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Dietary Tryptophan Modulation and Aggressive Behavior in Mice[1]

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KANTAK, K. M., L. R. HEGSTRAND AND B. EICHELMAN. *Dietary tryptophan modulation and aggressive behavior in mice.* PHARMAC. BIOCHEM. BEHAV. 12(5) 675–679, 1980.—The effects of a tryptophan-free diet on isolation-induced fighting and predatory cricket killing in mice were examined. The results demonstrated that consumption of a tryptophan-free diet for 18–20 days decreased both the number of fighters and duration of isolation-induced fighting; increased the number of cricket-killing mice and decreased the latencies to attack and the latencies to kill crickets; reduced brain serotonin 27%; increased water intake 38%; and decreased body weight 27% without affecting food intake. To determine if these effects were due specifically to the lack of dietary tryptophan, other groups of mice were fed a 5% tryptophan load in the standard chow; a 0.15% tryptophan supplement in the tryptophan-free diet; or a 3 grams/day restricted chow diet. The lack of tryptophan in the diet produced the marked inhibition in isolation-induced fighting, the reduction in brain serotonin, and the large decrease in body weight. The other non-specific effects appeared to be related to general factors such as dietary need for the cricket killing or diet composition (other than the lack of tryptophan) for the water intake.

Tryptophan-free diet Isolation-induced fighting Gryllicide

THE synthesis of serotonin (5-hydroxytryptamine, 5-HT) depends upon the availability of the essential amino acid tryptophan [34]. Therefore, limiting dietary tryptophan leads to depletion of brain 5-HT and elevating dietary tryptophan leads to enhancement of brain 5-HT [1,6]. Recently, there have been reports on the effects of dietary tryptophan modulation on behaviors which have been correlated, in part, with brain 5-HT [3, 8, 13, 16, 28, 31, 32]. With respect to aggressive behavior, tryptophan-free diets are reported to increase muricide [8,13] and shock-induced fighting [13] in male rats. Tryptophan loading in the diet, ranging from 0.5 to 5%, is without effects on rat aggression [8,13] and territorial fighting in male mice [31]. These reports are in general agreement with drug and lesion studies which show increases in aggressive behavior in rats following 5-HT depletion [5, 7, 12, 18, 29]. In male mice, isolation-induced fighting has been shown to be inhibited following 5-HT depletion produced by raphe lesions [15], p-chlorophenylalanine [17,33], 5,7-dihydroxytryptamine [19], 5,6-dihydroxytryptamine [25] and several serotonergic antagonists [17], although universal agreement with respect to 5-HT depletion and isolated mouse aggression is lacking [11]. In contrast, predatory cricket killing by mice is enhanced by p-chlorophenylalanine [23]. The present experiment reports the effects of dietary tryptophan modulation on isolation-induced fighting and predatory cricket killing (gryllicide) in male mice. Tryptophan-free (0%), tryptophan supplemented (0.15%), and tryptophan loaded (5%) diets were examined.

METHOD

Animals

CF-1 male mice (Sprague-Dawley, Madison, WI) were individually housed upon arrival and had free access to powdered Purina Laboratory Chow and water, unless otherwise stated. A continuous 12 hr light–12 hr dark cycle and constant humidity and temperature were maintained. Eighty mice, six weeks old on arrival, were used in this experiment. Live crickets *(Gryllus domestica)* were obtained from Wards Natural Science Establishment, Rochester, NY.

Apparatus

Clear 27.9×17.8×12.7 cm Plexiglas boxes which were used for home cages were also used as the testing chambers

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for isolation-induced fighting and gryllicide. A clean cage was used for each encounter.

Diets

The basic diets consisted of powdered Purina Laboratory Chow (CHOW) consisting of 0.28% tryptophan and 23% protein; or of a tryptophan-free diet (DIET) from Nutritional Biochemicals (Cleveland, OH) consisting of 0% tryptophan and 17% protein. In addition, powdered CHOW was loaded with 5% w/w L-tryptophan (CHOW+5% TRP) and powdered DIET was supplemented with 0.15% w/w L-tryptophan (DIET+0.15% TRP). Thus, the minimal requirements of protein (12.5%) and tryptophan (0.1%) for mouse, as set forth by the National Research Council, were met in each diet except, of course, for the 0% tryptophan in the tryptophan-free diet. A fifth group of mice was fed three grams/day CHOW (DEPRIVED) to group match the body weight loss of the DIET fed mice. Body weight was measured on Days 1, 11, 21 and 28 of the experiment. Food and water intakes were measured daily from the beginning of the experiment, except for the last week of isolation in order not to disturb the mice. Food was contained in 6 oz glass foodcups and water was contained in 8 oz glass water bottles with stainless steel spouts. Since spillage was almost negligible, it was not accounted for.

Procedure

Following 10 days of isolation, the five different diets were initiated and were continued until the end of the experiment on Day 30. There were 16 mice per dietary treatment. On Day 28, mice from each group were paired in a neutral test box. The latencies to attack were recorded and fighting duration was measured for 15 min. Aggressive attacks were defined as a directed movement toward the opponent mouse to make contact which resulted in biting or mutual fighting. On Day 29, one mouse was randomly selected from each fighting pair and tested for predatory cricket killing. Each mouse was paired with a cricket for 15 min and the latencies to attack and to kill the cricket were recorded. An attack was defined as striking the cricket with the forepaws or biting without consuming the prey. Nosing the cricket was not considered as an attack. This was considered to be investigatory and usually preceded attack behavior. A kill was defined as biting and consuming the cricket while holding the body in the forepaws. The tearing off and ingestion of the limbs was not considered as a kill. On Day 30, all mice were decapitated and brains were removed and assayed for 5-HT using a modification of the radioenzymatic assay of Saavedra *et al.* [27]. Qualification was made using the internal standardization method.

Statistical Analyses

All latency data, fight durations, 5-HT levels, food and water intakes and body weights were evaluated by the appropriate analysis of variance. Dunnett's tests and Neuman-Keuls procedure were used for all post hoc testing. Fisher exact probability tests were performed to analyze differences in the number of fighters and number of cricket attackers and killers in the various groups.

RESULTS

Isolation-Induced Fighting

Due to the variability both within and between groups,

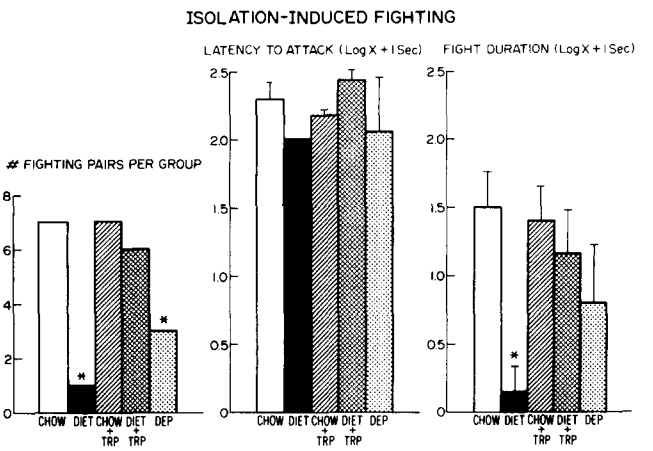

FIG. 1. The number of fighting pairs per group (left), the mean ± SEM latency to attack in log (x+1) sec (center) and mean ± SEM fight duration in log (x+1) sec (right) during isolation-induced fighting in the standard chow (CHOW), tryptophan-free diet (DIET), chow+5% tryptophan loaded (CHOW+TRP), tryptophan-free diet+0.15% tryptophan supplemented (DIET+TRP), and food restricted to 3 g/day chow (DEP) groups. *Significantly different from CHOW control, $p<0.055$.

attack latencies and fight duration were transformed to the log (x+1) sec prior to analysis. Mice which did not fight were not included in latency analysis. The number of fighting pairs of mice did differ with dietary condition. There were 1 of 8 fighting pairs in the DIET group which was a significantly lower number of fighting pairs than in the CHOW ($p=0.0$), CHOW+5% TRP ($p=0.0$) and DIET+0.15% TRP group ($p=0.02$). There were 3 of 8 fighting pairs in the DEPRIVED group which was also a significantly lower number of fighting pairs than in the CHOW ($p=0.055$) and CHOW+5% TRP ($p=0.055$) groups. The number of fighting pairs in the DEPRIVED group did not differ from DIET or DIET+0.15% TRP groups. The latencies to attack, which were not analyzed for the DIET group due to an insufficient number of data points, did not differ among the fighting pairs from various dietary groups. However, there were differences in fight duration, $F(4,35)=4.38$, $p<0.01$. DIET mice fought significantly less than CHOW ($p<0.01$), CHOW+5% TRP ($p<0.01$) and DIET+0.15% TRP ($p<0.05$). DEPRIVED mice differed neither from the DIET group nor from the CHOW, CHOW+5% TRP, and DIET+0.15% TRP groups. These data are presented in Fig. 1.

Gryllicide

The latencies to attack and to kill crickets were transformed to the log (x+1) sec due to between and within group variability. Mice which did not attack were not used in the attack latency analysis and mice which did not kill were not used in the kill latency analysis. The number of mich which attacked crickets did not differ with dietary condition whereas the latency to attack did differ, $F(4,31)=10.23$, $p<0.001$. DIET and DEPRIVED groups attacked significantly faster than did all other groups, $p<0.01$. These data are presented in Fig. 2A. The number of mice which killed crickets varied with dietary condition as did the latencies to kill, $F(4,22)=6.48$, $p<0.005$. There was an increased incidence of gryllicide in the DIET, DIET+0.15% TRP and DE-

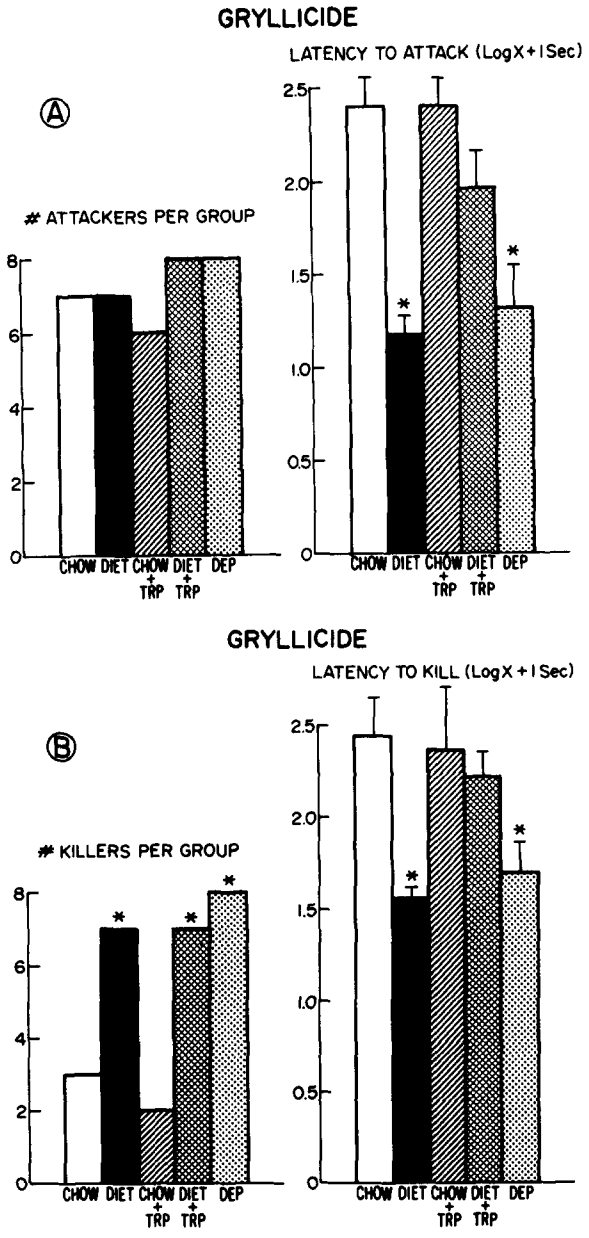

FIG. 2. A. The number of cricket attackers per group (left) and mean ± SEM latency to attack in log (x+1) sec (right) during gryllicide. *Significantly different from CHOW control, $p<0.01$. B. The number of cricket killers per group (left) and mean ± SEM latency to kill in log (x+1) sec (right) during gryllicide. *Significantly different from CHOW control, $p<0.055$.

PRIVED groups compared to CHOW ($p=0.055$, $p=0.055$ and $p=0.01$, respectively) and compared to CHOW+5% TRP ($p=0.02$, $p=0.02$ and $p=0.0$, respectively). The latencies to kill crickets were significantly reduced only in the DIET and DEPRIVED groups compared to all other groups ($p<0.01$). These data are presented in Fig. 2B.

Food, Water and Body Weight

Intake data from Days 8–10 and 18–20 were combined to form representative pre-diet and post-diet food and

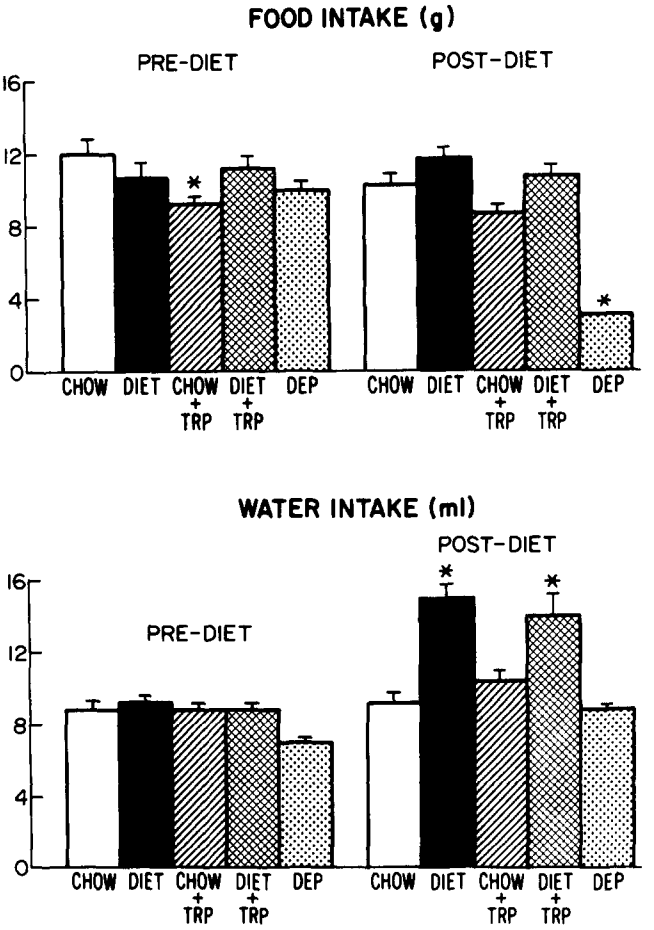

FIG. 3. Mean ± SEM food (top) and water (bottom) intakes prior to (Days 8–10, predict) and following initiation of the different diets (Days 18–20, post diet). *Significantly different from CHOW control, $p<0.05$.

water intake values, respectively. Analysis of food intakes revealed significant differences between groups, $F(4,75)=16.27$, $p<0.001$; days, $F(1,75)=24.0$, $p<0.001$; and groups×days, $F(4,75)=13.89$, $p<0.001$. Further testing indicated that groups did not differ in food intake before the initiation of the diets except for the CHOW+5% TRP group which ate slightly less chow initially, $p<0.05$. Subsequent to diet initiation, food intakes did not differ between or within the CHOW, DIET, CHOW+5% TRP, and DIET+0.15% TRP groups. The DEPRIVED group had to be fed significantly less chow, $p<0.01$, in order to group match body weight with the DIET group (see below). These data are presented in Fig. 3.

Analysis of water intakes revealed significant differences between groups, $F(4,75)=14.56$, $p<0.001$; days, $F(1,75)=104.33$, $p<0.001$; and groups×days, $F(4,75)=12.32$, $p<0.001$. Further testing indicated that groups did not differ in water intake before initiation of the diets, but differed subsequent to initiation of the diets. DIET and DIET+0.15% TRP groups drank significantly more water compared to their own baselines and the CHOW, CHOW+5% TRP, and DEPRIVED groups, $p<0.01$. These data are presented in Fig. 3.

Body weight analysis revealed significant differences

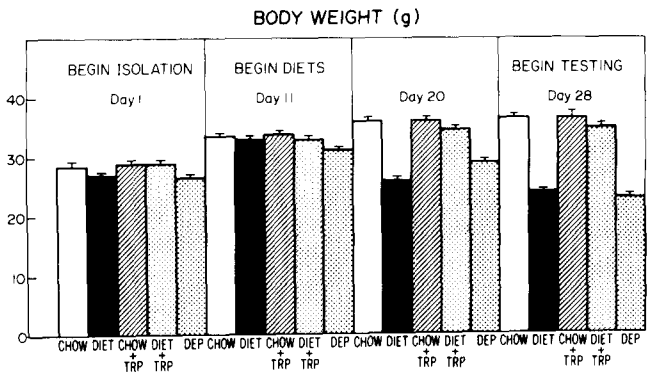

FIG. 4. Mean ± SEM body weight on Days 1, 11, 20 and 28.

between groups, $F(4,75)=39.33$, $p<0.001$; days, $F(3,225)=297.68$, $p<0.001$; and groups×days, $F(12,225)=93.75$, $p<0.001$. Further testing indicated that groups did not differ in body weight at the beginning of isolation (Day 1) or at the beginning of the test diets (Day 11). Following 10 days (Day 21) and 18 days (Day 28) on the different diets, body weight was significantly reduced in the DIET group, $p<0.01$, in spite of adequate food and water intakes. This necessitated testing a food DEPRIVED control group in order to group match the body weight loss of the DIET group and to examine the effects of reduced body weight alone on mouse aggression. The CHOW, CHOW+5% TRP and DIET+0.15% TRP, DIET+0.15% TRP and DEPRIVED groups. These data are presented in Fig. 5.

Brain 5-HT Levels

Analysis of whole brain 5-HT levels revealed significant differences between groups, $F(4,72)=166.56$, $p<0.001$. Further testing indicated that the DIET group had less whole brain 5-HT than all other groups, $p<0.01$. There were significant increases in whole brain 5-HT in the CHOW+5% TRP, DIET+0.15% TRP and DEPRIVED groups. These data are presented in Fig. 5.

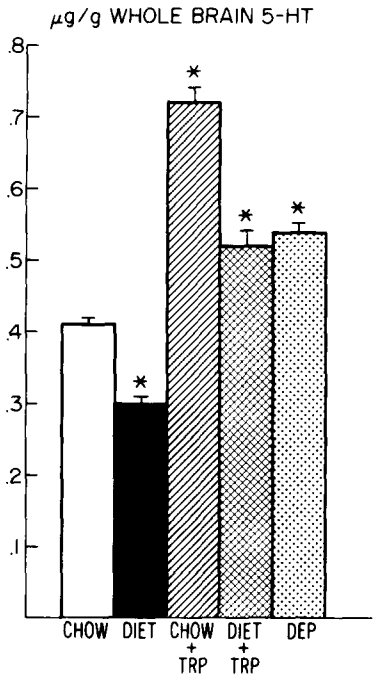

FIG. 5. Mean ± SEM μg/g whole brain 5-HT levels. *Significantly different from CHOW control, $p<0.01$.

DISCUSSION

These data indicate that a diet lacking the essential amino acid tryptophan decreases isolation-induced fighting and increases gryllicide in male mice. In addition, brain 5-HT levels are decreased and body weight is reduced without concomitant decreases in food and water intakes. The effects of the lack of dietary tryptophan on these behavioral and physiological measures do not appear to be entirely correlated with the level of 5-HT in the brain.

Body Weight

The large decreases in body weight in the DIET fed mice are clearly not related to reductions in food and water intakes. It is known that tryptophan is the rate limiting amino acid for liver [30] and possibly brain [2] protein synthesis. When tryptophan or the amount of food is deficient, there is a decrease in *de novo* protein synthesis. This could account for a reduction in body mass for protein utilization under conditions where the amount of ingested food is adequate but of poor quality. In general, disproportionate amino acid diets produce deficits in body weight [10,26].

Gryllicide

Although the incidence and rate of gryllicide were increased in the DIET group, the DEPRIVED group killed as many crickets as quickly as the DIET group. As expected, [4, 8, 13, 24] these two dietary conditions had differential effects on brain 5-HT levels; a decrease was measured in the DIET group and an increase was measured in the DEPRIVED group. Increases in 5-HT level were also found in the DIET+0.15% TRP and CHOW+5% TRP groups. These two diets had little influences on gryllicidal behavior. It is therefore concluded that the increase in predation was associated with dietary need brought about by an insufficient quantity of food for the DEPRIVED group and an insufficient quality of food for the DIET group. Since the number of mice which attacked crickets did not change with dietary treatment, mainly because most or all mice in each group attacked, cricket attack appears to be a natural, reliable and robust response even in the untreated group. The increase in predation is probably not related to the tryptophan deficiency induced brain 5-HT depletion in this study, although it has been shown that p-chlorophenylalanine, which depletes 5-HT [14], increases predatory cricket killing by mice [23]. It has also been previously demonstrated that food deprivation and tyrosine hydroxylase inhibition increase gryllicide [20,21]; whereas amphetamine, L-tyrosine and L-dopa decrease this behavior [20,22].

Isolation-Induced Fighting

Isolation-induced fighting was almost completely inhibited by the tryptophan-free DIET and only partially inhibited in the food restricted DEPRIVED group. The severity of these DIET effects might be related to 5-HT depletion superimposed upon an inadequate diet. However, drug and lesion induced 5-HT depletion has been shown to be suffi-

cient to inhibit isolation-induced fighting [15, 17, 19, 25, 33]. It should be noted that the reduction in isolation-induced fighting in the DIET and DEPRIVED groups cannot be attributed to general immobility and debilitation. Mice remained active throughout testing; test-cage and test-mate exploration occurred for the 15 min session. In addition, the mice were extremely quick to respond to the crickets.

These results are similar to but not identical with the effects of a tryptophan-free diet on rat aggression [8,13]. In both species, predatory killing is increased and brain 5-HT and body weight are reduced. In contrast, intraspecies defensive rat aggression (shock-induced) is enhanced; whereas, intraspecies offensive mouse aggression (isolation-induced) is inhibited by a tryptophan-free diet. The modulation of intraspecies aggression appears to be related to 5-HT depletion in both species although differential behavioral effects are found. Whether or not this differential behavioral effect on intraspecies aggression reflects actual species differences or is related to the type of aggression, that is, defensive versus offensive, remains to be determined.

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