

# Dopamine Sensitivity in Rats Selectively Bred for Increases in Cholinergic Function

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CROCKER, A. D. AND D. H. OVERSTREET. *Dopamine sensitivity in rats selectively bred for increases in cholinergic function.* PHARMACOL BIOCHEM BEHAV 38(1) 105–108, 1991.—Because of the extensive literature demonstrating an interaction between cholinergic and dopaminergic systems, the Flinders Sensitive (FSL) and Flinders Resistant (FRL) Lines of rats, selectively bred for differences in cholinergic function, were tested for differences in dopamine sensitivity. Large differences in sensitivity to dopamine agonists were detected, but the direction depended upon the function: The FSL rats were supersensitive to the hypothermic effects of dopamine agonists, but were subsensitive to the stereotypy-inducing effects. Measurement of dopamine receptors by either standard binding techniques or autoradiography failed to demonstrate any receptor differences in the FSL and FRL rats. Behavioural studies with dopamine antagonists were less clear-cut, but suggested that the FSL rats might be more sensitive to their catalepsy-inducing effects. These findings indicate that the changes in dopamine sensitivity which accompany cholinergic supersensitivity are function-dependent, but are not associated with parallel changes in dopamine receptor concentration.

Cholinergic supersensitivity    Dopamine sensitivity    Stereotypy    FSL and FRL rats    Temperature    Catalepsy

PREVIOUS studies have established that the Flinders Sensitive (FSL) and Flinders Resistant (FRL) Lines of rats, which were selectively bred for differences in cholinergic function (17,18), also differ in their sensitivity to a range of other compounds, including ethanol, diazepam, muscimol, and m-chlorophenyl-piperazine, a serotonin agonist (19, 21, 23). In each of these instances, the FSL rats, which are more sensitive to cholinergic agonists, are also more sensitive to the other drugs. Because of the well-documented interactions between the dopaminergic and cholinergic systems in the control of motor function (4,15), and in the regulation of core body temperature (5–7), it was decided to study the sensitivity of the FSL and FRL rats to dopamine agonists and antagonists. The present set of experiments provides evidence for large differences in dopamine sensitivity in the FSL and FRL rats, whose direction is dependent on the function.

## METHOD

### Animals

Male rats from the colonies of the FSL and FRL rats maintained in the School of Biological Sciences at Flinders University were used. They were at least 70 days old and weighed a mini-

mum of 300 grams at the beginning of the study and had been previously challenged with the cholinergic agonist, oxotremorine, to establish that differences in cholinergic sensitivity were present (18). They were housed in groups of 8–10 in a temperature ( $22 \pm 1^\circ\text{C}$ )- and humidity (50%)-controlled room with free access to food and water. To reduce circadian fluctuations in acetylcholine (11), the rats were bred and maintained under continuous lighting.

### Temperature Recording

Core body temperatures were recorded by the insertion of a thermistor probe 6–7 cm into the rectum. The probe was attached to a Accurex digital recorder. The output was read after the instrument stabilised, approximately one minute after insertion.

### Behavioural Recording

Rats were placed in an open field apparatus (60 × 30 cm) and the incidence of targeted behaviours was measured by the momentary sampling method, adapted for use in our laboratory by Cameron (3). In this method, the number of times a behaviour is exhibited at 20-second intervals over a 10-minute observation pe-

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riod are recorded and expressed as the percentage of the total of 30 observations. Stereotyped head-down sniffing was chosen as the primary target behaviour as it exhibits a clear dose-response relationship with both the mixed D1/D2 dopamine agonist apomorphine and the selective D2 agonist, quinpirole (3). This method was used because selective D2 and D1 agonists have been shown not to elicit the same behaviours as the mixed D1/D1 agonist, apomorphine, for which previous rating scales were designed [e.g., (8)]. In addition, the momentary sampling method is less subjective, has high interrater reliability and generates ratio level data which can be analysed parametrically.

#### Drugs

Apomorphine and quinpirole (LY171555) were used to elicit hypothermia and stereotyped head-down sniffing in the FSL and FRL rats. Apomorphine was obtained from commercial sources (Sigma), while quinpirole was a gift from Lilly laboratories. Raclopride, a selective D2 antagonist, was a gift from Astra and was used to induce catalepsy. Oxotremorine, a muscarinic agonist, and methylatropine, a peripherally acting muscarinic antagonist, were used to test the cholinergic sensitivity of the rats. All injections were given subcutaneously in volumes of one ml/kg.

#### D2 Dopamine Receptor Binding Assay

Rats were killed by cervical dislocation. Brains were removed and the striata were dissected out, weighed and homogenised in 10 volumes of ice-cold 50 mM Tris buffer (pH 7.4) and homogenates stored at  $-20^{\circ}\text{C}$ . Total and nonspecific [ $^3\text{H}$ ]spiperone binding was measured in the presence and absence of  $1\ \mu\text{M}$  raclopride. Aliquots containing 2 mg of striatal tissue were incubated with concentrations of [ $^3\text{H}$ ]spiperone (21 Ci/mmol, Amersham) ranging from 0.03–3.0 nM in a final volume of 4 ml for 15 min at  $37^{\circ}\text{C}$ . Incubation was terminated by the addition of 5 ml of ice-cold 50 mM Tris buffer (pH 7.7), followed by rapid vacuum filtration using GF/B filters. Filters were washed with three 5-ml aliquots of the same buffer and counted by liquid scintillation spectrometry. Binding data were analysed by an extended least squares modelling program, MKMODEL of Holford (12). Tissue protein concentration was determined using the method of Lowry (14).

#### Quantitative Autoradiography

Because of increasing evidence that the striatum is not a homogeneous structure [see (2)], quantitative autoradiography was employed since it permits the quantitative assessment of dopamine receptor concentrations in subregions of the striatum, as well as other brain regions. Rats were sacrificed by cervical dislocation. Brains were removed, embedded, sectioned ( $20\ \mu\text{m}$ ) by cryostat and thaw mounted onto slides, as described previously (2). Sections were incubated with [ $^3\text{H}$ ]sulpiride (15 nM, 65 Ci/mmol, New England Nuclear) and nonspecific binding determined in adjacent sections using  $1\ \mu\text{M}$  sulpiride (Sigma). Sections were opposed to Hyperfilm (Amersham) for a period of three weeks at  $-20^{\circ}\text{C}$  and the resulting autoradiograms analysed using the MD 20 computer-assisted image analysis system (13). The areas of the striatum examined represented the whole striatal area visible in sagittal section at 2.9 mm lateral to the midline (20) and a selected striatal area previously identified to contain the dopamine receptors responsible for stereotyped head-down sniffing (2), with its centre at coordinates (in mm) A +1.2, L 2.5, D 6.5 (20).

#### Procedure

Initially, FSL and FRL rats were pretreated with methylatro-

TABLE 1  
EFFECTS OF APOMORPHINE ON THE INCIDENCE OF HEAD-DOWN  
SNIFFING IN FSL AND FRL RATS

Dose of Apomorphine ( $\mu\text{mol/kg}$ )	Incidence of Head-Down Sniffing (mean % $\pm$ s.e.m.)	
	FRL (n=8–10)	FSL (n=8–10)
0 (saline)	9.6 $\pm$ 2.6	13.4 $\pm$ 3.1
0.5	45.6 $\pm$ 7.5	39.5 $\pm$ 5.1
0.65	64.3 $\pm$ 5.0	48.3 $\pm$ 3.3*
0.7	78.3 $\pm$ 6.3	58.4 $\pm$ 3.1*
1.0	80.8 $\pm$ 9.5	78.1 $\pm$ 6.4

\*Significantly different,  $p < 0.05$ , from FRL rats.

pine (2 mg/kg) and then given various doses of oxotremorine (0.1–0.3  $\mu\text{mol/kg}$ ) to determine their cholinergic sensitivities for temperature and locomotor activity responses. These experiments confirmed that the FSL rats exhibited a greater hypothermic response to oxotremorine and showed increased sensitivity to its locomotor suppressant effects as described previously (21,23).

After an interval of one week, the sensitivity of the rats to various doses (0.5–1.0  $\mu\text{mol/kg}$ ) of apomorphine and an  $\text{ED}_{50}$  dose of quinpirole (3) was assessed. Stereotyped head-down sniffing was observed for a 10-min period beginning 15 minutes after the injection of apomorphine and 25 minutes after the injection of quinpirole. Following completion of the behavioural recordings, body temperatures were recorded and subtracted from previously recorded baselines.

A separate group of rats was injected with raclopride (20  $\mu\text{mol/kg}$ ) and catalepsy was measured using the vertical grid method (16). Rats were placed individually on a metal grid fixed at an angle of approximately  $70^{\circ}$  and the time taken for the rat to move was recorded. Rats remaining in their originally placed position for longer than 180 s were considered to be maximally cataleptic.

After all behavioural measures were taken, the rats were sacrificed by decapitation and the brains were removed and prepared for standard receptor binding assays or for quantitative autoradiography.

#### Data Analysis

The behavioural and physiological data were analysed by Analysis of Variance, followed by Newman-Keuls tests. The binding data were analysed by Student's *t*-tests.

## RESULTS

#### Behavioural Responses to Apomorphine

Table 1 shows the effect of apomorphine on stereotyped head-down sniffing in the two lines of rats. The sniffing response of the FSL rats to each dose of apomorphine is less than that of the FRL and the differences are statistically significant at doses of 0.65 and 0.75  $\mu\text{mol/kg}$ . This confirms our earlier preliminary study (9) and shows that the cholinergically supersensitive FSL rats appear to be subsensitive to the stereotypy-inducing effects of this mixed D1/D1 agonist.

#### Thermic Responses to Apomorphine

In contrast to the above conclusion, the hypothermic effects of

TABLE 2  
EFFECTS OF APOMORPHINE ON CORE BODY TEMPERATURE IN  
FSL AND FRL RATS

Dose ( $\mu\text{mol/kg}$ )	Decrease in Temperature (mean $^{\circ}\text{C} \pm \text{s.e.m.}$ )	
	FRL (n=8-10)	FSL (n=8-10)
0.5	0.31 $\pm$ 0.12	0.53 $\pm$ 0.23
0.65	0.62 $\pm$ 0.20	1.90 $\pm$ 0.13*
0.75	1.77 $\pm$ 0.21	3.00 $\pm$ 0.13*
1.0	2.64 $\pm$ 0.26	3.45 $\pm$ 0.23*

\*Significantly different,  $p < 0.05$ , from FRL rats.

apomorphine (Table 2) are significantly greater in the FSL rats compared to the FRL rats at doses greater than 0.65  $\mu\text{mol/kg}$ . Again, these findings are consistent with previous preliminary studies (9) and suggest that the cholinergically supersensitive FSL rats are also supersensitive to the hypothermic effects of dopamine agonists.

#### Behavioural and Thermic Response to Quinpirole

Since both the stereotyped head-down sniffing and hypothermic responses to apomorphine are mediated through the D2 receptor subtype (2), the responses of eight rats from each of the two lines to an  $\text{ED}_{50}$  dose of quinpirole (0.65  $\mu\text{mol/kg}$ ) were assessed. Although this dose produced an incidence of  $53.3 \pm 6.1\%$  sniffing in the FRL rats, the response in the FSL rats was significantly less ( $p < 0.02$ ) and an incidence of only  $28.8 \pm 3.1\%$  was observed. Thus, the FSL rats appeared to exhibit greater subsensitivity to the selective D2 agonist. The FSL rats also showed a significantly ( $p < 0.02$ ) greater drop in body temperature ( $1.94 \pm 0.24^{\circ}\text{C}$ ) to this dose of quinpirole than the FRL rats ( $1.17 \pm 0.16^{\circ}$ ).

#### Behavioural Responses to Raclopride

The cataleptic responses of six rats from each of both lines to raclopride (20  $\mu\text{mol/kg}$ ) were assessed. It was found that FSL rats stayed on the grid for a significantly ( $p < 0.01$ ) longer period ( $178.4 \pm 1.5 \text{ s}$ ) than did the FRL rats ( $57.8 \pm 14.8 \text{ s}$ ). At this dose of raclopride five out of the six FSL rats were maximally cataleptic, i.e., they stayed on the grid for 180 s, whereas none of the FRL rats were. At lower doses of raclopride the large intragroup variabilities observed precluded any statistical conclusions about the differences between lines.

#### Receptor Binding

Preliminary receptor binding studies suggested that the FSL rats had a small (12%) but significant decrease in striatal D2 receptors compared to the FRL rats (9). However, our present studies have failed to support this finding and there were no differences between the FSL and FRL rats. The  $B_{\text{max}}$  values were  $22.6 \pm 4.0$  (n=6) and  $26.4 \pm 2.4$  (n=6) pmol/mg protein, respectively, for the FSL and FRL rats.

Because of the apparent discrepancy between these and previous findings and the appreciation that there is an unequal distribution of D2 receptors in the striatum, we conducted quantitative autoradiographic assessment of D2 receptor concentration in the striatum. However, there were no clear-cut differences between

the FSL and FRL rats, either in whole striatum or in the localised region which has been implicated in the head-down sniffing response to dopamine agonists. Thus, the concentration of D2 receptors is not different in the FSL and FRL rats.

#### DISCUSSION

The pattern of findings in this study is quite different than previously observed in studies of the serotonergic and benzodiazepine-GABAergic systems (21,23). In those studies, the FSL rats were more sensitive to all of the observed effects of the respective agonists: m-CPP, diazepam, and muscimol. In contrast, the FSL rats were more sensitive to the hypothermic effects of dopamine agonists in the present study but were less sensitive to their stereotypy-inducing effects. These findings clearly eliminate a pharmacokinetic mechanism underlying the differences in sensitivity. Various pharmacodynamic models to account for these findings will be considered in subsequent paragraphs.

The present findings strongly suggest that the differences in dopamine sensitivity between the FSL and FRL rats are not related to the concentrations of dopamine receptors. Although our initial studies suggested a small difference in striatal receptors in the predicted direction, further more extensive studies, using both standard receptor binding assays and quantitative autoradiography, have failed to support such a conclusion. Thus, it is apparent that modification of cholinergic function can affect the responses to dopamine agonists without there being any changes in dopamine receptor concentrations. Another recent report from our laboratory that behavioural responses to dopamine agonists were modified following alkylation of muscarinic receptors in normal rats, in the absence of changes in dopamine receptor concentration, also supports this conclusion (10).

A consideration of the relative roles of dopamine and acetylcholine in the regulation of temperature and motor function may help to elucidate why dopamine sensitivity in cholinergically supersensitive rats is function-dependent. It has been widely known for some time that the dopaminergic and cholinergic are opposing systems in the control of motor function (4,15): while stimulation of dopaminergic neurons activates, stimulation of cholinergic neurons inhibits. Further, with regard to stereotyped behaviour elicited by dopamine agonist, it has been shown that such responses are decreased by administration of muscarinic agonists (1) and increased by muscarinic antagonists (22). Thus, if cholinergic tone is up, as appears to be the case in FSL rats, it might be expected that stimulation of dopaminergic neurons may have a reduced effect.

In contrast, stimulation of both dopaminergic and cholinergic neurons in the hypothalamic areas leads to hypothermia, i.e., they are parallel systems (7). Cox and Lee (6) provided strong evidence that the two systems are not in series, because the respective antagonists only antagonised the respective agonists. We have unpublished findings that haloperidol, a dopamine antagonist, does not alter the differential hypothermic effects of oxotremorine in the FSL and FRL rats. If the two systems are in parallel and synapse on some common neuron, then animals with excess cholinergic tone might be expected to show a greater than normal response to dopamine agonists, as was found in the present study. Thus, the functionally dependent changes in dopamine sensitivity in the FSL rats may be the consequence of the type of interaction between the dopaminergic and cholinergic systems in particular functions.

These findings have important implications for studies of neurotransmitter functions in human psychiatric conditions. The fact that multiple neurotransmitter systems appear to have changed in the cholinergically supersensitive FSL rats indicates that investi-

gators of psychiatric patients should not make premature conclusions after challenging these patients with a single drug. More complete information on multiple neurotransmitter systems should be obtained. It is also important to know how the specific neurotransmitter systems interact to mediate the particular function being studied.

In conclusion, the cholinergic supersensitive FSL rats also vary in their sensitivity to dopamine agonist stimulation, but the direction is dependent on the function: When dopamine and acetylcho-

line are opposing systems, e.g., for stereotypy, the FSL rats are subsensitive; when dopamine and acetylcholine are parallel systems, e.g., for temperature, the FSL rats are supersensitive.

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