Reports

Catecholamine Uptake in Cerebral Cortex: Adaptive Change Induced by Fighting

Abstract. *Kinetics of the catecholamine uptake process in brain were altered by fighting. Significant increases in the apparent Michaelis constant* (K_m) *for the uptake of norepinephrine into cerebral cortical homogenates and significant increases in the inhibition constant* (K_i) *for* d-*amphetamine inhibition of this uptake occurred in group-caged mice living under chronic attack from aggressive cage mates. Also, significant increases in the apparent* K_m *and maximum velocity* (V_{max}) *for norepinephrine uptake were observed 18 to 20 hours after the last of a series of short intense daily fights between male mice previously made aggressive by long-term individual caging. These results suggest that the natural stress of fighting leads to (i) lowered affinity for reuptake of norepinephrine into nerve endings of the cerebral cortex, (ii) an increase in the number of uptake sites, and (iii) lowered affinity for* d-*amphetamine.*

After being released from nerve terminals in the brain, norepinephrine is thought to be inactivated primarily by reuptake across the neuronal membrane and rebinding (*1*). Tricyclic antidepressants and amphetamines that alter mood, alleviate depression, or induce schizoid-like psychoses have in common an ability to impair this natural reuptake, and this fact has motivated suggestions that some forms of depressive illness and schizophrenia may involve aberrations in the metabolism of brain catecholamines (*2*). Moreover, psychosocial stress, which often is a factor in the development of mental and emotional illness, dramatically alters the effects of these drugs and also accelerates the synthesis, release, and breakdown of brain (norepinephrine (*3*). If natural changes in the neuronal uptake of brain norepinephrine occur in response to different chronic conditions of nervous stimulation, this might have important implications for our understanding of the etiology and biochemical basis of some kinds of affective and ideational disorders. We report that the stress of chronic intermittent fighting in male mice produces alterations in the characteristics of the norepinephrine uptake system in brain.

We used synaptosome-rich homogenates of the cerebral cortex to measure the velocity of uptake of [^{3}H]norepinephrine in vitro by a modification of the method of Snyder and Coyle (*4*). The kinetic constants—apparent Michaelis constant (K_m) and maximum velocity (V_{max}) for norepinephrine uptake and the inhibition constant (K_i) for *d*-amphetamine inhibition of norepinephrine uptake—were calculated from Lineweaver-Burk plots (*5*) derived by least squares regression of the reciprocal of the velocity plotted against the reciprocal of the substrate concentration for [^{3}H]norepinephrine concentrations from 0.01 to 0.5 μM.

As a model of spontaneously occurring fighting behavior we used group-housed male mice (*6*) that exhibited evidence of severe bites on the rump and back (fight-stressed animals), sustained as a result of chronic aggressive attacks from dominant cage mates. Such fighting often occurs among certain incompatible groups of male mice despite adequate food, water, and living space (*3*). As controls we used similar group-housed males of the same age that were delivered from the supplier at the same time, but among which no evidence of fighting behavior was observed (group-housed controls). The apparent K_m for [^{3}H]norepinephrine uptake in grouped fight-stressed mice was significantly higher (64 percent) than in nonfighting controls (Table 1A and Fig. 1A); the K_m was higher for the fight-stressed mice in five of six pairs tested. Also, the K_i for *d*-amphetamine, a competitive inhibitor of norepinephrine uptake, was significantly elevated (51 percent) in fighting mice; the K_i was higher for the fight-stressed mice in all five pairs tested. After 4 to 6 weeks, when all evidence of fighting behavior had completely subsided, all apparent differences between former

Table 1. Uptake of *dl*-[^{3}H]norepinephrine in synaptosome-rich homogenates of mouse cerebral cortex. Kinetic constants were derived from linear regression analysis by the method of least squares; S.E.M., standard error of mean; *N*, number of animals. (A) Group-housed male mice 3 to 4 months of age were used. Controls were animals among which no fighting occurred. Fight-stressed animals were those among which spontaneous fighting repeatedly occurred; they were scarred by biting on the rump and back. (B) Individually housed male mice were either left undisturbed until killed (controls), or placed together with three to ten other isolated mice for 10 to 15 minutes of intense fighting per day for 14 consecutive days, then killed 18 to 20 hours after the last fight (fighters). Data from two separate experiments with similar results were combined; one with mice at 5 to 6 months of age and one with mice at 8 to 9 months.

Animals	Apparent K_m (μM)		V_{max} (nmole per gram per 5 minutes)		K_i for *d*-amphetamine (μM)	
	Mean ± S.E.M.	*N*	Mean ± S.E.M.	*N*	Mean ± S.E.M.	*N*
			(A) Group-housed			
Group-housed controls*	0.279 ± 0.040	6	1.67 ± 0.24	6	0.059 ± 0.008	5
Fight-stressed	.457 ± .055	6	2.20 ± .54	6	.089 ± .007	5
P, paired	$P < .07$†		$P > .05$		$P < .005$	
			(B) Isolated			
Isolated controls*	.397 ± .049	15	1.79 ± .27	15		
Fighters	.645 ± .093	15	3.21 ± .68	15		
P, paired	$P < .005$		$P < .025$			

* Group-housed and isolated controls are not directly comparable because they were not assayed concomitantly. † $P < .05$ by Student's unpaired two-tailed *t*-test ($t = 2.62$).

fighters and controls were abolished, which suggests that the alterations induced during fight stress were reversible.

The decreased affinity of synaptosomes for *d*-amphetamine (increased K_i) observed in fight-stressed mice may explain an earlier observation of a decreased sensitivity of such mice to the lethal effects of *d*-amphetamine (*3*). *d*-Amphetamine also causes the release of [^{3}H]norepinephrine from synaptosomal preparations in vitro (*7*). However, under the conditions of this study, even 1 μM *d*-amphetamine (ten times the concentration used to determine K_i) failed to increase the 5-minute efflux of [^{3}H]norepinephrine from cerebral cortical homogenates. In such release experiments, in which 1 μM desmethylimipramine was added to inhibit norepinephrine uptake by more than 90 percent, significant increases in the 5-minute efflux of [^{3}H]norepinephrine did occur when reserpine and KCl were used. Therefore, it is unlikely that the changes in K_i for *d*-amphetamine reported here are due to the releasing action of *d*-amphetamine.

Earlier studies employing this system of fight stress showed that greater magnitudes of change are induced in various physiological systems when male mice previously rendered uncommonly aggressive by long-term isolation are brought together for brief episodes of intense fighting than when mice live together continuously (*8*). Therefore, as a more severe model of fighting stress, male mice were caged individually from the time of weaning until 5 to 9 months of age, when they were brought together and allowed to fight for 10 to 15 minutes per day for 14 consecutive days (*9*). At 18 to 20 hours after the last of these intense fighting episodes, fighters and controls (undisturbed isolated animals) were killed in pairs, and the kinetic constants for norepinephrine uptake were determined. Combined data from two series of experiments with similar results (Table 1B and Fig. 1B) show that the apparent K_m was significantly elevated (62 percent) and the V_{max} increased by 79 percent in fighters as compared with undisturbed isolated mice. Of the 15 pairs of mice tested, K_m was higher for fight-stressed mice in 13 pairs, and V_{max} was higher for 12 pairs. Similar experiments conducted with isolated mice at 2 to 3 months of age failed to demonstrate significant increases in apparent K_m and V_{max}, a result suggesting that there may be specific hormonal requirements for full development of the apparent fighting-induced adaptive changes in catecholamine uptake which are not fulfilled at the earlier age.

Our results suggests that, in addition to biochemical changes occurring in brain biogenic amines as a consequence of intense fighting behavior (*3*), fundamental changes also occur in the characteristics of the norepinephrine uptake system. The elevation in V_{max} following intense episodic fighting is interpreted as an adaptive increase in the number of norepinephrine uptake sites in the nerve terminal membrane. This presumed increase in uptake sites could be due either to de novo synthesis of new terminal branches in the catecholamine neurons of the cerebral cortex or to a physical change in the carrier protein in the existing nerve terminals. If one accepts Michaelis-Menten kinetics as being appropriate for interpretation of these membrane transport phenomena (*10*), then the greater uptake of norepinephrine in synaptosomes observed at each substrate concentration in mice exposed to episodic fighting may be considered a reflection of the greater increase in V_{max} in these mice as compared with mice fighting less intensively in incompatible groups. In the same context, the elevation in apparent K_m observed in fighting mice would be interpreted to reflect a decreased affinity for the uptake of norepinephrine by the individual uptake sites in the nerve endings of the cerebral cortex. A decrease in the affinity of uptake sites for norepinephrine would mean that norepinephrine released from nerve endings would remain in the vicinity of the postsynaptic receptors for prolonged intervals.

To our knowledge, these data provide the first evidence that the norepinephrine uptake mechanism in brain is an adaptable, plastic, and probably reversible phenomenon. How rapidly these changes can be induced, how long they persist, how specific they are for the particular modes of stimulation used in these experiments, what implications they have for behavior, and what role, if any, hormonal factors play in producing them are questions now amenable to direct experimental testing. One might expect that sustained alterations in neurotransmitter biosynthesis (*3*, *11*) during fight stress may follow sustained alteration of neuronal catecholamine reuptake.

Earlier experiments of similar design showed that merely grouping hyperexcitable mice with strangers, or placing them in the presence of other fighting mice under conditions where actual physical contact is prohibited, causes changes in enzymes and neurotransmitters as large or larger than those caused by fighting (*8*). The possibility must be evaluated, therefore, that psychic stress per se may have a role in inducing alterations in the kinetics of

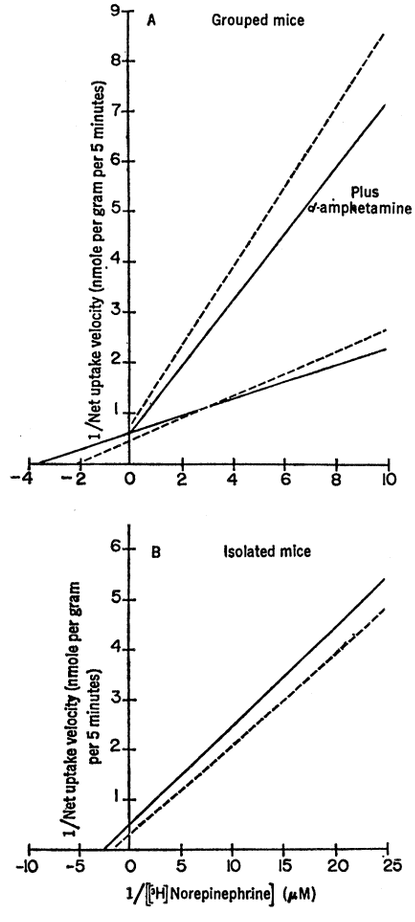

Fig. 1. Lineweaver-Burk analysis of changes in [^{3}H]norepinephrine uptake kinetics induced by fighting in male mice. Net uptake at 37°C less uptake at 0°C was determined in 5 minutes in homogenates of cerebral cortex from fighting mice (broken lines) and appropriate nonfighting controls (solid lines). Plotted values are averages derived from least squares regressions calculated for individual mice. (A) Fighting mice were group-housed animals that fought spontaneously over a period of 4 to 6 weeks until killed. Controls were group-housed mice with no apparent fighting behavior. Uptake velocity was measured with and without 0.1 μM *d*-amphetamine sulfate. (B) Mice made aggressive by long-term isolation were allowed to fight for 10 to 15 minutes per day for 14 days, then killed 18 to 20 hours after the last fight. Controls were undisturbed isolated animals.

norepinephrine uptake into brain nerve endings such as those observed in the two models of fighting stress reported here. It is possible that when "normal" or "abnormal" changes in mood or mental function occur as sequelae to chronic psychosocial stress, one important underlying mechanism may be a sustained alteration of the normal neuronal uptake of norepinephrine in the brain (*11*).

Note added in proof: After this report was submitted for publication, we found that the K_m for norepinephrine uptake in the cerebral cortex was increased immediately (68 percent, $P < .05$) after a single fighting episode in previously isolated mice, with no alterations in V_{max}; the difference in K_m no longer existed 24 hours after such single fighting episodes.

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Avalanche Mode of Motion: Implications from Lunar Examples

Abstract. *A large avalanche (21 square kilometers) at the Apollo 17 landing site moved out several kilometers over flat ground beyond its source slope. If not triggered by impacts, then it was as "efficient" as terrestrial avalanches attributed to air-cushion sliding. Evidently lunar avalanches are able to flow despite the lack of lubricating or cushioning fluid.*

An unusual feature at the Apollo 17 landing site has been interpreted as an avalanche deposit (*1*, *2*). The Apollo 17 avalanche, as it will be referred to herein, is a thin deposit of bright material that extends 5 km over a dark plain from the base of a mountain massif 2 km high (Fig. 1). This feature may hold important clues to mechanisms of debris transport where air or water are not available as lubricants. If it moved only by gravity, a low effective "friction coefficient" of only 0.2 is implied. This low friction is comparable to that of the Sherman (Alaska), Frank (Alberta), and other terrestrial avalanches in which the long runout has been attributed to air-cushion lubrication (*3*, *4*). In this report I compare the Apollo 17 avalanche with similar features on the earth and moon as a means of evaluating transport mechanisms.

The Apollo 17 avalanche covers 21 km^2, 2½ times the area of the Sherman avalanche (*3*). The thickness appears to decrease from perhaps 20 m or more at the mountain base to a few meters at the distal end, as determined from the size of small craters that penetrate the deposit so that their ejecta include darker underlying material (*2*). The thickness averages perhaps 10 m, so that the volume is approximately 200 × 10^6 m^3. Unlike bright crater rays, the margins of the deposit are fairly distinct against the adjacent dark material.

The thicker, proximal end of the deposit is ridged: low longitudinal ridges are spaced 100 to 200 m apart and are parallel or subparallel to the apparent direction of movement away from the slope. A narrow discontinuous moat several meters deep separates the avalanche deposit from the mountain slope, but it appears to be crossed by the longitudinal ridges. The moat may be analogous to distinctive troughs at some terrestrial rock avalanches (*5*).

A striking feature of the avalanche is that it appears to have hardly been influenced by preexisting topographic irregularities. An older crater 1 km in diameter and 60 m deep near the center of the deposit is clearly visible and apparently evenly mantled, as is also a fresh-appearing wrinkle-like ridge (Fig. 1).

The source of the avalanche was probably fine-grained regolith on the adjacent mountain slope (most of this slope is covered by such regolith). Two small isolated blocky areas of possible outcrop occur high on the slope above