for example, it would support the suggestion of Hruban *et al.* [6] that peroxisomes probably have a variety of functions in vertebrates depending on the tissue in which they occur.

In summary, feeding 0.25% CPIB, a hypolipidemic drug, caused significant increase in catalase activity and in the number of microperoxisomes in the intestinal mucosa of male rats. This increased catalase activity may be related to the important role of intestinal mucosa in cholesterol synthesis.

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Department of Pathology and Oncology,
University of Kansas Medical Center,
College of Health Sciences and Hospital,
Kansas City, KS 66103, *U.S.A.*

DONALD J. SVOBODA

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Dopamine—adaptive uptake changes in striatal synaptosomes after 30 sec of shock-induced fighting

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It has been found recently that adaptive kinetic changes in the neuronal uptake of norepinephrine (NE) take place in response to various forms of stress. These include electro-convulsive shock similar to that used in the treatment of depression [1, 2], acute head trauma,* and fighting [2–4].

In the latter instance, isolated mice subjected to chronic, daily fighting episodes exhibit an increased maximum velocity (V_{max}) for NE uptake in synaptosome-rich homogenates of cerebral cortex [3]. At the same time, there is a decreased carrier receptor affinity for this catecholamine, as indicated by an increased value for the Michaelis constant (K_m). When apparent Michaelis–Menten kinetics for NE uptake are also applied to acutely fighting isolated mice [2], there is little if any increase in V_{max}, but again the K_m is increased, a change which reverses itself after 24 hr. However, both V_{max} and K_m for NE uptake are strikingly increased in synaptosomes isolated from retired male breeding mice that fight vigorously for only 5 min [4].

In the present study, we investigated dopamine (DA) uptake to see whether this, too, was altered by fighting. We used a more intense fighting model than those previously referred to and we measured dopamine uptake in striatal synaptosomes after just 30 sec of combat to see if kinetic changes like those described above occurred almost immediately. Finally, we tested the effects of diphenylhydantoin (DPH, Dilantin) on DA uptake in our fighting animals because it has already been shown that DPH inhibits the enhanced uptake of NE induced by fight-

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ing in retired male breeding mice [4] and that it abolishes fighting in mice receiving electric foot-shock [5, 6].

We report that intense fighting produces virtually instantaneous changes in DA uptake and that DPH alters DA uptake in both fighting and control animals.

Adult (350 g) white male Wistar rats were individually caged for 1–3 weeks in an animal room where light, temperature and humidity were standardized. Before experimentation, the animals were transferred to the laboratory without removing them from their cages. After the move, they remained undisturbed for approximately 1.5 hr. Then they were quickly but gently lifted by pairs into a small plexiglass cage (21 × 21 × 13 cm) with a wired grid floor (Psychological Instruments, Richmond, Va.). Without delay, they received a 25 A foot-shock at 120 cps which lasted 30 sec. This aversive stimulus produced extreme fighting characterized by repeated biting and swift combative movements of all four extremities. Controls consisted of unshocked, non-fighting pairs.

The moment treatment was completed, the animals were lifted from the test boxes (two workers) and decapitated. The brains were removed within 30 sec and placed on chilled dental wax over ice in a deep, broad container. The corpora callosa were split and the striate bodies were shelled out [7], stripped of white matter, and dropped in chilled 0.32 M sucrose within 2 min. The cold, pooled tissue was rapidly blotted, weighed, and transferred to a glass homogenizing tube which contained chilled 0.32 M sucrose.

Synaptosome-rich homogenates were prepared from the pooled tissue and incubated with DA according to the modification by Welch, Hendley *et al.* [2, 3] of the method of Snyder and Coyle [8]. The concentration of [^{3}HG]DA HCl (sp. act. 8.6 Ci/m-mole, New England Nuclear Corp.) was held constant and cut with DA HCl (Sigma) to achieve four concentrations, 0.8, 0.4, 0.2 and 0.1 μM. DA uptake during 5 min was determined in triplicate at both 37° and 0° by liquid scintillation spectrometry. Net uptake (the 37° values less the 0° values) was used to determine kinetic constants. The incubation volume was 4 ml, and the aliquots contained 4 mg of original tissue. All experiments were replicated three times at each of the four substrate concentrations with the exception of the 0.2 μM concentration of the fighter experiments with DPH and all concentrations of the fighter experiments without DPH which were replicated twice. Identical incubations were also performed on the pooled striata of control resting rats and all experiments were again repeated after adding 0.1 mM DPH to the incubation medium. The experiments were carried out over a month and the control experiments were randomly distributed in relation to the treatment groups. V_{max} and K_m were calculated from Woolf plots [9] derived by linear regression of the velocity (V) plotted against the velocity over the substrate concentration ($V/[S]$).

The uptake for DA in striatal synaptosomes is increased in our fighting animals after just 30 sec of intense fighting, as compared with resting controls (Table 1 and Fig. 1). This is due to a significant increase in V_{max}. The K_m is not altered. On the other hand, DPH inhibits DA uptake in striatal synaptosomes obtained from both fighting and resting control animals (Table 1 and Fig. 1). However, this is accomplished by decreases both in (1) V_{max}, and (2) the membrane affinity for DA (increased K_m).

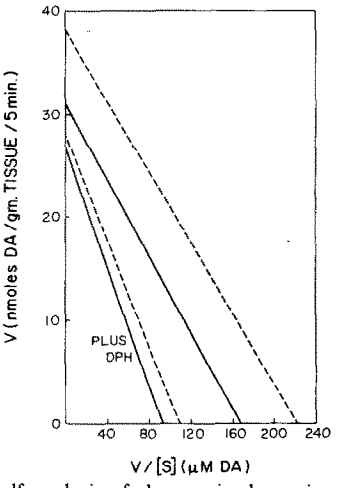

Fig. 1. Woolf analysis of changes in dopamine uptake induced by intense fighting in male rats receiving electric foot-shock (broken lines) and non-fighting controls (solid lines). The ordinate represents net (active) uptake velocity (V) in nmoles DA/g tissue/5 min and the "y" intercept indicates V_{max}. The abscissa represents the velocity over the μM concentration of DA ([S]). Therefore, the slope, V over $V/[S]$ is equal to [S] or the K_m. Plotted values are experimental averages derived from least squares regression calculated using four different concentrations of DA. Uptake velocity is measured with and without 0.1 mM DPH. Correlation coefficients for linearity and "t"values of slope (K_m) and intercept (V_{max}) data are all highly significant for each set of experiments ($P < 0.001$).

The results demonstrate that adaptive changes in membrane transport for DA can be measured almost instantaneously after intense fighting induced by electric foot-shock. To our knowledge, the shortest time interval previously reported to modify the kinetics of neurotransmitter uptake incident to a stressful situation is 5 min [1, 4]. In the present study, the increased V_{max} indicates that more DA receptor sites are available per unit time. This may reflect either unmasking or more efficient use of existing sites or creation of new ones. However, an alternate mechanism for the apparent change in V_{max} is exchange diffusion which could conceivably occur if DA levels were higher in the shocked fighting animals. Failure to observe a change in K_m as was reported earlier for NE after 5 min of fighting in mice [4] may simply mean that there was insufficient time to produce alterations in membrane affinity for DA. However, the present study also differs

Table 1. Uptake of dopamine in synaptosome-rich homogenates of rat striatum*

Experimental design	N	V_{max}† (nmoles/g/5 min)	Apparent K_m† (μM)
Non-fighters (NF)	12	31.08 ± 2.08‡	0.185 ± 0.028‡
Fighters (F)	8	38.25 ± 2.90	0.166 ± 0.030
Non-fighters + DPH (NFD)	12	27.01 ± 1.16	0.279 ± 0.023
Fighters + DPH (FD)	11	28.25 ± 3.26	0.258 ± 0.056

* Kinetic constants are derived from linear regression analysis; N indicates the number of determinations; and P is probability by t-test.

† For V_{max}, the following parallel comparisons are significant: NF vs F, $P < 0.05$; NF vs NFD, $P = 0.05$; and F vs FD, $P < 0.025$. Significant parallel comparisons for K_m are: NF vs NFD, $P < 0.01$; and F vs FD, $P < 0.05$.

‡ Mean ± standard error of mean.

from our 5-min fighting interval study in terms of the animal species used, the neurotransmitter employed, the areas of the brain examined and the intensity and induction of aggression, etc.

These data on DA uptake in fighting may be important, since they fit with other evidence that DA metabolism plays a role in mediating or modulating fighting behavior. Though not all studies are so clearly supportive of the role of DA in aggression as those cited below, and even contrary results have been obtained, the evidence includes the finding of unusually high levels of DA in the striata of aggressive animals [10] and the fact that intraventricular infusion of DA increases shock-induced fighting in rats [11]. Also, specific DA agonists such as apomorphine will induce aggressive behavior in rats [12–14] which can be subsequently abolished by administration of 6-hydroxydopamine, a DA antagonist. Finally, the direct precursor of DA, *l*-dopa, enhances fighting in experimental animals [10] and some patients who receive *l*-dopa for treatment of Parkinson's disease exhibit increased arousal and irritability [15].

The role of DA in fighting is of added interest, since this substance is richest in areas of the brain, such as the striate body and substantia nigra which are known to be the seat of the extrapyramidal system, a system which integrates and refines motor activity. Proper alterations in this system would be of obvious value to the intense motor activity of fighting.

Now let us analyze the effect of DPH on DA uptake. It is apparent that DPH produces both competitive (increase in K_m) and non-competitive (decrease in V_{max}) inhibition of DA uptake (Table 1 and Fig. 1). Competitive inhibition implies that DPH may interfere with DA uptake by binding with the DA receptor of the uptake carrier molecule in the pre-synaptic membrane. In contradistinction, we have previously reported that DPH inhibition of NE uptake is completely non-competitive, since in that instance V_{max} is decreased but the K_m is not changed.

The fact that DPH also inhibits DA uptake in part by non-competitive means inplies that DPH interferes with the metabolic transport of DA or impedes its passage through the membrane in some other manner. Indeed DPH has been shown (1) to inhibit ATPase [16, 17], an enzyme required for the active uptake of catecholamines [18], and (2) to alter ionic fluxes that may also effect neurotransmitter uptake [19, 20]. The present results confirm our earliest findings that DPH inhibits DA uptake in caudate putamen tissue slices of resting rats [21]. It should be noted that the inhibitory effect of DPH on DA uptake is directly antagonistic to the increased uptake of DA produced by fighting. This may represent a mechanism by which DPH abolishes fighting in electro-shocked mice, as reported in the laboratories of others [5, 6].

In conclusion, we have demonstrated an increased V_{max} for DA in rat striatal synaptosomes after just 30 sec of intense electro-shock-induced fighting. This alteration in neurotransmitter uptake may represent a virtually instantaneous plastic change in nerve ending membranes which could permit the central nervous system to respond immediately to stress by modulating neuronal firing. We have also demonstrated both a competitive and a non-competitive inhibitory effect of DPH on DA uptake which occurs not only at resting rates but also if uptake has been stimulated by fighting. Knowledge of this particular DPH effect may prove useful to those seeking to alter DA kinetics for clinical purposes or in the research laboratory.

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Divison of Neuropathology, Medical College of Virginia, Richmond, VA 23298, U.S.A.

M. GARY HADFIELD
WILLIAM F. C. RIGBY*

* Present address: Harvard Medical School, Boston, Mass.

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