Effects of dorsomedial hypothalamic nuclei lesions on intake of an imbalanced amino acid diet

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1Department of Biomedical Sciences, Baylor College of Dentistry, A Member of The Texas A & M University System Health Science Center, Dallas, Texas 75246; and 2Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine, and Food Intake Laboratory, University of California, Davis, California, 95616

Bellinger, Larry L., James F. Evans, Connie M. Tillberg, and Dorothy W. Gietzen. Effects of dorsomedial hypothalamic nuclei lesions on intake of an imbalanced amino acid diet. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R250–R262, 1999.—Within 3 h of ingesting an imbalanced amino acid diet (Imb), rats show attenuated intake, which can be ameliorated by prior administration of the serotonin receptor antagonist tropisetron (Trop). Earlier work in which the dorsomedial hypothalamic nucleus (DMN) was electrolytically lesioned (DMNL) determined that this structure plays a role in the early detection of and subsequent adaptation to Imb. However, that study did not address whether cell bodies in the DMN, fibers of passage, or both were involved in the DMNL response to Imb. In the present investigation in experiment 1, rats were given electrolytic DMNL or a sham operation (Sham). The rats were injected with saline (Sal) or Trop just before introduction of Imb. By 3 h Sal-DMNL rats consumed more Imb than did the Sal-Sham rats; intake was normal by 12 h. Trop enhanced Imb intake, with Trop and DMNL being additive. By day 4 the DMNL rats were eating and gaining weight less than were Sham rats. In experiment 2, DMN cell bodies were destroyed by ibotenic acid (Ibo). Sal-injected Ibo-lesioned and Sham rats showed similar food intake depression on Imb; Trop similarly increased Imb intake in both groups. By day 4 both Ibo-L rats were eating and gaining weight less than were Sham rats. In experiment 3, groups of rats were given knife cuts posterior, lateral, ventral, dorsal, or anterior to the DMN. During the first 3 h of consuming Imb, all cuts except posterior enhanced the intake of Imb. Over the next 24 h the anterior cut group continued to eat more Imb than did the Sham rats. In experiment 4 DMNL rats were given novel diets; the DMNL rats did not display a neophilic response. The data suggest that fiber tracts that pass through the DMN may be involved in the early detection of Imb. DMN cell bodies, or fibers of passage, are not involved in the Trop effect. Finally, DMN cell bodies are necessary for proper long-term adaptation to Imb.

Rats are able to detect slight imbalances in a diet's content of essential amino acids (18, 35). When given a choice between an imbalanced amino acid diet (Imb) and a protein-free diet, the rats invariably choose the protein-free diet (35). There appear to be at least three phases (13) in the rat's response to an Imb: 1) recognition that the diet is imbalanced (first 3 h after ingesting Imb) and the rat's development of hypophagia; 2) after recognition, a conditioned taste aversion develops (3–6 h after ingesting Imb) with continued hypophagia; and 3) in the absence of an alternate dietary choice, the rat adapts to the Imb (requires several days) and gains body weight.

The anterior piriform cortex (APC) plays an important role in the rat's ability to recognize an Imb, because lesioning this area permanently normalizes the animal's intake of an Imb (24, 31). A decrease in the limiting amino acid in the APC is associated with the decreased consumption of the Imb, and injection of the limiting amino acid into the APC increases food consumption from the usual 45–50% to 75–85% of baseline intake (10, 11).

The APC has connections with a variety of brain areas known to influence feeding behavior (32, 34, 38). One such area is the medial amygdala. Lesioning this area attenuates the hypophagia caused by an Imb (25), probably by eliminating a conditioned taste aversion (30, 42), which, as noted above, is the second phase of the anorectic response to an Imb (13). This second phase also has a peripheral serotoninergic (5-HT) component, because both the Imb-induced hypophagias (16, 20) and the taste aversion (42) can be attenuated by prior administration of a 5-HT3 receptor blocker that acts in the periphery.

Rats with electrolytic or ibotenic acid (Ibo) dorsomedial hypothalamic lesions (DMNL) are hypophagic and hypodipsic but competitively regulate their body weight, albeit at a lower level, when given complete diets (2, 4, 5, 7, 9, 12). The DMN has neural connections with the APC (see below), medial nucleus of the amygdala (27), hindbrain areas (9, 28, 40) that send and receive peripheral information, and other brain areas known to affect feeding behavior (9). In a recent study (6) it was found that rats with electrolytic DMNL demonstrated an attenuated ability to recognize that they were consuming an Imb during the first 3 h of consuming the diet, i.e., phase 1. Subsequently, the DMNL rats showed an inability to adapt to the Imb, i.e., phase 3.

The first experiment of the present study was conducted to verify the earlier Imb findings in electrolytically lesioned rats, with and without pretreatment with tropisetron (Trop), a 5-HT3 receptor blocker (13, 16, 20). In experiment 2 DMN cell bodies, but not fibers that pass through the DMN, were destroyed with Ibo before introduction of the Imb to determine whether destruction of cell bodies in the DMN was responsible for the...
recently presented with novel diets. Experiment 3 investigated the role of DMN fiber tracts in the rats’ response to Imb. The fourth experiment investigated whether electrolytically lesioned rats showed a neophobic response when presented with novel diets.

**METHODS**

General. Male Sprague-Dawley rats (Harlan Industries, Houston, TX) were housed individually in a light-cycle (12:12-h light-dark cycle with lights out at 0900) and temperature-controlled (23°C) room. On arrival, the animals were given a stock diet (Teklad 6% M/R Diet no. 7002; Harlan Industries, Houston, TX) and water ad libitum for 8–9 days. At the end of this period, the rats were given a stock diet (Teklad 6% M/R Diet no. 7002; Harlan Industries, Houston, TX) and water ad libitum for 8–9 days. The basal diet was used because rats demonstrate a dramatic and reliable suppression of food intake when subsequently switched to an isoleucine Imb (26).

Daily intake, corrected for spillage, was recorded, and on the last 2 days of basal diet, starting at lights out, cumulative 3-, 6-, 12-, and 24-h intakes were recorded. After the basal diet period the rats were presented at lights out with a severe isoleucine Imb (Table 1) (17), and on the first experimental day cumulative 3-, 6-, 12-, and 24-h intakes were recorded. Daily consumption of the Imb was measured for 6–7 additional days. Water was available ad libitum. Body weights of the animals were recorded at various times throughout the studies.

At the end of the study, the rats were killed with CO2, and their brains were saved for histological examination. In experiments 1, 2, and 4 the brains were removed, trimmed, dipped in 2-methylbutane, and then placed in liquid nitrogen. The frozen brains were next wrapped in foil and stored at −85°C until being cut for histology. During the cutting process the frozen brain was attached to a frozen specimen stage, and 16-µm-thick sections were cut with a cryostat (6). The sections were then stained with the Kluver-Barrera method. Three observations were performed on 20 rats by inserting the electrode to a depth of 1.0 mm dorsal to the DMN.

After rats were fed the basal diet for 2 days, food was removed at 0800, and the rats were injected intraperitoneally with saline (Sal). Food cups were returned at 0900. This sequence was repeated daily. On the last 2 days that the rats were on the basal diet, the rats were again injected with Sal at 0800. Food was presented at 0900, and cumulative intake was recorded 3, 6, 12, and 24 h later.

The following day at 0800, Sham and DMNL groups were injected intraperitoneally with Sal or with 9 mg/kg (16) of the 5-HT3 receptor antagonist tropisetron (Trop; 3-[3-tropinyl-1H-indole-3-carboxylic acid ester; Research Biochemicals International, Natick, MA). At 0900 the rats were presented with Imb, and cumulative intake was recorded as described. Comparisons among the groups were also made by periods, i.e., 0–3, 3–6, 6–12, and 12–24 h, and the data were expressed as a percentage of each animal’s basal diet intake during that time period. Daily intake of Imb was recorded for six additional days. At the end of this period, at 0900 the rats were switched to a corrected diet (Table 1; i.e., the Imb was corrected by adding 0.24 g isoleucine/100 g diet with a commensurate decrease in the carbohydrate content of the diet).

**Experiment 2:** Ibo DMNL. In this experiment the DMN of the 51 rats were infused with Ibo (3 µg/0.3 µl) as previously described (2). Briefly, the pH of the Ibo was adjusted to 7.4

**Table 1. Composition of diets used in experiments**

<table>
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<th>Ingredients</th>
<th>Isoleucine Diets</th>
<th>Casein Diets</th>
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Data are given as percentage of diet by weight. Composition of mixtures used are given in detail in Refs. 14 and 15. Amino acids used in diets were from Ajinomoto USA, Teaneck, NJ.
with NaHCO₃, and the final volume was adjusted with a solution of 0.01M NaH₂PO₄ and 0.15M NaCl. The Ibo was infused over 4 min, and the cannula was left in place another 4 min before it was removed. Sham operations (n = 19) were performed as in experiment 1. As described in experiment 1, the rats were injected with Sal or Trop, and Imb intake was recorded.

Experiment 3: knife cuts. Ninety-three rats were divided into six groups and given either bilateral knife cuts with a Scouter Knife (David Kopf Instruments, Tujunga, CA) or a sham surgery. In group 1 (n = 19) cuts were made just posterior (Post) to the DMN (anterior-posterior 3,800 µm, lateral to midline 0.8 mm, depth 2.5 mm (above ear bar zero)). The knife was extended 1.0 mm medially and then lowered 1.3 mm and retracted. In group 2 (n = 16) cuts were made lateral (Lat) to the DMN (anterior-posterior 3,800 µm, lateral to midline 0.7 mm, depth 2.5 mm). The knife was extended anteriorly 1.3 mm at an 11.5° angle lateral to the sagittal plane, lowered 1.2 mm, and retracted. In group 3 (n = 16) cuts were made ventral (Vent) to the DMN (anterior-posterior 3,800 µm, lateral to midline 0.7 mm, depth 1.4 mm). The knife was extended 1.4 mm at an 11.5° angle lateral to the sagittal plane. The knife was moved medially 45° so that the knife contacted the third ventricle. Next the knife was retracted 0.1 mm, and a medially 8° cut was made; this was repeated until a total 101.5° (11.5° + 90°) cut was made. In group 4 (n = 15) cuts were made dorsal (Dor) to the DMN. The anterior-posterior positioning of the knife shaft on the rat’s right side was 4,900 µm, 0.8 mm lateral to the midline, and 2.6 mm deep. The knife was extended 1.4 mm in the direction of the midline, rotated posteriorly 78.5°, and then retracted and brought back to the original starting position. The knife was then extended in the direction of the midline 1.9 mm and rotated 13° posteriorty. Next the knife shaft was inserted on the rats’ left side (anterior-posterior 3,800 µm, lateral to midline 0.7 mm, depth 2.6 mm), and the knife was extended to the midline. The cuts were made as noted above, but the knife was rotated in the anterior direction. In group 5 (n = 15) cuts were made just anterior (Ant) to the DMN (anterior-posterior 4,900 µm, lateral to midline 0.9 mm, depth 2.9 mm), and the knife was extended 1.1 mm medially and then lowered 1.6 mm and retracted. In group 6 (n = 12) Sham operations were made with the various anterior-posterior positions of groups 1–5. The knife shaft was lowered to a depth of 1 mm higher than used in groups 1–5. The groups were given the stock, basal, and Imb diets as described, but they did not receive drug treatment.

Experiment 4: electrolytic DMNL and various diets. The rats were given electrolytic DMNL (n = 16) or Sham operations (n = 9) as described in experiment 1. The rats were then given the stock diet, which contained 25% protein, 6% fat, and 45% nitrogen-free extract with a digestible energy of 3.34 kcal/g, for 7 days and then given the basal diet (4.01 kcal/g) at 0900. Basal diet intake was recorded 1, 3, 6, 12, and 24 h later. The rats remained on the basal diet for a total of 8 days. At the end of this period, the rats were presented at 0900 with a 17% casein diet (4.01 kcal/g; Table 1), and hourly intake was recorded. The next day the rats were returned to the basal diet for 4 days. At the end of this period, at 0900 the rats were given a high-fat diet [40% Crisco/60% powdered stock diet (wt/wt), 5.6 kcal/g], and hourly food consumption was measured for 1 day. The rats were then returned to the basal diet for 5 days. After this the rats were presented at 0900 with a 44% casein diet (4.01 kcal/g; Table 1), and hourly intake was recorded.

RESULTS

Experiment 1: electrolytic DMNL. Figure 1 shows a photomicrograph of a cross section of the lesions. The principal sites of bilateral destruction were located in the DMN. The lesions were dorsal to the ventromedial hypothalamic nucleus and did not extend laterally beyond the fornix or dorsal to the mammillothalamic tracts. After elimination of rats with improperly placed lesions, group sizes were Sal-DMNL, n = 7 and Trop-DMNL, n = 9. Group sizes for the control groups were Sal-Sham, n = 9 and Trop-Sham, n = 10. The DMNL rats were hypophagic on the stock diet [F(3,31) = 11.12, P < 0.001; Fig. 2] compared with the Sham animals. Figure 3 shows the averaged 3-, 6-, 12-, and 24-h cumulative basal diet intake (in grams) taken over the last 2 days the rats were on the basal diet. Again the DMNL groups were hypophagic compared with the Sham groups [F(3,124) = 22.0, P < 0.001], starting with the 6-h measurement.

Figure 4 shows the cumulative Imb intake, expressed as a percentage of the animal’s basal diet intake, for the DMNL and Sham rats after they received either Sal or Trop. Again there was a significant [F(3,124) = 126.50, P < 0.001] group effect; however, this time both the DMNL groups initially ate more than their respective control groups.

At the 3-h measurement the Sal-Sham group’s consumption of the Imb was 59% of their basal diet intake (Fig. 4). Remarkably, the Sal-DMNL group ingested 135% of their basal diet intake. At this measurement time the Trop-Sham group ate 158% of their basal diet intake. L, lesion; FX, fornix; MTT, mamillothalamic tract; III, 3rd ventricle. Magnification ×25. Luxol fast blue and cresyl violet were used.
intake level, whereas the Trop-DMNL group ingested 225% of their basal diet intake. The 6-h cumulative Imb intake (Fig. 4) of the Sal-DMNL group was 92% of their basal consumption, whereas cumulative Imb ingestion by the Sal-Sham group was 47% (P < 0.01). However, when Imb intakes during the 3- to 6-h period were compared, the Sal-DMNL group ate slightly, but not significantly, more than did the Sal-Sham group (Sal-Sham, 32.1 ± 7.1% vs. Sal-DMNL, 46.9 ± 12.9%; NS). Thus the main effect of the lesion occurred during the first 3 h of Imb ingestion. At the 6-h measurement point both Trop groups continued to increase (P < 0.01) their consumption of the Imb compared with their Sal groups, whether calculated either as cumulative intake (Fig. 4) or during the 3- to 6-h period (Trop-Sham, 151.8 ± 17%; Trop-DMNL, 198.4 ± 26.0%). The Trop-DMNL group's ingestion of the Imb was greater (P < 0.01) than that of the Trop-Sham group with either cumulative or period data.

The 12-h cumulative ingestion of the Imb by the Sal-DMNL group was slightly, but again not significantly, greater than that of the Sal-Sham group (Sal-Sham, 32.1 ± 7.1% vs. Sal-DMNL, 46.9 ± 12.9%; NS). Thus the main effect of the lesion occurred during the first 3 h of Imb ingestion. At the 6-h measurement point both Trop groups continued to increase (P < 0.01) their consumption of the Imb compared with their Sal groups, whether calculated either as cumulative intake (Fig. 4) or during the 3- to 6-h period (Trop-Sham, 151.8 ± 17%; Trop-DMNL, 198.4 ± 26.0%). The Trop-DMNL group's ingestion of the Imb was greater (P < 0.01) than that of the Trop-Sham group with either cumulative or period data.

The 12-h cumulative ingestion of the Imb by the Sal-DMNL group was slightly, but again not significantly, greater than that of the Sal-Sham group (Sal-Sham, 32.1 ± 7.1% vs. Sal-DMNL, 46.9 ± 12.9%; NS). The 12-h cumulative intake of the Imb by the two Trop-injected groups was greater (P < 0.01) than that of their respective control groups, and the Trop-DMNL group was still consuming (P < 0.05) more than the Trop-Sham group. However, when intake during the 6- to 12-h period was compared, the groups consumed similar amounts of Imb (Trop-Sham, 110.7 ± 11.2% vs. Trop-DMNL, 127.7 ± 12.2; NS). Thus the main drug-group effect occurred during the first 6 h of Trop treatment.

The 24-h cumulative measurement showed that the two Trop-treated groups consumed a similar percentage of Imb, which was still significantly higher than their respective control groups, which also consumed similar amounts (Fig. 4). When Imb intake during the 12- to 24-h period was compared, the Trop groups and Sal groups consumed similar amounts, respectively.

When the rats' intake of the Imb was compared over the next 7 days, there were group differences [F(3,31) = 12.79, P < 0.01; Fig. 5]. By the second day 24-h intake of all the groups except the Trop-DMNL group was similar. Interestingly, the latter group's consumption of Imb was significantly (P < 0.05) less than that of the Sal-DMNL group. On day 3 the 24-h intake of the four groups was similar and higher than on day 2. On day 4 the Sham groups continued to increase their consumption of Imb, whereas the intake of DMNL groups remained constant. This resulted in a significant differ-
ence between the DMNL and Sham animals that persisted on days 5–7. The Sham groups ate similar amounts of the Imb on days 4 and 5, but unexpectedly on days 6 and 7 the Trop-Sham group consumed less than did the Sal-Sham rats.

After being switched to the corrected diet (Fig. 6), the intake of all four groups was similarly increased [F(3,31) = 1.21, NS]. Thus all groups showed the expected compensatory hyperphagia with the corrected diet.

The body weights of the four groups were similar [F(3,31) = 0.18, NS] at the time of surgery (in grams: Sal-Sham, 162.3 ± 4.8; Trop-Sham, 162.2 ± 3.6; Sal-DMNL, 158.9 ± 3.2; Trop-DMNL, 159.9 ± 3.8). All groups gained weight on the stock diet. But, as expected, the DMNL rats weighed less than the Sham rats [F(3,31) = 10.19, P < 0.01] the day the groups were switched to basal diet (Sal-Sham, 195.0 ± 5.5 g vs. Sal-DMNL, 175.1 ± 2.6 g, P < 0.01; Trop-Sham, 200.9 ± 4.0 g vs. Trop-DMNL, 175.8 ± 3.2 g, P < 0.01).

Unexpectedly, all groups lost a similar percentage of body weight (P < 0.01) while on the basal diet, and the body weights of the four groups still differed [F(3,31) = 6.60, P < 0.01] on the day of Trop and Sal injections (Sal-Sham, 161.4 ± 4.7 g vs. Sal-DMNL, 144.8 ± 2.7 g, P < 0.01; Trop-Sham, 162.5 ± 3.3 g vs. Trop-DMNL, 146.3 ± 3.3 g, P < 0.01).

Over the next 7 days the body weights of the groups, expressed as a percentage of their weight the day they received Trop or Sal, differed [F(3,31) = 6.68, P < 0.01]. During the first 2 days of exposure to the Imb, the Sal groups lost weight with respect to their starting body weights. On the other hand, both Trop-injected groups gained weight, and they weighed significantly more than their respective Sal control groups on the first 2 days of ingesting Imb. By day 3 (Fig. 7) the body weights of the four groups were similar and around their weight at the start of Imb. The Sham groups stayed at that level until the end of the Imb period. In contrast, both DMNL groups showed weight loss and by days 6 and 7 weighed less than their respective Sham groups. Therefore, the DMNL rats were not able, as were the controls, to maintain their starting weight. When given the corrected diet the groups' body weights still differed [F(3,31) = 3.50, P < 0.03], with the lesioned animals weighing less than the controls (Fig. 8).

Experiment 2: Ibo DMNL. Representative Ibo-lesioned and Sham-operated rats are shown in Fig. 9, B and D, and Fig. 9, A and C, respectively. Note the absence of large neurons (Fig. 9D) and increased number of glia cells (Fig. 9, B and D) in the photomicrograph of the Ibo-lesioned rat. The third ventricle of all the Ibo-lesioned rats was expanded because of loss of neuronal tissue in the DMN. This is the reason the ventricle can be seen in Fig. 9B (lesioned) and not in Fig. 9A (Sham); both photomicrographs were taken at the same anterior-posterior position (3,900 µm) (22) and at the same magnification (×100). After eliminat-
ing those rats with misplaced lesions, the group sizes were Sal-Sham, n = 9; Trop-Sham, n = 10; Sal-DMNL, n = 11; and Trop-DMNL, n = 12. The DMNL rats were hypophagic on the stock diet [F(3,37) = 22.06, P < 0.001] compared with the Sham animals; group means over the 7 days on the stock diet were Sal-Sham, 20.1 ± 1.3 g vs. Sal-DMNL, 16.8 ± 1.6 g. The DMNL rats were also hypophagic on the basal diet [F(3,37) = 19.89, P < 0.001] compared with the Sham animals; group means over the 7 days on basal diet were Sal-Sham, 18.4 ± 0.7 g vs. Sal-DMNL, 14.8 ± 0.6 g, P < 0.01. The groups' hourly cumulative intake of the basal diet differed [F(3,148) = 14.77, P < 0.001]. Starting with the 12-h measurement, the DMNL groups ate less (P < 0.01) than did their respective Sham groups (3, 6, 12, and 24 h, respectively: Sal-Sham, 4.7 ± 0.4, 9.1 ± 0.4, 18.3 ± 0.7, and 20.7 ± 0.7 g; Trop-Sham, 4.8 ± 0.4, 9.3 ± 0.5, 17.3 ± 0.8, and 19.3 ± 1.0 g; Sal-DMNL, 4.3 ± 0.3, 8.3 ± 0.4, 14.3 ± 0.9, and 15.7 ± 1.2 g; Trop-DMNL, 4.4 ± 0.4, 8.4 ± 0.6, 13.8 ± 1.0, and 15.5 ± 0.8 g).

Figure 10 shows the first day cumulative Imb intake, expressed as a percentage of the animal's basal diet intake, for the DMNL and Sham animals after Sal or Trop injections. There was a significant [F(3,148) =

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**Fig. 8.** Experiment 1, body weights of rats when on corrected diet expressed as percentage of rat's body weight at time of injections. *a* Statistical effect of surgery, P < 0.05–0.01 for Sal-Sham vs. Sal-DMNL and Trop-Sham vs. Trop-DMNL. SE range, 0.68–1.10.

**Fig. 10.** Experiment 2, cumulative 24-h intake of Imb expressed as percentage of rat's basal intake. DMN lesions were produced with Ibo. *b* Statistical effect of drug, P < 0.05–0.01 for Sal-Sham vs. Sal-DMNL and Trop-Sham vs. Trop-DMNL. SE range, 0.68–1.10. SE range, 2.56–6.60.
74.44, \( P < 0.001 \) difference among the groups. The Imb intake of the two Sal groups was similar at every measurement point. The cumulative Imb intake of both Trop groups was alike from 3 to 24 h on day 1. As in experiment 1, the cumulative Imb intake of the Trop groups was significantly greater than that of the Sal groups during their first day of ingesting the Imb.

When the rats' daily intake of the Imb was compared, there were group differences \( [F(3,37) = 13.95, P < 0.01; \text{Fig. 11}] \). On day 2 the 24-h Imb intake of the Trop-Sham group remained significantly elevated compared with all other groups whose intakes were similar. On day 3 the intake of the Trop-DMNL group was significantly suppressed compared with all other groups. By day 4 the Sal-DMNL group's Imb intake was significantly less than that of the Sal-Sham group. Even though the Imb intake of both DMNL groups was expressed as a percentage of their basal diet consumption to account for their lesion-induced inherent hypophagia, the DMNL groups still ate less \( (P < 0.01) \) than did the Sham groups through day 7.

The body weights of the four groups were similar \( [F(3,37) = 0.91, \text{NS}] \) at the time of surgery (in grams: Sal-Sham, 161.0 \pm 4.3; Trop-Sham, 156.2 \pm 2.6; Sal-DMNL, 160.7 \pm 3.0; Trop-DMNL, 163.0 \pm 2.43). All groups gained weight while on the stock diet. However, the Sham groups increased their body weight more than did the DMNL groups \( [F(3,37) = 7.29, P < 0.01] \) by the day the groups were switched to basal diet (Sal-Sham, 197.3 \pm 4.3 g vs. Sal-DMNL, 176.3 \pm 3.1 g, \( P < 0.001 \); Trop-Sham, 194.6 \pm 3.5 g vs. Trop-DMNL, 182.9 \pm 3.4 g, \( P < 0.05 \)). As expected the groups also gained weight on the basal diet, but the DMNL groups still weighed less than the Sham groups \( [F(3,37) = 7.81, P < 0.01] \) on the day of Trop and Sal injection (Sal-Sham, 217.8 \pm 3.8 g vs. Sal-DMNL, 194.8 \pm 4.1 g, \( P < 0.01 \); Trop-Sham, 214.4 \pm 4.4 g vs. Trop-DMNL, 199.6 \pm 3.4 g, \( P < 0.05 \)).

The body weights (Fig. 12) of the groups, expressed as a percentage of their weight the day they received Trop or Sal, differed \( [F(3,37) = 13.3, P < 0.01] \) over the 7 days of ingesting Imb. During the first 2 days on Imb, the two Sal-injected groups lost weight compared with their starting body weight and weighed significantly less than the Trop groups. The body weight of the Sham groups reached a low on days 2 and 3 and then began to recover. As expected the DMNL groups continued to lose similar amounts of body weight and weighed significantly less than their Sham counterparts on days 4–7.

Experiment 3: knife cuts. Figure 13 shows a composite drawing of the greatest extent of the various knife cuts. After eliminating those rats with misplaced cuts, the group sizes were: Post, \( n = 8 \); Lat, \( n = 9 \); Vent, \( n = 9 \); Dor, \( n = 15 \); and Ant, \( n = 8 \); the Sham group was \( n = 9 \).

When on the stock diet the Post, Vent, Ant, and Dor groups were hypophagic on the first day postsurgery \( [F(5,52) = 4.33, P < 0.003] \). This hypophagia continued in the Post, Vent, and Dor groups over the next several days. When the groups were switched to the purified basal diet, only the Vent group was hypophagic \( [F(5,52) = 3.24, P < 0.02] \). On the last day of basal diet, the Post group ate more \( (P < 0.01) \) than did the Sham group.

This latter difference was also reflected \( [F(5,208) = 3.64, P < 0.004] \) in the averaged hourly cumulative intake (in grams) on the last 2 days of the basal diet, in which the Post group consumed more of the basal diet at the 24-h measurement point than did the Vent and Lat groups. At no measurement point did the 2-day average intake of any group differ significantly from the Sham group.

On the first day of ingesting Imb, cumulative intake differed \( [F(5,208) = 12.09, P < 0.001] \) among knife-cut and Sham groups (Fig. 14). At the 3-h measurement point the Lat, Vent, Dor, and Ant groups consumed significantly more of the Imb than did the Sham group.
At the 6-, 12-, and 24-h measurement points the Ant group continued to consume significantly more of the Imb compared with the Sham group. When the rats’ daily intakes of the Imb were compared on days 2–7, there were no significant group differences.

At the time of surgery, the body weights of the five experimental groups were statistically similar to the Sham group (in grams: Post, 176.8 ± 2.1; Lat, 163.9 ± 1.8; Vent, 178.1 ± 2.5; Dor, 178.4 ± 3.3; Ant, 176.0 ± 3.5; Sham, 171.4 ± 4.3). However, further analysis showed that the Lat group weighed less (P < 0.05) than the other experimental rats. For that reason it was necessary to express body weight changes in body weight gain from surgery. While consuming the stock diet the Sham group gained more weight [61.8 ± 3.3 g; F(5,52) = 8.71, P < 0.001] than did the Post (51.0 ± 3.5 g), Vent (39.3 ± 2.6 g), Dor (52.0 ± 2.1 g), and Ant (48.9 ± 5.4 g) groups, but not the Lat (67.8 ± 2.2 g) group. Body weight (Fig. 15), expressed as a percentage of weight on the last day the animals received the basal diet, did not differ significantly [F(5,52) = 1.09, P > 0.1] over the 7 days the groups were given Imb. There was, however, a trend for the Ant group to weigh more than the other groups.

Experiment 4: Electrolytic DMNL and various diets. After eliminating those rats with misplaced lesions, the group sizes were: Sham, n = 8 and DMNL, n = 7.

After surgery the rats were placed on the stock diet, and the DMNL rats displayed the typical lesion-induced hypophagia [F(1,13) = 5.15, P < 0.05]. Because the DMNL rats were hypophagic compared with the Sham group, the rats’ intake of the various diets had to be normalized before meaningful group comparisons could be made. Normalization was accomplished by comparing the amount of diet a rat consumed, in kilocalories, with that rat’s stock diet intake also.

At the 6-, 12-, and 24-h measurement points the Ant group continued to consume significantly more of the Imb compared with the Sham group. When the rats’ daily intakes of the Imb were compared on days 2–7, there were no significant group differences.

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The ingestion of the high-fat diet did not differ significantly between the groups. The DMNL and Sham groups' cumulative consumption (0–3, 3–6, 6–12, and 12–24 h) did not reveal any differences when intake of the groups was calculated by periods (phase 1). However, the DMNL group consumed less of the 44% casein diet over the 0- to 12-h and 0- to 24-h measurement periods. On the other hand, the casein diet over the 0- to 12-h and 0- to 24-h periods. The 12- to 24-h measurement period occurred during the light period, and the DMNL group consumed more than did the Sham group (24.1 ± 4.5 kcal vs. 23.6 ± 4.2 kcal).

Comparing the two groups' consumption by periods i.e., 0–1, 1–3, 3–6, 6–12, and 12–24 h, also revealed no differences between the DMNL and Sham animals. After changing to the 17% casein diet, cumulative intake of both groups was similar at 1, 3, and 6 h (Table 2). However, the DMNL group consumed less of the casein diet over the 0- to 12-h and 0- to 24-h cumulative measurement periods. On the other hand, when intake of the groups was calculated by periods (0–3, 3–6, 6–12, and 12–24 h) no significant differences were found. The DMNL and Sham groups' cumulative consumption of the high-fat diet did not differ significantly [F(1,65) = 0.14, NS; Table 2]. Intake comparisons by period were alike except for the 12- to 24-h measurement period. The 12- to 24-h measurement period occurred during the light period, and the DMNL group consumed more than did the Sham group (24 ± 4.1 kcal vs. 11.8 ± 3.4 kcal, P < 0.05). Both groups' cumulative consumption of the 44% casein diet was similar [F(1,65) = 2.38, NS; Table 2], as was their intake by periods.

The body weights of the two groups were similar at the time of surgery (Sham, 169.4 ± 2.7 g and DMNL, 170.9 ± 2.1 g). As expected, the body weight gains of the two groups differed (P < 0.05) by the day the animals were switched to basal diet (Sham, 22.5 ± 1.6 g vs. DMNL, 12.6 ± 3.2 g). Compared with their body weight on the last day they were given the stock diet, both groups gained a similar percentage of weight by the last day of basal diet (Sham, 10.0 ± 0.9% vs. DMNL, 10.4 ± 0.5%). The body weight gain of the DMNL group was still less (P < 0.01) at the end of the experiment (Sham, 40.0 ± 2.0 g vs. DMNL, 26.7 ± 4.8 g).

**DISCUSSION**

Intact rats display a very low first-day consumption of Imb, which is caused by (13) a combination of the recognition phase (phase 1) and the aversive phase (phase 2). Pretreatment with Trop increases the intake of Imb by attenuating phase 2 (42).

In the electrolytic lesion study Sal-treated DMNL rats showed an attenuation of the food intake suppression normally observed during the first 3 h of ingesting an Imb. Thus the electrolytic lesion interfered with the early recognition of an amino acid deficiency (phase 1) caused by eating Imb. After 3 h both Sal-DMNL and Sal-Sham consumed similar amounts of Imb. These data confirm an earlier report (6), in which electrolytic lesions were placed in the DMN. In contrast, the Ibo-lesioned rats during the first 3 h of ingesting Imb showed a normal suppression of Imb intake. This contrasts with what occurred in electrolytically lesioned rats and suggests that loss of DMN cell bodies was not responsible for the electrolytic DMNL attenuation of the food intake suppression caused by Imb. The data further suggest that electrolytic lesions destroyed fiber tracts that were responsible for the feeding response.

The knife-cut experiment suggests that some, but not all, pathways to or from the DMN are involved in the rats' initial response to Imb. Rats with Post cuts responded like control rats to the Imb; thus fiber tracts (9, 43, 44) using this route are probably not important in attenuating the rats' response to Imb. However, at the 3-h measurement point rats with Dor, Lat, Vent, and Ant cuts showed similar attenuation of the normal food intake suppression caused by the Imb. This attenuation at the 3-h measurement point was similar to that observed after electrolytic DMNL (6), which suggests that redundant, but not additive, pathways are involved in the response. These redundant pathways may have interfered with the rats' recognition that it was consuming an Imb, i.e., phase 1. On the other hand, it is also possible that one or more pathways enter the DMN from one direction, pass through it, and exit uninterrupted in another direction. Cutting this pathway as it either entered or exited the DMN would give the same behavioral results.

At 6 h the intake of the rats with Dor, Lat, and Vent cuts decreased. The intake of the rats with Dor cuts...
decreased to control levels and remained there the rest of the day. This ingestion pattern by the Dor-cut rats was identical to rats with electrolytic DMNL (6); thus the development of phase 2 was not disrupted by this Dor cut or by electrolytic DMNL. Rats with the Vent and Lat cuts remained somewhat above the control levels the rest of the day, but significance was not reached. Interestingly, the Imb intake of the Ant group remained significantly elevated throughout the first day. It is possible that two separate tracts are involved in this response. One tract when cut interfered with the rat’s early recognition that diet was amino acid imbalanced, i.e., phase 1. The second tract that was cut may normally be involved in the later development of the Imb-induced taste aversion, i.e., phase 2.

It should be noted that knife cuts disrupt all neuronal pathways that travel through the area being cut. For example, it is possible that the cut could remove both positive and negative pathways with no resultant change (see Ref. 2 for an earlier discussion of this issue). For this reason caution must be exercised in interpreting the findings of such cuts. Nevertheless, there are anatomi cal findings that lend support to the possibility that pathways that course to, from, or through the DMN may be involved in the animal's recognizing a diet as amino acid deficient and its subsequent adaptation to Imb (see Fig. 4 of Ref. 14 for a schematic anatomical diagram). As mentioned in the introduction, the APC plays an important role in the animal’s response to Imb, and the APC has indirect neuronal connections with the DMN. The APC has efferent connections with the amygdalar cortex and secondarily with the central nucleus of the amygdala (32, 34), which shows activation in response to Imb at 3 h in c-fos studies (45). The amygdala in turn sends efferent fibers to the DMN (27–29) (the Vent and Lat cuts would have disrupted this pathway) and to the paraventricular nucleus (PVN) (15), which sends fibers to the DMN (27) (the Ant and Dor cuts would disrupt this connection). The amygdala also sends fibers to the lateral hypothalamus (LH) (33), and the LH in turn sends fibers to the PVN (37, 39). The APC also has efferent connections with the bed nucleus of the stria terminalis (38), which in turn has both efferent (27) and afferent (40) connections with the DMN [the Lat cut would have disrupted these pathways (43)] and the PVN (37, 39). Finally, the APC sends efferent fibers to the infralimbic cortex (29). The infralimbic cortex has been studied in this model with mixed results (1). The infralimbic cortex sends neurons to the DMN [where they probably enter the DMN from either lateral and/or ventral direction (44)] and the amygdala (21, 36). Therefore, there are a number of pathways by which signals from the APC can reach or travel through the DMN.

The DMN also has direct efferent connections with the posterior piriform cortex (36) (the Lat and Vent cuts would interfere with this pathway), which in turn projects directly to the APC (29). Additionally, the DMN has efferent connections through the lateral and/or ventral pathways with the amygdala (40), insular cortex, infralimbic cortex, and retrosplenial cortex (36). The DMN is also connected to the PVN (41) via anterior and dorsal pathways, and the latter is connected to the insular cortex (23). These five areas in turn send fibers to the APC (21, 29, 38). Signals from the insular cortex can also be reached indirectly by the DMN through its connections with the LH (41). The LH sends projections to the insular cortex (36) (the Lat and Vent cuts would have disrupted this pathway). PVN also has connections with the LH (28); thus signals from the DMN can also reach the insular cortex via the PVN. Finally, the PVN has connections with the amygdala (23), which in turn sends projections to the APC (33, 38). Thus there are several routes by which information processed in or traveling through the DMN could reach and possibly influence the APC.

The electrolytic (6) and Ibo DMNL did not affect the onset of the development of aversion (phase 2) (13, 16, 19, 20, 42); i.e., the initial Imb intake was normally attenuated between 3 and 6 h in Sal-treated DMNL rats. This is also supported by the unaltered feeding response to Trop in the electrolytic and Ibo DMNL groups.

As noted above, blockade of peripheral 5-HT₃ receptors with Trop has been shown to attenuate the initial hypophagia that occurs after introduction of an Imb (20, 46), presumably by blocking or delaying the aversive reaction to Imb (phase 2). With regard to the Sham groups, Trop may have alleviated the aversive phase 2 response. This would have resulted in the Trop rats consuming more Imb than the Sal-Sham group, which experiences both phases 1 and 2.

Notably, treatment of the DMNL rats with Trop apparently caused an additive lesion and drug effect during the first 3 h of ingesting Imb. This could occur if the lesion initially removed the recognition phase (phase 1) while the Trop blocked any signals pertaining to nausea or malaise that were being generated in the periphery or routed through the periphery (phase 2) in the intact and electrolytic or Ibo DMNL rats. It should also be recalled that DMNL rats respond like controls to a quinine-adulterated diet (9); thus DMNL rats do have the capacity to react to at least some aversive substances.

The 24-h intake of the Imb by the Trop-treated groups decreased on day 2. Interestingly, on this day in both the electrolytic and Ibo-lesioned experiments, the intake of the Trop-DMNL groups was suppressed compared with all other groups. Whether this is a compensatory response for their initial high intake on day 1 is unclear.

When Imb hourly intake data from the electrolytic lesion experiment were compared with an earlier finding (6), it was apparent that the responses of the electrolytic DMNL rats to Trop and Imb in the two studies were very similar. However, the magnitude of the response of the DMNL and Sham groups was greater in the present study. This was caused by a very low intake of the basal diet by all the groups of the electrolytic lesion experiment compared with an earlier study (6). In the electrolytic lesion experiment all
groups lost body weight on the basal diet. The rats' low intake of the basal diet and their weight loss are not the norm (Refs. 6 and 8; experiments 2 and 3) and indicated that something was amiss with the basal diet. Nevertheless, the electrolytic lesion experiment still confirms the earlier findings (6) and actually extends them by showing that mildly deprived DMNL animals demonstrate an additive lesion and deprivation effect when presented with Imb. It should be recalled that electrolytic DMNL rats on stock diet respond like controls to food deprivation (4, 9).

All groups in the electrolytic lesion, Ibo lesion, and knife-cut experiments increased their intake of the Imb on day 3, the beginning of phase 3. Nevertheless, by day 4 the electrolytic and Ibo DMNL groups of the electrolytic and Ibo lesion experiments, but not the knife-cut rats, consumed less Imb than did their Sham counterparts. This occurred despite the fact that consumption was calculated as a percentage of the DMNL rats' basal diet intake to account for the inherent lesion-induced hypophagia. Thus the electrolytic and Ibo DMNL rats were taking relatively less of the Imb than the controls, which suggests a decreased adaptation to the Imb by the Ibo-lesioned rats. The attenuated Imb consumption by the electrolytic and Ibo DMNL rats was also reflected in their body weight gain while ingesting the Imb. Even when body weight change was expressed as a percentage of the rat's weight on the last day of basal diet to account for the lower body weight of the DMNL rats, the electrolytic and Ibo-lesioned rats still gained less weight on the Imb than the Sham groups. This was not seen in the knife-cut groups. Therefore, it is apparent that loss of cell bodies in the DMN, and not fibers of passage, was responsible for their low intake of the Imb and attenuated body weight gain during the normal adaptation period (phase 3). This lack of adaptation by the DMNL rats is not caused by a generalized inability of DMNL rats to regulate their lower-than-normal body weight. Previous studies (2, 4, 9) have demonstrated that electrolytic and Ibo DMNL rats will actively regulate their lower-than-normal body weight. Previous studies (2, 4, 9) have demonstrated that electrolytic and Ibo DMNL animals demonstrate an additive lesion and deprivation effect when presented with Imb. It should be recalled that electrolytic DMNL rats on stock diet respond like controls to food deprivation (4, 9).

During the first 3 h of ingesting Imb, the DMNL rats were not likely overresponding to the increased dietary nitrogen in Imb. Several early studies (see Ref. 9) have shown that DMNL rats, given a choice of macronutrients, take a normal percentage of protein in their diet when fed either ad libitum or after a fast. Complementing these findings, the data of the electrolytically lesioned rats given a variety of diets (experiment 4) demonstrated that DMNL rats did not show any abnormal eating behaviors when first introduced to a variety of novel diets varying in protein, carbohydrate, and fat content. Therefore, the increased consumption of Imb by the DMNL rats during the first 3 h of ingesting the novel Imb is most likely not attributable to a neophilic response.

In the Ibo-lesioning experiment the DMNL rats were hypophagic and had reduced body weight compared with Sham, which confirms an earlier report (2) that loss of cell bodies in the DMN produces much of the electrolytic "DMN Lesion Syndrome." In the knife-cut experiment over the 7-day stock diet measurement period, rats with Post and Vent cuts in particular showed sustained hypophagia, which confirms an earlier study (3). It should be recalled that rats with the fully developed electrolytic or excitotoxin (kainic acid or Ibo) DMNL syndrome consume 70–85% of the food and water taken by Sham rats (2, 5, 7; see Ref. 9 for review). They also demonstrate reduced body weight and linear growth but have normal body composition. In the present knife-cut study the magnitude of stock diet and basal diet intake suppression caused by the individual Post and Vent cuts (data not shown) was less than that observed after electrolytic or Ibo DMNL. However, the combined food intake suppression of both cuts is similar to that observed after DMNL. The decreased intake caused by the cuts was reflected in significantly attenuated body weight gain. The DMN has connections (40, 43) with the ventromedial hypothalamic nucleus through ventral projection and with the LH through lateral and ventral projections. Both of these areas are known to affect feeding behavior and body weight gain (9). The DMN has both afferent and efferent connections with hindbrain areas that also affect feeding behavior (9, 28, 38, 40, 43). The Post and Vent cuts would have disrupted many, but not all (44), of these hindbrain connections. It is also of interest that ANT cuts that would have partially disrupted pathways to or from the PVN did not affect the rats' intake of the stock or basal diet. The above data suggest that multiple DMN pathways may be responsible for the hypophagia and reduced body weight observed after electrolytic DMNL.

Clearly, the role of the DMN in the rat's ingestion of Imb is twofold. First, fiber tracts that pass through the DMN appear to be important in the rat's recognition that it is consuming an Imb. Second, cell bodies in the DMN apparently play a role in the rat's adaptation to Imb as the loss of cell bodies in the DMN attenuates the rat's adaptation to Imb. Thus the DMN must be considered in future studies of the mechanisms underlying the animal's responses to Imb.

**Perspectives**

The ability of generalist feeders to compose a diet from natural foods that meets their nutritional needs is an important adaptation when they forage foods that may be deficient or imbalanced in some nutrients. Recognition of a deficiency or imbalance can lead to a search for other food types. Adaptive mechanisms also serve to prevent the ingestion of excess amino acids. From a clinical view deficiencies and imbalances may occur when people take amino acid supplements in the hope that they will improve their health, body composition, memory, strength, etcetera. Moreover, unless their diet selection is knowledgeably made, individuals on vegetarian diets may also suffer from imbalances or deficiencies. Accordingly, Imb has been used to study the responses to plasma amino acid deficiencies in omnivores, particularly in rodents and birds (reviewed...
in Ref. 13). Whether these responses are mediated centrally or peripherally has been a matter of interest over the years. The present results suggest that fibers that course through the DMN, especially to or from the anterior direction, may be a component of central sites that recognize amino acid deficiency. The loss of the DMN cell bodies attenuates the adaptation to Imb that occurs in the absence of food choices.

Clearly, maintenance of essential amino acid precursors for protein synthesis is an important homeostatic mechanism, and would be served by multiple systems, such as multiple brain sites and brain-periphery interactions. These interactions will provide an important focus for future studies.

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