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Functional Development of Dopamine Receptors in the Rat Forebrain

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ORMOND, D. L. AND C. VAN HARTESVELDT. *Functional development of dopamine receptors in the rat forebrain* PHARMAC. BIOCHEM. BEHAV. 10(6) 855–860, 1979.—Dopamine (DA) was injected unilaterally into the dorsal caudate-putamen (D-CPU), ventral caudate-putamen (V-CPU), piriform cortex (PIR), olfactory tubercle (OTU) and frontal cortex (FC) of two day old rats and rotational behavior observed. Injection of DA into D-CPU, PIR, and OTU produced a contralateral postural deviation which differed significantly from the ipsilateral deviation produced by control injections. Only DA injections into PIR and OTU produced contralateral turning differing significantly from the effects of control injections. These results suggest that the DA receptors in D-CPU, PIR, and OTU involved in rotational behavior are functionally mature at two days of age and that the two components of rotation, postural deviation (direction) and turning (locomotion), involve different neural systems at this age. The developing rat is suggested as a valuable tool for understanding the neural circuitry and pharmacology of rotational behavior.

Dopamine receptors Rotational behavior Development Striatum Mesolimbic Cannulations

SINCE the use of the histofluorescent technique for visualizing monoaminergic neurons in the brain [4, 12, 29] there has been extensive research on the dopamine (DA) systems of both the adult and developing rat. In adult animals there are two major DA systems ascending to the forebrain: a nigrostriatal DA system, arising from the substantia nigra, pars compacta (Area A9) and innervating the caudate-putamen (CPU) [1, 2, 3, 18]: and a mesolimbic system arising from the ventral tegmental area, VTA (Area A10) and innervating such limbic structures as nucleus accumbens, septum, olfactory tubercle (OTU), and frontal cortex (FC) [6, 14, 18, 19]. These two systems may not be completely distinct. Autoradiographic tracing of the projections of VTA shows a substantial projection from this area to ventromedial CPU [5] and the glyoxylic acid histofluorescent method shows a small dopaminergic projection connecting the two structures [18].

Rotational behavior has been used as an index of nigrostriatal dopaminergic function. Systemic injection of amphetamine in animals with unilateral lesions of the nigrostriatal tract (NST) or CPU will cause the animal to rotate to the side ipsilateral to the lesion [9, 15, 23, 30, 31, 32]. This behavior has been attributed to an imbalance in the release of DA from the two NST's. In support of this hypothesis, unilateral microinjection of DA directly into CPU results in rotation contralateral to the site of the injection, the opposite direction from that produced by a lesion [33].

In the developing rat, both the nigrostriatal and mesolimbic DA systems show the adult pattern of histofluorescence by the fourth postnatal week [20, 21, 24]. Neurochemical markers of presynaptic maturity of DA neurons, such as endogenous DA concentration, ^{3}H-DA uptake, and activity of tyrosine hydroxylase, do not reach adult levels in CPU until this same age [10,22]. A recent study using rotational behavior suggested that the presynaptic dopamine neurons may become functional earlier than this [36]. Amphetamine-induced rotation was obtained at 12 days after birth in animals with unilateral biochemical lesions in CPU.

Although the DA input to CPU matures over a long postnatal period, there is both biochemical and behavioral evidence to suggest that the postsynaptic receptors are functional at birth. The stimulation of adenyl cyclase activity by DA in CPU at birth has been reported to be at least equal to [10] or twice that in the adult [10]. Furthermore, unilateral lesions of CPU at two days of age produce an ipsilateral rotation remarkably similar to the behavior seen in adults with such lesions [12]. Thus, the implication is that DA receptors in CPU and the output pathways involved in the rotational response are functional at two days of age.

The present study examined the functional development of postsynaptic DA receptors in CPU by directly injecting DA unilaterally into this structure in the two day old rat and observing rotational behavior. It was expected that if the DA receptors were functional, the pups would show postural deviation and/or turning contralateral to the side of the injection. Since a lesion would result in ipsilateral rotation, animals injected with saline were expected to rotate ipsilaterally or randomly. Since DA fibers project into other areas of the forebrain, DA was also injected into piriform cortex (PIR), OTU, and frontal cortex (FC).

METHOD

Animals

One hundred and twenty male and female Long-Evans hooded rats, 48–72 hr old were obtained from our breeding

colony. Mothers and fathers were purchased from Charles River Laboratories and maintained on a 16/8 hour light/dark cycle upon delivery.

Apparatus

A dental cement head mold was mounted on a Plexiglas platform adjustable in three dimensions and mounted in a stereotaxic frame. An electrode carrier attached to the stereotaxic frame held a 1 μl Hamilton syringe filled with distilled water and connected to a 27 ga needle with polyethylene tubing containing the drug.

For behavioral observations the animals were placed in a circular arena 17.8 cm in dia. Running time meters and electric counters were used to record 0.01 min and one-quarter turns, respectively.

Drugs

Animals were injected with 10 μg of dopamine in a volume of 0.25 μl or 0.9% saline in 0.25 μl. The dopamine (3-hydroxytyramine) was purchased from Sigma Chemical Company.

PROCEDURE

Pretest

Rat pups were removed from their nest, weighed and observed for two 5-min intervals to determine side preference. Postural deviation and turning were recorded. The side to which the pup deviated and/or turned most in that 10-min period was considered the preferred side. The cannula was placed on the preferred side so that the drug would have to counteract the preference and any lesion effect due to damage made by the cannula.

Cannulation

Each pup was anesthetized with ether and immediately secured in the head mold with strips of tape over the snout and back of the neck. The platform was adjusted so that bregma and lambda, which are visible through the skin, were at equal horizontal levels and aligned in the anterior-posterior plane. Coordinates for CPU placements were derived from previous experiments and coordinates for OTU, PIR, and FC were empirically determined. Bregma was used as the landmark for the medial-lateral and anterior-posterior measurements. Coordinates for D-CPU and V-CPU were 0.5 mm anterior and 2.0 mm lateral to bregma, and 3.0 and 4.0 mm ventral to the skull surface, respectively. For OTU and PIR placements the coordinates were 0.5 mm anterior and 2.0 and 2.5 mm lateral to bregma, respectively. The ventral coordinate was 4.5 mm below the skull surface. FC placements were 1.5 mm anterior and 1.0 mm lateral to bregma. The ventral coordinate was 2.0 mm ventral to the skull surface.

With the exception of FC injections, the cannula was lowered directly into the brain through the skin and skull and the drug injected at a rate of 0.25 μl/min. It was impossible to drop the cannula directly through the skin and skull of animals receiving FC injections because of the curved surface of the skull in this area. Pressure on the flexible skull compressed the underlying tissue producing distortion of the ventricles and surrounding brain. Thus, an incision was made in the scalp and a small hole scraped into the skull over the frontal pole with a No. 10 scalpel blade before securing the pup into the head mold. The cannula was then lowered through the hole and the drug injected. In all cases, after the drug was injected the cannula was allowed to sit in place for an additional minute to help prevent diffusion back up the track. After withdrawing the cannula the pup was placed into a warming tray until it regained its righting response (approximately 10 min). The animals were then placed in the testing arena for behavioral recording.

Behavioral Testing

The animals were observed during six consecutive 5-min intervals. Postural deviation was recorded whenever an imaginary line between the tip of the nose and the base of the tail was not straight. Durations of ipsilateral and contralateral postural deviation were recorded during each 5-min interval. Also recorded were the number of one-quarter turns in either direction during each interval. A pup was considered to make a one-quarter turn whenever it changed the orientation of its longitudinal axis by 90°. A difference score was obtained for both behaviors by subtracting ipsilateral scores from contralateral scores.

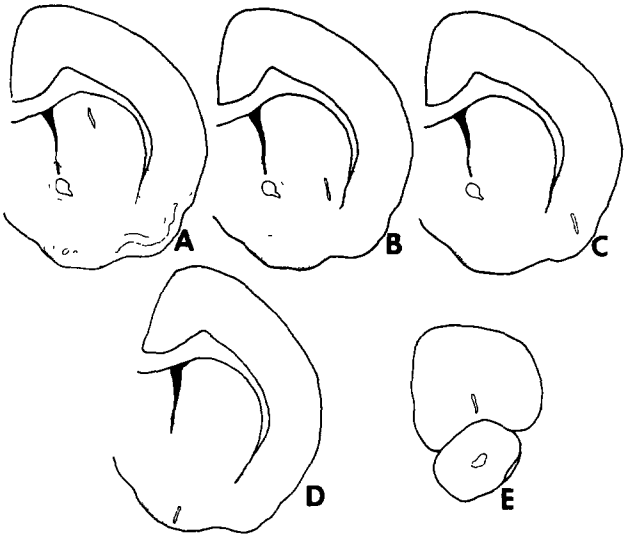

FIG 1. Placement of track of cannula tips for determining groups. A) D-CPU, B) V-CPU, C) PIR, D) OTU, E) FC.

Histology

Immediately after the behavioral observations the pups were sacrificed with ether, perfused intracardially with 10% Formalin and their brains removed. The brains were embedded in egg-yolk gelatin using a modified Snodgrass-Dorsey procedure [11], sectioned frozen at 50 μ, and stained with cresyl violet. On the basis of histology animals were assigned to the following groups (Fig. 1): D-CPU, the tip of the cannula was in the dorsal half of that part of the caudate-putamen nucleus that lies anterior to the globus pallidus and posterior to the head of the caudate (DA, N=9: saline, N=5): V-CPU, the tip of the cannula was in the ventral half of the same anterior-posterior extent of the caudate-putamen nucleus included in the previous group (DA, N=13: saline, N=13): OTU, the tip of the cannula was in the olfactory tubercle ventral to the same anterior-posterior extent of

the caudate-putamen as above (DA, N=5: saline, N=7): PIR, the tip of the cannula was in piriform cortex ventrolateral to the same anterior-posterior extent of the caudate-putamen and dorsolateral to the olfactory tubercle (DA, N=8: saline, N=6): FC, the tip of the cannula was in the neocortex anterior to the forceps minor of the corpus callosum (DA, N=4; saline, N=10).

Data Analysis

Difference scores were obtained for each interval by subtracting ipsilateral from contralateral scores for both deviation (D) and turning (T). These dependent variables were analyzed separately using a multivariate analysis of variance (MANOVA) at $p<0.025$, giving a total experiment-wise error $p<0.05$. The 10 groups were compared over the 6 time intervals as a 6-variate MANOVA using Wilks' multivariate statistic [26] to look for group differences. Given significant differences, a one-way analysis of variance for each time period was run revealing which time periods showed group differences. Kramer's modification of Duncan's procedure [17] was run at those time periods shown to have group differences.

A trend analysis was also performed to test whether there was a significant upward or downward trend over intervals. The slope for each animal within each group was obtained by linear regression. The multivariate analysis simultaneously tested the 10 population values as zero under the null hypothesis.

RESULTS

Behavior

The multivariate analysis of variance revealed that there was a significant difference among the groups on postural deviation (D) over time ($F(54,336)=1.47, p<0.025$). One-way analysis of variance showed that group differences existed within the following intervals: 2 ($F(9,70)=2.32, p<0.025$), 4 ($F(9,70)=3.89, p<0.01$), 5 ($F(9,70)=4.03, p<0.01$), and 6 ($F(9,70)=2.44, p<0.025$). Duncan's multiple range test, as modified for unequal sample sizes, then revealed those groups which showed differences at these specific intervals ($p<0.05$). Scores for each group have been averaged over intervals and are presented in Fig. 2. The specific intervals showing differences between specific groups are included in the parentheses in the text.

Pups injected with DA into D-CPU (2,4,5) PIR (4,5,6) and OTU (4,5) deviated contralaterally and differed significantly from their saline controls which deviated ipsilaterally. Animals injected with DA into V-CPU and FC also deviated contralaterally but did not differ significantly from their controls (Fig. 2). An overall trend analysis revealed no significant upward or downward trend to differences over intervals.

A multivariate analysis of variance on turning (T) revealed significant differences among groups ($F(54,336)=2.01, p<0.01$) over time. Univariate analysis of variance showed that only three intervals showed group differences on this variable: 1 ($F(9,70)=2.59, p<0.025$), 4 ($F(9,70)=4.39, p<0.01$), and 5 ($F(9,70)=2.05, p<0.05$). Duncan's multiple comparisons, modified as before, demonstrated that animals injected with DA into OTU (1) and PIR (4,5) turned contralaterally significantly more than their saline controls. These data are presented in Fig. 3 as averages over intervals.

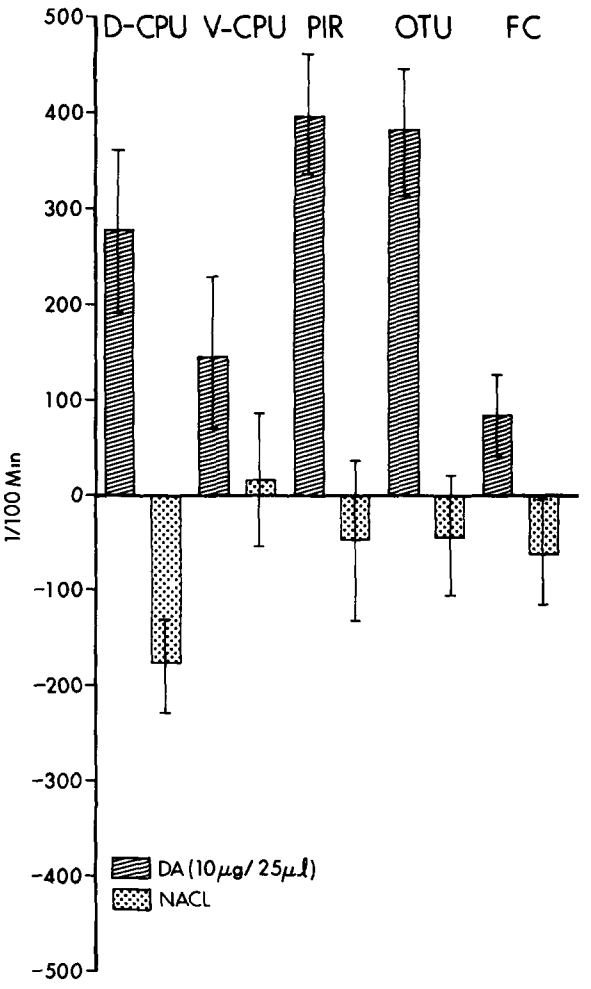

FIG. 2. The average duration of postural deviation per 5 min interval expressed in 0.01 min. Ipsilateral deviation was subtracted from contralateral deviation to give a difference score. Positive scores represent a predominantly contralateral deviation and negative scores an ipsilateral deviation. Standard errors of the mean (SEM) are indicated.

An overall trend analysis revealed no significant upward or downward trend to the differences over time.

Histology

Placement of the cannula tip was easily located under low power light microscope. The cannula track was visible in several sections posterior to the cannula tip. Damage in these adjacent sections was limited to the area that the cannula track had penetrated. Under high power the section containing the track of the tip of the cannula showed minimal damage to the tissue, damage being limited to a small circumscribed area that the tip had reached (Fig. 4).

DISCUSSION

The results of this experiment show that DA injected unilaterally into the rat brain at two days of age can produce contralateral postural deviation, turning, or both, depending upon the brain region. These results have important implica-

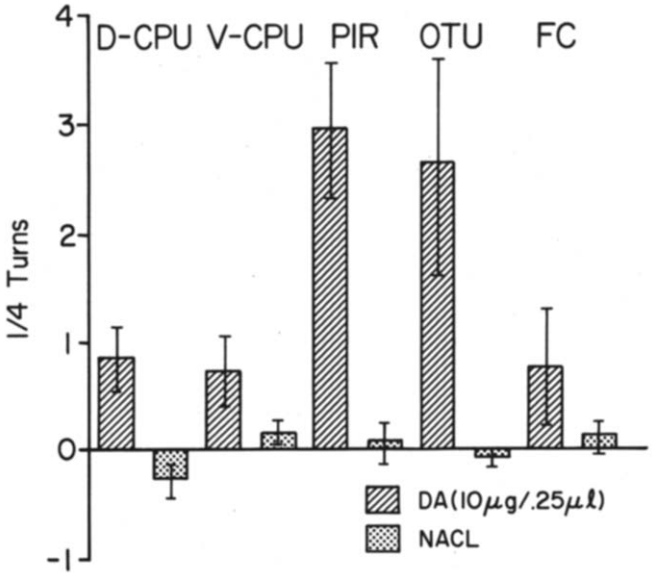

FIG 3 The average number of one-quarter turns per 5 min interval Ipsilateral turns were subtracted from contralateral turns to give a difference score. Positive scores represent a predominance of contralateral turns and negative scores ipsilateral turns. Standard errors of the mean (SEM) are indicated

tions both with respect to development and to the neural circuitry and pharmacology of rotational behavior. With respect to development, the present results extend the findings of previous research which showed that unilateral basal ganglia lesions at this age [34], as in the adult, result in ipsilateral postural deviation. Thus, even at two days of age, some forebrain mechanisms and their efferent neural paths are capable of mediating posture and rotation. Whether afferent pathways to these regions are functionally mature at this age is yet to be determined.

With respect to known mechanisms of postural deviation and rotation, the results of this experiment showed an unexpected lack of effect of DA in V-CPU, and unexpected postural and rotational effects in OTU and PIR. The failure to find significant effects of DA in V-DPU and the failure of cannula damage in this area to produce significant ipsilateral deviation (see Figs. 2 and 3) raises the question whether the caudate nucleus is regionally organized with respect to postural deviation, at this age or in the adult. There are several possibilities for the lack of an effect of DA in V-CPU in this experiment: (1) there are no or few DA receptors in this region: (2) the DA receptors in this region are not involved in this behavioral response. or (3) the DA receptors and related efferent paths are not mature at two days of age. The first

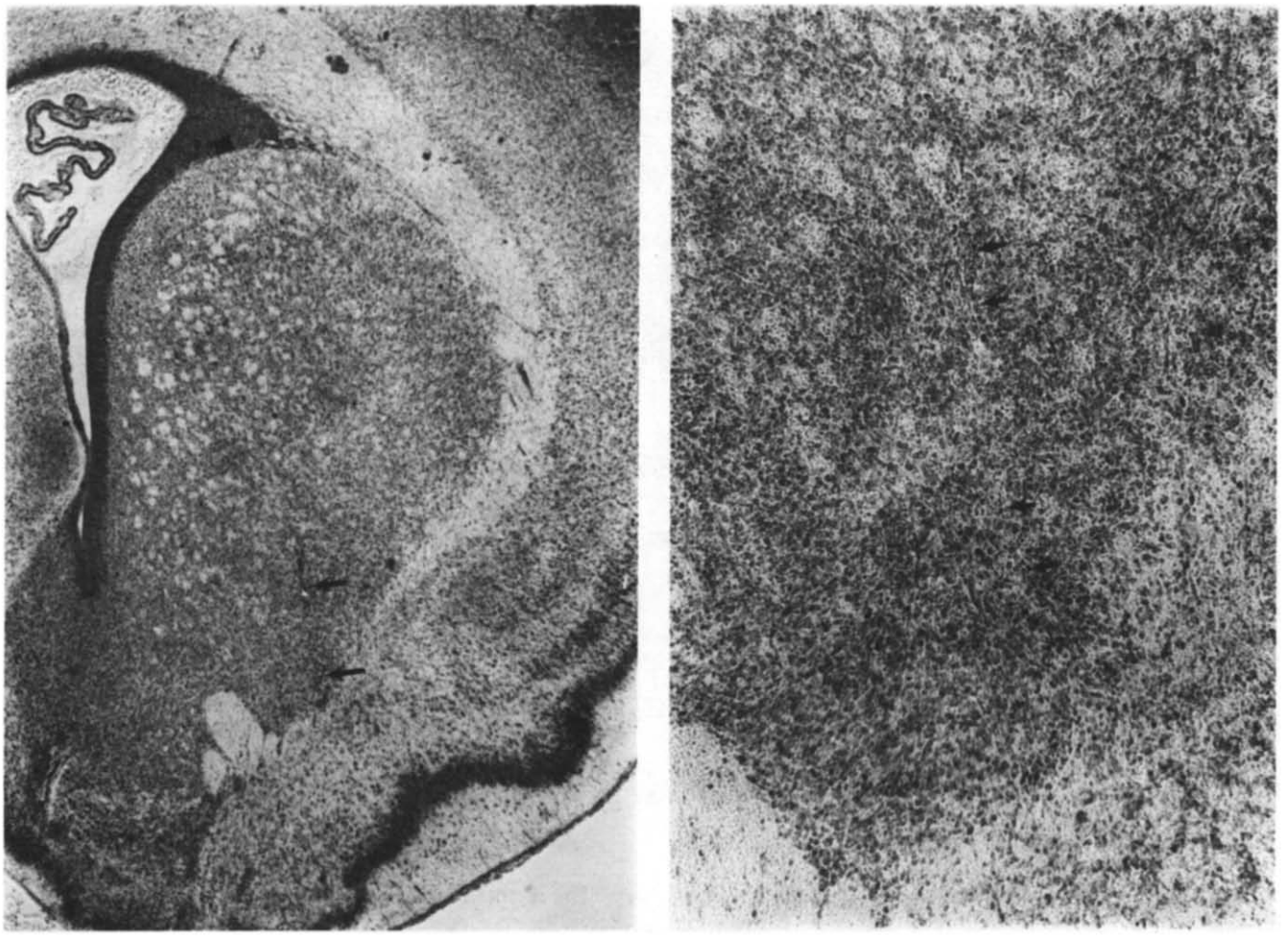

FIG 4 Light micrograph of track of cannula tip in V-CPU: (a) low power, 50×, (b) high power, 200× Black arrows point to track of cannula tip

alternative is unlikely since studies of the distribution of DA content, uptake, and stimulation of adenyl cyclase activity in the adult show a homogeneity in the dorsoventral plane of the caudate-putamen [7,28]. Further work with DA cannulations and lesions in the CPU of adults and developing rats must be carried out in order to determine which of the other alternatives is correct.

The effect of DA in PIR was unexpected since studies looking at regional distribution of DA to date have reported only small amounts of endogenous DA or DA terminals in this region [8, 13, 14, 18, 35]. The effect does not appear to be due to diffusion of the DA into CPU, since DA injected into V-CPU, which lies between PIR and D-CPU, shows a minimal effect and DA in PIR shows the strongest effect of any group. The drug could have diffused into OTU, however, and exerted its effect there. With respect to contralateral deviation this may be a likely explanation, since DA had an immediate effect in OTU but took several intervals to have an effect when injected into PIR: however, significant contralateral turning appeared at about the same time when DA was injected into either structure. Alternatives to the diffusion explanation are: (1) the few DA receptors that are in this area are involved in rotational behavior or (2) the DA injected into this area is acting non-specifically and stimulating noradrenergic or serotonergic receptors.

The effects of DA in OTU and PIR give further impetus to the idea that mesolimbic as well as nigrostriatal DA projections are involved in postural deviation and turning. Mesolimbic structures such as OTU [37] and nucleus accumbens [15,16] have been implicated in rotational behavior in adults, although direct cannulation studies have not been done. The results of the present study suggest that while the deviational component of the response may be represented in the caudate nucleus, OTU, and PIR, the locomotor component is found only in OTU and PIR. This latter finding is in accord with work showing that bilateral infusion of DA agonists into mesolimbic structures of adult rats produces increased locomotion [25]. Thus, the postural and locomotor component of rotational behavior involve different neural systems, at least at this age.

The developing rat represents a valuable tool for exploring the neural paths involved in the expression of rotational behavior, as well as the role of various neurotransmitters in this behavior. By taking advantage of the different rates of development of the various neurotransmitter systems it may be possible to determine where these neurotransmitters exert their influence on rotational behavior.

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