Effect of dorsomedial hypothalamic nuclei knife cuts on ingestive behavior

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Bellinger, Larry L., and Lee L. Bernardis. Effect of dorsomedial hypothalamic nuclei knife cuts on ingestive behavior. *Am. J. Physiol.* 276 (*Regulatory Integrative Comp. Physiol.* 45): R1772–R1779, 1999.—Previous findings show that rats with electrolytic or excitotoxic lesions in the dorsomedial hypothalamic nucleus (DMN) are hypophagic and hypodipsic and have reduced ponderal and linear growth but normal body composition. DMN-lesioned (DMNL) rats also show altered ingestive responses to naloxone. The present study investigated the intrahypothalamic nerve pathways involved in these DMNL effects and the response of the pathways to deprivation challenges by placing knife cuts posterior (Post), lateral (Lat), ventral (Vent), dorsal, or anterior to the DMN or by administering sham operations. One major finding was that rats with Post or Vent were hypophagic ($P < 0.05$) and had reduced body weight but responded normally to deprivation challenges. Post and Lat groups were hypodipsic ($P < 0.05$), but plasma Na^+, K^+, and osmolality and 24-h post-water-deprivation drinking responses were similar in all groups. Naloxone did not suppress the intake of Post rats. It appears that the hypophagia and the reduced body weight after DMNL involve fibers entering or leaving the DMN from ventral and posterior directions, and they may be part of an opioid feeding system.

food intake; water intake; 2-deoxy-D-glucose; naloxone; cholecystokinin; body weight; opioids

SMALL ELECTROLYTIC LESIONS of the dorsomedial hypothalamic nucleus (DMN) of rats produce animals that are hypophagic and hypodipsic and show reduced body weight and linear growth but have normal body composition when compared with sham-operated (Sham) rats (10–12, 16). Although the DMN-lesioned (DMNL) rats are hypodipsic, they regulate water consumption just as well as Sham rats (2). Similar effects have been noted after kainic acid (9) and ibotenic acid (1) excitotoxin destruction of neuronal cell bodies, but not of fibers of passage, in the DMN. Notably, the secretion of anabolic hormones and intermediary metabolism is normal in DMNL rats (11). The above changes have been termed the DMNL syndrome (11).

Although DMNL rats are hypophagic, they consume adequate calories for their body size, show normal efficiency of food utilization (11), and actively defend their lower body weights (5, 11). These findings indicate that DMNL rats adequately regulate long-term energy intake and body weight. Nevertheless, after food deprivation, excitotoxin-lesioned, but not electrolytic-lesioned, DMNL rats initially consume more food relative to body size than do Sham rats (1, 9, 11). This suggests that DMNL rats have deficits in mechanisms that regulate short-term food intake (1, 11). This was supported by the finding that the DMN contains glucosensitive and glucose-responsive neurons (see Ref. 11) and that DMNL rats have deficiencies (4, 11) in their ability to respond to short-term changes in glucose utilization caused by infusions of insulin, glucose, or 2-deoxy-D-glucose (2-DG). Additionally, the short-term food intake suppressor (14, 23) CCK does not affect the intake of DMNL rats (3, 6), and DMNL rats initially overconsume an imbalanced amino acid diet (8). Therefore, the initial overconsumption of food after food deprivation may be caused by a loss of one or more of these short-term regulatory mechanisms. As noted above, despite these short-term deficits in food intake regulation after food deprivation, DMNL rats competently regulate their intake over longer periods, i.e., days. This indicates that DMNL rats have other intact regulatory mechanisms that adequately control long-term caloric intake (7, 11).

The DMN contains a variety of neurotransmitters and receptors (11, 12), including norepinephrine, neuropeptide Y (NPY), CCK, opioids, and, as discovered most recently, leptin receptors (17). All of these are known to affect feeding behavior and may work, at least in part, through the DMN (11, 12, 24). With regard to opioids, naloxone (NOX) does not suppress the food intake of DMNL rats (6), and it was suggested that the DMN contains an opioid system, which in turn inhibits a satiety system. In support of this was the later finding (24) that activation of μ- and δ-receptors in the DMN increases the animal's food intake. Still later, it was suggested that DMN opioids play a role in eating after food deprivation (13).

The DMN is thought to contain one or more systems controlling ingestive behavior. However, the functional brain connections of the DMN that produce the DMNL syndrome are unknown. The present study investigated the effects of knife cuts of fiber tracts entering or leaving the DMN on production of the DMN syndrome.

MATERIALS AND METHODS

In *trial 1*, 134 male Sprague-Dawley rats (Harlan Industries, Houston, TX) were caged individually in a light-controlled (12:12-h light-dark cycle, lights off at 1545) and temperature-controlled (23°C) room. The rats were given laboratory chow (Purina) and tap water (in Wahmann cali-

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brated bottles) ad libitum unless otherwise specified. Food intake, corrected for spillage, and water intake were measured daily, and body weight was recorded as noted in Fig. 4. At the time of surgery, the rats were anesthetized with pentobarbital sodium (4.2 mg/100 g body wt). Body weight and nasoanal length were recorded (Table 1) for indirect computation of body composition via the Lee obesity index method (see Ref. 4). The rats were divided into six groups and given either bilateral knife cuts or sham operations with a Scouten knife (David Kopf Instruments, Tujunga, CA). In *group 1*, cuts were made (19) just posterior (Post) to the DMN [anterior-posterior 3.8 mm, 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*, cuts were made lateral (Lat) to the DMN (anterior-posterior 3.8 mm, 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*, cuts were made ventral (Vent) to the DMN (anterior-posterior 3.8 mm, 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 medial 8° cut made; this was repeated until a total 101.5° (11.5° + 90°) cut was made. In *group 4*, cuts were made dorsal (Dor) to the DMN. The anterior-posterior positioning of the knife shaft on the rat's right side was 4.9 mm, 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° posteriorly. Next the knife shaft was inserted on the rats' left side (anterior-posterior 3.8 mm, 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*, cuts were made just anterior (Ant) to the DMN (anterior-posterior 4.9 mm, 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*, sham operations were made with the various AP positions of *groups 1–5*. The knife shaft was lowered to a depth of 1 mm higher than was used in *groups 1–5*. The incisions were closed with stainless steel wound clips, and the rats were returned to their cages.

Nineteen days after surgery, the rats were deprived of food, but not water, for 24 h to ascertain whether the knife-cut rats would drink in the absence of food. Food was returned, and 1- and 24-h consumptions were recorded to determine the rats' feeding response after a period of food deprivation (1, 9). Twenty-three days after surgery, 1.0 ml of blood was removed from the rats by heart puncture under light ether anesthesia. The heparinized blood was chilled, and plasma was separated by centrifugation. Sodium and potassium concentrations of the plasma were determined using a flame photometer, model 51 (Perkins Elmer, Norwalk, CT). Plasma osmolality was determined with an Osmomat 300 (UIC, Joliet, IL). Next, a test for glucoprivic feeding was conducted. Rats were given intraperitoneal injections of saline (~1.8 ml) at 1000 for several days. Food intake, corrected for spillage, was recorded for 1-, 2-, and 3-h cumulative totals. The rats were then injected with 2-DG (ICN Pharmaceuticals, Cleveland, OH) at a dose of 300 mg/kg body wt that was in a concentration of 5% wt/vol (4). Food intake was recorded as described above. Five days later the rats were tested to observe whether they would eat in the absence of water, and, on return of water, their drinking response to the water deprivation, i.e., combination of intracellular and extracellular dehydration, was determined (1, 2, 9). Therefore, the rats were deprived of water for 24 h, and at the end of this period their food cups were removed, water was returned, and water consumption was recorded for a 1-h duration. Food was then returned, and water intake was recorded for a 23-h total. Several days later a trial was started to test the rats' feeding response to CCK. Food cups were removed at 0830, and the rats were injected with saline (0.5 ml ip) at 1600. The rats' food was returned, and their intake, corrected for spillage, was recorded at 30 min. After this familiarization period, one half of each group was injected intraperitoneally with saline, whereas the other half received CCK octapeptide (Bachem, Torrance, CA) at a dose of 3.0 μg/kg body wt (3, 6). Two days later the trial was repeated in a counterbalanced design. Several days later the food cups were removed at 1300. The rats were injected with saline (0.3 ml) at 1515, and food cups were returned at 1545. Food intake, corrected for spillage, was recorded for 1-, 2-, and 3-h cumulative totals. This was repeated the following day, whereas on the third day the rats were injected with NOX dissolved in saline at a dose of 1 mg/kg body wt (6). Food intake was recorded as described above. Forty-nine days after surgery, the rats were anesthetized to determine body weight and nasoanal length and then killed by decapitation. The rats' brains were saved for histological evaluation with Luxol fast blue and cresyl echt violet stain as previously described (1). Serial 8-μm sections were cut, and every sixth section was stained. If more sections were needed, they were obtained from the unstained saved sections and stained. Only those animals with correctly placed transections were used for statistical analysis. At this point it was determined that there were not enough rats with Ant cuts to perform statistical analyses. Therefore, the above experiment was repeated

Table 1. *Body measurements of DMNL and Sham rats at operation and 49 days postsurgery*

	Post ($n = 7$)	Lat ($n = 8$)	Vent ($n = 7$)	Dor ($n = 7$)	*Trial 1* Sham ($n = 13$)	Ant ($n = 7$)	*Trial 2* Sham ($n = 9$)
At surgery							
Body wt, g	149.7 ± 2.3	160.3 ± 2.7	154.9 ± 1.3	154.9 ± 1.6	153.6 ± 2.0	184.4 ± 4.5	184.3 ± 4.8
Lee index	304.9 ± 3.9	313.4 ± 3.4	305.1 ± 1.9	309.1 ± 3.8	312.0 ± 1.9	310.3 ± 1.9	305.3 ± 1.9
49 days postsurgery							
Body wt, g	314.6 ± 6.2*	362.4 ± 8.1	324.4 ± 6.5*	349.7 ± 9.1	359.8 ± 7.3	360.9 ± 9.9	368.7 ± 6.0
Nasoanal length, mm	50.1 ± 1.4	55.1 ± 2.3	47.3 ± 2.2†	54.3 ± 2.0	55.1 ± 2.1	45.8 ± 1.7	43.9 ± 1.1
Lee index	303.0 ± 1.9	311.9 ± 1.9	307.3 ± 1.7	308.7 ± 2.5	313.6 ± 2.4	310.6 ± 1.8	310.1 ± 1.4

All values are means ± SE; n = no. of rats. A second trial was conducted with an anterior knife-cut (Ant) group and a sham-operated (Sham) group (see MATERIALS AND METHODS). All statistical comparisons for Ant group are made with *trial 2* Sham group; all other statistical comparisons are made with *trial 1* Sham group. Lee index values 295–315 indicate normal body composition. *$P < 0.01$ vs. *trial 1* Sham group; †$P < 0.05$ vs. *trial 1* Sham group. DMN, dorsomedial hypothalamic nucleus; Post, posterior knife cut; Lat, lateral knife cut; Vent, ventral knife cut; Dor, dorsal knife cut.

(*trial 2*) with 26 Ant and Sham rats. Data from the Vent, Post, Dor, Lat, and Sham groups of *trial 1* were analyzed together, as were the Ant and Sham groups of *trial 2*. Data were compared by means of one- and two-way ANOVA with and without repeated measurements, Duncan's multiple range test, and Student's *t*-test.

RESULTS

A few animals died during or became ill shortly after surgery and were eliminated from the study. Two individuals separately determined the location and extent of the knife cuts. Composite drawings were then made and any disagreements resolved. A very conservative approach was used in accepting correct knife cut placement; thus only 58 of the 134 animals used met the criteria. Histology (Fig. 1) revealed that in *trial 1* correct knife cuts were placed in 7 Post, 8 Lat, 7 Vent, and 7 Dor rats, and there were 13 Sham rats. In *trial 1*, correct knife cuts were placed in too few Ant rats to make any analysis possible (see MATERIALS AND METHODS). When the experiment was repeated in *trial 2*, correct knife cuts were placed in seven Ant rats, and there were nine Sham rats.

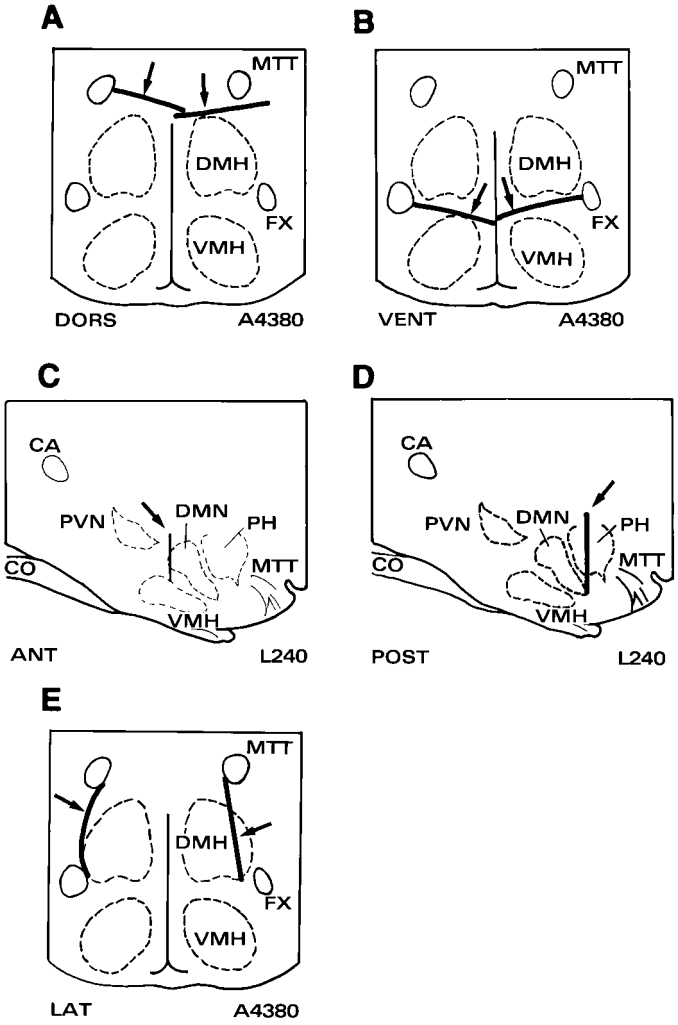

Fig. 1. Line drawing of composite knife cuts (*arrows*). Cuts were made dorsal (*A*; Dors), ventral (*B*; Vent), anterior (*C*; Ant), posterior (*D*; Post), and lateral (*E*; Lat) to dorsomedial hypothalamic nucleus (DMH). Location of drawing is denoted in μm anterior (A) to ear bar zero or lateral (L) from midline (21). MTT, mammillo-thalamic tracts; VMH, ventromedial hypothalamic nucleus; FX, fornix; CA, anterior commissure; CO, optic chiasm; PVN, paraventricular nucleus; PH, posterior hypothalamic area.

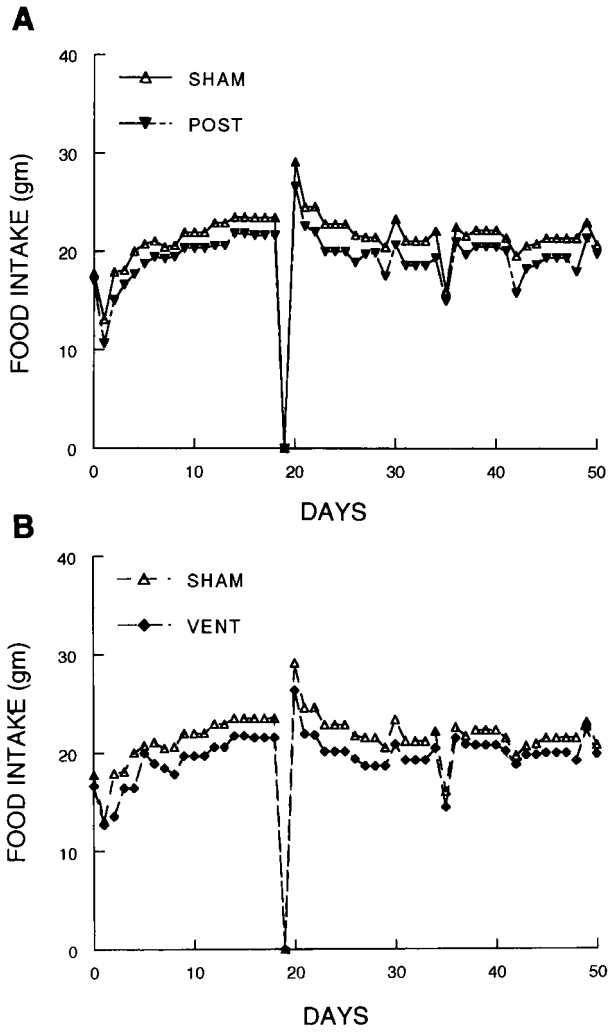

Fig. 2. Food intake of sham-operated (Sham) rats and Post (*A*) and Vent (*B*) knife-cut rats. Post and Vent groups were significantly ($P < 0.05$–0.01) hypophagic compared with Sham group over period of measurement. On *day 19* rats were food deprived for 24 h. SE range, 0.24–2.23.

At the time of surgery (Table 1), body weight and body composition, as determined by the Lee index, were similar in the five groups of *trial 1* and the two groups of *trial 2*.

In *trial 1* there was a significant ($P < 0.001$) group effect on food consumption after the surgery. Further analysis revealed that the Post (Fig. 2*A*) and Vent (Fig. 2*B*) groups consumed significantly ($P < 0.05$–0.01) less food than did the Sham group throughout the experiment [mean daily food intake during experiment: Sham 22.0 ± 0.6 vs. Post 19.0 ± 0.6 g ($P < 0.01$) and vs. Vent 19.6 ± 0.5 g ($P < 0.01$)]. The food intake of the Ant group of *trial 2* did not differ significantly [$F(1,14) = 0.74$; NS] from its Sham group over the course of the study.

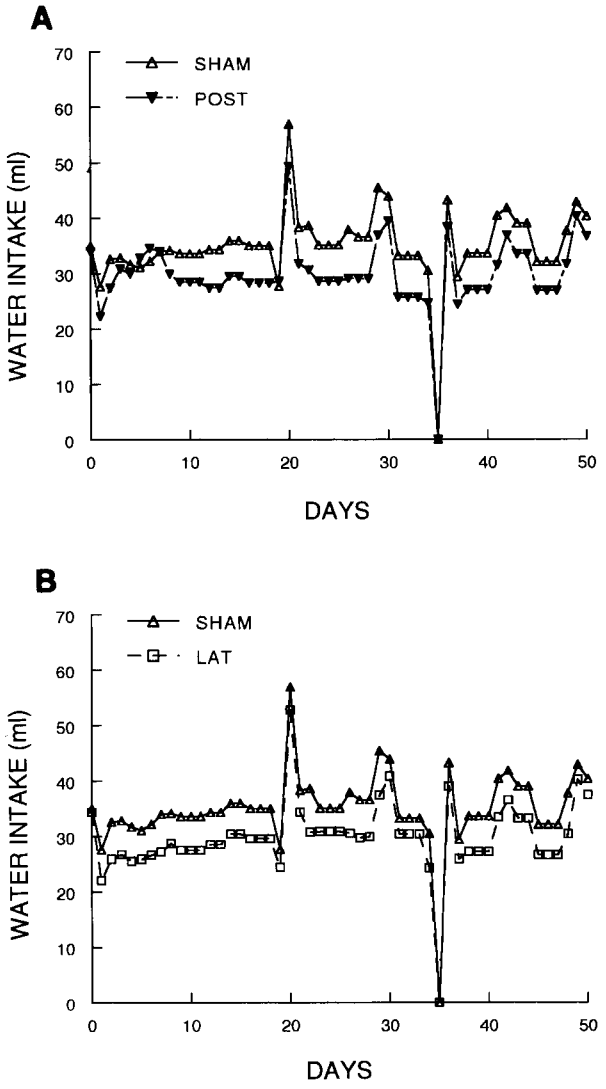

Fig. 3. Water intake of Sham and Post (*A*) and Lat (*B*) knife-cut rats. Post and Vent groups were significantly ($P < 0.05$–0.01) hypodipsic compared with Sham group over period of measurement. On *day 35* rats were water deprived for 24 h. SE range, 0.24–5.93.

In *trial 1* there was also a significant ($P < 0.001$) group effect on water intake subsequent to the operations. Additional analysis showed that the Post (Fig. 3*A*) and the Lat (Fig. 3*B*) groups, but not the Vent group, were hypodipsic ($P < 0.05$–0.01) compared with the Sham group throughout the experiment [mean daily water intake during the experiment: Sham 34.9 ± 1.0 vs. Post 29.6 ± 0.9 ml ($P < 0.01$), vs. Lat 29.4 ± 1.0 ml ($P < 0.01$), and vs. Vent 33.6 ± 0.8 ml (NS)]. The water intake of the Ant group of *trial 2* did not differ significantly [$F(1,14) = 0.26$; NS] from its Sham group throughout the study.

In *trial 1* the lower food intake of the Post and Vent groups manifested itself in a reduced growth rate (Fig. 4) in these two groups compared with the Sham group (group effect, $P < 0.001$). Further analysis revealed that the body weight of the two groups differed ($P < 0.05$–0.01) from the controls by *day 18* and thereafter. The Post and Vent groups remained at ~88–90% of the weight of the controls throughout the study. By the end of the study the Vent group was significantly shorter than the controls (Table 1). The Post group also had reduced body length, but significance was not reached. The body weight of the Ant group did not differ significantly [$F(1,14) = 1.00$; NS] from the Sham group over the course of the study. The body composition, as determined by the Lee index, of the groups of *trials 1* and *2* was normal (Table 1).

The reduced body weight of the Post and Vent groups was apparently not a result of dehydration, inasmuch as plasma analyses of Na^+, K^+, and osmolality revealed normal values in all groups (Table 2).

During food deprivation in *trial 1*, water consumption was reduced suggestively in the Sham, Lat, and Dor groups (Table 3), whereas it was reduced significantly, but not eliminated, in the Post group and elevated slightly in the Vent group. In *trial 2* water consumption was significantly reduced in the Sham and Ant groups and for unexplained reasons was greater than that observed in the Sham group of *trial 1*. However, when water intake during food deprivation was expressed as a percentage of baseline intake, the five groups of *trial 1* did not differ significantly [$F(4,37) = 1.68$; NS], but the variation was large. The Ant group of *trial 2* also showed a percentage intake similar to that of its Sham group (Table 3). On refeeding, all groups significantly increased their 24-h food intake [$F(1,74) = 110.90$; $P < 0.001$]. Although the Post and Vent groups remained hypophagic compared with the Sham group [$F(4,74) = 3.71$; $P < 0.01$], all groups of *trial 1* showed similar percent increases (compared with their baseline intakes) at 1 h and 24 h postfeeding. The Ant group of *trial 2* consumed slightly more than did its Sham group at 1 h but not at 24 h, and both showed an increase intake during the 24 h postdeprivation.

After 24 h of water deprivation (Table 4), during the initial hour of access to water all groups consumed a similar amount of water. Thus the hypodipsic Post rats, on a percentage of baseline intake, actually consumed more ($P < 0.05$) water than the control animals. A

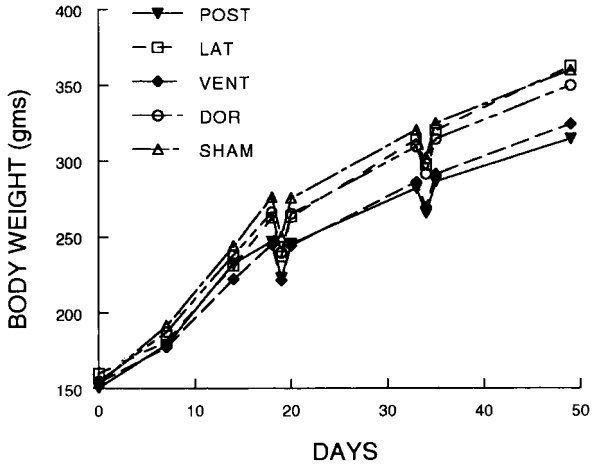

Fig. 4. Body weight of Sham DMN knife-cut groups. Post and Vent groups were significantly ($P < 0.05$–0.01) different from Sham group from *days 18–49*. SE range, 1.6–7.0.

Table 2. *Plasma Na^+, K^+, and osmolality of DMNL and Sham rats*

	Post ($n=7$)	Lat ($n=8$)	Vent ($n=7$)	Dor ($n=7$)	*Trial 1* Sham ($n=13$)	Ant ($n=7$)	*Trial 2* Sham ($n=9$)
Na^+, meq/l	160 ± 5.7	160 ± 2.7	145 ± 5.2	147 ± 2.5	154 ± 2.5	138.0 ± 0.9	137.5 ± 1.3
K^+, meq/l	4.0 ± 0.1	4.3 ± 0.3	5.0 ± 0.7	4.4 ± 0.2	4.2 ± 0.1	3.8 ± 0.1*	3.5 ± 0.1
Osmolality, mosmol	294 ± 2.6	294 ± 2.2	285 ± 12.0	298 ± 5.8	295 ± 1.3	311.0 ± 1.3	317.6 ± 13.0

All values are means ± SE; n = no. of rats. A second trial was conducted with an Ant group and a Sham group (see MATERIALS AND METHODS). All statistical comparisons for Ant group are made with *trial 2* Sham group; all other statistical comparisons are made with *trial 1* Sham group. *$P < 0.05$ vs. *trial 2* Sham group.

similar increase was seen in the Lat group, but significance was not reached. Subsequent 24-h water intakes exceeded [$F(1,73) = 54.40$; $P < 0.001$] 24-h consumption before deprivation in all groups, with the Post group, but not the Lat group, remaining hypodipsic compared with the Sham rats [group effect, $F(4,73) = 5.45$; $P < 0.001$]. Notably, the Vent group also consumed significantly less water than the Sham group. When the 24-h water intake postdeprivation was calculated as a percentage of baselines, all groups showed similar increases. During the 24 h of water deprivation, the groups consumed between 70 and 76% of their mean daily food intake before deprivation. Thus all groups in *trial 1* continued to eat in the absence of water and to a similar degree. The Ant and Sham groups displayed the exact same pattern of response as did the Sham group of *trial 1*; however, for unexplained reasons, during the first hour postdeprivation both groups consumed more water than did the Sham rats of *trial 1*.

The data from the 2-DG and CCK trials are not shown because the findings were equivocal. In the case of the 2-DG trial, the baseline data showed differences among the groups, and in the CCK trial the Sham group of *trial 1* did not respond to CCK.

In the NOX experiment, all groups in *trial 1* showed similar intakes over the 3-h measurement period after saline injection. Injection of NOX (Table 5) significantly [drug effect, $F(1,222) = 54.49$; $P < 0.001$] reduced the food intake of the Sham group during all 3 h of measurement. NOX did not cause a significant suppression of the Post group's food intake [group effect, $F(4,222) = 5.49$; $P < 0.001$]. The Lat and Dor groups had attenuated food intakes during the first hour and a significant depression of food consumption during the second hour. The Ant group of *trial 2* did not show a significant attenuation in food intake after treatment with NOX, whereas the Sham group's intake was significantly decreased at the 2- and 3-h measurement points.

DISCUSSION

Rats with electrolytic or excitotoxic lesions of the DMN are hypophagic and have reduced body weights but normal body compositions (7, 11). The present study demonstrates that transection of fiber tracts that enter or leave the DMN from the posterior and ventral directions also produces a reduction in food consumption, a decrease in body weight, and a reduction in linear growth. The magnitude of the food intake reductions after these individual cuts was about half that observed after electrolytic or excitoxic destruction of the DMN (1, 9, 11). Thus if the observed food intake reductions after Post and Vent cuts are cumulated, they approach levels observed after destruction of the DMN. Despite the reduced body weight of the Post and Vent groups, all groups in *trials 1* and *2* had normal body compositions as measured by the Lee obesity index.

The afferent and efferent neural connections of the DMN have been extensively investigated (21, 22, 25–28). Posterior DMN cuts would disrupt fibers originating in the premammillary, tuberomammillary, supramammillary, and posterior hypothalamic nuclei and the periaqueductal gray area. Ventral DMN cuts would

Table 3. *Effects of food deprivation on water intake and refeeding food intake in DMNL and Sham rats*

	Post ($n=7$)	Lat ($n=8$)	Vent ($n=7$)	Dor ($n=7$)	*Trial 1* Sham ($n=13$)	Ant ($n=7$)	*Trial 2* Sham ($n=9$)
24-h WI							
Before FD, ml	28.3 ± 1.4	29.6 ± 1.2	32.5 ± 2.3	34.7 ± 4.1	35.0 ± 1.8	30.9 ± 0.9	31.2 ± 1.0
During FD, ml	19.5 ± 2.5	24.4 ± 2.7	35.1 ± 5.9	27.3 ± 3.3	27.7 ± 2.0	16.1 ± 2.7	18.6 ± 1.9
P	<0.05	NS	NS	NS	NS	<0.01	<0.01
During FD, %	69.7 ± 9.1	82.7 ± 8.6	110.3 ± 20.7	84.4 ± 13.8	80.3 ± 6.0	51.7 ± 8.3	59.0 ± 5.9
1-h FI post-FD, %	24.4 ± 3.2	27.9 ± 2.6	31.9 ± 5.3	22.7 ± 5.0	29.1 ± 2.7	27.2 ± 0.9‡	23.6 ± 1.1
24 h FI							
Before FD, g	21.7 ± 0.7†	23.0 ± 0.8	21.5 ± 0.6†	23.1 ± 0.4	23.5 ± 0.5	24.7 ± 0.5	25.6 ± 0.4
Post-FD, g	26.6 ± 0.9*	28.7 ± 1.0	26.3 ± 0.7*	27.3 ± 1.1	29.1 ± 0.6	28.9 ± 0.7	30.1 ± 0.7
P	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Post-FD, %	122.9 ± 1.8	124.7 ± 3.5	122.5 ± 1.7	117.9 ± 3.7	124.4 ± 2.6	119.1 ± 1.3	117.5 ± 1.1

All values except P values are means ± SE; n = no. of rats. A second trial was conducted with an Ant group and a Sham group (see MATERIALS AND METHODS). All statistical comparisons for Ant group are made with *trial 2* Sham group; all other statistical comparisons are made with *trial 1* Sham group. Water intake (WI) during food deprivation (FD) is expressed as percentage of 24-h baseline WI. One-hour refeeding food intake (FI) post-FD and 24-h FI post-FD are expressed as percentage of 24-h baseline FI. *$P < 0.05$ vs. *trial 1* Sham group; †$P < 0.052$ vs. *trial 1* Sham group; ‡$P < 0.05$ vs. *trial 2* Sham group. NS, not significant.

Table 4. *Effects of water deprivation on subsequent 1-h and 24-h water intake of DMNL and Sham rats*

	Post ($n=7$)	Lat ($n=8$)	Vent ($n=7$)	Dor ($n=7$)	*Trial 1* Sham ($n=13$)	Ant ($n=7$)	*Trial 2* Sham ($n=9$)
1-h WI post-WD							
ml	6.7 ± 0.3	5.1 ± 1.2	4.9 ± 0.8	4.9 ± 0.6	5.2 ± 0.4	8.5 ± 0.6	10.2 ± 0.9
ml/100 g body wt	2.5 ± 0.1	1.6 ± 0.4	1.8 ± 0.3	1.7 ± 0.2	1.7 ± 0.2	2.6 ± 0.2	3.2 ± 0.3
ml (%)	26.6 ± 1.5*	22.4 ± 6.0	18.4 ± 3.1	18.1 ± 3.4	17.1 ± 1.8	29.4 ± 2.0	38.2 ± 3.9
ml/100 g body wt (%)	27.8 ± 1.5*	23.4 ± 6.5	19.7 ± 3.3	19.2 ± 3.6	18.2 ± 1.9	29.2 ± 2.1	38.0 ± 3.9
24-h WI							
Before WD, ml	24.7 ± 1.1*	24.6 ± 1.4*	26.7 ± 1.6	29.3 ± 2.5	30.5 ± 1.4	28.9 ± 0.8	27.1 ± 0.9
Post-WD, ml	30.5 ± 1.7†	36.6 ± 1.8	33.6 ± 1.0*	37.6 ± 1.6	39.5 ± 1.8	45.5 ± 2.0	45.0 ± 1.3
P	<0.05	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01
ml (%)	124.4 ± 7.3	151.2 ± 9.3	127.3 ± 5.9	131.7 ± 7.6	130.4 ± 4.4	157.5 ± 5.0	166.5 ± 3.1
Before WD, ml/100 g body wt	8.7 ± 0.4	7.9 ± 0.4	9.3 ± 0.5	9.5 ± 0.9	9.5 ± 0.4	9.0 ± 0.3	8.6 ± 0.2
Post-WD, ml/100 g body wt	10.6 ± 0.6	11.6 ± 0.8	11.4 ± 0.4	12.0 ± 0.6	12.2 ± 0.5	14.2 ± 0.7	14.2 ± 0.2
P	<0.05	<0.01	<0.05	<0.01	<0.01	<0.01	<0.01

All values except *P* values are means ± SE; *n* = no. of rats. A second trial was conducted with an Ant group and a Sham group (see MATERIALS AND METHODS). All statistical comparisons for Ant group are made with *trial 2* Sham group; all other statistical comparisons are made with *trial 1* Sham group. Percent values are expressed as percentage of 24-h baseline WI. *$P < 0.05$ vs. *trial 1* Sham group; †$P < 0.01$ vs. *trial 1* Sham group. WD, water deprivation.

remove input fibers from the ventral subiculum and lateral septal, suprachiasmatic, subparaventricular, arcuate, and ventromedial hypothalamic nuclei and amygdala. Ventral cuts would also remove fibers entering the DMN from the lateral hypothalamic area that course under the fornix. It is also possible that some fibers arising from the ventral medulla (A1/C1), nucleus of the solitary tract, parabrachial nucleus, mesencephalic reticular nucleus, and superior central nucleus would be interrupted after ventral cuts.

The knife cuts posterior to the DMN would disrupt efferent fibers from the DMN to the posterior hypothalamic nucleus, superior colliculus, mesencephalic reticular nucleus, retroruberal area, dorsal nucleus of the raphe, periaqueductal gray, cuneiform nucleus, Barrington's nucleus, and parabrachial nucleus. The cuts ventral to the DMN would apparently disrupt some descending efferent fibers from the DMN to the ventral and dorsal premammillary nuclei, dorsal and ventral tuberomammillary nucleus, posterior periventricular nucleus, nucleus of the solitary tract, area postrema, nucleus raphe globus pallidus, and medullary reticular nucleus. Lateral ascending connections from the DMN exit both ventral and dorsal to the fornix and at this time cannot be distinguished from one another. The Vent cuts would transect only those lateral fibers leaving the DMN ventral to the fornix. With this in mind, some laterally directed fibers innervate the perifornical region. Other fibers enter the medial forebrain bundle to innervate the parvocellular region of the paraventricular nucleus, anterior hypothalamic area, preoptic area, bed nucleus of the stria terminalis, lateral septal nucleus, nucleus accumbens, amygdala, layer 1 of the entorhinal area, the ventral subiculum, and areas CA1 and CA3 of the hippocampus. Another efferent DMN pathway is directed ventrally in the periventricular region and would be disrupted by the ventral cuts. This pathway innervates the ventromedial nucleus, arcuate nucleus, anterior hypothalamic nucleus, preoptic region, retrochiasmatic area, supraoptic nucleus, and plexus around the suprachiasmatic nucleus.

Many of these brain areas that are connected to the DMN have been shown to be involved in the control of feeding behavior and body weight (11, 12, 21, 22, 26–28). It should be remembered that most of the

Table 5. *Effect of intraperitoneal injections of naloxone (1 mg/kg) or saline on cummulative food intake in DMNL or Sham rats*

	Post ($n=7$)	Lat ($n=8$)	Vent ($n=7$)	Dor ($n=7$)	*Trial 1* Sham ($n=13$)	Ant ($n=7$)	*Trial 2* Sham ($n=9$)
1 h							
Saline	3.3 ± 0.5	2.7 ± 0.4	2.8 ± 0.3	3.3 ± 0.5	3.3 ± 0.3	3.1 ± 0.4	3.1 ± 0.4
Naloxone	2.8 ± 0.6	2.1 ± 0.2	1.4 ± 0.3	2.2 ± 0.3	2.3 ± 0.3	2.2 ± 0.4	2.1 ± 0.3
P	NS	NS	<0.05	<0.06	<0.05	NS	NS
2 h							
Saline	6.4 ± 0.5	6.1 ± 0.4	5.8 ± 0.3	6.2 ± 0.4	7.0 ± 0.5	6.8 ± 1.1	7.6 ± 0.3
Naloxone	5.1 ± 0.9	4.7 ± 0.4	3.7 ± 0.4*	4.2 ± 0.4	5.1 ± 0.3	5.6 ± 1.0	5.5 ± 0.4
P	NS	<0.05	<0.01	<0.01	<0.01	NS	<0.01
3 h							
Saline	8.8 ± 0.7	8.4 ± 0.5	8.1 ± 0.3	8.6 ± 0.3	9.0 ± 0.5	8.3 ± 1.3	8.9 ± 0.5
Naloxone	8.4 ± 0.9	7.9 ± 0.2	6.4 ± 0.4*	7.6 ± 0.6	7.7 ± 0.3	7.3 ± 0.7	7.5 ± 0.3
P	NS	NS	<0.01	NS	<0.05	NS	<0.05

All values except *P* values are means ± SE; *n* = no. of rats. A second trial was conducted with an Ant group and a Sham group (see MATERIALS AND METHODS). All statistical comparisons for Ant group are made with *trial 2* Sham group; all other statistical comparisons are made with *trial 1* Sham group. *$P < 0.05$ vs. *trial 1* Sham group.

DMNL syndrome is reproduced by loss of cell bodies in the DMN (1, 9). Therefore, cell bodies in the DMN are actively participating in the regulation of feeding behavior and body weight through their efferent connections with other brain areas. The DMN may also have a role in integrating afferent signals from other brain areas controlling ingestion and body weight (11, 12). It is noteworthy that Ant and Dor cuts would have disrupted most connections between the DMN and the paraventricular nucleus. The paraventricular nucleus is thought to have a major role in the control of feeding behavior (15, 18, 24, 29), and yet disconnecting the DMN and paraventricular nucleus did not affect food or water intake or body weight.

The DMN of rats contains both opioid fiber tracts and cell bodies (see Refs. 6, 11), and infusion of μ- and δ-receptor agonists into the DMN increases food intake of rats (24). In the present study, rats with Post cuts, and to a lesser degree rats with Ant cuts, did not show food intake suppression after NOX. On the other hand, rats with Vent cuts overresponded to the food intake-suppressive effects of NOX. In a previous study (6) rats with electrolytic DMN lesions showed no food intake suppression when given 1.0 mg/kg of NOX. It was proposed that an opioid system in the DMN increases feeding by inhibiting a satiety system (6). Destruction of the DMN would leave the satiety system uninhibited and thus would produce hypophagia. Hypophagia is a characteristic of the DMNL syndrome. It was further (6) suggested that lesions of the DMN do not destroy a feeding system. This was supported by the observation that DMNL rats enhance their food intake after food deprivation (1, 9, 11). Additional evidence (5) comes from the finding that if the body weight of the rat before DMNL is reduced below the lesion-induced lowered body weight "set point," the DMNL rat actually becomes hyperphagic. The rats' intake remains elevated until they reach the DMNL-induced body weight "set point." Finally, DMNL rats are not aphagic, and thus feeding system(s) remain operational (6) but are unable to maintain food intake at control levels. Lambert et al. (20) reported that giving NOX to intact rats caused them to decrease their food intake while at the same time increasing NPY concentrations in the DMN. Recently, Carr et al. (13) suggested a role for DMN opioids and NPY in the control of feeding behavior. They noted that NPY from the arcuate nucleus may modulate DMN-induced feeding and that a feedback loop from the DMN to the arcuate nucleus may exist. Whether the enhanced NOX response in the Vent group is caused by a loss of efferent or afferent connections between the arcuate nucleus and DMN awaits further investigation. Finally, Carr et al. (13) proposed that the DMN may be one of multiple brain sites involved in NPY and opioid orexigenic activity.

Like electrolytic- or excitotoxic-lesioned DMN rats, the knife-cut groups elevated their intake after food deprivation. This demonstrates that although DMNL, Post, and Vent rats are hypophagic, they retain the ability to respond to caloric deficits and regulate their body weight, albeit at a lower "set point."

Rats with Post and Lat cuts were hypodipsic, but the magnitude of the decrease in water consumption was less than that observed after electrolytic or excitotoxic DMNL (1, 2, 9). However, the additive decreases observed after these two types of cuts approximated that measured after DMNL. Earlier, it was demonstrated (2) that the drinking responses of DMNL rats to a variety of extra- and intracellular dehydration challenges were normal. Like DMNL rats, the knife-cut groups ate in the absence of water, drank in the absence of food, and responded normally to water deprivation.

The DMN knife-cut data indicate that the same neural pathways appear to be involved in the attenuation of both feeding and body weight. The hypodipsia observed after DMNL involve some neural pathways separate from feeding systems. The data also suggest that the loss of DMN opioid pathways that enter or leave the DMN from the posterior direction are involved in the attenuation of feeding after DMNL. The data indicate that the DMN influences ingestive behavior through complex neural connections.

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