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Dietary Tryptophan Reversal of Septal Lesion and 5,7-DHT Lesion Elicited Shock-Induced Fighting[1]

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KANTAK, K. M., L. R. HEGSTRAND AND B. EICHELMAN. *Dietary tryptophan reversal of septal lesion and 5,7-DHT lesion elicited shock-induced fighting.* PHARMAC. BIOCHEM. BEHAV. **15**(3) 343–350, 1981.—Using two procedures known to enhance shock-induced defensive fighting (SIF) and mouse-killing—septal lesions and 5,7-DHT lesions—we determined if a 5% tryptophan-loaded diet could reverse the lesion effects. The results indicated that SIF, but not mouse-killing, could be maintained at normal levels following dietary tryptophan loading in both septally lesioned and 5,7-DHT lesioned rats. This behavioral reversal was independent of pain sensitivity, feeding, drinking and body weight levels. Regional brain analysis of monoamines and metabolites indicated that the lesions produced substantial depletions in 5-HT and 5-HIAA with minimal reduction or no change in catecholamines. Dietary tryptophan loading elevated 5-HT and 5-HIAA in unlesioned animals and partially restored 5-HT and 5-HIAA levels in lesioned animals. These patterns of depletion and repletion were confined to the hippocampus following septal lesions and distributed trhoughout the brain following 5,7-DHT lesions. The results are discussed in terms of a possible hippocampal mediation of the dietary tryptophan reversal in shock-induced defensive fighting following lesioning.

Dietary tryptophan Shock-induced fighting Mouse-killing Septal lesions 5,7-Dihydroxytryptamine Brain monoamines and metabolites

WE have previously reported that a chronically fed tryptophan-deficient diet produces increases in shock-induced defensive fighting (SIF) and mouse-killing in rats [18]. In contrast a chronically fed 5% tryptophan-loaded diet produces little change in both behaviors. In a study by Jones *et al.* [16], septally lesioned rats failed to show an increase in SIF when pretreated with parachlorophenylalanine (PCPA). Because of the rapid onset of the PCPA reversal of the septal lesion-induced irritability and fighting (30 min to four hours post-injection), the effect could be attributed to the serotonin-enhancing properties of PCPA [14] rather than to the serotonin-inhibiting properties of PCPA [19].

In the present study, we used two procedures known to enhance SIF and mouse-killing: septal lesions [1, 6, 21] and 5,7-dihydroxytryptamine (5,7-DHT) lesions [3,17]. Following lesioning, we determined if a 5% tryptophan-loaded diet could reverse the lesion effects. The results indicated that SIF, but not mouse-killing, could be maintained at normal levels following dietary tryptophan loading in both septally lesioned and 5,7-DHT lesioned rats. Analysis of brain monoamines and metabolites indicated that serotonergic mechanisms within the hippocampus may be critical for the reversal in defensive fighting.

METHODS

Animals

Male albino rats (Holtzman, Madison, WI) were housed individually and had free access to food and water. A continuous 12 hr light-12 hr dark cycle and constant humidity and temperature were maintained. Sixty-two rats, 350–450 g on arrival, were used in the first experiment to study the effects of 5,7-DHT lesions, and 76 rats, 350–450 g on arrival, were used in the second experiment to study the effects of septal lesions.

Apparatus

The testing chamber for shock-induced fighting and jump-flinch thresholds consisted of a 32×25×30 cm Coul-

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bourn Instruments (Lehigh Valley, PA) Model E10-10 small animal test cage. A Coulbourn Instruments solid state shocker/distributor and power supply were programmed to deliver scrambled footshock. For shock-induced fighting [6], footshock was 2 mA for a duration of 0.5 sec which was presented every 15 sec (Experiment 1; see also [17]) or 7.5 sec (Experiment 2; see also [6]) for 50 shocks. Jump-flinch thresholds for pain sensitivity were determined as previously described [6] with shock intensities of 0.1, 0.2, 0.3, 0.4 and 0.5 mA.

Diets

The diets consisted of Purina Laboratory Chow (CHOW) with and without the addition of 5% L-tryptophan (TRP, ICN Nutritional Biochemicals, Cleveland, OH). The powdered diets were mixed with enough distilled water to make a dough which was pressed onto a foil sheet, air-dried overnight, and refrigerated to retard spoilage. In the home cage, food blocks were contained in pre-weighed 6 oz glass food cups and fed ad lib. Food intakes (corrected for spillage), water intakes, and body weights were recorded daily for all groups.

Surgery

Stereotaxic infusions and lesions were performed under 3 cc/kg Equi-Thesin anesthesia. For infusions, 96 μg of 5,7-dihydroxytryptamine (5,7-DHT, expressed as free base of creatinine sulfate salt; Sigma, St. Louis, MO) were dissolved in 10 μl of a 0.1% ascorbic acid solution made with 0.9% bacteriostatic saline and infused into the right lateral cerebroventricle according to predetermined DeGroot [5] coordinates: AP+5.4, L+2.0, V+8.0 mm from the interaural line. Ten μl of the 0.1% ascorbic acid solution were used as the control VEHICLE. All rats treated with 5,7-DHT received intraperitoneal injections of 25 mg/kg desmethylimipramine HCL (USV Pharmaceuticals; Tuckahoe, NY) 1 hr prior to infusion to prevent uptake of 5,7-DHT into noradrenergic neurons [2].

For septal LESIONS, 2 mA of anodal DC current were bilaterally delivered through a platinum epoxilite-insulted electrode for 45 sec into the septal region according to predetermined DeGroot [5] coordinates: AP+8.6, L±0.7, V+5.5 mm from the interaural line. Control animals received SHAM lesions 2 mm above the septal region.

Procedures

Experiment 1: 5,7-DHT lesions and tryptophan loads. Following a seven-day adaptation period to CHOW, rats were paired and subjected to three days of shock-induced fighting (Baseline SIF). On day 11, animals were divided into two groups: one group continued to be fed CHOW and the other group was fed TRP. On the day of surgery, day 14, each diet group was further subdivided into two groups: CHOW/VEHICLE (n= 8 pair) and CHOW/5,7-DHT (n= 9 pair), or TRP/VEHICLE (n=7 pair) and TRP/5,7-DHT (n=7 pair). On post-infusion days 4, 5 and 6, the rats that were paired during Baseline SIF were fought again (Post-Infusion SIF). All rats were tested for mouse-killing on day 7 post-infusion by placing a mouse into the home cages for the subsequent 24 hours. On day 9 post-infusion, jump-flinch thresholds for pain sensitivity were measured (n=8 per group). At the end of the experiment, day 10 post-infusion, the animals were killed and the levels of norepinephrine (NE), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) were measured in the hippocampus, the forebrain (diencephalon, amygdala, striatum, septum), and the rest of brain (cortex, cerebellum, brain stem) by high performance liquid chromatography (HPLC) with electrochemical detection. The levels of NE and DA were measured using the alumina absorbtion method described by Hegstrand and Eichelman [12], and the levels of 5-HT, 5-HIAA and DOPAC were measured using the supernatant analysis method described by Kantak *et al.* [17].

Experiment 2: Septal lesions and tryptophan loads. Rats used in this second experiment were maintained as described above for the first 14 days. On the day of surgery, day 14, each diet group was further subdivided into two groups: CHOW/SHAM (n=8 pair) and CHOW/LESION (n=6 pair), or TRP/SHAM (n=8 pair) and TRP/LESION (n=10 pair). All rats were tested for mouse-killing on day 1 post-lesion for a 24 hr period. On post-lesion days 2, 3 and 4, the same rats that were paired during Baseline SIF were fought again (Post-Lesion SIF). On day 6 post-lesion, jump-flinch thresholds were measured (n=8 per group). At the end of the experiment, day 7 post-lesion, brain monoamines and metabolites were measured as above.

Statistical Analyses

Data from shock-induced fighting, jump-flinch thresholds, brain amines and metabolites, food and water intakes, and body weights were evaluated by the appropriate analysis of variance [29]. The Neuman-Keuls procedure was used for post-hoc testing. Fisher exact probability tests [11] were performed to analyze differences in the number of mouse-killers.

RESULTS

Experiment 1: 5,7-DHT Lesions and Tryptophan Loads

Shock-induced fighting. Analysis of the mean number of attacks per 50 footshocks revealed significant differences between groups, $F(3,24)=13.0$, $p<0.001$; days, $F(3,72)=2.97$, $p<0.05$; and groups×days, $F(9,72)=10.55$, $p<0.001$. Further testing revealed that groups did not differ during Baseline SIF, but did differ during Post-Infusion SIF (Fig. 1). On post-infusion days 5 and 6, 96 μg of 5,7-DHT (CHOW/5,7-DHT) enhanced the level of fighting compared to the CHOW/VEHICLE control and its own baseline, $p<0.01$. In unlesioned TRP/VEHICLE animals the level of fighting remained stable and was not different from the CHOW/VEHICLE control. The addition of 5% L-tryptophan to the diet reversed the facilitative effects of 5,7-DHT lesions on shock-induced fighting, $p<0.01$ (TRP/5,7-DHT). The level of fighting in the TRP/5,7-DHT group remained at baseline levels and was not different from the CHOW/VEHICLE and TRP/VEHICLE groups.

Mouse-killing and pain sensitivity. Ninety-six μg of 5,7-DHT failed to substantially influence mouse-killing (Table 1) as we have previously found with this moderate intraventricular dose [17]. Tryptophan loading was ineffective as well, whether or not rats were treated with 5,7-DHT. There were no diet or lesion effects on flinch or jump thresholds indicating no changes in sensitivity to footshock among groups.

Food, water and body weight. Analysis of mean food intakes (Table 2) during baseline (days 1–7), diet pre-lesion

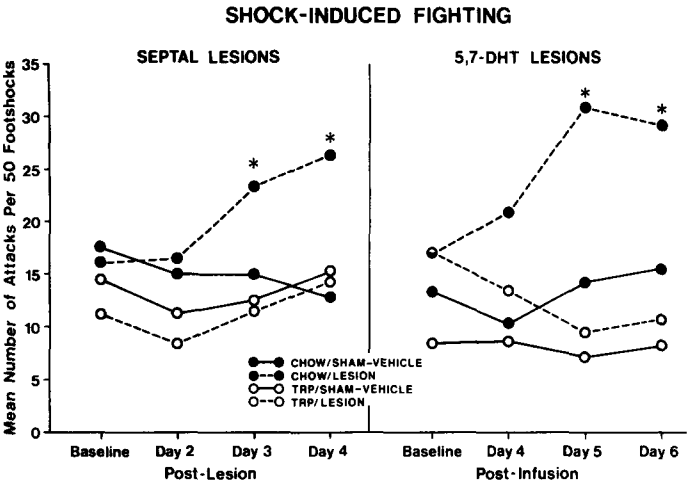

FIG. 1. Mean number of attacks per 50 footshocks in septal lesion experiment (left) and 5,7-DHT lesion experiment (right). Data were recorded during Baseline and days 2–4 Post Septal Lesion and during Baseline and days 4–6 Post 5,7-DHT Infusion in CHOW/SHAM-VEHICLE (solid circles with solid lines), CHOW/LESION (solid circles with broken lines), TRP/SHAM-VEHICLE (open circles with solid lines), and TRP/LESION (open circles with broken lines) groups. *Significantly different from all other groups, and own Baseline, $p \leq 0.01$.

(days 11–14) and diet post-lesion (days 1–6) periods revealed significant differences between groups, $F(3,57)=6.23$, $p<0.001$; days, $F(2,114)=25.93$, $p<0.001$; and groups×days, $F(6,114)=6.52$, $p<0.001$. Due to baseline differences in food intakes between groups, comparisons were made within groups. Further testing revealed that food intakes decreased in all groups during the diet post-lesion period relative to their own baselines, $p<0.01$ and relative to the diet pre-lesion period, $p<0.01$. There were no major effects on food intakes between groups during the diet post-lesion period when shock-induced fighting was markedly affected.

Analysis of mean water intakes (Table 2) revealed significant differences between groups, $F(3,57)=5.71$, $p<0.005$; days, $F(2,114)=14.40$, $p<0.001$; and groups×days, $F(6,114)=4.81$, $p<0.001$. Due to baseline differences in water intakes between groups, comparisons were made within groups. Further testing revealed that 5,7-DHT (CHOW/5,7-DHT) produced a hyperdipsia relative to baseline and diet pre-lesion water intake levels, $p<0.01$. This lesion effect on water intake could not be reversed by the addition of 5% L-tryptophan to the diet, $p<0.01$. (TRP/5,7-DHT) which under normal conditions does not influence

TABLE 1

MOUSE KILLING

	%	No.
5,7-DHT		
CHOW/VEHICLE	38%	6/16
CHOW/5,7-DHT	61%	11/18
TRP/VEHICLE	50%	7/14
TRP/5,7-DHT	57%	8/14
Septal Lesions		
CHOW/SHAM	44%	7/16
CHOW/LESION	85%	17/20*
TRP/SHAM	41%	7/17
TRP/LESION	86%	18/21*

*$p=0.01$ Compared to CHOW/SHAM control.

TABLE 2

5,7-DHT LESIONS: FOOD, WATER AND BODY WEIGHT

	Baseline	Diet Pre-Lesion	Diet Post-Lesion
Food Intake*			
CHOW-VEHICLE	48.9 ± 1.8	39.5 ± 1.4	34.0 ± 1.5‡
CHOW-5,7-DHT	51.4 ± 2.0	43.5 ± 2.5	36.4 ± 12.7†‡
TRP-VEHICLE	54.2 ± 1.1	53.0 ± 1.6	36.0 ± 3.3†‡
TRP-5,7-DHT	56.1 ± 1.0	53.4 ± 1.5	29.9 ± 3.4†‡
Water Intake*			
CHOW-VEHICLE	28.4 ± 2.1	33.8 ± 1.7	26.6 ± 1.6
CHOW-5,7-DHT	28.5 ± 2.9	32.7 ± 2.9	43.0 ± 3.6†‡
TRP-VEHICLE	20.6 ± 1.0	24.1 ± 0.7	25.7 ± 3.1
TRP-5,7-DHT	21.8 ± 1.5	27.6 ± 1.8	37.6 ± 4.8†‡
Body Weight*			
CHOW-VEHICLE	402 ± 11	419 ± 9	406 ± 10†
CHOW-5,7-DHT	404 ± 10	421 ± 6	382 ± 6†‡
TRP-VEHICLE	361 ± 3	372 ± 3	358 ± 5†
TRP-5,7-DHT	371 ± 3	392 ± 4	351 ± 4†‡

*Values are the mean ± S.E.M. for Baseline (days 1–7), Diet Pre-Lesion (days 11–14) and Diet Post-Lesion (days 1–6) conditions.
†$p<0.01$ compared to Diet Pre-Lesion days.
‡$p<0.01$ compared to Baseline days.

water intake (TRP/VEHICLE). Thus, 5,7-DHT lesions produced increases in water intakes which were not responsive to tryptophan treatment.

Body weight analysis (Table 2) revealed significant differences between groups, $F(3,56)=10.87$, $p<0.001$; days, $F(2,112)=96.95$, $p<0.001$; and groups×days, $F(6,112)=9.37$, $p<0.001$. Due to baseline differences in body weights between groups, comparisons were made within groups. Further testing revealed that body weights decreased in all groups during the diet post-lesion period compared to the diet pre-lesion period, $p<0.01$. However, body weight was reduced below baseline levels in CHOW/5,7-DHT and TRP/5,7-DHT groups only, $p<0.01$, during the diet post-lesion period. Thus, the 5,7-DHT lesion-induced decreases in body weights were not responsive to tryptophan treatment.

Brain monoamines and metabolites. Analysis of mean ng/g wet weight levels in the hippocampus (Table 3) revealed significant group differences in 5-HT, $F(3,38)=41.94$, $p<0.001$; and 5-HIAA, $F(3,36)=50.14$, $p<0.001$. The levels of NE did not significantly differ between groups and the hippocampal levels of DA and DOPAC were below the sensitivity of our assay. Further testing revealed that 5,7-DHT (CHOW/5,7-DHT) produced a 96% depletion in 5-HT and 88% depletion in 5-HIAA in the hippocampus, compared to the CHOW/VEHICLE control, $p<0.01$. The addition of 5% L-tryptophan to the diet (TRP/VEHICLE) produced a significant 46% elevation in 5-HIAA, $p<0.01$. In 5,7-DHT treated animals given 5% L-tryptophan in the diet (TRP/5,7-DHT) the levels of 5-HT and 5-HIAA were 75% and 65% depleted relative to CHOW/VEHICLE, $p<0.01$. However, the level of 5-HT was 21% restored in the TRP/5,7-DHT group relative to CHOW/5,7-DHT, $p<0.05$.

TABLE 3

BRAIN MONOAMINES IN 5,7-DHT LESIONED ANIMALS*

	NE	DA	DOPAC	5-HT	5-HIAA
Hippocampus					
CHOW-VEHICLE	494 ± 28	N.D.#	N.D.	240 ± 18	682 ± 37
CHOW-5,7-DHT	408 ± 35	N.D.	N.D.	10 ± 5†	79 ± 19†
TRP-VEHICLE	452 ± 56	N.D.	N.D.	250 ± 18	995 ± 75†
TRP-5,7-DHT	393 ± 21	N.D.	N.D.	60 ± 22†¶	242 ± 52†
Forebrain					
CHOW-VEHICLE	347 ± 10	868 ± 38	577 ± 39	368 ± 17	867 ± 46
CHOW-5,7-DHT	282 ± 11†	771 ± 38	600 ± 26	95 ± 9†	247 ± 28†
TRP-VEHICLE	291 ± 13†	837 ± 33	514 ± 25	555 ± 40‡	1319 ± 85†
TRP-5,7-DHT	281 ± 9†	781 ± 35	554 ± 35	217 ± 59‡¶	441 ± 45†
Rest of Brain					
CHOW-VEHICLE	316 ± 7	144 ± 11	63 ± 5	277 ± 31	293 ± 37
CHOW-5,7-DHT	265 ± 18†	154 ± 21	63 ± 7	71 ± 8†	90 ± 7†
TRP-VEHICLE	280 ± 9‡	144 ± 15	57 ± 4	365 ± 13†	469 ± 21†
TRP-5,7-DHT	267 ± 6†	166 ± 23	73 ± 9	141 ± 22†§	205 ± 34†§

*Values are the mean ± S.E.M. ng/g wet weight based upon 8–14 determinations.
†$p<0.01$ and ‡$p<0.05$ compared to CHOW-VEHICLE control.
§$p<0.01$ and ¶$p<0.05$ compared to CHOW-5,7-DHT Lesion.
#Not detectable.

Analysis of mean ng/g wet weight levels in the forebrain (Table 3) revealed significant group differences in NE, $F(3,41)=6.13$, $p<0.001$; 5-HT, $F(3,39)=20.03$, $p<0.001$; and 5-HIAA, $F(3,37)=64.24$, $p<0.001$. The levels of DA and DOPAC did not significantly differ between groups in the forebrain. Further testing revealed that 5,7-DHT (CHOW/5,7-DHT) produced a 74% depletion in 5-HT and 72% depletion in 5-HIAA in the forebrain compared to the CHOW/VEHICLE control, $p<0.01$. The addition of 5% L-tryptophan to the diet (TRP/VEHICLE) produced a significant 51% elevation in 5-HT, $p<0.05$, and 52% elevation in 5-HIAA, $p<0.01$. In TRP/5,7-DHT treated animals the levels of 5-HT, $p<0.05$, and 5-HIAA, $p<0.01$, were 41% and 49% depleted relative to CHOW/VEHICLE. However, the level of 5-HT was 33% restored in the TRP/5,7-DHT group relative to CHOW/5,7-DHT, $p<0.05$. The levels of NE in the forebrain were reduced 19%, 16% and 19% in the CHOW/5,7-DHT, TRP/VEHICLE and TRP/5,7-DHT groups, respectively, relative to CHOW/VEHICLE, $p<0.01$.

Analysis of mean ng/g wet weight levels in the rest of brain (Table 3) revealed significant group differences in NE, $F(3,41)=4.34$, $p<0.01$; 5-HT, $F(3,40)=51.28$, $p<0.001$; and 5-HIAA, $F(3,40)=37.62$, $p<0.001$. The levels of DA and DOPAC did not significantly differ between groups in the rest of brain. Further testing revealed that CHOW/5,7-DHT treatment produced a 74% depletion in 5-HT and 69% depletion in 5-HIAA in the rest of brain compared to CHOW/VEHICLE, $p<0.01$. TRP/VEHICLE treatment produced a significant 32% elevation in 5-HT and 60% elevation in 5-HIAA, $p<0.01$. In TRP/5,7-DHT treated animals, the levels of 5-HT and 5-HIAA were 49% and 30% depleted relative to CHOW/VEHICLE, $p<0.01$. However, the level of 5-HT was 25% restored and the level of 5-HIAA was 39% restored in the TRP/5,7-DHT group relative to CHOW/5,7-DHT, $p<0.01$. The levels of NE in the rest of brain were reduced 26%, $p<0.01$, 11%, $p<0.05$, and 15%, $p<0.01$, in the CHOW/5,7-DHT, TRP/VEHICLE, and TRP/5,7-DHT groups, respectively, relative to CHOW/VEHICLE.

Experiment 2: Septal Lesions and Tryptophan Loads

Shock-induced fighting. Analysis of the mean number of attacks per 50 footshocks revealed significant differences between groups, $F(3,28)=3.95$, $p<0.01$; days, $F(3,84)=5.42$, $p<0.01$; and groups×days, $F(9,84)=3.10$, $p<0.005$. Further testing revealed that groups did not differ during Baseline SIF, but did differ during Post-Lesion SIF (Fig. 1). On post-lesion days 3 and 4, septal lesions (CHOW/LESION) enhanced the level of fighting compared to the CHOW/SHAM control and its own baseline, $p<0.01$. In unlesioned TRP/SHAM animals, the level of fighting remained stable and was not different from the CHOW/SHAM control. However, the addition of 5% L-tryptophan to the diet reversed the facilitative effect of septal lesions on shock-induced fighting, $p<0.01$ (TRP/LESION). The level of fighting in the TRP/LESION group remained at baseline levels and was not different from the CHOW/VEHICLE and TRP/VEHICLE groups.

Mouse-killing and pain sensitivity. Septal lesions (CHOW/LESION) produced an 85% incidence of mouse-killing which was significantly different from CHOW/SHAM $p=0.01$ (Table 1). Tryptophan loading (TRP/SHAM) was ineffective in altering the incidence of mouse-killing. Tryptophan feeding in lesioned animals (TRP/LESION) did not reverse the effects of septal lesions on mouse-killing, and produced an 86% incidence in mouse-killing, $p=0.01$ compared to CHOW/SHAM. There were no diet or lesion effects on flinch or jump thresholds.

TABLE 4

SEPTAL LESIONS: FOOD, WATER AND BODY WEIGHT

	Baseline	Diet Pre-Lesion	Diet Post-Lesion
Food Intake*			
CHOW-SHAM	43.0 ± 1.0	38.1 ± 1.3	31.1 ± 2.4†‡
CHOW-LESION	46.5 ± 1.0	44.6 ± 2.1	25.6 ± 3.2†‡
TRP-SHAM	50.1 ± 2.0	48.9 ± 1.7	33.7 ± 2.4†‡
TRP-LESION	48.2 ± 1.2	46.1 ± 1.5	23.2 ± 2.0†‡
Water Intake*			
CHOW-SHAM	29.8 ± 1.4	32.1 ± 1.4	26.8 ± 1.6
CHOW-LESION	27.2 ± 1.4	26.9 ± 1.3	52.7 ± 8.1†‡
TRP-SHAM	28.9 ± 1.4	26.2 ± 1.3	29.7 ± 1.9
TRP-LESION	28.5 ± 1.2	27.0 ± 1.5	58.9 ± 15.8†‡
Body Weight*			
CHOW-SHAM	418 ± 4	427 ± 5	412 ± 5†
CHOW-LESION	417 ± 3	436 ± 4	381 ± 7†‡
TRP-SHAM	420 ± 7	428 ± 7	411 ± 6†
TRP-LESION	418 ± 3	424 ± 4	381 ± 6†‡

*Values are the mean ± S.E.M. for Baseline (days 1–7), Diet Pre-Lesion (days 11–14) and Diet Post-Lesion (days 1–4) conditions.
†$p<0.01$ compared to Diet Pre-Lesion days.
‡$p<0.01$ compared to Baseline days.

Food, water and body weight. Analysis of mean food intakes (Table 4) during baseline (days 1–7), diet pre-lesion (days 11–14) and diet post-lesion (days 1–4) periods revealed significant differences between groups, $F(3,64)=5.29$, $p<0.005$; days, $F(2,128)=147.1$, $p<0.001$; and groups×days, $F(6,128)=4.63$, $p<0.001$. Further testing revealed that food intakes decreased in all groups during the diet post-lesion period relative to their own baselines, $p<0.01$, and relative to the diet pre-lesion period, $p<0.01$. There was a small reduction in food intakes in CHOW/LESION and TRP/LESION groups during the diet post-lesion period relative to CHOW/SHAM and TRP/SHAM conditions. Thus, the small reductions in food intake which were produced by septal lesions were not responsive to tryptophan treatment.

Analysis of mean water intakes (Table 4) revealed significant differences between groups, $F(3,64)=3.96$, $p<0.05$; days, $F(2,128)=20.3$, $p<0.001$; and groups × days, $F(6,128)=9.91$, $p<0.001$. Further testing revealed that septal lesions (CHOW/LESION) produced a hyperdipsia relative to baseline and diet pre-lesion water intake levels, $p<0.01$. This lesion effect on water intake levels could not be reversed by the addition of 5% L-tryptophan to the diet, $p<0.01$ (TRP/LESION) which under normal conditions does not influence water intake (TRP/SHAM). Thus, like 5,7-DHT lesions, septal lesions produced increases in water intakes which were not responsive to tryptophan treatment.

TABLE 5
BRAIN MONOAMINES IN SEPTALLY LESIONED ANIMALS*

	NE	DA	DOPAC	5-HT	5-HIAA
Hippocampus					
CHOW-SHAM	429 ± 24	N.D.¶	N.D.	132 ± 11	1338 ± 116
CHOW-LESION	361 ± 18‡	N.D.	N.D.	37 ± 2†	430 ± 34†
TRP-SHAM	317 ± 17	N.D.	N.D.	169 ± 7†	2013 ± 80†
TRP-LESION	336 ± 22‡	N.D.	N.D.	64 ± 5†§	692 ± 34†§
Forebrain					
CHOW-SHAM	210 ± 8	619 ± 29	744 ± 85	596 ± 60	926 ± 100
CHOW-LESION	186 ± 9	591 ± 32	750 ± 63	557 ± 35	1000 ± 79
TRP-SHAM	193 ± 6	572 ± 15	1044 ± 62†	755 ± 44‡	2010 ± 130†
TRP-LESION	182 ± 4	570 ± 23	931 ± 46	727 ± 47‡	1784 ± 134†
Rest of Brain					
CHOW-SHAM	320 ± 24	143 ± 32	123 ± 16	212 ± 22	416 ± 42
CHOW-LESION	330 ± 13	150 ± 15	109 ± 7	222 ± 14	416 ± 10
TRP-SHAM	349 ± 18	154 ± 16	145 ± 8	320 ± 20†	740 ± 50†
TRP-LESION	345 ± 11	183 ± 16	127 ± 8	309 ± 19†	697 ± 31†

*Values are the mean ± S.E.M. ng/g wet weight based upon 8–10 determinations.
†$p<0.01$ and ‡$p<0.05$ compared to CHOW-SHAM control.
§$p<0.05$ compared to CHOW-LESION.
¶Not detectable.

Body weight analysis (Table 4) revealed significant differences between days, $F(2,128)=102.6$, $p<0.001$; and groups×days, $F(6,128)=12.6$, $p<0.001$. The group factor was not significant. Further testing revealed that body weights decreased in all groups during the diet post-lesion period compared to the diet pre-lesion period, $p<0.01$. However, body weight was reduced below baseline levels in CHOW/LESION and TRP/LESION groups only, $p<0.01$, during the diet post-lesion period. Thus, like 5,7-DHT lesions, the septal lesion-induced decreases in body weight were not responsive to tryptophan treatment.

Brain monoamines and metabolites. Analysis of mean ng/g wet weight levels in the hippocampus (Table 5) revealed significant group differences in NE, $F(3,34)=3.77$, $p<0.05$; 5-HT, $F(3,34)=90.2$, $p<0.001$; and 5-HIAA, $F(3,32)=107.98$, $p<0.001$. The hippocampal levels of DA and DOPAC were below the sensitivity of our assay. Further testing revealed that septal lesions (CHOW/LESION) produced a 72% depletion in 5-HT and a 68% depletion in 5-HIAA in the hippocampus compared to the CHOW/SHAM control, $p<0.01$. The addition of 5% L-tryptophan to the diet (TRP/SHAM) produced a significant 28% elevation in 5-HT and 50% elevation in 5-HIAA, $p<0.01$. In septally lesioned animals given 5% L-tryptophan in the diet (TRP/LESION), the levels of 5-HT and 5-HIAA were 52% and 48% depleted relative to CHOW/SHAM, $p<0.01$. However, the levels of 5-HT and 5-HIAA were 20% restored in the TRP/LESION group relative to CHOW/LESION, $p<0.05$. The levels of NE in the hippocampus were reduced 16% in the CHOW/LESION group and 22% in the TRP/LESION group relative to CHOW/SHAM, $p<0.05$.

Analysis of mean ng/g wet weight levels in the forebrain (Table 5) revealed significant group differences in 5-HT, $F(3,33)=5.28$, $p<0.005$; 5-HIAA, $F(3,35)=25.5$, $p<0.001$ and DOPAC, $F(3,32)=5.91$, $p<0.005$. The levels of NE and DA did not differ between groups in the forebrain. Further testing revealed that septal lesions (CHOW/LESION) did not affect the levels of 5-HT and 5-HIAA in the forebrain. Tryptophan loading in non-lesioned (TRP/SHAM) and lesioned (TRP/LESION) groups produced significant elevations in 5-HT, $p<0.05$, and 5-HIAA, $p<0.01$ compared to CHOW/SHAM control. DOPAC levels in the forebrain were elevated following 5% L-tryptophan loading, $p<0.01$. However, this change was not replicated in Experiment 1 (see Table 3).

Analysis of mean ng/g wet weight levels in the rest of brain (Table 5) revealed significant group differences in 5-HT, $F(3,35)=9.71$, $p<0.001$; and 5-HIAA, $F(3,33)=24.88$, $p<0.001$. The levels of NE, DA and DOPAC did not significantly differ between groups in the rest of brain. Further testing revealed that septal lesions (CHOW/LESION) did not affect the levels of 5-HT and 5-HIAA in the rest of brain. Tryptophan loading in non-lesioned (TRP/SHAM) and lesioned (TRP/LESION) groups produced significant elevations in 5-HT, $p<0.01$, and 5-HIAA, $p<0.01$ compared to CHOW/SHAM control.

DISCUSSION

These data indicate that shock-induced defensive fighting can be maintained at normal levels following 5% dietary tryptophan loading in both septally lesioned and 5,7-DHT lesioned rats. This reversal was independent of pain sensitivity, mouse-killing, feeding, drinking and body weight levels. Furthermore, the lesion effects on most of these other measures could not be reversed by the addition of dietary

tryptophan. Most striking are the similarities in the behavioral changes following the two independent, dissimilar lesioning procedures. This suggests the possibility of common neurochemical changes which are affected by both these procedures and direct the behavior.

The fact that tryptophan loading reversed the facilitative effects of lesions on SIF and partially restored the levels of 5-HIAA and/or 5-HT in these animals strongly supports a serotonergic influence on defensive behavior under these conditions. Both septal lesions and 5,7-DHT lesions produced substantial depletions in 5-HT and 5-HIAA with minimal reduction or no change in catecholamines. Serotonergic depletions following 5,7-DHT lesions were distributed throughout the brain; whereas the depletions of 5-HT and 5-HIAA following septal lesions were confined to the hippocampus among the regions examined. This suggests that serotonergic mechanisms within the hippocampus may be critical for this behavioral reversal. There is substantial evidence that hyperreactivity resulting from septal lesions [7,24], median raphe lesions [8,15], and parachlorophenylalanine administration [15,24] is mediated by the hippocampus. Furthermore, there is a temporal relationship between the depletion and repletion of 5-HT in the hippocampus and the development and disappearance of hyperreactivity, respectively [7]. All available evidence suggests that there is a causal relationship between hyperreactivity which develops following central 5-HT depletion and the facilitation of SIF (see [4] for review). Mouse-killing behavior, on the other hand, can be independent from reactivity [1]. This suggests a non-hippocampal mediation of this behavior. Others [9] have indicated that a reduction in serotonergic activity within the septum is of primary importance for maintaining mouse-killing. If septal, this could account for the lack of a reversal in mouse-killing in tryptophan-loaded, septally lesioned rats since electrolytic lesioning would destroy the neuronal elements in this area which probably would prevent the synthesis of 5-HT from excess tryptophan.

Although partial repletion of 5-HIAA and/or 5-HT occurred in the hippocampus following 5% dietary tryptophan loading in lesioned animals, it is unlikely that the partially restored levels alone could account for the reversal in shock-induced fighting unless serotonergic neurons were rendered supersensitive following lesioning. From previous work we know that a similar or less amount of 5-HT depletion, as was found in tryptophan-loaded lesioned animals, still results in facilitated shock-induced fighting [17,18]. It has been demonstrated that following destruction of serotonergic neurons by various means [22,23] there is an increase in the number of post-synaptic 5-HT receptors exclusively within the hippocampus. This increase in receptor number may compensate for diminished 5-HT levels to maintain normal functioning, positing sufficient 5-HT remaining to stimulate these receptors. In the present study, nearly complete depletion of 5-HT in the hippocampus occurred following septal and 5,7-DHT lesions. The moderate amount of 5-HT repletion in the hippocampus following 5% dietary tryptophan loading in lesioned animals could have been sufficient to stimulate a supersensitive serotonin system while the amount of 5-HT in lesioned chow-fed animals was not sufficient. In addition, Wang *et al.* [27] have demonstrated a presynaptic neuronal supersensitivity to exogenously applied 5-HT following intraventricular 5,7-DHT treatment. The firing rate in serotonergic neurons was facilitated under these conditions. This phenomenon could have contributed to the reversal in shock-induced fighting as well, with the 5-HT synthesized from the excess tryptophan acting as the exogenous source.

Perhaps the most intriguing question raised by the present study is how excess tryptophan modulates defensive-aggressive behavior under pathological conditions, but not under normal conditions. In general under normal conditions, excess tryptophan does not markedly influence behavior although it substantially elevates brain 5-HT and 5-HIAA [10, 13, 18, 20, 28]. Following drug or lesion procedures which deplete brain 5-HT, enhancing serotonergic activity restores behavior to normal [20, 25, 26]. It has been suggested that excess tryptophan can be intraneuronally metabolized without the transmitter being available to the postsynaptic receptor and therefore not behaviorally active [10]. It is also postulated that when central 5-HT stores are depleted, excess tryptophan is converted to 5-HT which is stored and thus available for release to receptors [20]. The mechanisms which regulate this dualistic utilization of 5-HT metabolized from excess tryptophan are not precisely known. By elucidating these molecular mechanisms we can gain further insight into how the brain functions in expressing behavioral actions, and potentially how best to modulate abnormal behavior.

REFERENCES

1. Albert, D. J. and K. N. Brayley. Mouse killing and hyperreactivity following lesions of the medial hypothalamus, the lateral septum, the bed nucleus of the stria terminalis, or the region ventral to the anterior septum. *Physiol. Behav.* **23**: 439–443, 1979.
2. Bjorklund, A., H. G. Baumgarten and A. Rench. 5,7-Dihydroxytryptamine: improvement of its selectivity for serotonin neurons in the CNS by pretreatment with desipramine. *J. Neurochem.* **24**: 833–835, 1975.
3. Breese, G. R. and B. R. Cooper. Behavioral and biochemical interactions of 5,7-dihydroxytryptamine with various drugs when administered intracisternally to adult and developing rats. *Brain Res.* **98**: 517–527, 1975.
4. Davis, M. Neurochemical modulation of sensory-motor reactivity: acoustic and tactile startle reflexes. *Neurosci. Biobehav. Rev.* **4**: 241–263, 1980.
5. DeGroot, J. The rat forebrain in stereotaxic coordinates. *Trans. R. Neth. Acad.* **52**: 1–40, 1959.
6. Eichelman, B. Effect of subcortical lesions on shock-induced aggression in the rat. *J. comp. physiol. Psychol.* **74**: 331–339, 1971.
7. Gage, F. H., R. G. Thompson and J. J. Valdes. Endogenous norepinephrine and serotonin within the hippocampal formation during the development and recovery from septal hyperreactivity. *Pharmac. Biochem. Behav.* **9**: 359–367, 1978.
8. Geyer, M. A., A. Puerto, D. B. Menkes, D. S. Segal and A. J. Mandell. Behavioral studies following lesions of the mesolimbic and mesostriatal serotonergic pathways. *Brain Res.* **106**: 257–270, 1976.
9. Gibbons, J. L., M. Potegal, A. D. Blau, S. Ross and M. Glusman. Quipazine and metergoline alter thresholds for septal inhibition of muricide. *Soc. Neurosci. Abstr.* **6**: 366, 1980.
10. Grahame-Smith, D. G. Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.* **18**: 1053–1066, 1971.

11. Hays, W. L. *Statistics for the Social Sciences*, 2nd ed. New York: Holt, Rinehart, Winston, 1973.
12. Hegstrand, L. R. and B. Eichelman. Determination of rat brain tissue catecholamines using liquid chromatography with electrochemical detection. *J. Chromat.* **222:** 107–111, 1981.
13. Hole, K. and C. A. Marsden. Unchanged sensitivity to electric shock in L-tryptophan treated rats. *Pharmac. Biochem. Behav.* **3:** 307–309, 1975.
14. Hwang, E. C. and M. H. Van Woert. Behavioral and biochemical properties of parachlorophenylethylamine (p-CPEA) in mice. *Life Sci.* **24:** 595–602, 1979.
15. Jacobs, B. L., C. Trimbach, E. E. Eubanks and M. Trulson. Hippocampal mediation of raphe lesion- and PCPA-induced hyperactivity in the rat. *Brain Res.* **94:** 253–261, 1975.
16. Jones, A. B., J. D. Barchas and B. Eichelman. Taming effects of p-chlorophenylalanine on the aggressive behavior of septal rats. *Pharmac. Biochem. Behav.* **4:** 397–400, 1976.
17. Kantak, K. M., L. R. Hegstrand and B. Eichelman. Facilitation of shock-induced fighting following intraventricular 5,7-dihydroxytryptamine and 6-hydroxydopa. *Psychopharmacology*, in press.
18. Kantak, K. M., L. R. Hegstrand, J. Whitman and B. Eichelman. Effects of dietary supplements and a tryptophan-free diet on aggressive behavior in rats. *Pharmac. Biochem. Behav.* **12:** 173–179, 1980.
19. Koe, B. K. and A. Weissman. P-Chlorophenylalanine: a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
20. Marsden, C. A. and G. Curzon. Studies on the behavioral effects of tryptophan and p-chlorophenylalanine. *Neuropharmacology* **15:** 165–171, 1976.
21. Miczek, K. A. and S. P. Grossman. Effects of septal lesions on inter- and intra-species aggression in rats. *J. comp. physiol. Psychol.* **79:** 37–45, 1972.
22. Nelson, D. L., H. S. Bourgoin, J. Glowinski and M. Hamon. Characteristics of central 5-HT receptors and their adaptive changes following intracerebral 5,7-dihydroxytryptamine administration in the rat. *Molec. Pharmac.* **14:** 983–995, 1978.
23. Seeman, P., K. Westman, D. Coscina and J. J. Warsh. Serotonin receptors in hippocampus and frontal cortex. *Eur. J. Pharmac.* **66:** 179–191, 1980.
24. Smith, R. F. Mediation of footshock sensitivity by serotonergic projection to the hippocampus. *Pharmac. Biochem. Behav.* **10:** 381–388, 1978.
25. Stewart, R. M., J. H. Growdon, D. Cancian and R. J. Baldessarini. 5-Hydroxytryptophan induced myoclonus: increased sensitivity to serotonin after intracranial 5,7-dihydroxytryptamine in the adult rat. *Neuropharmacology* **15:** 449–455, 1976.
26. Trulson, M. E., E. E. Eubanks and B. L. Jacobs. Behavioral evidence for supersensitivity following destruction of central serotonergic nerve terminals by 5,7-dihydroxytryptamine. *J. Pharmac. exp. Ther.* **198:** 23–32, 1976.
27. Wang, R. Y., C. deMontigny, B. I. Gold, R. H. Roth and G. K. Aghajanian. Denervation supersensitivity to serotonin in rat forebrain: single cell studies. *Brain Res.* **178:** 479–497, 1979.
28. Weinberger, S. B., S. Knapp and A. J. Mandell. Failure of tryptophan load-induced increases in brain serotonin to alter food intake in the rat. *Life Sci.* **22:** 1595–1602, 1978.
29. Winer, B. J. *Statistical Principles in Experimental Design*, 2nd ed. New York: McGraw-Hill, 1971.