primary role in the orientation of foragers of *P. tarsatus*.

We can tentatively conclude that when *P. tarsatus* workers leave the exits they take a "snapshot picture" (*8*) of the surroundings of which the canopy is a significant part. The ants then orient within the coordinate system of this picture. If sufficient information is unavailable from this picture, the ants fall back on a second system, possibly chemical (*7*). Such an orientation mechanism of course limits the range of accurate orientation. In fact, the home range of *P. tarsatus* foragers above ground is not greater than about 5 m and is usually considerably smaller ($\bar{X}$ = 1.9 m, S.D. = 1.4, N = 25). Marking and recapture tests indicate that individual ants show a high fidelity in the use of specific exits. The ants travel along subterranean tunnels to these exits. What was originally considered to be nest entrances are actually exits of foraging tunnels that lead from the central nest area well into the foraging grounds. Only after the ants have left the tunnels do they conduct the individual foraging excursions during which they are guided by visual orientation cues.

Canopy orientation, which appears to have escaped attention previously, is well suited to the peculiarly restrictive lighting conditions of tropical forests. It seems likely to occur in other forest-dwelling insects and perhaps even in some vertebrates.

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6. The fieldwork was carried out in the Shimba Hills Wild Life Reserve, Kenya, during June, July, and August 1978.
7. Foragers of *P. tarsatus* can lay chemical recruitment and orientation trails with sternal gland secretions [B. Hölldobler and H. Engel, *Psyche* **85**, 285 (1979)].
8. The mechanism might be similar to that originally suggested by K. V. Frisch for the honey bees' ability to use polarized light to locate the sun. Recent data, obtained by J. L. Gould and his collaborators (personal communication), tend to support the photograph hypothesis.
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Long-Term Antidepressant Treatment Decreases Spiroperidol-Labeled Serotonin Receptor Binding

Abstract. *Antidepressants compete at several neurotransmitter receptor binding sites, but drug affinities do not correlate with clinical efficacy. Long-term, but not short-term, antidepressant treatment decreases the numbers of both serotonin and β-adrenergic receptors. The decrease in the number of receptor sites is most marked for [^{3}H]spiroperidol-labeled serotonin receptors and is characteristic for antidepressants of several classes.*

The behavioral depression following biogenic amine depletion by reserpine in animals and man and the potentiation of amine action by antidepressants suggest a functional deficiency of biogenic amines in depressive disorders (*1*). Although the actions of norepinephrine and serotonin are potentiated by tricyclic antidepressants and monoamine oxidase (MAO) inhibitors, these effects occur immediately, whereas there is a 1- to 3-week lag before therapeutic response (*2*). Moreover, drugs such as cocaine block the uptake of amines but are not clinically effective antidepressants (*3*), and drugs such as iprindole relieve depression but do not inhibit amine uptake (*4*). Antidepressant agents are also fairly potent blockers of muscarinic cholinergic (*5*), α-adrenergic (*6*), histamine (*7*), and serotonin (*8*) receptors. However, receptor blockade also occurs immediately and thus does not correlate with the time course of therapeutic responses. In contrast, long-term, but not short-term, treatment with antidepressants reduces the activity of a norepinephrine-stimulated adenylate cyclase in brain tissue (*9*) and reduces the number of β-adrenergic receptor sites in brain membranes (*10–12*). We now report that long-term treatment with tricyclic antidepressants, the atypical antidepressant iprindole, and the MAO inhibitor pargyline decreases the number of serotonin receptors labeled by [^{3}H]spiroperidol.

Male Sprague-Dawley rats (6 weeks old, weighing 150 to 175 g) were given daily 0.5-ml intraperitoneal injections of various drugs or saline for 21 days. Doses of all drugs were 10 mg/kg, except for pargyline (25 mg/kg). Twenty-four hours after the final injection, rats were decapitated; the brains were removed, dissected over ice, and stored at −70°C until assay. For studies of drug competiton, the fresh brains of rats that had not been given injections were used immediately for assay. The frontal cerebral cortex was used for all of the binding studies except the binding of [^{3}H]-spiroperidol to dopamine receptors, for which the corpus striatum was used. The tissues were homogenized in 10 volumes of 0.32M sucrose and centrifuged at 700g for 10 minutes. The P_1 fraction was discarded and the supernatant was centrifuged at 20,000g for 10 minutes; the sediment was suspended in tris-HCl buffer (pH 7.7 at 25°C) and centrifuged again at 20,000g for 10 minutes. The sediment was then suspended in 80 volumes of tris-HCl buffer, and the tissue was immediately used in binding assays. Briefly, 0.1 ml of ^{3}H-labeled ligand, 0.1 ml of displacing drug, and 0.8 ml of tissue suspension were incubated for varying periods (*13, 14*). The reaction was terminated by rapid filtration under vacuum through Whatman GF/B filters, which were washed three times with 5 ml of tris-HCl buffer. The labeled material retained on the filters was counted by liquid scintillation spectrometry.

The antidepressants examined compete for several receptor binding sites (Table 1). In general, the greatest potencies occur at histamine H_1 receptors labeled by [^{3}H]mepyramine. However, relative potencies of drugs at these binding sites do not correlate with clinical potencies; amitriptyline is used clinically at dosages similar to those of desipramine, but at histamine H_1 receptors, it is 75 times more potent. The antidepressants are fairly potent at muscarinic cholinergic receptors labeled by [^{3}H]quinuclidinylbenzilate ([^{3}H-]QNB), which correlates well with clinical anticholinergic side effects (*5*). Relative potencies of tricyclic antidepressants at α-adrenergic receptors labeled by [^{3}H]WB-4101 (2-([2,6-dimethoxy]phenoxyethylamino)-methylbenzodioxan) are similar to effects at muscarinic receptors and correlate with sedation and relief of psychomotor agitation (*6*). Two distinct populations of serotonin receptors are labeled, one with [^{3}H]serotonin and the other with [^{3}H]spiroperidol; [^{3}H]lysergic acid diethylamide ([^{3}H]LSD) labels both sites to the same extent (*14*). Antidepressants are much more potent at the [^{3}H]spiroperidol-labeled receptors (serotonin-2) than at the [^{3}H]serotonin-labeled receptors (serotonin-1), effects on [^{3}H]LSD binding being intermediate. The antidepressants are substantially less potent at dopamine receptors labeled by [^{3}H]spiroperidol in the corpus

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Table 1. Antidepressant affinities (K_i) for neurotransmitter receptor binding sites. For each drug, inhibition of ^{3}H-labeled ligand binding was measured at four concentrations, and the median inhibitory concentration (IC_{50}) was determined by log-probit analysis. Apparent K_i values were calculated from the equation $K_i = IC_{50}/(1 + [^3\text{H-ligand}]/K_D)$, where K_D is the dissociation constant of the ^{3}H-labeled ligand (*12, 13*). Each value is the mean ± standard error of three to six experiments, each conducted in triplicate.

Drug	K_i (n*M*)							
	Histamine H_1 ([^{3}H]mepyramine)	Muscarinic cholinergic ([^{3}H]QNB)	α-Adrenergic ([^{3}H]WB-4101)	Serotonin-1 ([^{3}H]serotonin)	Serotonin-1 + serotonin-2 ([^{3}H]LSD)	Serotonin-2 ([^{3}H]spiroperidol)	Dopamine ([^{3}H]spiroperidol)	β-Adrenergic ([^{3}H]DHA)
Tertiary amines								
Amitriptyline	3.2 ± 0.6	11 ± 2.1	22 ± 4.0	1,700 ± 70	170 ± 60	13 ± 2.8	290 ± 77	6,800 ± 360
Imipramine	18 ± 3.9	89 ± 20	54 ± 17	10,000 ± 3,400	1,800 ± 600	245 ± 69	610 ± 170	38,000 ± 2,000
Secondary amines								
Desipramine	240 ± 19	210 ± 37	130 ± 29	9,500 ± 3,000	3,200 ± 420	540 ± 190	980 ± 270	4,200 ± 95
Nortriptyline	27 ± 5.3	81 ± 21	70 ± 19	640 ± 150	310 ± 79	41 ± 17	800 ± 200	15,000 ± 3,700
Atypical antidepressants								
Iprindole	110 ± 8	37,000 ± 2,200	9,600 ± 480	21,000 ± 7,700	12,000 ± 1,600	1,900 ± 480	6,300 ± 2,100	21,000 ± 4,300
Fluoxitene	780 ± 74	13,000 ± 360	8,000 ± 1,200	7,400 ± 450	6,700 ± 2,500	1,300 ± 250	6,600 ± 1,300	11,000 ± 720
Neuroleptics								
Haloperidol	1,600 ± 730	110,000 ± 28,000	14 ± 2.3	16,000 ± 2,000	960 ± 210	45 ± 2.5	4.2 ± 0.80	28,000 ± 940
Chlorpromazine	28 ± 6.8	5,700 ± 1,700	4.3 ± 1.1	3,500 ± 930	270 ± 71	15 ± 1.9	25 ± 4.0	13,000 ± 1,200

Table 2. Effect of long-term drug treatment on neurotransmitter receptor binding in rat brain. The relative amount of ^{3}H-labeled ligand bound (saline control = 100) was determined in homogenates of rat brain after 21 consecutive days of treatment with equivalent volumes of drugs or saline. Each value is the mean ± standard error from three to six separate animals. The experiment was replicated twice. The concentrations of the ligands were 0.36 n*M* [^{3}H]spiroperidol, 3.7 n*M* [^{3}H]LSD, 2.0 n*M* [^{3}H]serotonin, 0.8 n*M* [^{3}H]DHA, 0.2 n*M* [^{3}H]QNB, and 0.9 n*M* [^{3}H]WB-4101. Specific binding was defined as the excess over blanks taken in the presence of 1 μ*M* *d*-LSD for serotonin receptors, 1 μ*M* (±)-propranolol for β-adrenergic receptors, 0.1 m*M* oxotremorine for muscarinic cholinergic receptors, 0.1 m*M* (−)-norepinephrine for α-adrenergic receptors, and 1 μ*M* (+)-butaclamol for dopamine receptors. All experiments were performed in triplicate. Student's *t*-test was used for statistical analysis of the raw data before conversion to percentages.

Drug	Serotonin-2 ([^{3}H]spiroperidol)	Serotonin-1 + serotonin-2 ([^{3}H]LSD)	Serotonin-1 ([^{3}H]serotonin)	β-Adrenergic ([^{3}H]DHA)	Muscarinic cholinergic ([^{3}H]QNB)	α-Adrenergic ([^{3}H]WB-4101)	Dopamine ([^{3}H]spiroperidol)
Tricyclic antidepressants							
Amitriptyline	57 ± 3.0*	80 ± 1.7*	90 ± 3.7	82 ± 3.9†	103 ± 2.6	97 ± 6.8	101 ± 4.5
Imipramine	60 ± 4.5*	74 ± 7.0†	80 ± 3.5†	82 ± 3.5†	100 ± 0.72	104 ± 6.1	96 ± 4.4
Desipramine	79 ± 4.1*	82 ± 5.2†	94 ± 2.6	71 ± 6.2†	105 ± 1.9	97 ± 8.8	97 ± 4.2
Atypical antidepressants							
Iprindole	66 ± 4.1*	79 ± 6.6†	92 ± 6.4	88 ± 1.9†	95 ± 2.2	107 ± 3.8	100 ± 2.8
Fluoxitene	87 ± 6.3	92 ± 9.7	98 ± 3.8	97 ± 5.8	97 ± 2.1	102 ± 4.3	99 ± 4.0
MAO inhibitor							
Pargyline	65 ± 5.8*	65 ± 0.65*	58 ± 2.7*	85 ± 3.1†	101 ± 0.54	97 ± 2.9	104 ± 1.5
Serotonin antagonist							
Methysergide	90 ± 7.2	95 ± 1.2	102 ± 1.3	98 ± 3.9	104 ± 6.3	100 ± 2.6	100 ± 6.0
Neuroleptics							
Chlorpromazine	90 ± 4.7	90 ± 4.4	105 ± 7.4	100 ± 4.2	100 ± 2.8	102 ± 1.5	132 ± 2.0*
Haloperidol	98 ± 6.1	102 ± 4.5	93 ± 3.2	102 ± 5.4	102 ± 0.79	101 ± 2.7	139 ± 6.4*

*$P < .01$. †$P < .05$.

striatum and at β-adrenergic receptors labeled by [^{3}H]dihydroalprenolol ([^{3}H]DHA) than at any other receptor binding sites. The neuroleptics haloperidol and chlorpromazine have considerable affinities for several receptors, being similar to the more active antidepressants at α-adrenergic and serotonin-2 sites and the most potent drugs at dopamine receptors. Antidepressant potencies also vary widely at the [^{3}H]imipramine binding sites described by Raisman *et al.* (*15*). Blockade of any one of these receptors cannot simply account for antidepressant efficacy.

Long-term treatment with the tricyclic antidepressants amitriptyline, imipramine, and desipramine, the atypical antidepressant iprindole, and the MAO inhibitor pargyline reduces the binding of [^{3}H]DHA to β-adrenergic receptors; these results confirm and extend those previously reported (*10–12*) (Table 2). A substantially greater reduction of [^{3}H]spiroperidol binding to serotonin-2 receptors occurs after long-term treatment with antidepressants. Amitriptyline and imipramine reduce [^{3}H]DHA binding by 20 percent, but reduce [^{3}H]-spiroperidol binding to serotonin-2 receptors by 40 percent. Iprindole and pargyline also reduce binding to serotonin-2 receptors more than they reduce [^{3}H]DHA binding. The secondary amine desipramine is the only antidepressant tested in which the decline in [^{3}H]DHA binding (29 percent) exceeds the decline in binding to serotonin-2 receptors (21 percent). The reductions in [^{3}H]DHA binding observed here are similar to those obtained by others (*11, 12*) at the same doses and durations of treatment. More prolonged treatment may elicit a greater reduction of both [^{3}H]DHA (*10*) and [^{3}H]spiroperidol (*16*) binding. In marked contrast, binding to β-adrenergic and serotonin receptors is unaffected by the serotonin antagonist methysergide or the neuroleptics chlorpromazine and haloperidol. Fluoxitene, a potent inhibitor of serotonin but not of norepinephrine uptake, whose antidepressant efficacy is questionable (*17, 18*), does not affect [^{3}H]DHA or [^{3}H]spiroperidol binding.

The binding of [^{3}H]serotonin to serotonin-1 receptors also declines with chronic administration of imipramine and pargyline, but not with any other antidepressant; these results resemble those of others (*12, 19*) and suggest that effects on serotonin-1 receptors are unrelated to antidepressant efficacy. Consistent with its labeling of both serotonin-1 and serotonin-2 receptors (*14*), [^{3}H]LSD binding is affected by long-term treatment with antidepressants in a fashion intermediate to effects on [^{3}H]spiroperidol and [^{3}H]serotonin binding. In contrast to the significant reductions of serotonin and β-adrenergic receptors, long-term drug treatment has no effect on muscarinic cholinergic receptors labeled by [^{3}H]QNB or α-adrenergic receptors labeled by [^{3}H]WB-4101. Binding to dopamine receptors labeled in the corpus striatum by [^{3}H]spiroperidol is increased by neuroleptic treatment (*20*), but is unaffected by antidepressants.

Decreases in the binding of ^{3}H-labeled ligand may be due to changes in the number of sites or in the dissociation constant (K_D). Scatchard analysis of the binding of [^{3}H]spiroperidol at a wide range of concentrations indicates that the antidepressants decrease the number of serotinin-2 binding sites with no change in the affinity constant (data not shown). Similarly, a reduction in the number of [^{3}H]DHA binding sites with no change in K_D has been reported after antidepressant treatment (*10–12*). It is unlikely that changes in receptor binding are due to residual drug in the brain, since short-term injections of antidepressant fail to alter the binding to serotonin-2 or β-adrenergic receptors 24 hours after a single dose (*16*).

Clinically effective antidepressants have substantial affinity for a number of neurotransmitter receptor binding sites, although no correlation exists with clinical dosages. However, long-term treatment with antidepressant reduces binding to both serotonin-2 and β-adrenergic receptors, the reduction in the binding to serotonin-2 receptors being the more marked for all drugs except desipramine. Moreover, this effect appears selective, because fluoxitene, the serotonin antagonist methysergide, and the neuroleptics chlorpromazine and haloperidol fail to alter serotonin-2 or β-adrenergic binding. In neurophysiological studies (*18*), long-term treatment with antidepressant increases neuronal responsiveness to the inhibitory effect of serotonin. It is unclear whether this effect is related to the decrease in binding to serotonin-2 receptors. The decrease in the binding to β-adrenergic receptors is correlated with a decrease in the sensitivity of the β-adrenergic-sensitive adenylate cyclase (*11*).

How might these findings fit with other data on the pharmacology of depression? Depletion of monoamines by reserpine is associated with clinical depression (*1*). The tricyclic antidepressant blockade of uptake systems and the inhibition of MAO by pargyline both enhance amine activity. Though iprindole is considered an atypical antidepressant because of its failure to block uptake and to inhibit MAO, it presumably potentiates central biogenic amines, since it increases the central effects of amphetamine (*21*). Subsensitivity of serotonin-2 and β-adrenergic receptors might be secondary to chronic enhancement of amine activity. Whether the antidepressant actions stem primarily from effects on synaptic monoamines or from changes in receptor sensitivity is not clear. However, the fact that long-term treatment is required to elicit changes in receptor density whereas enhancement of monoamine action occurs immediately favors the role of receptor sensitivity in mediating antidepressant action.

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