Table 1. Conversion of T4 to T3 in normal human subjects; SA, specific activity.

Day after first injection	Subject 4		Subject 5	
	Serum ^{14}C as T3 (%)	SA T3/T4	Serum ^{14}C as T3 (%)	SA T3/T4
2	0.72	0.25		
3			1.53	0.49
4	1.04	0.36		
5			1.72	0.52
6	1.29	0.44		
7			2.20	0.69
8	1.17	0.40		
9			0.95	0.28
10	1.23	0.42		
11			1.16	0.36
12	1.11	0.39		
13			1.47	0.46
15			1.11	0.36
17			1.69	0.54
19			1.73	0.55
21			1.59	0.49
Mean	1.2*	0.40*	1.5	0.48
S.D.	±0.10*	0.03*	±0.36	±0.12

* From day 4 on.

remained, and there had been no appreciable conversion of T4 to T3 in vitro. From day 3 on, [^{14}C]T3 was present in the serum, and by this time it had reached an apparent plateau. The data were interpreted to support the conversion of administered labeled T4 to T3 in vivo. In this set of serums from subject 3, corrections were required for residual ^{131}I.

The serums of subjects 4 and 5 had no detectable ^{131}I at the time of ^{14}C assay, which showed mean values of 1.2 and 1.5 percent of the total serum radioactivity in the T3 zones (Table 1).

Again, the principal inference from the data obtained was the conversion of T4 to T3 in normal human volunteers, a result in agreement with the demonstrated conversion of T4 to T3 in athyreotic human subjects maintained on T4 (*1*).

The percentages of [^{14}C]T3 in the serums of subjects 4 and 5 were 1.2 and 1.5 percent. Since T3 has a much more rapid fractional turnover than T4, even as little as 1.5 percent of radioactivity in the serum suggests appreciable conversion. Despite the fact that the normal concentration of T3 in serum is only about 1/30 that of T4 (*3*), the absolute removal rate is almost as great (*3, 4*). Thus the normal rate of disposal of T4 approximates 80 μg/day, while that of T3 is approximately 60 μg/day (*3, 5*). The volume clearance of T4 is about 1 liter/day, whereas T3 has a clearance of approximately 22 liter/day (*5*). On this basis, one may estimate that 1.5 percent of serum radioactivity as T3 could signify 0.33 liter/day (0.015 × 22) as the probable fraction converted of the daily T4 clearance of 1 liter. This would then suggest that as much as one-third of the T4 disposal could occur by conversion to the rapidly metabolized T3 in normal humans. Obviously further kinetic data would be needed to verify the extent of this route of transformation.

The converse problem is the relative contribution of T3 formed by conversion from T4 to the circulating T3 in comparison with that secreted by the thyroid gland. If all T3 were made from T4, the ratio of the specific activity of T3 to that of T4 after equilibration would be 1.00. If half the T3 were from T4, the ratio would be 0.5. In subjects 4 and 5 (Table 1) the ratios were 0.40 and 0.48, signifying that more than one-third of T3 may arise from conversion in normal human subjects.

Inasmuch as T3 is three to four times as potent as T4 and has recently been considered to produce the major thyroid hormonal effect (*3*) our findings raise the question whether T4 itself has any primary action or exerts its effect only after transformation to T3.

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Serotonin-Containing Neurons in Brain: Depression of Firing by Monoamine Oxidase Inhibitors

Abstract. *Monoamine oxidase inhibitors were administered to rats while the activities of single, serotonin-containing neurons of the midbrain raphe nuclei were being monitored with microelectrodes. All the inhibitors tested (pargyline, tranylcypromine, phenelzine, iproniazid) caused depression of raphe unit firing rate. The ability of monoamine oxidase inhibitors to depress raphe units was impaired by prior treatment with* p-*chlorophenylalanine, an inhibitor of serotonin synthesis.*

Inhibition of monoamine oxidase (MAO) markedly increases the concentration of serotonin (5-hydroxytryptamine; 5-HT) in brain (*1*). By the histochemical fluorescence method for monoamines, at least some of this increase has been shown to take place within the 5-HT–containing neurons of the midbrain raphe nuclei (*2*). We now report the monitoring of the activity of single neurons in the raphe nuclei during the administration of MAO inhibitors to determine whether this observed increase in 5-HT content is associated with an alteration in rate of unit firing. We found that MAO inhibitors of various chemical structures produce a marked depression of raphe unit firing; these results demonstrate for the first time that MAO inhibitors modify the physiological activity of monoamine-containing neuronal units in the brain.

Extracellular spikes of single, midbrain raphe neurons were recorded (*3*). In brief, male rats (Charles River, cesarean delivered; 250 to 275 g) were anesthetized with chloral hydrate and mounted in a stereotaxic apparatus. A tungsten microelectrode, with a tip diameter of approximately 1 μm, was lowered through a burr hole into the midbrain raphe area by means of a hydraulic microdrive. Electrode signals were fed into a high impedence amplifier and then displayed on an oscilloscope screen. The firing rate of an individual unit was followed with an electronic counter whose analog output was plotted on a potentiometric re-

corder. Under the experimental conditions employed, raphe units typically are regular in rhythm and slow in rate (~ 1 to 2 spike/sec) (*3*).

Monoamine oxidase inhibitors of various structural types were tested to insure that any effects on raphe units were common to the class of drugs and not limited to a member with unique properties. Two nonhydrazines, pargyline and tranylcypromine, and two hydrazines, phenelzine and iproniazid, were studied. Tranylcypromine and phenelzine have amphetamine-like or catecholamine-releasing effects in addition to being MAO inhibitors (see *4*).

Previous studies have shown that parenterally administered amphetamine does not depress raphe units and that norepinephrine, even in doses sufficient to cause a maximum increase in blood pressure, does not alter raphe firing rate (*5*). After the rate of a unit had been recorded for a base-line period of about 10 minutes the drugs were given by intraperitoneal injection. After the completion of each experiment, a small anodal lesion was made through the microelectrode, and the precise location of the electrode tip was later determined by histological examination. In separate experiments the concentration of 5-HT in brain was assayed (*6*) at various intervals following injections of drugs.

Beginning 5 to 10 minutes after an intraperitoneal injection of pargyline hydrochloride (100 mg/kg), tranylcypromine sulfate (20 mg/kg), or phenelzine sulfate (20 mg/kg), the rate of firing of dorsal or median midbrain raphe units decreased gradually until, 30 to 45 minutes later, there was almost a total cessation of activity (Fig. 1).

In contrast, the MAO inhibitors had little or no effect on the firing rate of 20 midbrain units outside the raphe (reticular formation; cranial nerve nuclei III and IV; tectum; pontine nuclei). Iproniazid (100 mg/kg) also depressed raphe units, but the initial slowing did not begin until 30 to 45 minutes had elapsed after injection, and minimum rates (< 1 spike/10 sec) were not reached for 1 to 2 hours. At 4 and 24 hours after pargyline or tranylcypromine no spontaneously firing units were found in the raphe nuclei. However, 4 and 24 hours after phenelzine or iproniazid some spontaneously active units were found firing at very slow rates (~ 1 to 2 spike/10 sec). In such cases, a second dose of phenelzine or iproniazid produced further depression of firing. Although detailed dose-response experiments were not carried out, lower doses of pargyline, tranylcypromine, and phenelzine than those mentioned above also depressed raphe units, but the onset of effects was delayed or the reduction in firing rate was less marked.

The possible role of increased concentrations of brain monoamines in mediating the action of MAO inhibitors on raphe units was investigated by giving "loading" doses of monoamine precursors or by "selectively" inhibiting the synthesis of 5-HT or catecholamines, respectively, with *p*-chlorophenylalanine (*7*) or α-methyl-*p*-tyrosine (*8*). When given alone, *p*-chlorophenylalanine and α-methyl-*p*-tyrosine did not alter raphe firing rate appreciably. In 27 out of 29 cases, prior treatment with *p*-chlorophenylalanine totally or partially blocked depression of raphe units by pargyline (Fig. 2A), tranylcypromine, or phenelzine. On the other hand, D-lysergic acid diethylamide, which previously has been shown to inhibit raphe units (*3*), was inhibitory despite prior treatment with *p*-chlorophenylalanine (Fig. 2B). In terms of effects on raphe units, this experiment represents one means of differentiating between MAO inhibitors and D-lysergic acid diethylamide. α-Methyl-*p*-tyrosine methyl ester (50 to 75 mg/kg) given 1 to 3 hours prior to the MAO inhibi-

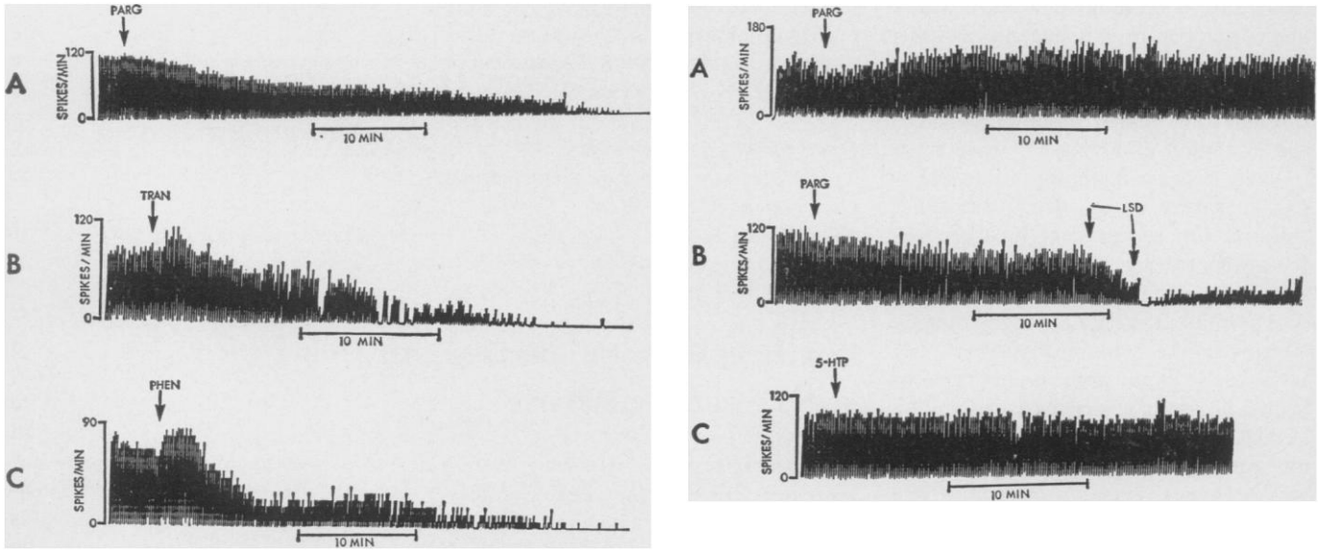

Fig. 1 (left). Effect of MAO inhibitors on the rate of firing of midbrain raphe units. Pargyline hydrochloride (100 mg/kg), tranylcypromine sulfate (20 mg/kg), and phenelzine sulfate (20 mg/kg) were injected intraperitoneally as indicated by the arrows. The depression of firing seen in these experiments was typical of all the units tested ($n = 27$, pargyline; $n = 10$, tranylcypromine; $n = 11$, phenelzine). The small, transient increase in rate after tranylcypromine and phenelzine was not a constant finding with these drugs and may merely represent a response to the local irritant effect of injection. Fig. 2 (right). Block by *p*-chlorophenylalanine of pargyline-induced depression of raphe unit firing and failure of L-5-hydroxytryptophan to alter raphe unit rate. In A and B *p*-chlorophenylalanine methyl ester hydrochloride (400 mg/kg) was given intraperitoneally 48 hours prior to pargyline (100 mg/kg). In both A and B, pargyline failed to produce its usual inhibition of firing. In B, after pargyline, D-lysergic acid diethylamide given intravenously (LSD; 15 μg/kg, twice) produced its typical cessation of firing. Partial recovery from LSD occurred despite prior injection of pargyline. In C, L-5-hydroxytryptophan (100 mg/kg) failed to appreciably depress firing rate.

Table 1. Effect of prior treatment with *p*-chlorophenylalanine or α-methyl-*p*-tyrosine on the pargyline-induced increase in rat brain serotonin. The number of animals used in each group was eight.

Prior treatment	Serotonin		
	After saline (ng/g ± S.E.)	After pargyline (ng/g ± S.E.)	Change (ng/g)
Control	346 ± 11	603 ± 11	+ 257*
p-Chlorophenylalanine†	111 ± 15	124 ± 11	+ 13
α-Methyl-*p*-tyrosine‡	357 ± 5	604 ± 8	+ 247*

* $P < .0005$. † DL-*p*-Chlorophenylalanine methyl ester hydrochloride (400 mg/kg) was given intraperitoneally 24 hours prior to saline or pargyline. ‡ DL-α-Methyl-*p*-tyrosine methyl ester hydrochloride (75 mg/kg) was given intraperitoneally 1 hour prior to saline or pargyline. Brains were taken for assay 1 hour after saline or pargyline.

tors did not prevent depression of raphe unit firing. Biochemical studies done in parallel with the unit recordings showed that the expected pargyline-induced increase in brain 5-HT was prevented by *p*-chlorophenylalanine but not by α-methyl-*p*-tyrosine (Table 1). These results suggest an association between an increase in brain 5-HT and inhibition of raphe units by MAO inhibitors. However, L-5-hydroxytryptophan, which produces a rapid elevation in brain 5-HT (*9*), caused slight if any depression of raphe firing even in doses up to 100 mg/kg (Fig. 2C). Similarly, injections of the catecholamine precursor L-3,4-dihydroxyphenylalanine of up to 100 mg/kg had little or no effect on raphe firing rate.

Our results demonstrate that MAO inhibitors have a profound depressant effect on the rate of firing of 5-HT–containing neurons in the brain. There are a number of possible mechanisms that might explain this phenomenon. (i) The drugs could depress raphe units through some shared action unrelated to the inhibition of MAO; this may seem unlikely because of the disparate chemical structures of the drugs tested but there is ample precedent in other systems affected by MAO inhibitors for considering such a possibility (*10*). (ii) The inhibition of MAO might directly depress raphe units independently of increases in brain monoamines; against such a notion is evidence that inhibition of MAO by pargyline, tranylcypromine, and phenelzine is extremely rapid after intraperitoneal injection (that is, within 5 to 10 minutes) (*11*), but depression of raphe firing is delayed. (iii) The accumulation of one or more endogenous monoamines secondary to MAO inhibition might result in depressed raphe firing, possibly through a negative feedback mechanism. It is reported that MAO inhibitors increase the output of 5-HT from perfused cerebral ventricles (*12*), and leakage of 5-HT from terminals onto postsynaptic receptive sites could thus lead to a compensatory reduction in the firing of 5-HT neurons. Such a possibility fits in with our finding that preventing an increase in 5-HT concentration with *p*-chlorophenylalanine also blocks the MAO inhibitor-induced depression of raphe units. However, the hypothesis is not supported by the observation that loading doses of 5-hydroxytryptophan failed to produce inhibition. (iv) Finally, as has been proposed for peripheral adrenergic nerves (*13*), the accumulation in 5-HT nerve endings of an amine such a tryptamine, which is not normally present in large amounts (*14*), may alter raphe activity by acting as a false transmitter. No final judgment can be made as to the relative contribution, if any, of these various proposed mechanisms in accounting for the observed depression of raphe units by MAO inhibitors.

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Panting in Dogs: Unidirectional Air Flow over Evaporative Surfaces

Abstract. *In dogs which are panting due to a heat load, most of the respired air enters through the nose and leaves through the mouth. Different patterns of flow are, however, possible. The unidirectional flow over the evaporative (nasal) surfaces is an important mechanism for regulating the amount of heat dissipated in panting.*

Panting in dogs is often described as a rapid in-and-out breathing through the open mouth, with evaporation taking place from the moist oral surfaces and the large hanging tongue. In such a system much of the air would not flow in immediate proximity of the moist surfaces; it is difficult to imagine how the entire volume of air could become saturated with water vapor. If the air is not fully saturated, correspondingly larger volumes of air