ter mechanisms involved with receptor recognition sites and cyclic nucleotide coupling (*22*).

Clearly, more work is needed to identify which factors contribute to the behavioral effects induced by choline alterations, and how these factors are related to similar changes occurring naturally with age. By examining these relations, it might be possible to gain some insight into the etiology of the neurobehavioral impairments that occur with old age, and how they might be alleviated.

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14. Purified diet 785 (Bio-Serv) was used for the deficient group. Independent blind bioassays (Raltech) revealed less than 1 mg of choline per gram of chow in the deficient diet and 12 to 15 mg of choline per gram of chow in the enriched diet. Control chow (Purina) was estimated to contain approximately 1.6 mg of choline per gram.
15. Drinking water for the control groups was supplemented with choline chloride (1.5 mg/ml), making their daily consumption of choline normal. The choline-enriched group was given 4.0 mg of choline per milliliter of water, or 2.67 times the normal amount. Mice in all three groups consumed approximately 5 ml of water per day. Chow consumption averaged approximately 3 g per mouse per day. The mean weight of the mice during the last month of the study was 40 g.
16. An analysis of variance across the three choline conditions yielded F (2, 24) = 57.1, $P < .0001$; individual t-tests demonstrated significant effects of both choline-deficient and choline-enriched conditions [for choline controls (mean latency, 218.4 seconds) versus choline-deficient mice (mean latency, 65.7 seconds), t (12) = 6.70, $P < .001$; for choline controls versus choline-enriched mice (mean latency, 277.7 seconds), t (12) = 2.60, $P < .02$].
17. It has already been documented that the activity of choline acetyltransferase and the number of muscarinic receptor binding sites decrease significantly in the aged brain (*5*). Either of these factors could contribute to a dampened response to precursor availability. Still other possibilities involve unknown dysfunctions in postsynaptic, cyclic nucleotide coupling mechanisms (*6*) or in presynaptic choline uptake mechanisms.
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Tritiated Imipramine Binding Sites Are Decreased in Platelets of Untreated Depressed Patients

Abstract. *The high-affinity binding of tritiated imipramine to platelet membranes was compared in samples from 16 untreated depressed women and 21 age-matched controls of the same sex. The maximal binding in the depressed group was significantly lower than that of the controls, although the affinity constants were similar. These results suggest that binding of tritiated imipramine in human platelets may represent a biochemical index of depression, possibly reflecting similar changes in the brain.*

Until recently, tricyclic antidepressant drugs were thought to act by inhibiting neuronal uptake of monoamines (*1*). This monoamine hypothesis was challenged by the advent of "atypical" antidepressant drugs, such as iprindole, that do not inhibit neuronal monoamine uptake (*2*). Furthermore, the demonstration that tricyclic antidepressant drugs can have direct postsynaptic effects on β-adrenoceptor-linked adenyl cyclase (*3*) suggested that their actions are more complex than previously supposed (*4*).

The high-affinity binding site for [^{3}H]imipramine possesses many of the properties expected for the site of action of tricyclic antidepressant drugs (*5*), thus providing a possible direct approach to the study of the mode of action of these drugs. Recent work has shown that high-affinity binding sites for [^{3}H]imipramine exist in human platelets and that their properties are apparently identical to those of high-affinity binding sites for [^{3}H]imipramine in rat brain (*6*). These findings make it possible to test the working hypothesis that the high-affinity binding site for [^{3}H]imipramine may be involved in the pathogenesis of depression. An essential requirement for such a study is the availability of a homogeneous population of clearly diagnosed depressed patients who have had no antidepressant treatment.

Previously we had shown that maximal binding of [^{3}H]imipramine in human platelets decreases with age in a control population, whereas the values of affinity constants do not change with age (*7*). In this study, we compared a group of female volunteers (20 to 65 years old) with 16 untreated depressed female patients of the same age range and demonstrated that the maximal binding of [^{3}H]imipramine is significantly decreased in the depressed patients, while the affinity constant is unaltered.

Platelet membranes were prepared as already described (*6*). Twenty milliliters of blood was withdrawn by antecubital venipuncture and collected into plastic tubes containing sodium citrate (final concentration, 0.38 percent). Platelets were obtained from platelet-rich plasma by centrifugation at 16,000*g* for 10 minutes. The platelets were washed twice with buffer (5 m*M* tris-HCl, 20 m*M* EDTA, 150 m*M* NaCl, *p*H 7.5), and membranes were prepared by hypotonic lysis (5 m*M* tris-HCl, 5 m*M* EDTA, *p*H 7.5), homogenization (Polytron), and centrifugation at 39,000*g* for 10 minutes.

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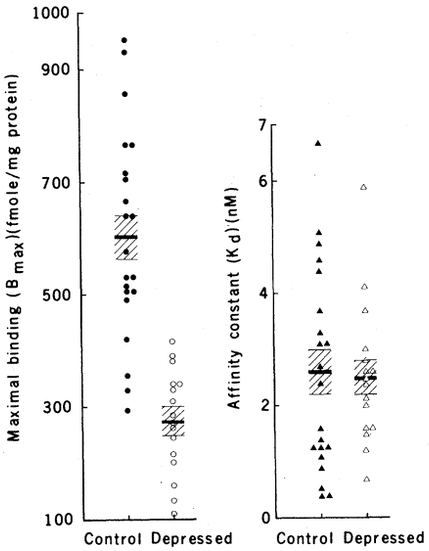

Fig. 1. Comparison of maximal binding (B_{max}) and affinity constant (K_d) for [^{3}H]imipramine in platelet membranes from control and depressed subjects. Each point represents an individual subject. Binding parameters were calculated by Scatchard analysis with at least six concentrations of [^{3}H]imipramine between 0.4 and 8 n*M*. Solid lines and hatched areas represent the means ± standard errors of each group. The difference in B_{max} values is statistically significant ($P < .001$).

The pellet was washed with buffer (70 m*M* tris-HCl, *p*H 7.5) and finally resuspended in buffer (50 m*M* tris-HCl, 5 m*M* KCl, 120 m*M* NaCl, *p*H 7.4) at a concentration of 0.7 to 0.9 mg of protein per milliliter.

[^{3}H]Imipramine binding was carried out as previously described (*5*), with concentrations of 0.4 to 8 n*M* [^{3}H]-imipramine. Specific binding was defined as that inhibited in the presence of 100 μM desipramine and represented 70 percent of the total binding at 5 n*M* [^{3}H]imipramine.

Control donors (21 females) were between 25 and 64 years old and had a mean age of 41.1 ± 2.8 years. Only donors receiving no psychoactive medication in the past month were included in the study. All blood samples were taken between 9 and 10 a.m. from August 1979 to February 1980. The donors did not fast. For both control and depressed donors, platelet membranes were prepared between 30 minutes and 1 hour after sampling, and [^{3}H]imipramine binding was measured the same day.

Clinical data on the 16 female depressed patients are given in Table 1. Patients were between 23 and 65 years old and had a mean age of 44.8 ± 3.5 years. The criterion for inclusion in the study was a clear diagnosis of depression (either mono- or bipolar endogenous or reactive) of sufficient severity to require hospitalization. Scores on the Hamilton scale (National Institute of Mental Health version, 1967, 25 items) varied from 39 to 67 (mean, 50.8 ± 2.2). The patients had received no drugs except those mentioned in Table 1 for at least 4 weeks before the blood sample was taken and were totally drug-free for 24 hours immediately before sampling. Patients with atypical or complex symptoms and patients who had received any form of antidepressant treatment were excluded from the study. Blood samples were taken from depressed patients when they entered the hospital, so that both control and depressed patients were ambulatory at the time of sampling.

Scatchard plots from both control and depressed patients were linear over the range studied, suggesting a single noninteracting site in each case. The binding parameters for both control and depressed donors are summarized in Fig. 1. Each point represents an individual donor and is calculated from a Scatchard plot of at least six points determined in duplicate. The mean value of maximal [^{3}H]imipramine binding (B_{max}) for the depressed donors was significantly lower than the mean value for the control population (depressed, 275 ± 24 fmole per milligram of protein; control, 604 ± 42 fmole/mg). The value of the mean affinity constant (K_d) for [^{3}H]imipramine in the depressed group was similar to the value for the control group (depressed, 2.5 ± 0.3 and control, 2.6 ± 0.4 n*M*).

For 8 of the 16 depressed patients, blood samples were also taken 9 days after treatment with tricyclic antidepressant drugs. The depressions were improved as judged by clinical assessment and a decreased Hamilton score. There was a small increase in the K_d values of [^{3}H]imipramine binding compared to the values obtained 9 days earlier at the time of admission to the hospital. The B_{max} values of [^{3}H]imipramine binding were essentially unchanged (*8*).

Patients suffering from mono- and bipolar endogenous depression and those suffering from reactive depression had decreased numbers of [^{3}H]imipramine binding sites on their platelet membranes (Table 1 and Fig. 1). The eight patients with reactive depression had B_{max} values similar to those of patients suffering from both mono- and bipolar endogenous depression.

Table 1. Clinical details of the 16 depressed patients used in the study and the parameters of [^{3}H]imipramine binding to their platelet membranes. Patients were women, aged 20 to 65, with a well-characterized depression (mono- or bipolar endogenous or reactive types) serious enough to require hospitalization, who had received no antidepressant treatment within the last month. Binding parameters were determined by Scatchard analysis, using a minimum of six concentrations of [^{3}H]imipramine between 0.4 and 8 n*M*, each point being determined in duplicate.

Patient	Age	Diagnosis*	Medication†	HDRS‡	B_{max} (fmole per milligram of protein)	K_d (n*M*)
Lep	23	Reactive	Trimeprazine§ + benzodiazepine	47	332	0.7
Fle	31	Reactive	Benzodiazepine	50	340	1.5
Fra	32	Reactive	Trimeprazine + benzodiazepine	41	263	3.0
Duf	32	Endogenous	Benzodiazepine	44	202	2.6
Gig	35	Reactive	Benzodiazepine	40	390	5.9
Ric	35	Reactive	Chloral hydrate	55	110	2.4
Jol	39	Endogenous (B)	Li_2CO_3 + benzodiazepine	51	384	2.0
Pla	42	Endogenous	Chloral hydrate	57	340	1.5
Sel	47	Reactive	Trimeprazine + benzodiazepine	43	135	3.7
Cou	47	Endogenous		54	160	2.6
Zil	47	Endogenous	Benzodiazepine	39	216	1.2
Har	52	Reactive	Laudanum	56	310	2.8
Haz	59	Reactive	Benzodiazepine	44	284	2.1
Bar	65	Endogenous (B)	Benzodiazepine	62	418	1.6
Sai	65	Endogenous (B)	Laudanum	62	243	2.5
Duh	65	Endogenous	Trimeprazine + benzodiazepine	67	269	4.1
Mean ± S.E.M.	44.8 ± 3.5			50.8 ± 2.2	275 ± 24	2.5 ± 0.3

*Endogenous depressions were all of the monopolar type except those marked (B), which were bipolar. †Only patients receiving no antidepressant therapy were included; details of any nonantidepressant medication is given in this column. All drug treatment was stopped 24 hours before the first blood sample was taken. ‡HDRS, Hamilton depression rating scale (National Institute of Mental Health version, 1967, 25 items). §Trimeprazine is a noncataleptogenic, nonantipsychotic phenothiazine.

At this stage there appears to be no relationship between the severity of depression as estimated by the Hamilton score and the parameters for [^{3}H]imipramine binding (Table 1); however, more patients should be studied before conclusions about this relationship are drawn.

Recent studies have demonstrated [^{3}H]imipramine binding sites in postmortem and neurosurgical samples of human brain (*9*). In the regions studied in detail—A_{34} (hippocampus), A_{10} (cortex), hypothalamus, and caudate nucleus—the K_d and B_{max} values closely resemble those found in rat brain (*9*). Thus the decrease in [^{3}H]imipramine binding sites seen in the platelets of depressed subjects may be a reflection of similar changes in the brain. It has been recently demonstrated that changes in platelet monoamine oxidase activity are correlated with the levels of 5-hydroxyindoleacetic acid in cerebral spinal fluid in human control volunteers (*10*), suggesting that changes in platelets may indeed reflect similar changes in the central nervous system.

[^{3}H]Imipramine binding in platelets from depressed patients may be decreased in response to the biochemical or humoral changes that trigger or maintain the depressive state. It is tempting to speculate that the differences in the binding of [^{3}H]imipramine observed in platelets may reflect sensitivity changes resulting from altered levels of a circulating substance having a role in mood regulation. This unknown substance could act directly on the [^{3}H]imipramine binding site. Compatible with the possible existence of a mood-regulating endogenous ligand for the [^{3}H]imipramine binding site is the fact that similar endogenous ligands have been found for (i) the [^{3}H]morphine binding site (*11*), now accepted to be the opiate receptor, and, more recently, for (ii) the [^{3}H]diazepam binding site (*12*), thought to be involved in the anxiolytic effect.

Although the significance of [^{3}H]imipramine binding is not yet clear, several possibilities can be suggested. (i) The binding site may be a specific receptor for an endogenous ligand. (ii) A direct or indirect relationship with the site of neuronal serotonin uptake has been suggested (*5*) on the basis of the high affinity of certain serotonin-uptake blockers. (iii) Finally, the [^{3}H]imipramine binding site could be a modulatory site for one of the established neurotransmitter receptors in analogy to the interaction between the γ-aminobutyric acid receptor and the benzodiazepine binding site (*13*).

In summary, maximal [^{3}H]imipramine binding is significantly lower in the platelets of untreated depressed patients than in the control group, whereas the affinity constant is unchanged. The study of [^{3}H]imipramine binding in depressed patients (before treatment and after treatment with tricyclic or atypical drugs, electroconvulsive shock, lithium, and even social and psychological therapies) represents a new approach in biological psychiatry. At this stage there are still many questions to be answered concerning the pathogenesis and therapy of depression, but it would appear that [^{3}H]imipramine binding both in platelets and in postmortem brain samples will be a powerful tool in our attempts to answer them.

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Long-Lasting Depletion of Striatal Dopamine by a Single Injection of Amphetamine in Iprindole-Treated Rats

Abstract. *A single injection of amphetamine given to rats treated concurrently with iprindole so that they could not metabolize the amphetamine by* para*-hydroxylation resulted in a decrease in the concentration of striatal dopamine 1 week later. The decrease was antagonized by amfonelic acid, an inhibitor of uptake into dopamine neurons. The long-lasting depletion of cerebral dopamine by amphetamine may be analogous to the depletion of cerebral serotonin by halogenated derivatives of amphetamine.*

Certain analogs of amphetamine are neurotoxic. For example, when a single dose of *p*-chloroamphetamine is injected into rats, the concentration of cerebral serotonin is decreased and remains decreased for months (*1*). Along with this reduction, there are long-lasting reductions in 5-hydroxyindoleacetic acid concentration, tryptophan hydroxylase activity, and high-affinity serotonin uptake (*1*), all of which imply the destruction of serotonin-containing neurons. Supporting this idea are histological findings that cell bodies degenerate in the serotonin-rich raphe region of rat brain (*2*).

Two characteristics of *p*-chloroamphetamine seem necessary for this neurotoxic effect on serotonin neurons: persistence of *p*-chloroamphetamine for many hours because of its slow rate of metabolic removal, and continued active accumulation of *p*-chloroamphetamine in serotonin neurons during this time because of its high affinity for the membrane uptake system on the neurons (*3*, *4*). Amphetamine itself has a high affinity for the membrane uptake system on dopamine neurons (*4*, *5*). Amphetamine is metabolized more rapidly than *p*-chloroamphetamine in the rat because amphetamine is hydroxylated in the *para* position of the phenyl ring, the position occupied by the chlorine substituent in *p*-chloroamphetamine (*6*). This hydroxylation can be inhibited by drugs like iprindole; in iprindole-treated rats the persistence of amphetamine in tissues resembles that of *p*-chloroamphetamine (*7*).

We conducted the present experiments to ascertain whether a single dose of amphetamine causes long-lasting effects on dopamine neurons in iprindole-treated rats (*8*). It has been shown that, although depletion of serotonin by halogenated amphetamines is initially re-

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