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Effects of Lateral Hypothalamic Lesions on the Anorexia Induced by Ethanolamine-O-Sulfate

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COSCINA, D. V. AND J. N. NOBREGA. *Effects of lateral hypothalamic lesions on the anorexia induced by ethanolamine-O-sulfate*. PHARMACOL BIOCHEM BEHAV 32(1) 275–281, 1989.—Intracisternal (IC) injection of the GABA-transaminase inhibitor, ethanolamine-O-sulfate (EOS), has been previously shown to induce dose-dependent anorexia in normal rats as well as to reverse overeating in several rodent models of acute and chronic hyperphagia. To determine if such anorexia might be mediated by cells within or fibers of passage which traverse the lateral hypothalamus (LH), adult female rats received bilateral radiofrequency heat lesions of the LH vs. anesthesia control injections and were allowed to recover normal feeding and drinking responses. Using a longitudinal design, all animals then received 100, 0, and 200 μg EOS in 20 μl deionized water IC with 1 week separating each injection. In addition to daily measures of feeding, drinking and body weight, all animals were screened 24 hr after injections for sensorimotor competence and general health by testing open-field activity, catalepsy, paw-lick responses on a hot-plate and rectal temperature. As reported previously, IC EOS induced dose-dependent hypophagia and weight loss. However, the magnitude and duration of these effects were equivalent in lesioned and control rats. In addition, open-field activity and body temperature were reliably lowered as a function of dosage while catalepsy was increased. Again, this effect was equivalent in lesioned and control rats. Subsequent tests of drinking and feeding in response to hyperosmotic and hypoglycemic challenges, respectively, confirmed that lesioned rats were deficient compared to controls. These findings suggest that an intact LH axis is not required for the anorexigenic effects of IC EOS.

Rat Lateral hypothalamus Lesion Feeding GABA Ethanolamine-O-sulfate GABA-transaminase Anorexia

GAMMA-AMINOBUTYRIC acid (GABA) is a major inhibitory neurotransmitter with widespread distribution in mammalian brain (1). Recent studies have demonstrated that systemic, intraventricular or intracisternal injections of drugs which prevent the catabolism of brain GABA by inhibiting GABA-transaminase (GABA-T) produce anorexia in experimental animals [for recent summary see (15)]. In keeping with these findings, past work from this laboratory has shown that intracisternal injections of the GABA-T inhibitor ethanolamine-O-sulfate (EOS) produce dose-dependent anorexia in normal female rats, block the acute eating induced by systemic insulin or 2-deoxyglucose (2-DG), and reverse the chronic overeating induced by highly palatable diets, genetic predisposition, or damage to the medial hypothalamus (2, 3, 8). Since EOS-induced anorexia can occur at doses which do not alter indices of motoric or sensory competence (9,10), these findings imply that elevating brain GABA within specified ranges might affect CNS mechanisms specific to feeding regulation. What remains unclear is exactly where in brain such anorexia is mediated.

GABA has been shown to exist in uniformly high concentrations throughout the hypothalamus (16). The lateral hypothalamus (LH) is one brain site which has been classically linked with appetite and hunger control (17,19). Within the context of GABAergic regulations over feeding, Kelly and co-workers have shown that GABA agonists applied locally to the LH can lower feeding while GABA antagonists can enhance it (5,6). These findings were interpreted to mean that inhibitory GABA interneurons can suppress the feeding signals which originate in or impinge upon LH sites (5). If this interpretation is correct, it is possible that the anorexia which follows central administration of EOS is mediated within the LH. To test that possibility, we studied the capacity of relatively low intracisternal doses of EOS to suppress feeding in rats that had recovered from bilateral LH lesions. If the integrity of the LH is important for the ex-

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pression of such anorexia, damaging it should partially or wholly prevent the feeding suppression which follows this drug treatment.

METHOD

Subjects

Thirty adult female Wistar rats were purchased from Charles River Laboratories (Montreal, Quebec). Upon arrival in our laboratory, animals weighed approximately 240 g. Throughout these investigations they were housed in single cages with stainless steel wire-mesh fronts and bottoms in a colony room with controlled lighting (on 0800–2000 hr) and temperature (22±1°C). At all times rats had free access to powdered Purina Lab Chow (4% fat) in Wahmann nonspill food cups and water in Wahmann graduated water tubes.

Procedure

Surgery. Following 7 days adaptation to colony conditions, rats were divided into the following groups based on equivalent body weights: 1) small LH lesions (n=9); 2) large LH lesions (n=9); 3) anesthesia controls (n=12). All animals were anesthetized with sodium pentobarbital (Nembutal; 45 mg/kg/2 ml IP) and rats in the first two groups received stereotaxically placed bilateral radiofrequency heat lesions of the LH using a Radionics RFG4 device connected to an electrode (0.8 mm diameter; 1.0 mm uninsulated tip) with a thermistor imbedded in its tip. For small lesions, 50°C was generated for 1 min in each hemisphere at the following coordinates (head flat): 5.6 mm anterior to the interaural line; 1.8 mm lateral to the midsagittal sinus; 8.5 mm below the dura. For large lesions, 55°C was generated for 1 min in each hemisphere using the same coordinates. Scalp wounds were closed with stainless steel clips and animals were returned to their home cages for a 2-week postoperative recovery period, during which 24-hr food intakes, water intakes and body weights were recorded daily.

EOS tests. Using a longitudinal design, all rats were monitored for daily food intakes, water intakes and body weights after the following sequence of intracisternal (IC) injections: 1) 100 μg EOS (Calbiochem, San Diego, CA), 2) vehicle, 3) 200 μg EOS. In each case, animals were lightly anesthetized with methoxyflurane, placed in a steroeotaxic frame, and received the indicated IC injection. All injections were made in a volume of 20 μl of deionized water. Animals recovered the ability to locomote approximately 3 min after each injection. All IC treatments were performed between 0900 and 1100 hr.

Twenty-two to twenty-four hr after each injection, animals were assessed for general health and sensorimotor competence using the following tests: 1) rectal temperature, 2) catalepsy, 3) exploratory activity, 4) analgesia. To measure rectal temperature, each rat was removed from its home cage and a thermistor probe (Y.S.I. Model 43TA, Yellow Springs, OH) was inserted 5 cm beyond the anus. Stabilized temperatures were read from an analog scale to the nearest $^1/_{10}$ degree centigrade. To measure catalepsy, rats' front paws were gently placed onto a platform 10 cm above the surface that their hindlimbs rested on and the latency (tenths of sec) to remove their forelimbs from this position or to climb onto the elevated platform was recorded. For measures of exploratory activity each animal was placed for 15 min in a 0.49 m^2 open field constructed of Plexiglas® walls and a 0.25 in^2 stainless-steel mesh floor. The apparatus was located in a small room illuminated by a 15-watt bulb located 1 M above the center of the floor. Crossings of infrared photo beams positioned every 15 cm along the walls generated counts of horizontal motor activity which were recorded every min throughout this test. Each activity session was also recorded on video tape to subsequently score the number of rearings which occurred in each session. Measures of analgesia were obtained by placing each rat on a thermoelectric hot-plate (Omnitech Analgesiometer Model AMTI, Columbus, OH) and recording the latency (nearest tenth of a sec) to lick a paw. The surface temperature of this device was set at 52°C. If paw licking did not occur within 60 sec, the animal was removed and 60 sec was recorded as its score. The sequence of testing was randomized across subjects except for the hot-plate test which was always conducted last to minimize stress effects on other behavioral measures. In addition to these tests, food intake over the first 6 hr after IC injection was recorded. This was done based on other work in this laboratory which indicated that feeding may initially be enhanced during this time perhaps as a consequence of short-term agonistic effects of IC EOS on GABA-A receptors (10).

The criterion used to determine the interval of time between injections was the recovery of group feeding and weight gains to normal for at least 3 consecutive days. Using this requirement, 7 days intervened between all IC injections. Following the 200 μg treatment, rats were monitored for 11 days of ingestive and weight responses.

Acute ingestive challenges. To determine if LH lesions were effective in producing long-term ingestive impairments, all rats were tested for acute drinking responses to a hyperosmotic challenge as well as for acute feeding responses to a glucoprivic challenge [see (13) and (18) as examples of the rationale for these tests]. To assess the former, rats were removed from their cages, weighed and injected with 1 M NaCl IP in a volume equal to 1% of their body weights. Water intakes were recorded 15, 30, 60, 120 and 360 min after injection. Seven days later, glucoprivic responsivity was tested by removing rats from their cages, weighing them, and injecting 400 mg/kg 2-deoxy-d-glucose (2-DG; Sigma Chemicals, St. Louis, MO) IP in a volume of 200 mg/ml. Food intake was measured 1, 2, 4 and 6 hr after injection. Both tests were conducted between 1000 and 1600 hr.

Sacrifice. Rats were sacrificed with an overdose of Nembutal and perfused transcardially with 0.9% saline followed by 10% formalin. Brains were removed, fixed in 10% formalin, and later sectioned coronally at 20 microns. Every 5th section was retained for staining and examination of lesion sites by light microscopy and quantitation of lesion volume using a computer-assisted imaging system (MCID, Imaging Research Inc., St. Catherines, Ontario).

Statistical analysis. The data obtained were analyzed by two-way analyses of variance (ANOVAs) or *t*-tests for independent samples. In addition, quantitative measures of lesion volume were correlated with all behavioral measures obtained 24 hr after the 200 μg dose of EOS.

RESULTS

Postoperative Recovery From LH Lesions

Of the 9 animals designated to receive large LH lesions, 1 was not lesioned by mistake, so it was reassigned to the control group. All 17 lesioned rats which remained showed clear signs of LH injury the first day after surgery. This

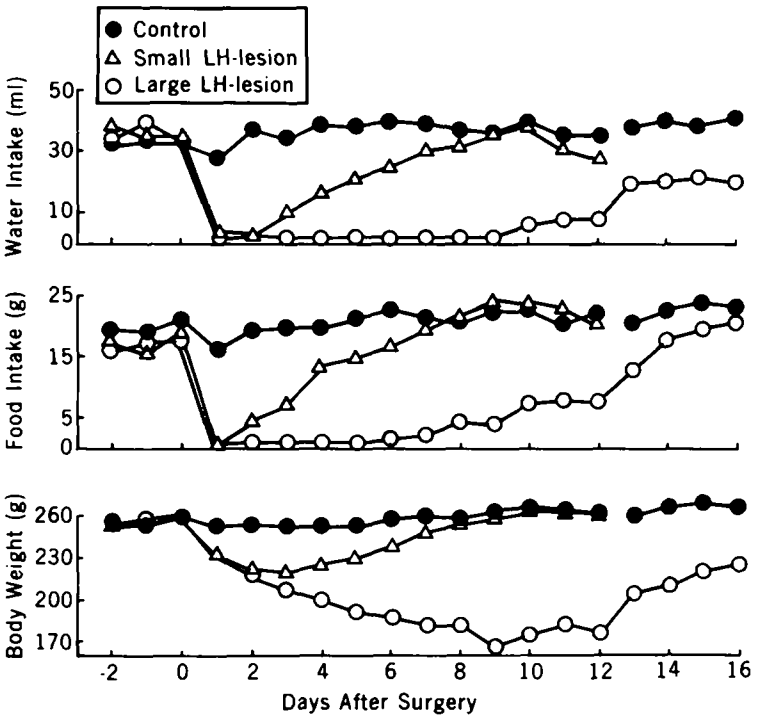

FIG. 1. Mean daily body weights (lower frame), food intakes (middle frame) and water intakes (upper frame) during postoperative recovery for Control, Small LH-lesioned and Large LH-lesioned rats (see inset for definition of group symbols). The numbers of animals summarized in these plots are: n=8 for Small LH lesions; n=2 for Large LH lesions; n=8 for Controls from day −2 to day 12; n=2 for Controls from day 13–16. See text for description of different postoperative time courses for these groups.

included anorexia, adipsia, weight loss, poorly groomed fur, reduced motor activity and/or sedation, and porphyrin deposits around the eyes and nose. Over the next several days, most rats in the large-lesion group developed signs of vaginal infections and respiratory problems in addition to persisting anorexic and adipsic. By the 14th postoperative day, six rats in the large-lesion group and one in the small-lesion group had died despite daily IP injections of isotonic saline to minimize dehydration, starting on postoperative day 2, and daily intragastric feeding, starting on day 5.

By the end of the second postoperative week, the 8 small-lesioned rats had fully recovered and were ready for EOS injections. However, the 2 large-lesioned rats which survived required an additional week of recovery before they could receive IC injections. All subsequent testing of these animals, as well as that of 2 nonlesioned rats included for control, was delayed 1 week. This meant that only the 8 small-lesioned rats were tested on the first dose of EOS. To maintain equal sample sizes, only 8 of the 11 remaining controls were selected for this and subsequent testing. Figure 1 depicts the daily weights and intakes over the first 16 postoperative days for all rats used in this study. Since no reliable differences were found among small- and large-lesioned animals on any of the subsequent tests, their data were combined.

Responses to Intracisternal EOS vs. Vehicle

Twenty-four hr after injection. The results of all tests for each drug dosage are summarized in Fig. 2. A two-way ANOVA (Group × Drug Dose) for each measure revealed a reliable effect of drug dosage to suppress both indices of activity, body temperature and food intake for hr 6–24 after injection. Also, there was a reliable enhancement of catalepsy as drug dosage increased. In addition, there was a reliable effect of lesion to suppress feeding during the first 6 hr after injection. No effect of either factor was seen on paw-lick latencies. In no case were interactions found between these two factors.

Daily intake and weight changes. Figure 3 depicts daily feeding, drinking and weight responses for each drug dosage tested. Separate *t*-tests on each time point revealed no reliable differences in feeding or weight measures between groups for any IC treatment. However, lesioned rats showed reliable ($ps<0.05$) depressions of water intake at the times indicated.

Acute Ingestive Challenges

Figure 4 illustrates the drinking responses to the hyperosmotic saline challenge and the feeding responses to the 2-DG challenge. In the former case, a two-way repeated

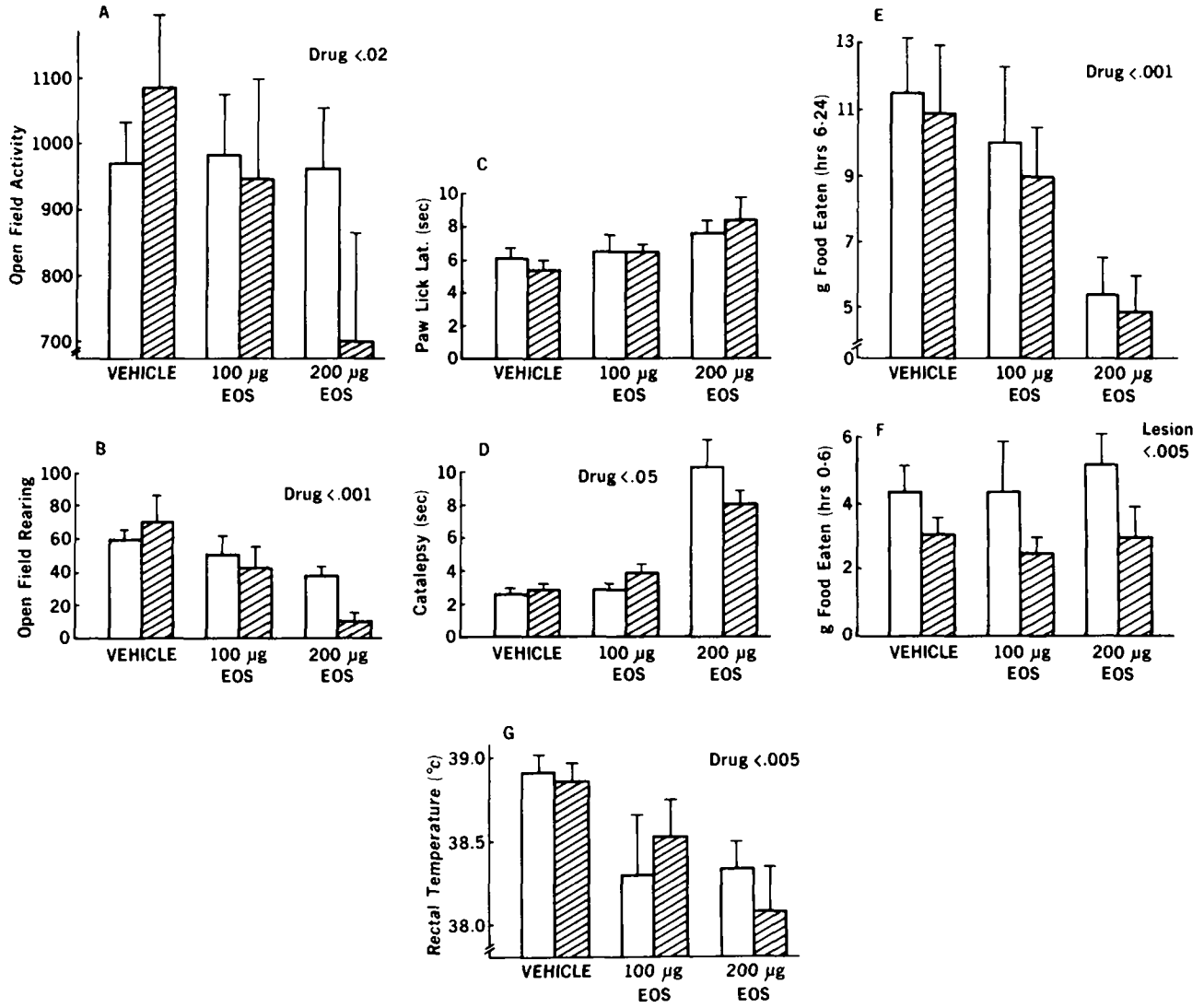

FIG. 2. Mean responses to sensorimotor and general health tests conducted 24 hr after each intracisternal injection. Open bars represent the mean of all controls; hatched bars represent the mean of all lesioned rats. Vertical capped lines represent the standard errors of each mean. Variables depicted are (upper left corner per bar graph): (A) Cumulative photobeam crossings in the 15-min open-field test; (B) Cumulative vertical rearings in the 15-min open-field test; (C) Latency to lick a paw on the hot-place test; (D) Latency to respond in the catelepsy test; (E) Grams of food eaten 6–24 hr after IC injection; (F) Grams of food eaten 0–6 hr after IC injection; (G) Rectal temperature. See text for further explanation of how each variable was measured. The results of significant two-way ANOVAs are included in the upper right-hand corner of each bar graph. In all cases, only main effects were found. No reliable effects were seen on paw-lick latencies (bar graph: C).

measures ANOVA (Group × Time) revealed reliable Group ($p<0.05$), Time ($p<0.001$) and Group × Time ($p<0.05$) effects. In the latter case, a separate two-way ANOVA revealed reliable Group ($p<0.005$) and Time ($p<0.001$) effects. Subsequent t-tests revealed reliable ($ps<0.05$) deficits at all time points shown.

Histology

Lesions varied considerably in their size, location and symmetry (see Fig. 5). In general, lesions were situated at the lateral edge of the LH. The centers of these lesions ranged from the anterior to the posterior limits of the hypothalamus. In one case, the lesion appeared unilateral rather than bilateral.

To determine if variations in lesion size and/or location might be associated with any of the effects observed, area measurements of cavitation throughout the extent of these lesions were summated to provide an index of lesion size. In addition, the ratio of smaller to larger lesion volume per hemisphere was computed for each rat as an index of lesion symmetry. These measures were then correlated with all sensorimotor measures taken 24 hr after the 200 µg dosage of EOS as the latter seemed most efficacious in modifying these variables. No reliable correlations were found between these

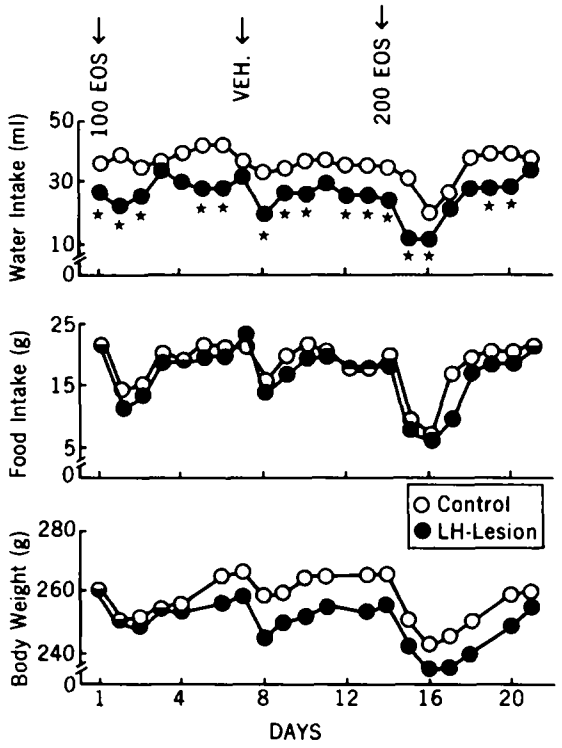

FIG. 3. Mean daily body weights (lower frame), food intakes (middle frame) and water intakes (upper frame) after each intracisternal injection. The dosage and day of EOS injection are indicated along the top of the figure. VEH=vehicle. See text for description of sample sizes per treatment condition. Stars mark significantly lower water intakes by LHs on the days indicated. No differences were found between groups on any measures of feeding or body weight.

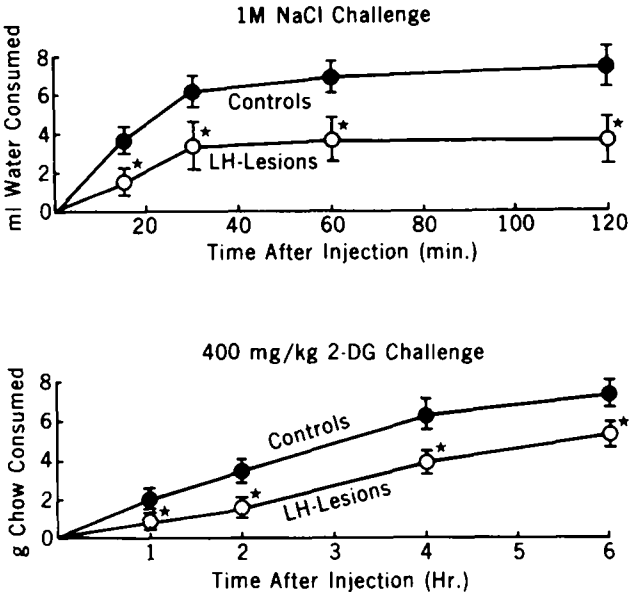

FIG. 4. Mean±SEM cumulative water intakes after hyperosmotic challenge (upper frame) and food intakes after hypoglycemic challenge (lower frame). Stars mark significantly lower intakes by LHs on all days shown as determined by *t*-tests. Not included in the upper tracing are values for the 360 min time point tested as both groups showed equivalent responses by then (i.e., 24.3±5.7 ml for LHs; 25.7±5.8 ml for Controls). See text for description of sample sizes per condition.

indices of lesion size or symmetry and any of the behavioral variables measured.

DISCUSSION

Intracisternal EOS injections produced essentially the same functional effects in both LH-lesioned and control rats. The nature and magnitude of alterations to feeding and other behavioral states were the same as seen in other recent experiments (10). Therefore, the present results indicate that the locus of EOS effects is distinct from those sites which are responsible for the classic LH syndrome. If the full integrity of the LH were important for the expression of such anorexia, then damage to it should have lessened or prevented any feeding suppression. This clearly did not occur since LH-lesioned rats showed the same anorexia and weight loss as nonlesioned controls across the drug conditions tested. If anything, it appeared that LH-lesioned rats were generally more rather than less sensitive to treatment effects. That possibility is based on their relatively greater suppression of feeding during the first 6 hr after all IC injections. Another finding which implies enhanced rather than blunted sensitivity was lower daily water intake. While statistical analyses confirmed that this decrement was associated with EOS, this response was not specific as LH rats also drank less just before the first EOS injection, drank less after vehicle injection, and continued to drink less even after the drug's anorexic effects had worn off. Therefore, this deficit may reflect a more enduring feature of LH lesions, which is a chronic impairment in consuming fluids [see (4, 14, 17, 19)].

The lesions in most of our animals were quite small and varied in symmetry as well as location. However, the qualitative aspects of these animals' postoperative recovery syndrome was quite similar to that described for "classic" LH-lesioned rats (17). In addition, post-EOS tests of behavioral responsivity to hyperosmotic and glucoprivic challenges demonstrated that these rats were deficient in both spheres. Thus, it is difficult to argue that our findings are due to functionally inadequate brain injury. In addition, the documentation of consistent dose-dependent deficits on both measures of activity as well as catalepsy and feeding 18 hr after IC injections indicates the efficacious nature of our EOS treatment regimen to modify several functional states. However, the lack of interaction between drug dosage and lesion factors further supports the main finding of this study, which is the comparable level of daily feeding observed after IC injections.

Taken together these data argue against the likelihood of a major involvement by the lateral hypothalamus in EOS-induced anorexia. It is possible that other sites within the LH which were spared by our lesions are more involved in this process. Nevertheless, there was a fair amount of variation in our lesion placements and in no case did we observe alterations in responsivity to EOS. As to the possibility that other major hypothalamic sites implicated in feeding control might be involved in such anorexia, an earlier report from this laboratory showed that large medial lesions which

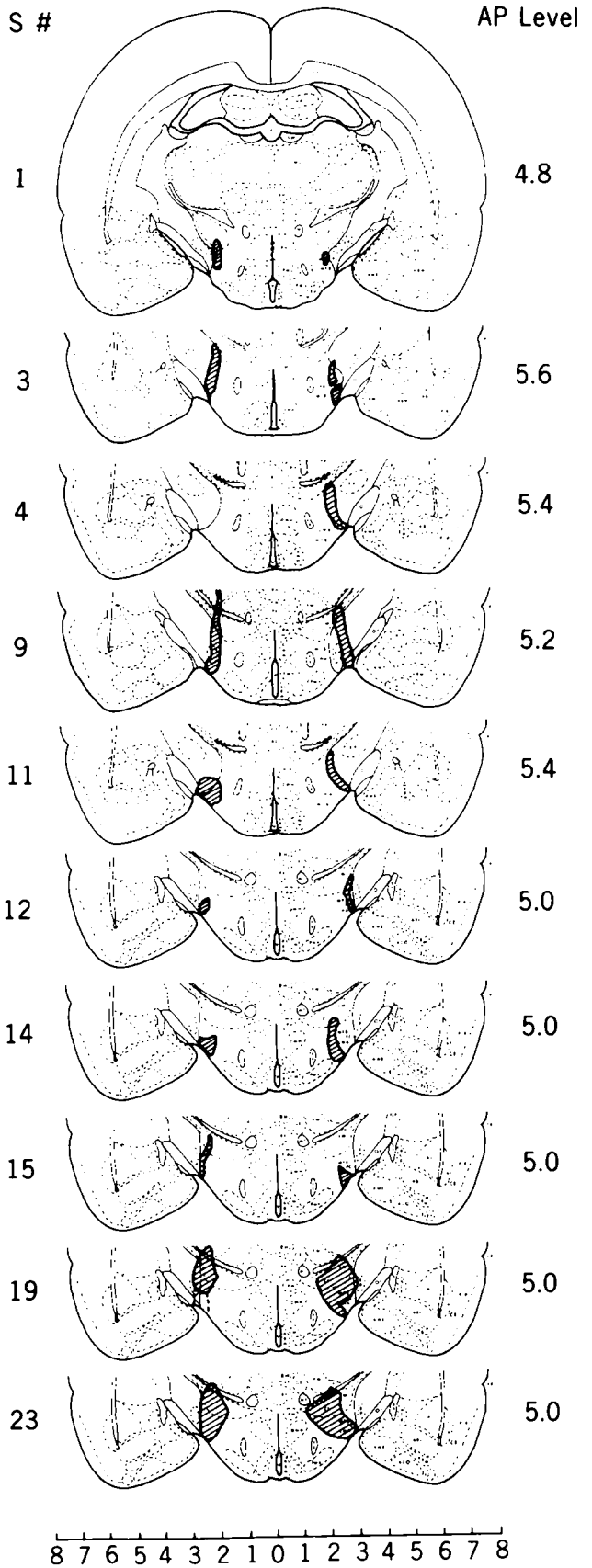

FIG. 5. Schematic representation of lesion cavitation at the center of all LH sites. Subject numbers (S #) are indicated along the left; the location of each section in mm rostral to the interaural line is shown along the right (AP Level). S #4 appeared to have only unilateral LH injury. S #19 and #23 were the 2 surviving large-lesioned LHs. All lesions were reconstructed on plates from the Pellegrino and Cushman Atlas (12).

produced substantial hyperphagia and obesity were also not critical for the expression of EOS anorexia (3). Other researchers who have employed local injections of GABA agonists and antagonists have suggested that ventral sites between the lateral and medial hypothalamus may be important in the GABAergic regulation of feeding (5,7). Additional work will be required to determine if such sites might be involved in the anorexia which follows IC EOS. At present, the exact brain site(s) which mediates such feeding suppression remains unknown.

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