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Strain Differences in Dopamine Receptor Function and the Initiation of Movement

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WOLF, M. D., R. E. WILCOX, W. H. RIFFEE AND L. D. ABRAHAM. *Strain differences in dopamine receptor function and the initiation of movement.* PHARMAC. BIOCHEM. BEHAV. **13**(1) 5–7, 1980.—The relationship between voluntary movement initiation (VMI) and caudate nucleus dopamine receptor dynamics was analyzed in two rat strains. Charles River CD/F F-344 (CR-CD/F) and Zivic-Miller CD (ZM-CD) rats (male, 125–150 g) were trained to rapidly release and reset a response lever to avoid electric shock. Whereas 86% of all CR-CD/Fs completed training, only 43% of the ZM-CDs were able to do so. Of those rats completing training, the CR-CD/Fs showed marginally higher avoidance percentage and significantly faster VMI latencies. Physiologically, the more behaviorally-successful CR-CD/Fs showed significantly higher affinity for binding than the trained ZM-CDs and the large group of ZM-CDs which could not be successfully trained. In contrast, the trained ZM-CDs showed significantly higher density of dopamine receptors B_{max} than the ZM-CDs which failed to train and the trained CR-CD/Fs. The behavior-physiology continuum is summarized as follows: CR-CD/F Rats=highest affinity and lowest B_{max}—rapid, highest percentage avoidance; Trained ZM-CD Rats =lowest affinity and highest B_{max}—slower, high percentage avoidance; ZM-CD rats that failed training=intermediate affinity and B_{max}—avoidance failure.

Dopamine receptors Caudate nucleus Movement initiation Conditioned avoidance

THE neural organization of voluntary movement initiation (VMI) has been an issue of intense interest involving repeated demonstrations of VMI impairment produced by pathological processes and experimentally-induced lesions [8, 10, 11]. Several recent reports have ascribed a role in VMI to the nigrostriatal dopamine system [5,6], and additional evidence for this system's role in movement initiation has come from studies correlating neurochemical indices with motor behavior. For example, Kempf *et al.* [7] demonstrated in mice a positive correlation between dopamine turnover rate in pooled cortical tissue and conditioned avoidance performance.

Two major issues confound the interpretation of these preliminary correlations. First, responses within the standard conditioned avoidance framework must be initiated within 5 to 10 sec for shock to be averted, making it intuitively unlikely that organismic initiation capacities are being assessed. Second, the value of the dopamine turnover measure is clouded by the fact that, by virtue of the complex feedback networks in the striatum, both lesioning and stimulating the nigrostriatal system may result in an increase in turnover [3]. This study addresses these issues in an attempt to extend the correlation between neurophysiology and behavior.

METHOD

Behavior

Experimentally-naive male rats (125–150 g), Charles River CD/F F-344 (CR-CD/F) and Zivic-Miller CD (ZM-CD) were trained in an operant chamber to keep a 2×5 cm response lever depressed with their forepaws. The response (rapid lever release and redepression) was developed during the first two days of training, and then shaped over the next four-to-five days; the duration of the interval between conditioned stimuli (CS; 101 dB buzzer and 70 W lamp) and unconditioned stimulus (UCS; 300 W, 2.0–3.0 mA scrambled shock) was gradually reduced in an experimenter-set order from 4 sec (Day 3) to 250 msec (Day 6 or 7). In this manner, lever release/reset responses were shaped for increasing speed. By the conclusion of training, lever release must have occurred within 250 msec of CS for shock to be averted. Receptor binding experiments as described below were carried out on trained CR-CD/F, trained ZM-CD, untrainable ZM-CD, and on nontrained CR-CD/F and ZM-CD animals.

Receptor Binding

Rat striata were dissected rapidly over ice using a blunt dissection which made possible the removal of the caudate-putamen complex with sparing of mesolimbic-mesocortical structures, via medial visualization of lateral ventricle and anterior commissure. Dopamine receptor binding studies were carried out after completion of behavioral testing using the basic procedure of Creese ([4] Wilcox, Clement-Cormier, *et al.*, in preparation). Striatal tissue was homogenized (Polytron, Brinkman Instruments) in 10 ml ice cold 50 nM Tris-HCl buffer (pH=7.7 at 25°C; containing 0.1%

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ascorbic acid) using 15 smooth up and down motions at the lowest possible rotor speed. The volume was adjusted to 20 ml with the same buffer and the tissue centrifuged twice at 50,000×g for 10 min (J21C centrifuge; Beckman Instruments) with an intermediate wash in the same buffer. The resulting P_2 pellet of tissue (representing tissue containing dopamine receptors relatively free from contaminants) was homogenized in a second buffer (Tris HCl, pH=7.1 at 37°C with 10 μM pargyline, 0.1% ascorbic acid, and ions as follows: 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM Mg Cl_2). This volume was adjusted to 20 ml with the pH 7.1 buffer and the tissue incubated at 37°C for 10 min (to allow the pargyline to inactivate the monoamine oxidase present). The suspension was returned to ice for the binding assay. Dopamine receptor binding was determined by incubating 800 μl of the tissue homogenate with 200 μl of buffer, and ascorbate and (^{3}H) spiroperidol (26 Ci/mmol, 0.05 to 1.2 nM; New England Nuclear) and in some tubes dopamine, in a final volume of 1.0 ml. Specific binding for striatal tissue was defined as excess over blanks [2] minus that occurring in the presence of 1 mM dopamine [1]. This mixture was incubated at 37°C for 15 min and the incubation terminated by rapid filtration of the mixture under vacuum through Whatman GF/B glass microfiber filters using a Millipore filtration manifold. Following three 5 ml rinses of ice-cold buffer, the filters were placed in 10 ml of liquid scintillation cocktail (GP Ready-Solv., Beckman Instruments) and counted by liquid scintillation spectrometry (Beckman LS 8000) with a counting efficiency of approximately 40%.

RESULTS

Behaviorally, while 86% (n=19) of CR-CD/Fs completed training by responding with a lever press within 250 msec of CS, only 43% (n=23) of ZM-CDs were able to do so. That is, 57% (n=31) of ZM-CDs failed to meet training criteria and were treated as a separate group for receptor binding assays (as compared to only 14% or n=3 CR-CD/Fs, which were not tested for receptor binding). This resulted in three, rather than two groups for analysis: trained CR-CD/F, trained ZM-CD, and ZM-CD rats that were untrainable. CR-CD/Fs that did not reach the established training criteria occurred at a frequency such that there were insufficient numbers to be pooled for binding analysis (i.e., each assay utilized pooled tissue from three animals).

Trained CR-CD/Fs showed significantly faster response initiation latencies at 250 ms CS-UCS interval than did the trained ZM-CDs, 140±17 msec vs 203±26 msec respectively, mean±SEM; $t(40)=8.80$, $p<0.05$. Avoidance percentages at the 250 ms interval did not differ significantly, 81±9% vs 77±13%, mean±SEM; $t(40)=1.32$, $p>0.05$.

Physiologically, receptor binding affinity (Table 1) was significantly higher in the trained CR-CD/Fs than in the trained ZM-CDs, K_D=0.28±0.02 nM vs 0.41±0.08 nM, mean±SEM; $t(12)=3.31$, $p<0.05$, and in untrainable ZM-CDs, 0.38±0.13 nM, $t(10)=1.90$, $p<0.05$. Affinity was low in the ZM-CD controls (rats not subjected to training at all) (K_D=0.48±0.2 nM), and high in the CR-CD/F controls (K_D=0.24±0.02 nM), paralleling the difference between trained rats of two strains. The small number of animals in the nontrained control groups precluded statistical comparison with the trained groups.

Receptor density (B_{max}) however, was significantly higher in the trained ZM-CDs than in (1) the untrainable rats from this strain, 48.0±10.4 pmol/g vs 33.0±6.9 pmol/g respectively mean±SEM; $t(10)=2.55$, $p<0.05$, and (2) the trained CR-CD/Fs, 48.0±10.4 pmol/g vs 31.7±1.2 pmol/g respectively, $t(12)=3.38$, $p<0.05$. It can be seen that variability of both affinity and density measures was higher in the trained and untrainable ZM-CDs than in the trained CR-CD/Fs. Receptor density was low in both the ZM-CDs control rats (34.5±0.6 pmol/g) and the CR-CF/Fs control animals (28.2±0.7 pmol/g).

TABLE 1

BINDING OF (^{3}H)-SPIROPERIDOL TO STRIATAL HOMOGENATES OF CR-CDF/F VS ZM-CD RATS AFTER AVOIDANCE TRAINING

Group	K_D (nM)	B_{max} (pmol/g)
CR-CD/F trained: efficient avoidance responders	0.28 ± 0.02	31.7 ± 1.2†
ZM-CD trained: efficient avoidance responders	0.41 ± 0.08*	48.0 ± 10.4
ZM-CD nontrainable: poor avoidance responders	0.38 ± 0.13*	33.0 ± 6.9†

*$p<0.05$ (t-test) CR-CD/F vs others.
†$p<0.05$ (t-test) trained ZM-CD vs others.

DISCUSSION

The correlation between behavioral and physiological data is described by the following continuum: Trained CR-CD/F rats=rapid, high-percentage avoidance—highest receptor affinity—lowest B_{max}; Trained ZM-CD rats=slower, high percentage avoidance—lowest receptor affinity—highest B_{max}; Untrainable ZM-CD rats=avoidance failure—low receptor affinity—low B_{max}.

Examination of the across-strain behavioral differences suggests the possibility that high receptor affinity (CR-CD/F) has a stronger correlation with response initiation speed capacity than does high B_{max} (ZM-CD trained). The presence of low receptor affinity and low B_{max} in the untrainable ZM-CDs, paired with their performance failure, leads to speculation that high receptor density in the ZM-CD strain may be a "compensation" enabling high avoidance capacity. Where such a compensation does *not* exist (i.e., the untrainable ZM-CDs), avoidance capacity is low or non-existent. This speculation is in a sense supported by widespread reports of behavioral compensation due to receptor supersensitivity, which appears to reflect an increase in receptor density after nigrostriatal lesions [9].

A salient but at present unanswered question is whether or not the behavioral training had an effect on binding characteristics. For the CR-CD/Fs, the question is being pursued (Wolf, in preparation). The existence of two behaviorally categorized sub-populations within the Zivic-Miller strain, however, confounds the interpretation of the training issue. In any given sample of ZM-CD rats, there are unknown proportions of "avoiders" and "nonavoiders." (In the present study, the proportion was 43%/57%.) A control group in a study of training effects might by chance contain all avoiders (high density-low affinity) or all nonavoiders (low density-low affinity). The simple comparison of a trained group with a group which contained an unknown proportion of rats of each type would be fruitless. This problem is, however, likewise being currently addressed. (Wolf *et al.*, in progress).

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