-inhibition by dopaminergic neurons, Science 19, 522.

Hartley, E.J. and P. Seeman, 1983, Development of receptors for dopamine and norepinephrine in rat brain, Eur. J. Pharmacol. 91, 391.

Herregodts, P., B. Velkeniers, G. Ebinger, Y. Michotte, L. Vanhaelst and E. Hooghe-Peters, 1990, Development of monoamine neurotransmitters in fetal and postnatal rat brain: Analysis by HPLC with electrochemical detection, J. Neurochem. 55, 774.

Hu, X.-T. and R.Y. Wang, 1988, Comparison of the effects of D_1 and D_2 dopamine receptor agonists on neurons in the rat caudate-putamen: an electrophysiological study, J. Neurosci. 8, 4340.

Kalsbeek, A., P. Voorne, R.M. Buijs, C.W. Pool and H.B.M. Uylings, 1988, Development of the dopaminergic innervation in the prefrontal cortex of the rat, J. Comp. Neurol. 269, 58.

Kinsbourne, M., 1973, Minimal brain dysfunction as a neurodevelopmental lag, Ann. N.Y. Acad. Sci. 205, 263.

Kolta, M.G., F.M. Scalzo, S.F. Ali and R.R. Holson, 1990, Ontogeny of enhanced behavioral responses to amphetamine in amphetamine-pre-treated rats, Psychopharmacology 100, 377.

Lanier, L.P. and R.L. Isaacson, 1977, Early developmental changes in the locomotor response to amphetamine and their relation to hippocampal function, Brain Res. 126, 567.

Lauder, J.M. and F.E. Bloom, 1974, Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. I. Cell differentiation, J. Comp. Neurol. 155, 469.

Lauder, J.M. and F.E. Bloom, 1975, Ontogeny of monoamine neurons in the locus coeruleus, raphe nuclei and substantia nigra of the rat. II. Synaptogenesis, J. Comp. Neurol. 163, 251.

Levine, M.S., C.D. Hull and N.A. Buchwald, 1974, Pallidal and entopeduncular intracellular responses to striatal, cortical, thalamic and sensory inputs, Exp. Neurol. 44, 448.

Levine, M.S., E. Cherubini, G.D. Novack, C.D. Hull and N.A. Buchwald, 1979, Development of responses of globus pallidus and entopeduncular nucleus neurons to stimulation of the caudate nucleus and precruciate cortex, Exp. Neurol. 66, 479.

Loizou, L.A. and P. Salt, 1970, Regional changes in monoamines of the rat brain during postnatal development, Brain Res. 20, 467.

Morris, R., M.S. Levine, E. Cherubini, N.A. Buchwald and C.D. Hull, 1979, Intracellular analysis of the development of responses of caudate neurons to stimulation of cortex, thalamus and substantia nigra in the kitten, Brain Res. 173, 471.

Murrin, L.C. and W. Zeng, 1986, Postnatal ontogeny of dopamine D_2 receptors in rat striatum, Biochem. Pharmacol. 35, 1159.

Nakamura, S., F. Kimura and T. Sakaguchi, 1987, Postnatal development of electrical activity in the locus ceruleus, J. Neurophysiol. 58, 510.

Napier, T.C., S. Coyle and G.R. Breese, 1985, Ontogeny of striatal unit activity and effects of single or repeated haloperidol administration in rats, Brain Res. 333, 35.

Noisin, E.L. and W.E. Thomas, 1988, Ontogeny of dopaminergic function in the rat midbrain tegmentum, corpus striatum and frontal cortex, Dev. Brain Res. 41, 241.

Olson, L. and A. Seiger, 1972, Early prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations, Z. Anat. Entwicklungsgesch. 137, 301.

Pardo, J.V., I. Creese, D.R. Burt and S.H. Snyder, 1977, Ontogenesis of dopamine receptor binding in the corpus striatum of the rat, Brain Res. 125, 376.

Pitts, D.K., A.S. Freeman and L.A. Chiodo, 1990, Electrophysiological studies of nigrostriatal dopamine neuron ontogeny, Soc. Neurosci. Abstr. 16, 1046.

Rebec, G.V., 1987, Electrophysiological pharmacology of amphetamine, in: Monographs in Neural Sciences, ed. M.M. Cohen (Karger, Basel) p. 1.

Sasaki, K., H. Suda, H. Watanabe and H. Yagi, 1990, Involvement of the entopeduncular nucleus and the habenula in methamphetamine-induced inhibition of dopamine neurons in the substantia nigra of rats, Brain Res. Bull. 25, 121.

Seiger, A. and L. Olson, 1973, Late prenatal ontogeny of central monoamine neurons in the rat: Fluorescence histochemical observations, Z. Anat. Entwicklungsgesch. 140, 281.

Tepper, J.M., F. Trent and S. Nakamura, 1990a, Postnatal development of the electrical activity of rat nigrostriatal dopaminergic neurons, Dev. Brain Res. 54, 21.

Tepper, J.M., F. Trent and J.S. Rankin, 1990b, Morphological development of the substantia nigra in the postnatal rat, Soc. Neurosci. Abstr. 16, 1045.

Trent, F. and J.M. Tepper, 1991, Postnatal development of synaptic responses, membrane properties and morphology of rat neostriatal neurons in vivo, Soc. Neurosci. Abstr. 17, 938.

Trent, F., S. Nakamura and J.M. Tepper, 1990, Amphetamine exerts anomalous effects on dopaminergic neurons in neonatal rats, Soc. Neurosci. Abstr. 16, 1047.

Voorn, P., A. Kalsbeek, B. Jorritsma-Byham and H.J. Groenewegen, 1988, The pre- and postnatal development of the dopaminergic cell groups in the ventral mesencephalon and the dopaminergic innervation of the striatum of the rat, Neuroscience 25, 857.

Wender, P.H., 1987, The Hyperactive Child, Adolescent and Adult – Attention Deficit Disorder Through the Lifespan (Oxford University, New York).

Wilson, C.J., H.T. Chang and S.T. Kitai, 1983, Disfacilitation and long-lasting inhibition of neostriatal neurons in the rat, Exp. Brain Res. 51, 227.

Xu, Z.C., C.J. Wilson and P.C. Emson, 1991, Synaptic potentials evoked in spiny neurons in rat neostriatal grafts by cortical and thalamic stimulation, J. Neurophysiol. 65, 477.

Zeng, W., J. Hyttel and L.C. Murrin, 1988, Ontogeny of dopamine D_1 receptors in rat striatum, J. Neurochem. 50, 862.

Zetterstrom, T., T. Sharp, A.K. Collin and U. Ungerstedt, 1988, In vivo measurement of extracellular dopamine and DOPAC in rat striatum after various dopamine-releasing drugs; implications for the origin of extracellular DOPAC, Eur. J. Pharmacol. 148, 327.