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8. Since switching bees leave groove nectar when pool nectar is abundant, direct measurement of the time required to remove all nectar is not possible in the field. However, during each of the five 1-hour periods of observation from 0530 to 1030 hours, I know the mean standing crop of nectar per flower (N), the mean nectar removed per flower (R), and (T), the time required to remove that fraction of the total nectar per flower (mean time per flower—mean time per empty flower). By using the formula

$$\sum_{i=1}^{5} T_i(N_i - N_{i+1})/R_i$$

when $N_6 = 0$, I can calculate the time required to remove each fraction of the total nectar. The sum of these fractions is the feeding time required to empty a full flower. This formula assumes linear and time-independent extraction rates of pool and groove nectar. To check the validity of this approach, laboratory experiments were performed in which I measured foraging times of caged bees (starved for 1 hour) with single *C. linearis* flowers that contained measured quantities of 14.7 percent honey syrup. After each trial, flowers were checked for remaining nectar, but bees tested in this manner removed all nectar. Handling time (mean time spent on empty flowers) was subtracted from the total time spent per flower to give feeding time.
9. Theory (*4*) predicts that forager response to a food type will be all or nothing. The data from 0630 to 0930 hours suggest partial preferences, because bees still leave nectar even though the mean standing crop indicates that flowers contain only groove nectar. However, Fig. 2 shows means only. By 0700 hours, 66 percent of all flowers have been visited, but 34 percent are still full. Since the pollinator's only estimate of nectar availability comes from encounter frequency, one is not surprised that the mean response (nectar left) is not a step function of mean nectar available. These data do not allow an empirical test of this foraging problem.
10. The caloric cost of flight is 3.75 cal per 0.6-g queen per minute [B Heinrich, *J. Comp. Physiol.* **96**, 155 (1975); _____ and P. H. Raven, *Science* **176**, 597 (1972)]. If a bee is not producing additional heat for thermoregulation there is still a basic cost to nonflight foraging estimated to be 50 cal g^{-1} $hour^{-1}$. For the ambient temperatures encountered in this study (14° to 31°C) and the amount of cooling that would occur during a nonflight interval (1° to 2°C), the cost of thermoregulation is much less than the 0.5 cal per 0.6-g queen per minute of nonflight foraging. Since both of these costs result in the production of heat, for the purposes of these calculations, I have used that cost which is highest.
11. The caloric reward of nectar per flower is (microliter of nectar per flower) (0.147) (3.7 cal/mg) where 0.147 is the sugar concentration of desert willow nectar and 3.7 cal/mg is the caloric yield of glucose combustion.
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Dopamine Receptor Binding Enhancement Accompanies Lesion-Induced Behavioral Supersensitivity

Abstract. *The binding of [^{3}H]haloperidol to rat striatal dopamine receptors increases after lesion (made by injection of 6-hydroxydopamine) of the nigrostriatal dopamine pathway in those rats which are behaviorally supersensitive, as reflected by apomorphine-induced contralateral rotations. The enhanced binding is associated with an increased number of receptor sites with no change in their affinity.*

The behavioral changes that occur in rats in which specific lesions have been made in the nigrostriatal dopamine pathway suggest that postsynaptic dopamine receptors in the corpus striatum become supersensitive to dopamine after removal of their normal innervation. After bilateral lesions (induced by 6-hydroxydopamine) have been made in the dopamine cell bodies of the substantia nigra, rats display increased stereotyped behavioral responses to apomorphine, a dopamine receptor stimulant, and respond to previously subthreshold doses (*1*, *2*). After unilateral lesions (induced by 6-hydroxydopamine) have been made within the nigrostriatal system, behavioral supersensitivity is manifested by the animal rotating after treatment with apomorphine in a direction contralateral to the side of the lesion (*3*). This rotation provides a readily quantified index of behavioral supersensitivity (*4*). The nigrostriatal lesion induced by 6-hydroxydopamine may be a useful model of Parkinson's disease in which the nigrostriatal dopamine pathway is degenerated, and the supersensitivity of the dopamine receptors in the corpus striatum could account for the dramatic therapeutic response to β-(3,4-dihydroxyphenyl)-L-alanine (L-dopa) (*5*).

The enhanced behavioral response to dopamine receptor stimulants after nigrostriatal lesions have been induced might result, however, from changes distal to the dopamine receptor or in other neuronal systems. Alternatively, it could reflect a true alteration in the dopamine receptor itself. Activity of a striatal dopamine-sensitive adenylate cyclase, which appears to be associated with the dopamine receptor, has been reported to be unaffected by nigrostriatal lesions (*6*) or to show some enhanced activity (*7*). The response of striatal cells to iontophoretically applied dopamine and apomorphine is enhanced by nigrostriatal lesion (*8*). Recently, dopamine receptor binding has been demonstrated in brain membranes by labeling the receptor with both the agonist [^{3}H]dopamine and the antagonist [^{3}H]haloperidol (*9*). The binding sites of the two tritiated ligands have a similar regional distribution, their greatest densities occurring in brain regions with high dopamine levels (*10*). Dopamine agonists and phenothiazine antagonists have the same relative potencies in displacing both [^{3}H]dopamine and [^{3}H]haloperidol binding, indicating that both ligands label sites that have the characteristics expected of the dopamine receptor (*10*). However, it is only in displacing [^{3}H]haloperidol binding that the relative drug potencies for all classes of dopamine antagonists (phenothiazine, butyrophenone, thioxanthene, for example) parallel clinical and behavioral effects in man and animals (*11*). We have interpreted this result to indicate that the dopamine receptor may exist in two states, one of which has a high affinity for [^{3}H]dopamine and the other a high affinity for [^{3}H]haloperidol. The [^{3}H]haloperidol binding site [to which dopamine binds with an affinity in the 0.5 to 1.0 μ*M* range paralleling its EC_{50} (effective concentration for 50 percent stimulation) for stimulating adenylate cyclase activity] thus appears to be the physiologically active form of the dopamine receptor while [^{3}H]dopamine may be labeling a high-affinity, desensitized, and perhaps physiologically inactive form of the dopamine receptor (*12*). We now report enhanced dopamine receptor binding of [^{3}H]haloperidol in the corpus striatum of rats in which lesions of the nigrostriatal pathway have been made with 6-hydroxydopamine.

Binding assays were performed as described (*10*). Homogenates (Brinkmann Polytron) of fresh rat corpus striatum in cold tris buffer, *p*H 7.7 at 25°C, were washed twice by centrifugation. The final pellet was resuspended in cold 50 m*M* tris buffer containing 0.1 percent ascorbic acid, 10 μ*M* pargyline, and ions as follows: 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ (giving a final *p*H of 7.1 at 37°C). This mixture was warmed to 37°C for 5 minutes and returned to ice. Each tube received 1.0 ml of tissue suspension (4 to 6.4 mg, wet weight) and contained 0.2 to 4 n*M* [^{3}H]haloperidol (9.6 c/mmole; Janssen Pharmaceutica). The tubes were incubated at 37°C for 10 minutes and triplicate 0.3-ml portions were rapidly filtered under vacuum through Whatman G F/B filters with three 5-ml rinses of cold buffer. The filters were counted by liquid scintillation spectrometry. Specific binding of [^{3}H]haloperidol, measured as the excess over blanks containing 100 μ*M* dopamine, represented about half of the total binding. Previous experiments have demonstrated that 100 μ*M* dopamine displaces [^{3}H]haloperidol binding to the same extent as the maximum stereo-

specific displacement by the isomers of butaclamol. Moreover, these maximum displacements are not additive, indicating that both drugs are displacing [^{3}H]haloperidol from the same sites (*10*).

Sprague-Dawley rats (150 g) were used. Lesions of the nigrostriatal pathway were induced with 6-hydroxydopamine as described previously (*1*), except that the injection site was just rostral to the substantia nigra [coordinates AP + 3.2 mm, L 1.4 mm, V 7.6 mm (*13*)] and 3 μl of 6-hydroxydopamine (4 μg of free base per microliter) were injected.

Two to seven months after the lesions were made, all the rats were injected subcutaneously with apomorphine (0.25 mg/kg), and motor rotation was measured manually 10 minutes later for 3 minutes in an opaque plastic hemisphere (38 cm in diameter). The rats showed a variable degree of contralateral rotation after the lesion was made, presumably reflecting the success of the lesioning procedure. The binding of [^{3}H]haloperidol in the striatum was assayed between 1 and 10 weeks after the behavioral tests were conducted.

Behaviorally supersensitive rats, defined as those exhibiting at least six contralateral rotations per minute (mean rate, 16 ± 1 turns per minute), showed a mean increase in [^{3}H]haloperidol binding of 50.0 ± 5.5 percent (range, − 3.0 to 117 percent, N = 27) on the lesioned side compared to their own contralateral unlesioned striatum. In some of the rats the binding was more than doubled on the lesioned side. Rats that did not turn displayed essentially no augmentation in binding on the lesioned side (mean, 7.6 ± 5.5 percent, range, − 19 to 20 percent, N = 10). Figure 1 shows that with increasing receptor "supersensitivity" there is a concomitant increase in behavioral "supersensitivity." However, rotational behavior does not increase in direct proportion to the augmentation of receptor binding. The apparent decrease in behavioral supersensitivity in the rats with the most pronounced increase in receptor binding may be more apparent than real. The rats with the highest levels of receptor binding showed greater stereotyped gnawing of their fore and hind contralateral paws and tail, and this caused them to fall and stumble so that their rotational rate was physically decreased. There was no apparent trend for the rats that had lesions for the longest periods to show a degree of rotation or increase in [^{3}H]haloperidol binding on the lesioned side greater than that for those rats that had lesions for shorter periods. Previous behavioral studies have also demonstrated that the greatest increase in behavioral supersensitivity occurs within the first 3 to 4 weeks after lesions have been made (*3*).

Table 1. Effect of unilateral lesions (induced by 6-hydroxydopamine) in the nigrostriatal dopamine pathway on the binding of [^{3}H]haloperidol in the rat. The rats were injected unilaterally with 6-hydroxydopamine in the substantia nigra. At least 2 months after the lesions were made, the left and right striatum of each rat were assayed separately with four concentrations of [^{3}H]haloperidol between 0.4 and 4 nM. The K_d and B_{max} (maximum number of binding sites) values were calculated from Scatchard plots of [^{3}H]haloperidol binding data and analyzed by t-tests (two tailed) of the paired differences between the lesioned and control striatum of each rat.

Tissue	K_d (nM)	B_{max} (pmole/g*)
Control striatum†	0.69 ± 0.08	16.1 ± 1.1
Lesioned striatum†	0.75 ± 0.07	22.7 ± 1.2
Paired differences	0.06 ± 0.08‡	6.6 ± 1.2§

*Wet weight of tissue. †N = 24. ‡Not significant. §Significant at $P < .001$.

Increased [^{3}H]haloperidol binding associated with dopamine receptors could be caused by a change in the affinity of binding or by a change in the number of binding sites. To discriminate between these two possibilities, we assayed each corpus striatum for [^{3}H]haloperidol binding at four concentrations of the tritiated ligand (0.4 to 4.0 nM) (Table 1). Again, only rats that exhibited at least six contralateral turns per minute (mean 16 ± 1, range 6 to 31) to injections (0.25 mg/kg) of apomorphine were included. The dissociation constant (K_d) was unaffected by the lesion (Table 1), whereas a highly significant 40 percent increase in the number of binding sites was apparent in the lesioned striata ($P < .001$).

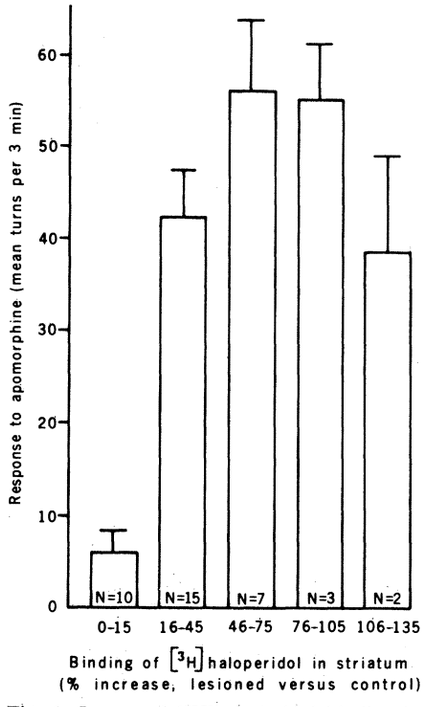

Fig. 1. Increased [^{3}H]haloperidol binding in the lesioned striatum and behavioral supersensitivity to apomorphine in rats with unilateral nigrostriatal lesions induced by 6-hydroxydopamine. Rotational behavior in response to subcutaneous injection of apomorphine (0.25 mg/kg) was measured 2 to 7 months after the lesions were made. The binding of [^{3}H]haloperidol was assayed in the lesioned and control striatum of each rat separately 1 to 10 weeks later and is expressed as the percentage increase in radioactivity (counts per minute) of specific [^{3}H]haloperidol bound in the lesioned compared with the control striatum at four concentrations of [^{3}H]haloperidol (0.4 to 4.0 nM).

It is conceivable that depletion of endogenous dopamine by the lesion could reduce the amount of dopamine in the striatal membranes available to compete for the binding of [^{3}H]haloperidol, thus producing only an apparent increase in binding. This is unlikely for several reasons. The striatal membranes used for [^{3}H]haloperidol binding assays were washed twice and subjected to over a 300-fold dilution in buffer for homogenization and assay, so that the amount of dopamine in the membranes was very reduced (*14*). Moreover, if this increased [^{3}H]haloperidol binding was simply caused by the depletion of endogenous dopamine on the lesioned side, this would be reflected by a change in the K_d, and not in the number of the binding sites. To determine whether short-term depletion of endogenous dopamine could change [^{3}H]haloperidol binding, we injected groups of six control rats with reserpine (5 mg/kg) or saline and assayed [^{3}H]haloperidol binding in the striatal membranes 18 hours after drug administration. The K_d values for the control rats and the rats injected with reserpine were 0.65 ± 0.11 and 0.61 ± 0.04 nM, respectively, and the number of binding sites were 27.7 ± 2.2 and 29.6 ± 0.9 pmole per gram of tissue, respectively. Thus a dose of reserpine that causes more than a 90 percent depletion of endogenous dopamine (*15*) failed to alter significantly the affinity or number of binding sites for [^{3}H]haloperidol. However, if the dopamine depletions were maintained by long-term treatment with reserpine (0.25 mg/kg per day) for 3 weeks, [^{3}H]haloperidol binding was increased by 25 percent (*16*), this effect again paralleling the enhanced behavioral sensitivity to apomorphine that occurs at this time (*17*). The apparent lower number of total binding sites in the control striata of the lesioned rats compared

to the number of sites in the control rats from the short-term reserpine experiment is a function of age. We have previously demonstrated that [^{3}H]haloperidol binding significantly decreases with age (*18*), and the lesioned rats in the present experiments were 2 to 7 months older than the rats used in the reserpine experiment. In the rats with unsuccessful lesions (such rats may be considered equivalent to sham-injected rats of the same age), binding in their control striata (B_{max} = 18.7 ± 2.3 pmole per gram of tissue, N = 10) was comparable to that in the unlesioned striata of the behaviorally supersensitive rats. This indicates that rotation is associated with an increase in [^{3}H]haloperidol binding on the lesioned side rather than a decrease in binding on the control side.

The major finding of this study is that [^{3}H]haloperidol binding sites increase in rats with lesions of the nigrostriatal dopamine pathway. This increased number of dopamine receptors could account for the behavioral supersensitivity to dopamine agonists which results from the lesion. The increased sensitivity to apomorphine in rats with bilateral lesions of the nigrostriatal pathway (in which the same behavioral response can be measured before and after the lesions are made) is many times greater than the 20 to 120 percent increase in binding seen here (*1*). This indicates that other components in the overall system determining the behavioral response may also be changed by the lesion and thus produce additive effects. However, measurements of striatal dopamine-sensitive adenylate cyclase after nigrostriatal lesions have been made have proved equivocal (*6*, *7*). In studies of butyrophenone neuroleptic drugs we have also observed a similar poor correlation between their influences on dopamine receptor binding and on the cyclase (*11*). Recently, Kelly and Moore (*19*) have demonstrated that although effects of 6-hydroxydopamine in the caudate nucleus determine the direction of rotation and the sensitivity to apomorphine, the nucleus accumbens is also involved in determining the rate of rotation. Since lesions induced by 6-hydroxydopamine in the region of the substantia nigra probably destroy the dopamine innervation in the nucleus accumbens because they also destroy the neighboring A10 cell bodies, the apparent greater behavioral supersensitivity as compared to augmented receptor binding may result from enhanced receptor activity in the nucleus accumbens as well as the caudate.

Behavioral supersensitivity to apomorphine also occurs in rats subjected to long-term treatment with neuroleptic drugs, and such rats provide a model for tardive dyskinesia, the increased bizarre motor activity that occurs in patients treated for long periods with neuroleptics (*17*). The behavioral supersensitivity to apomorphine in such rats is also associated with enhanced [^{3}H]haloperidol binding to dopamine receptors, resulting from an increased number of binding sites with no change in K_d value (*16*).

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Electrochemical Concentration Cells

I would like to add a few thoughts on the extraction of energy from seawater by means of electrochemical concentration cells (*1*). A few simple analyses will illustrate the critical role of membrane resistance in any practical system for the extraction of energy from seawater based on the use of concentration cells.

In the analysis by Clampitt and Kiviat, R_i, the internal cell resistance, is taken to be the resistance of the low-concentration (freshwater) side of the membrane. However, if one considers a reasonable geometry comprising closely spaced parallel plates separated by intervening membranes and electrolyte compartments, the following relationship describes R_i:

$$R_i = (n/a)(r_m d_m + r_{C_A} d_{C_A} + r_{C_B} d_{C_B}) \quad (1)$$

where n is the number of concentration cells in series (as in a voltaic pile); a is the area of the membrane (assuming electrode plates and membranes of equal areas); d_m is the membrane thickness; d_{C_A} and d_{C_B} are the electrode-to-membrane distances on each side of the membrane; and r_m, r_{C_A}, and r_{C_B} are the specific resistivities of the membrane and each solution.

The maximum theoretical power of a series of such "seacells" based on Na^+ transport is

$$P_{max} = \frac{2an\left[\frac{t_{Na}RT}{F}\ln\left(\frac{C_A\gamma_A}{C_B\gamma_B}\right)\right]^2}{d_m r_m + 1000(H_A + H_B)}$$

where $H_A = d_{C_A}(\Lambda_\infty C_A - bC_A^{1.5})^{-1}$ and $H_B = d_{C_B}(\Lambda_\infty C_B - bC_B^{1.5})^{-1}$; in Eq. 2 t_{Na}