predicts that the response magnitude will vary as a function of time according to:

$$R = \frac{S}{S + k_2/k_1}\left(k_2 + k_1 S e^{-(k_1 S + k_2)t}\right) \quad (3)$$

where t is the time, and S, k_1, and k_2 are as defined in Eq. 1. Predictions for Beidler's theory were computed from Eq. 2. A nonlinear regression program fit Paton's theory to the averaged response to 0.1*M* NaCl, and predictions for other concentrations were then computed from Eq. 3 (*13*). Product moment correlation coefficients between observed and predicted responses for both theories for the concentrations shown in Fig. 1 are given in Table 1. Neither theory adequately describes the relation between the observed response and time. Paton's theory does predict phasic responses, but encounters great difficulty with the range of stimulus concentrations employed.

The phasic response, which is so poorly predicted by Beidler's and Paton's theories, has been observed in many investigations (*9, 11*). It has sometimes been argued that the phasic portion of the response is due to a property of the nerve since recordings from taste bud receptor cells do not show a phasic response. However, the receptor cell responses are all much slower than the "subsequent" neural responses (*17, 18*), and consequently such slow potentials cannot be the actual receptor potentials.

The significance of the phasic portion of the neural response is emphasized by the results of the behavioral decision experiment (*5*). Taking both the response latency and the time required for central processing into account, we suggest that the rising portion of the responses, which is nearly linear with respect to time, may convey the information required for rapid taste quality discrimination (*19*). Thus, the phasic portion of gustatory neural responses deserves further study in theoretical terms also.

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6-Hydroxydopa Depletion of Brain Norepinephrine and the Facilitation of Aggressive Behavior

Abstract. *A significant increase in shock-induced aggression occurs in the rat 4 days after an intraventricular injection of 90 micrograms of 6-hydroxydopa. Both fluorescent histology and biochemical assay demonstrate that brain norepinephrine is reduced by 90 micrograms of 6-hydroxydopa, while brain dopamine remains unaltered. This suggests that one form of aggressive behavior (shock-induced aggression) is modulated through a central noradrenergic system.*

Central administration of 6-hydroxydopamine has been shown to produce a long-lasting depletion of brain catecholamines (*1*) as well as a central degeneration of catecholamine terminals (*2*). Intracisternal injection of this drug into rats produces a progressive increase in shock-induced aggression that persists for as long as 6 months after a single 200-μg dose (*3*). Prior administration of desmethylimipramine, a drug that blocks uptake of amines into catecholamine terminals alters both the catecholamine-depleting and the behavioral effects of 6-hydroxydopamine (*4*). Since both brain norepinephrine (NE) and dopamine (DA) are affected by 6-hydroxydopamine, it is unclear as to which neurotransmitter is responsible for the facilitation in aggressive behavior that is observed. The introduction of 6-hydroxydopa pro-

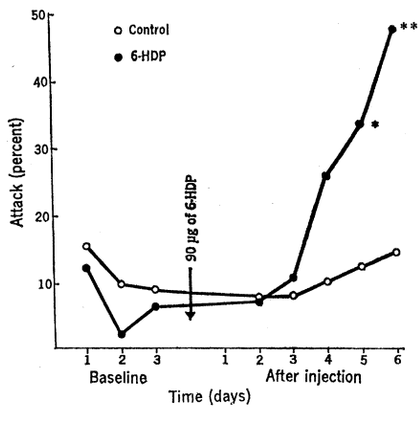

Fig. 1. Time course of the development of facilitated shock-induced aggression after 6-hydroxydopa (*6-HDP*). Symbols: * $P < .05$, ** $P < .01$; two-tailed *t*-test, control versus treated animals.

Table 1. Effect of 6-hydroxydopa (6-HDP) on brain norepinephrine (NE) and dopamine (DA) content. Brain catecholamines were assayed 7 days after an intraventricular injection of 6-HDP (90 μg) or of the vehicle. Number of animals is indicated in parentheses.

	NE (ng/g)					DA (ng/g)
	Whole brain	Hypothalamus	Brain stem	Cortex	Rest of brain	Whole brain
Vehicle	490 ± 54 (12)	1691 ± 74 (8)	556 ± 12 (8)	318 ± 15 (8)	364 ± 13 (8)	568 ± 24 (12)
6-HDP	323 ± 17 (14)	795 ± 95 (8)	408 ± 18 (8)	207 ± 12 (8)	254 ± 18 (8)	620 ± 16 (14)
	Percentage of amine remaining					
	66*	48*	73.4*	65*	70*	109

* $P < .001$, ± two-tailed t-test, 6-hydroxydopa-treated group versus control.

vides a means of studying the relation between brain NE depletion and shock-induced fighting. A single intravenous injection of 6-hydroxydopa into mice produces a selective depletion of brain NE with no change in the brain DA concentration (*5*). We now report that intraventricular administration of 6-hydroxydopa to rats causes both an increase in shock-induced fighting and a depletion only of brain NE.

Male Sprague-Dawley rats (170 to 200 g) were randomly paired and ear-punched. During the first 3 days of the study they were subjected, in pairs, to daily sessions of 50 electric footshocks (2-ma intensity, 0.4-second duration every 7.5 seconds) as described (*6*). The fighting behavior was then quantified by scoring the number of attack responses per 50 footshocks. This allowed the percentage of attacks to be calculated (the number of attacks divided by the number of shocks administered 100). Attacks were defined by rigorous criteria (*6*). On day 4, the rats were anesthetized intraperitoneally with pentobarbital (40 mg/kg). One group received 90 μg of 6-hydroxydopa intraventricularly (in 10 μl of saline containing 0.1 percent ascorbic acid). The control group received the same volume of vehicle. The groups were then tested again for shock-induced aggression on days 2 to 6 after the injection. On day 7 the animals were decapitated. The brains were rapidly removed; were kept whole; or were dissected into brainstem, hypothalamus, cortex, and rest of brain, excluding cerebellum. Three 6-hydroxydopa-treated and two control brains were frozen in liquid nitrogen for fluorescent histological studies according to the method of Falck and Hillarp *et al.* (*7*). The brains or brain portions were homogenized in 10 ml of 0.4*N* perchloric acid, and the homogenates were frozen overnight. They were then thawed and centrifuged in a refrigerated Sorvall centrifuge at 15,000 rev/min for 15 minutes. Norepinephrine and DA in the supernatants were then isolated over alumina columns (*8*). Both catecholamines were eluted with 6 ml of 0.2*N* acetic acid. Norepinephrine was assayed according to the trihydroxyindole method of von Euler and Lishajko (*9*), and DA was assayed by the method of Laverty and Taylor (*10*).

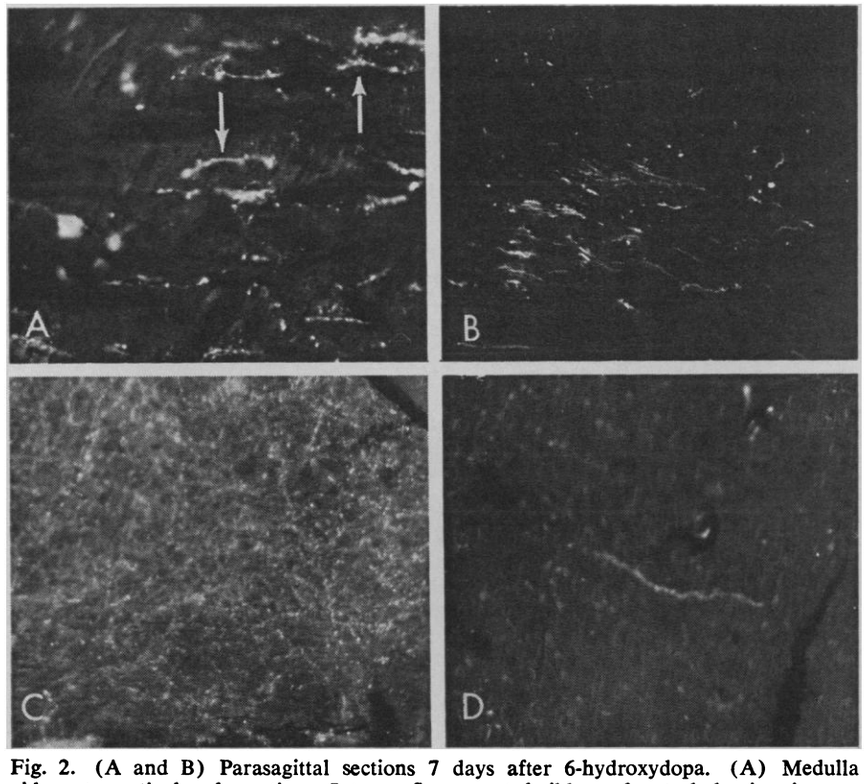

Fig. 2. (A and B) Parasagittal sections 7 days after 6-hydroxydopa. (A) Medulla oblongata, reticular formation. Intense fluorescent buildup of catecholamine in preterminal axons (arrows) (× 146). (B) Reticular formation, level of red nucleus. Appearance of preterminal axons with dense accumulation of catecholamine (× 58). (C and D) Parasagittal sections: hypothalamus, nucleus dorsomedialis (× 92). (C) Control, normal number of noradrenergic varicose terminals. (D) Seven days after 6-hydroxydopa. A reduction in the number of terminals is observed. One preterminal axon with accumulated catecholamine is also noted.

No change in fighting was observed during the first 3 days after 6-hydroxydopa administration (Fig. 1). From day 4 to day 6, however, there was a progressive increase in fighting in the 6-hydroxydopa-treated group. Mean fighting levels significantly rose from 7 percent on day 2 to 47 percent on day 6, while there was an increase of only 6 percent in fighting in the vehicle controls over this same period of time. The increase in fighting after 6-hydroxydopa was significant ($P <$ .001). If the baseline number of attacks over the initial 3 days was compared with the number on days 4, 5, and 6 after the 6-hydroxydopa injection [as done in studies with 6-hydroxydopamine (*3, 4*)], the attack percentage rose from 5.2 to 38.4 percent for the 6-hydroxydopa-treated group ($P < .01$), and from 11.4 percent to only 12.3 percent for the controls. Rats treated with 6-hydroxydopa demonstrated increased irritability commencing about 48 hours after the injection. Some rats frequently attempted to bite the experimenter when handled and often attempted to escape from the home cages if they were opened. There was, however, little or no intraspecific aggression when the animals were in their home cages.

Seven days after the injection of 6-hydroxydopa, whole brain NE was significantly decreased ($P < .001$), while whole brain DA remained un-

changed (Table 1). The NE decrease affected all parts of the brain assayed, with the hypothalamus being most depleted (Table 1).

Correlative fluorescent histochemical observations were also obtained 7 days after 6-hydroxydopa treatment. There was a moderate to marked reduction of varicosities in the neocortex, septum, preoptic area, and hypothalamus (Fig. 2), but not in the caudatus putamen. The most pronounced alteration was the presence of numerous green fluorescent preterminal axons that were not observed in control brains. These fluorescing preterminal axons (Fig. 2) were observed in noradrenergic areas of the medulla oblongata, reticular formation, cerebellar peduncle, hypothalamus, preoptic region, septum, and cingulum. This is similar to that seen in the mouse brain after an intravenous injection of 6-hydroxydopa (*5*). Analogous to 6-hydroxydopamine treatment, the appearance of intensely fluorescent axons is highly suggestive of a proximal accumulation of catecholamines within monoaminergic neurons after degeneration of nerve terminals (*11*).

Thus, selective depletion of brain NE without DA depletion can be produced by 6-hydroxydopa. When it occurs, it is accompanied by an increase in shock-induced fighting. The time course of the onset of increased fighting behavior suggests that NE depletion is not the sole cause of the facilitation of shock-induced fighting. On the second day after an injection of 90 μg of 6-hydroxydopa, at a time when no increase in shock-induced aggression is evident, brain NE is already as low as on day 7 (*12*).

In addition, however, 6-hydroxydopa produces degeneration of central NE terminals, as noted by accumulation of intensely fluorescent noradrenergic preterminal axons (*5*). This is an effect similar to that produced by 6-hydroxydopamine (*11*). Ungerstedt (*13*) has shown that intracerebral injections of 6-hydroxydopamine produce supersensitivity of central DA receptors to exogenously administered L-dopa and apomorphine. It is plausible that, as with 6-hydroxydopamine, the NE terminal degeneration produced by 6-hydroxydopa is accompanied by an increase in sensitivity of NE receptors to endogenous amine which in turn induces an increase in shock-induced aggression (*14*).

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Uranium in Runoff

In the report by Spalding and Sackett (*1*) we have to assume that the P/U ratios are correct. However, if we compare the phosphate and uranimum values from the Brazos River (*1*, table 1) the resulting correlation coefficient would be very low, an indication that the two are not related. The relations shown in figure 2 (*1*) raise some questions.

Spalding and Sackett arrive at the erroneous value of 285 short tons of U_3O_8 as being applied to those U.S. regions draining into the Gulf of Mexico because of the large error they made in the amount of P_2O_5 applied in 1967. They stated that ". . . this amounted to over 2.9 billion short tons of P_2O_5. . . ." The facts are that the total P_2O_5 used in the 48 contiguous states was only 4.7 million tons for fiscal 1971. The amount used in the Gulf region would only be a fraction of that. Whether or not the initial error was made in the *Chemical Economics Handbook* (*1*, reference 6) or in their use of the published figures is not clear.

With respect to their assumption that the difference between the P/U ratio in fertilizers and rivers suggests that more than 90 percent of the applied phosphate has been taken up by plants, a look at the reaction product between soil and fertilizer would indicate that rarely is more than 20 percent of the applied phosphorus recovered in a given year. The remaining portion becomes an integral part of the soil, some of which will be available to subsequent crops.

The agricultural and environmental implications of Spalding and Sackett's work could be explored further, perhaps by the scientists at some public agency like the Tennessee Valley Authority's National Center for Fertilizer Development and Soils and Fertilizer Research Branch.

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Reference

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Munson points out a mistake in our article that occurred in copying; however, this mistake was not used in the calculation of 285 short tons. The article should read as follows: In a 5-year span from 1962 to 1965 fertilizer consumption increased from 25 million to 36 million short tons per year. In 1967 this amounted to over 2.9 million short tons of P_2O_5 applied to United States regions draining into the Gulf of Mexico. If we assume an average value of uranium in phosphate fertilizer to be 100 μg/g, this amounts to approximately 290 short tons of uranium.

Since 100 μg/g is a minimum uranium concentration in phosphate fertilizer, especially when one considers that all tabulations of tons of P_2O_5 applied are in terms of 100 percent P_2O_5, a considerably higher uranium concentration could have been used in this calculation and still have been realistic. In short, the mistake noted by Munson did not change the uranium estimates or the implications of the report.

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