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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References and Note

1. B. B. Brodie, A. Pletscher, P. A. Shore, *J. Pharmacol. Exp. Ther.* **116**, 9 (1956).
2. A. Dahlström and K. Fuxe, *Acta Physiol. Scand.* **62**, Suppl. 232, 1 (1965); G. K. Aghajanian and A. W. Graham, unpublished data.
3. G. K. Aghajanian, W. E. Foote, M. H. Sheard, *Science* **161**, 706 (1968); *J. Pharmacol. Exp. Ther.* **171**, 178 (1970).
4. W. C. Lee, Y. H. Shin, F. E. Shideman, *J. Pharmacol. Exp. Ther.* **131**, 180 (1961); T. H. Tsai and W. W. Fleming, *Biochem. Pharmacol.* **14**, 369 (1965); R. M. Atkinson and K. S. Ditman, *Clin. Pharmacol. Ther.* **6**, 631 (1965); V. Clineschmidt and A. Horita, *Brit. J. Pharmacol.* **30**, 67 (1967).
5. W. E. Foote, M. H. Sheard, G. K. Aghajanian, *Nature* **222**, 567 (1969).
6. M. H. Sheard and G. K. Aghajanian, *J. Pharmacol. Exp. Ther.* **163**, 425 (1968).
7. B. K. Koe and A. Weissman, *ibid.* **154**, 499 (1966).
8. S. Spector, A. Sjoerdsma, S. Udenfriend, *ibid.* **147**, 86 (1965).
9. S. Udenfriend, H. Weissback, D. F. Bodanski, *J. Biol. Chem.* **224**, 803 (1957); E. Costa and F. Rinaldi, *Amer. J. Physiol.* **194**, 214 (1958); M. K. Paasonen and N. J. Giarman, *Arch. Int. Pharmacodyn. Ther.* **114**, 189 (1958).
10. S. Shanberg, *Biochem. Pharmacol.* **1**, 301 (1958); J. Axelrod, G. Hertting, R. W. Patrick, *J. Pharmacol. Exp. Ther.* **134**, 325 (1961); B. Dubnick, G. A. Lesson, G. E. Phillips, *J. Neurochem.* **9**, 299 (1962); M. D. Davey, J. B. Farmer, H. Reinert, *Brit. J. Pharmacol.* **20**, 121 (1963); I. J. Kopin and J. Axelrod, *Ann. N.Y. Acad. Sci.* **107**, 848 (1963); G. L. Gessa, E. Cuenca, E. Costa, *ibid.* p. 35; G. R. Pscheidt, *ibid.*, p. 1057; K. F. Gey, A. Pletscher, W. Burkard, *ibid.*, p. 1147; M. J. Antonaccio and C. B. Smith, *J. Pharmacol. Exp. Ther.* **170**, 97 (1969).
11. T. N. Tozer, N. H. Neff, B. B. Brodie, *J. Pharmacol. Exp. Ther.* **153**, 177 (1966).
12. M. B. E. El Hawary, W. Feldberg, V. J. Lotti, *J. Physiol. London* **188**, 131 (1967); C. A. Goodrich, *Brit. J. Pharmacol.* **37**, 87 (1969).
13. I. J. Kopin, J. E. Fischer, J. M. Musacchio, W. D. Horst, V. K. Weise, *J. Pharmacol. Exp. Ther.* **147**, 186 (1965).
14. D. Eccleston, G. W. Ashcroft, T. B. B. Crawford, R. Loose, *J. Neurochem.* **13**, 93 (1966).
15. We thank Chas. Pfizer & Co., Warner-Lambert Research Institute, Abbott Laboratories, Smith Kline & French, and the FDA–PHS Psychotomimetic Agents Advisory Committee for providing, respectively, *p*-chlorophenylalanine methyl ester hydrochloride, phenelzine sulfate, pargyline hydrochloride, tranylcypromine sulfate, and D-lysergic acid diethylamide bitartrate. Supported in part by PHS grant K3-MH-14,459.

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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

must be moved in and out, thus increasing the expenditure of metabolic energy and the heat load.

A priori, it would seem more advantageous for the moist surfaces of the nose to be used for evaporation, for here the air flows over the large surfaces of the nasal turbinates and comes to nearly complete equilibrium with them with respect to temperature and water vapor (*1*). A difficulty in dissipating heat from the nose is that, on exhalation, a large part of the heat and water vapor that was added to the air inhalation is again recovered on exhalation through a counter-current exchange system between the air stream and the nasal surfaces (see *2*).

One way to circumvent this counter-current system is to arrange for a one-way flow in the exchanger. This would be achieved if inhalation could take place through the nose, and exhalation through the mouth. This pattern of breathing during panting was in fact proposed by Negus, based on his anatomical studies (*3*). It cannot be regarded as established, however, for studies of dead specimens do not give conclusive information about air flow in the living animal.

To determine whether air flow in the nose is unidirectional during panting, we measured the volumes of air inhaled and exhaled through the nose and through the mouth of panting dogs. Two small mongrel dogs (2.5 and 3.5 kg) were trained to wear specially molded fiberglass masks which separated nose and mouth air flow (with minimum dead space). To avoid restraining the dogs and to reduce discomfort, we did not attempt simultaneous measurements of nasal and oral flows. The mask was connected to an Otis-McKerrow low-impedance valve, and small heated thermistors were placed in the intake and exhaust streams to serve as hot-wire anemometers for measurements of air flow. Calibration was achieved by using a reciprocating respiratory pump which was adjusted to reproduce the signals obtained from the panting dogs. Sources of error in this calibration system consist of reading of the record, leakage in the valve, differences in temperature and humidity of the air, as well as conventional errors inherent in amplification and recording of signals. All the errors of which we are aware add up to less than 10 percent of the stated volume. The experiments reported here were carried out at 23°C and 30 percent relative humidity. Panting was induced by wrapping the dog in an electric blanket and adjusting the heating to obtain steady-state frequencies. Rectal temperature was recorded with thermistor probes (± 0.2°C).

The typical pattern of normal, shallow thermal panting in our dogs was predominantly inhalation through the nose and exhalation through the mouth (Fig. 1). Over a prolonged period we found that, on the average, only about one-quarter of the air inhaled through the nose was exhaled through the nose, the remaining three-quarters being exhaled through the mouth. The amount exhaled through the nose could, however, vary from 0 to 100 percent of the inhaled volume. The record in Fig. 1 shows a selected (but typical) example of such variations. The record for the mouth indicates exhaled air volumes about equal to the nasal inhalation, while inhalation through the mouth was practically nil. This was the common pattern in ordinary panting.

If the animals' body temperatures increased and the animals appeared

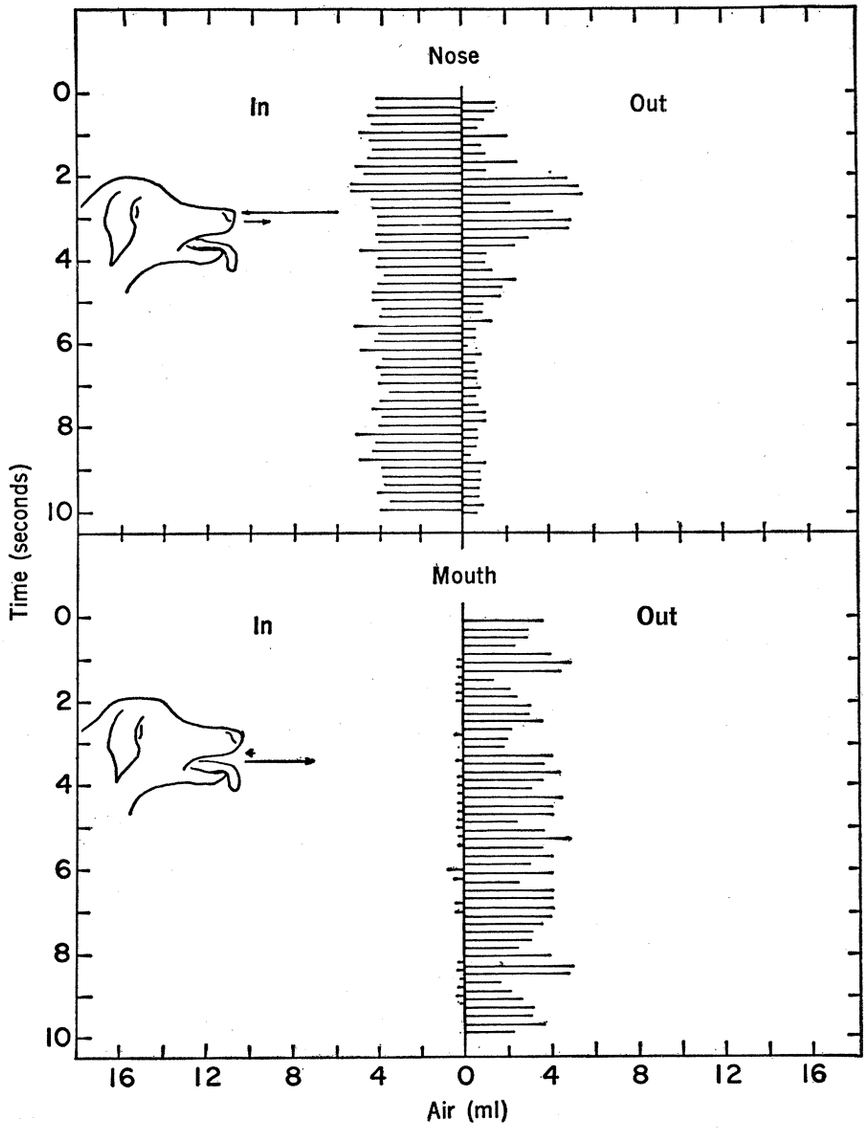

Fig. 1. Volume of air entering and leaving the nose (upper graph) and the mouth (lower graph) during shallow thermal panting. Measurements were not made simultaneously, and typical patterns are presented here. Mean inspired and expired volumes for 10 seconds are indicated by vectors adjacent to the dog head. (Volumes are expressed as milliliters of dry air at 23°C and 760 mm-Hg.)

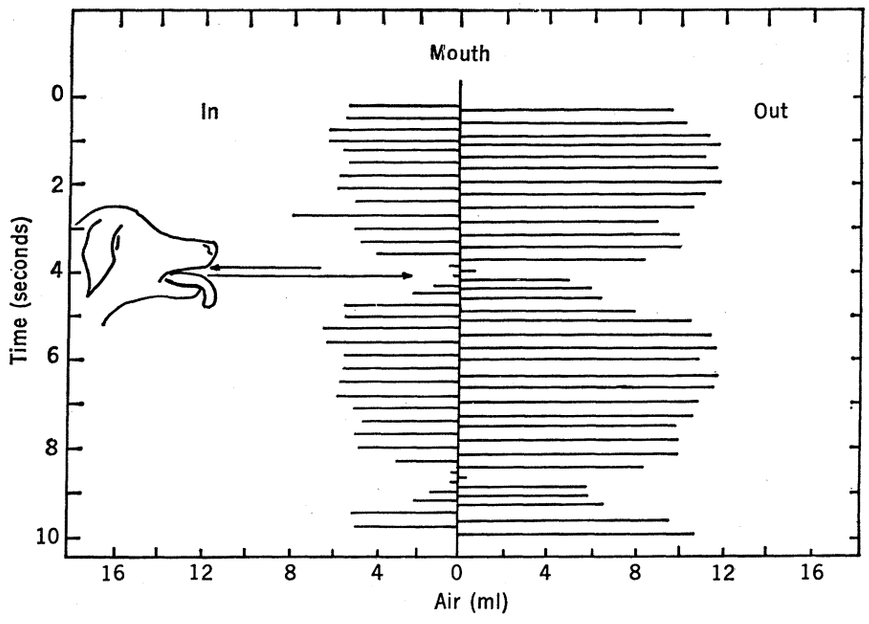

Fig. 2. Volume of air entering and leaving the mouth during "deep" panting (see text). Mean inspired and expired volumes for 10 seconds are indicated by vectors adjacent to the dog head. (Volumes expressed as milliliters of dry air at 23°C and 760 mm-Hg.)

more distressed, the panting tended to change to a somewhat lower frequency (from 300 to 220), and inhalation through the mouth began to appear in the recordings (Fig. 2). At the same time the total tidal volume increased, and the volume exhaled through the mouth was now approximately twice as large as that inhaled through the mouth. Thus air inhaled through the nose was still exhaled through the mouth. The statement of Negus (*3*) that man and anthropoid apes are the only mammals capable of inhalation through the mouth is therefore incorrect.

The importance of the described pattern of air flow can be estimated from the temperature of the exhaled air. A dog may pant with closed mouth, both inhalation and exhalation taking place through the nose. Under these circumstances the temperature of the exhaled air was 29°C (*4*). If the same dog, while still panting at the same frequency, changed to exhalation through the mouth, the temperature of the exhaled air was nearly identical to body temperature (38°C). For a given volume of air, exhalation at 38°C instead of 29°C substantially increases the amount of heat carried away. In our experiments the amount of heat carried away by 1 liter of air was 14.9 cal for exhalation through the nose and 27.7 cal from exhalation through the mouth (calculated from the water added to saturate air and the heat added to warm the air to exhaled air temperature). By changing the relative amounts of air exhaled via the nose or via the mouth, the dog can thus, without changing frequency or tidal volume, modulate the amount of heat dissipated. The advantage of a constant frequency of panting was discussed by Crawford (*5*), who emphasized that dogs seem to pant at the resonant frequency of the respiratory system, thus reducing the energy expenditure (and heat load) of panting. Modulation of tidal volume, especially an increase in tidal volume, may be undesirable because of its effect on hyperventilation of the lungs and the ensuing alkalosis. It seems to us that a modulation of evaporation by a mere change in the flow pattern of the air is, within its limitations, a simple and effective mechanism.

An important consequence of the described pattern of panting is that the nasal mucosa, rather than the oral surfaces and the tongue, is the primary site of evaporation. Consequently, these surfaces must be supplied with sufficient quantities of moisture. It is quite possible that the large serous-type nasal gland which is found in the dog is the major source of the necessary secretion. This gland was first described by Steno in 1664 and has since received considerable attention from anatomists (*6*), but no specific function has been ascribed to it. If this gland is indeed of importance for supplying the water required for heat dissipation during panting, its function is in a sense analagous to that of sweat glands in man. It can therefore be expected that secretory activity may be under control of the thermoregulatory system, and this should be investigated.

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References and Notes

1. F. Verzár, J. Keith, V. Parchet, *Pfluegers Arch. Gesamte Physiol. Menschen Tiere* **257**, 400 (1953).
2. K. Schmidt-Nielsen, F. R. Hainsworth, D. E. Murrish, *Resp. Physiol.* **9**, 263 (1970).
3. V. E. Negus, *The Comparative Anatomy and Physiology of the Larynx* (Grune and Stratton, New York, 1949).
4. Ambient conditions 23°C, 30 percent relative humidity. Temperature of the exhaled air was measured as described in (*2*).
5. E. C. Crawford, Jr., *J. Appl. Physiol.* **17**, 249 (1962).
6. C. Kangro, *Z. Anat. Entwicklungsgesch.* **85**, 376 (1928); W. Meyer, *Anat. Anz.* **24**, 369 (1934).
7. K. S.-N. is supported by NIH research career award 1-K6-GM-21,522 and NIH research grant HE-02228. W.L.B. is supported by an NSF predoctoral fellowship and NIH postdoctoral fellowship 1 FO2 GM43875-01.
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Alcohol Addiction and Tetrahydropapaveroline

Davis and Walsh (*1*) proposed that heavy consumption of alcohol may result in a diversion of dopamine (DA) metabolism in the central nervous system from a pathway producing dihydroxyphenylacetic acid to a nonenzymatic condensation of dihydroxyphenylacetaldehyde with DA to produce tetrahydropapaveroline (THP). They further hypothesized that THP has addictive liability or is further metabolized to morphine-like compounds which are then responsible for the development of the syndrome of alcohol addiction. In support of this formulation, they have (i) extended to rat brain homogenates the findings in vitro of Holtz *et al* (*2*) as well as our own (*3*) with guinea pig liver preparations with respect to the formation of THP from milligram quantities of DA; (ii) shown a slight increase in the amount of THP formed as a per-