*REDUCTION OF NOREPINEPHRINE IN THE LOWER BRAINSTEM BY PSYCHOLOGICAL STIMULUS**

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It is rather obvious from day-to-day experience that animals may modify one another physiologically without actually coming into physical contact. However, there appear to have been no clear-cut demonstrations of the production of changes in specific brain chemicals as a result of purely psychological stimuli. The importance of attempting to detect and understand the nature of such changes is apparent when one considers that many mental and somatic illnesses may have their etiology, at least partially, in social environmental pressures. The desirability of looking for possible sociopsychological effects upon norepinephrine in the brain is obvious, for there is evidence suggesting its role as a neurotransmitter,[1–3] and further, a substantial body of experimental evidence has related differences in its metabolism to differences in normal and abnormal affective states.[4, 5] In this paper, we will report evidence that merely witnessing fighting, without opportunity for actual physical contact, may markedly lower norepinephrine in the pons and medulla oblongata of the brain of mice.

Methods.—Male ICR white Swiss mice were employed. Littermates weighing 18–20 gm were weaned at 6 weeks of age and were individually housed under filter tops in opaque solid-bottomed polycarbonate cages 7 × 11.5 × 5 in. in size. They were not shipped, but were housed at the breeder's in a quiet room maintained at 25–27°C. Unmodified Wayne Feed for Laboratory Mice was available to them *ad libitum*. Cages were changed once each week. The mice presumably could hear and smell other mice in adjoining cages, but they could not see, touch, or otherwise interact with them. Such mice become aggressive and will normally initiate vigorous fighting within 5–60 sec after they are placed with another mouse.

The experiment was terminated 10 weeks after the mice were isolated, at which time they weighed 33–35 gm. Half of the animals were sacrificed between 9:00 P.M. and midnight on one evening, and the remainder during the same hours on the following evening. When the animals were sacrificed, a strict systematic rotation was maintained from one treatment to the other every two animals in order to balance the effects of normal rhythmic or otherwise uncontrollable variations, and an identical pattern of rotation was later employed for all phases of amine extraction and analysis in the laboratory.

There were three experimental conditions with 20 mice in each: (1) For *controls*, cages were not moved from their original location on the shelf until time for sacrifice; when a cage was moved, the filter top was immediately removed and the mouse was sacrificed without delay. (2) As a control for the possible effect of a shift in environment, some mice were gently placed in an empty wire-mesh feed basket that was positioned over an empty *strange cage*, and a second feed basket was inverted over it to contain them; these mice were removed and sacrificed exactly 75 min later. (3) For *exposure to fighting*, individual mice were treated identically, except that the cage over which they were suspended contained four to six other preisolated mice that were fighting vigorously; physical contact between the experimental mice and the fighting mice was prevented by positioning the basket containing the experimental mice in a second feed basket, thereby producing a double wire-mesh separation of about $1/2$ in.

Sacrifice was by decapitation. The brains were immediately removed, rinsed in cold isotonic saline, and transferred to a bakelite platform over dry ice in order to keep them

chilled below 0°C during the period of dissection (approx. 30 sec). Our dissection was identical to that employed by J. Glowinski[6] for rat brains, except that we retained the diencephalon and mesencephalon as a single unit. The cerebellum and the bulbus olfactorius were discarded and the remainder of the brain was subdivided into three pieces, the telencephalon, the combined diencephalon and mesencephalon, and the combined pons and medulla oblongata. The three dissections weighed 259 ± 0.9 mg, 82 ± 0.4 mg, and 57 ± 0.3 mg, respectively (mean ± s.e.m.). The brains were immediately frozen on dry ice and maintained at −50°C until analysis.

Norepinephrine was analyzed in brain parts from individual animals. Tissues were homogenized, while still frozen, in 2.5 ml of ice-cold 0.01 *N* HCl containing 10 mg ethylenediaminetetraacetate (EDTA) for stabilization of the amine during extraction. Norepinephrine was extracted into 25 ml of salt-saturated n-butanol by rapid shaking for 10 min on a reciprocating shaker; after centrifugation at 3000 rpm in the cold, 23 ml of the butanol was transferred to 40 ml of washed heptane, and the norepinephrine was reextracted into 1 ml of 0.01 *N* HCl. For each set of extractions, three concentrations of norepinephrine (50, 100, 150 ng) were added in duplicate to brain homogenates and carried throughout the entire procedure to serve as internal standards. Recovery of the internal standards averaged 79% with an s.e.m. of ±0.3–0.6% within a given analytical run. Separate extraction and analytical runs were made for each brain part. The norepinephrine was measured in a final volume of 1.6 ml by the trihydroxyindole procedure using I_2 as the oxidant.[7] Fluorescence was developed by exposure for 35 min to our standard lighting conditions consisting of a fluorescent desk lamp positioned at a distance of 10 cm above the tubes, which were placed on a reflecting background. Relative fluorescent intensity was measured with an Aminco-Bowman spectrophotofluorometer equipped with adapter for semimicro (0.6-ml) cuvettes. Slit setting No. 3 was used with an excitation wavelength of 400 mμ and an emission wavelength of 510 mμ. Spectra of representative samples were checked against the spectrum of authentic norepinephrine. Where the norepinephrine was measured in the pons and medulla oblongata of individual mice, the actual amount of norepinephrine in the final control samples was 20–24 ng.

Results.—The mice that were placed individually into wire baskets above empty strange cages initially demonstrated a moderate amount of exploratory activity, but they did not appear to be greatly disturbed; all were quiet and a few were asleep at the time of sacrifice 75 minutes later. However, the mice that were kept individually in wire baskets above the fighting mice for 75 minutes appeared to be somewhat excited. They maintained their orientation towards the fighting animals throughout most of the experimental period, and most of them gnawed at the separating wire and "dug" at it with their forefeet in an apparent attempt to join the fighting mice. They showed mild piloerection about the neck and a distinct tachycardia. The fighting mice were largely preoccupied with each other, and we noticed no attempt on their part to attack a mouse suspended above them in a wire cage. Further, none of the experimental mice showed any evidence of having been bitten. Only their tails could possibly have extended through the double wire-mesh barrier, and it is unlikely that this happened, particularly in view of their general orientation towards the fighting and in view of the fact that, even when they lie down, mice maintained on wire do not allow their tails to protrude through the wire but fold it under them instead. Results of the brain analyses are shown in Table 1. Simply allowing the mice to witness fighting for 75 minutes markedly lowered norepinephrine in the pons and medulla oblongata; norepinephrine levels were lowered about 24 per cent ($p < 0.002$) below levels in mice exposed to the strange cage alone, and about 32 per cent ($p < 0.001$) below levels in undisturbed controls. Exposure to

TABLE 1. *Reduction of brain norepinephrine in male mice that have witnessed fighting among other male mice made aggressive by long-term isolation.**

	Norepinephrine (ng/gm)		
	Undisturbed controls (n = 20)	Strange cage (n = 20)	Witness fighting (n = 20)
Telencephalon	568 ± 17.4	557 ± 11.4	531 ± 17.0
Diencephalon and mesencephalon	524 ± 25.2	583 ± 20.5	522 ± 26.6
Pons and medulla oblongata	472 ± 24.9	421 ± 22.4	319 ± 23.4†

* Values are expressed as mean ± s.e.m. Mice were placed in a feed basket above an empty strange cage or above a strange cage that contained fighting mice for 75 min. All brain parts were analyzed separately for individual animals. Differences were evaluated by Student's t test (two-tailed).

† Differs $p < 0.001$ from controls, $p < 0.002$ from strange cage.

a strange cage elevated norepinephrine in the combined diencephalon and mesencephalon approximately 11 per cent, but this increase was not significant ($p < 0.07$).

Discussion.—A number of experimental studies have shown that sociopsychological events may modify peripheral autonomic and endocrine functions both in animals and in humans. Of particular note are the demonstrations by Elmadjian *et al.*[8] and by Mason,[9] indicating that such factors may rather dramatically influence the activity of the adrenal medulla and the adrenal cortex in humans, and the well-controlled studies conducted by Bronson and Eleftheriou,[10] demonstrating that male mice that had previously experienced physical defeat by a "trained fighter" had a marked increase in unbound plasma corticosterone when they were subsequently faced with the same fighter, even though they were separated from him by a wire partition. Clearly, all such phenomena must be mediated through the brain. Other studies that are of interest, although physical effects cannot be so unequivocally separated from the psychological aspects of the experimental situation, include the observation by Barnett[11] that sometimes wild rats, which are very excitable, die without any sign of being wounded while under attack in the home territory of another rat, and a report by Bliss and Zwanziger[12] that brain norepinephrine was lowered in mice that were under attack by more aggressive mice, even though the mice were removed before any apparent injury had been inflicted.

Our study appears to provide the first evidence for a specific neurochemical change in the central nervous system following a mildly stimulating, purely psychological event, namely the exposure of naïve mice to the sights, sounds, and odors of vigorous fighting among other mice while physically separated from participation in the fighting by a wire-mesh barrier. We have shown elsewhere that mice actually allowed to engage in fighting may, under some circumstances, show similar reductions of brain norepinephrine in the pons and medulla oblongata (article submitted for publication). These observations suggest that psychological and physical stimuli, or combinations thereof, may have at least grossly similar effects on noradrenergic systems in the brain.

Fluorescence histochemical studies have demonstrated that a majority of noradrenergic nerve terminals in bulbospinal, higher subcortical, and cortical areas of the brain derive from cell bodies in the pons and medulla oblongata, and

further, that some of the largest groups of neurons involved are functional components of the formatio reticularis,[3, 13] the primary controller of the level of nonspecific activation of the brain.[14] The behavioral arousal observed in the mice exposed to fighting and the preferential reduction of norepinephrine in the pons and medulla oblongata fit well with an assumed activation of the brainstem reticular formation and an increased rate of release of amine neurotransmitter onto activating receptors within it.

Stimulation has recently been shown to increase the rate of transport of glutamate, a presumed neurotransmitter in snails, from the nerve cell body to nerve terminals.[15] Inasmuch as norepinephrine granules originate in the neuronal cell body and are transported distally to nerve terminals,[13, 16, 17] our findings are compatible with a possible acceleration of the proximodistal transport of norepinephrine by increased nervous activity.

Summary.—Norepinephrine was reduced by 32 per cent in the pons and medulla oblongata of the brain by allowing mice that previously had been made hyperexcitable by long-term isolation to observe for 75 minutes through a wire-mesh barrier the sights, sounds, and odors of vigorous fighting among other mice that had similarly been made aggressive by long-term isolation. Since the mice exposed to fighting had no opportunity for physical contact with the fighters, these data provide direct evidence suggesting that purely psychological stimuli may enhance the release of a specific neurotransmitter in the brain.

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