Lateral parabrachial lesions attenuate ingestive effects of area postrema lesions

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Edwards, Gaylen L., and Robert C. Ritter. Lateral parabrachial lesions attenuate ingestive effects of area postrema lesions. Am. J. Physiol. 256 (Regulatory Integrative Comp. Physiol. 25): R306-R312, 1989.—Lesions of the area postrema and adjacent nucleus of the solitary tract (AP lesions) cause rats to consume increased amounts of palatable food in short duration tests. Because the lateral parabrachial nucleus (IPBN) receives a prominent afferent projection from the AP and adjacent nucleus of the solitary tract, it is possible the IPBN plays a role in the altered ingestive behaviors observed in AP-lesioned rats. The present study examines the role of the IPBN in overingestion of highly palatable foods subsequent to AP lesions. We found that lesions of the IPBN alone did not cause rats to consume increased amounts of palatable food. Rather, when IPBN lesions were produced before AP lesions, increased intake of highly palatable food did not occur. Moreover, when AP-lesioned rats received subsequent IPBN lesions, the previously established overingestion of palatable foods was abolished. These results indicate that the IPBN is necessary in the pathogenesis of AP lesion-induced overingestion of highly palatable foods.

parabrachial nucleus; feeding behavior; hindbrain; rats

Ablation of the area postrema (AP) and the adjacent nucleus of the solitary tract (NST) produces several behavioral changes, which suggest that these structures are involved in the control of ingestion (1, 3-8, 12-15, 21-23). For example, subsequent to lesions that destroy the AP and damage the immediately adjacent NST (AP lesions), rats consume increased quantities of highly palatable or novel foods during short (30-120 min) presentations (6-8, 21-23). This increase in the intake of highly palatable foods is dramatic. Lesioned rats often consume two to three times the quantity of a sweet food or solution as is eaten by intact control rats (6). Moreover, the increased intake is selective to palatable foods, as lesioned rats do not overingest their maintenance diet either ad libitum or after food deprivation (7, 21, 22).

Overingestion by AP-lesioned rats might be produced by a lesion-induced deficit in visceralosensation or a change in responsiveness to specific gustatory cues. Structures typically damaged by AP lesions include not only the AP but also varying amounts of the medial and commissural subnuclei of the NST. These NST subnuclei, as well as the AP, are recipients of primary vagal sensory terminals (for review see 22) that convey general visceral sensory modalities from the gastrointestinal tract and other viscera. Gustatory nerves from the oral cavity also synapse in the NST. The gustatory nerves, however, terminate mostly in the rostral lateral portions of the NST (2), which are not directly damaged by AP lesions. Thus AP lesions do produce some damage in areas involved in gastrointestinal sensation, but do not impinge on major gustatory afferent areas. Our previous work, however, indicates that overingestion of palatable foods by AP-lesioned rats is not due to a simple impairment of visceral sensory function but suggests that a change in gustatory responsiveness causes overingestion (7).

The neural connections through which AP lesions might influence the behavioral response to specific gustatory cues are uncertain. However, the AP and caudal NST and gustatory portions of the rostral NST both project to the parabrachial nuclei in the pons (16-19, 27, 28). Furthermore, recent electrophysiological data indicate that visceral afferent signals from the caudal NST and gustatory signals from the rostral NST converge on cells in the lateral parabrachial nucleus (11). Therefore the selective removal of the AP and/or caudal NST portion of such a convergent projection might account for the alteration of orosensory responsiveness observed after AP lesions. As an initial test of this possibility, we lesioned the IPBN before placing AP lesions. We found that IPBN lesions abolished increased intake of highly palatable foods by AP-lesioned rats. Moreover, when the IPBN was lesioned subsequent to an AP lesion, the established increase in intake of highly palatable foods was reversed. These findings suggest that connections between the AP and/or caudal NST and the IPBN may be involved in the altered response to specific foods observed after AP lesion.

METHODS

Adult male Sprague-Dawley rats weighing 300-350 g at the start of the experiments were used in all procedures. The animals were housed in a temperature-controlled room in suspended stainless steel cages with pelleted food (Purina) and water available ad libitum except during actual testing when the pelleted chow was removed. The room was maintained on a 12:12-h light-dark cycle, and all testing was done between 0900 and 1400 h.

Experimental subjects were assigned to one of five surgical groups. Rats in the group designated PAP re-
received a lesion in the IPBN (IPBN lesion) first, followed by lesion of the AP and adjacent NTS (AP lesion). Animals in the APB group received an AP lesion before the IPBN lesion. Rats in the PBD group received a sham AP lesion first, followed by a sham IPBN lesion. Rats in the AP group received sham IPBN lesion first, followed by an AP lesion. The SH group was composed of animals that received two sham lesion surgeries. The sham surgeries in the SH group were arranged such that 50% of the group received the AP lesion sham surgery first and 50% received the IPBN lesion sham surgery first. All groups were allowed to fully recover from the first surgery and were tested for intake of a palatable food (cookies) during short duration tests before the second surgical procedure. Therefore the two surgical procedures were separated by at least 6 wk.

The AP was removed by aspiration, and the surgical approach was based on the AP lesion procedure of Ritter et al. (29). Briefly, the procedure involved anesthetizing each rat with methoxyflurane and clipping and cleaning the dorsum of the head and neck. The rat was then positioned in a stereotaxic instrument with the neck flexed. A 3-cm skin incision was then made from the occipital crest to the midcervical level. The musculature was dissected using blunt and sharp dissection to expose the atlantooccipital ligament and underlying dura mater and arachnoid membrane. These structures were incised, and the foramen magnum was enlarged with rongeurs to expose the dorsal hindbrain. The pia mater was then incised and the cerebellum elevated, if necessary, to expose the caudal end of the rhomboid fossa. The AP was visualized with an operating microscope and aspirated with a blunted 23-gauge hypodermic needle. The musculature was sutured with chromic gut and the skin with silk to close the surgical wound. Sham AP lesions were prepared by exposing the dorsal hindbrain and blotting the region of the obex with a cotton-tipped swab.

Lesions of the IPBN were produced using Nichrome electrodes with 0.5-mm uninsulated tips, attached to the anode of an electrolytic lesion-making device (Stoelting, Chicago, IL). Each rat was anesthetized with methoxyflurane and the dorsum of the head was clipped and cleaned. The animal was positioned in a stereotaxic apparatus with the skull level. A 3- to 4-cm incision was made from the level of the orbits to just caudal to the occipital crest. The underlying fascia and periosteum were then elevated from the calvarium and allowed to retract laterally. The skull was leveled between bregma and lambda, and bilateral drill holes were placed 9.2 mm caudal and 1.8 mm lateral from bregma. The dura was incised, and an electrode was lowered at 9.2 mm posterior and 1.8 mm lateral from bregma and 5.4 mm ventral from dura. Electrolytic lesions were produced by passing 0.8-mA anodal current for 10 s on each side. Sham-lesioned rats were produced by incising the skin and drilling skull holes. The dura was incised but the electrodes were not lowered. After lesioning, the rats were allowed at least a 15-day recovery period before behavioral testing began. Body weight and food and water intake were also monitored after surgery.

After recovery from surgery, consumption of a palatable food, cookies (Nilla Wafers, Nabisco), was measured during 2-h presentations during the light phase of the light-dark cycle. Intake of cookies was monitored after the first surgery as well as after the second surgical procedure, allowing a comparison of intakes before and after AP lesions in the same rats. Before cookie intake measurements were begun, the rats were familiarized with vanilla wafer cookies by allowing them access to the cookies during the dark phase of the light-dark cycle for 1 or 2 days. Additionally, the rats were given one 2-h exposure to cookies at the start of each series of tests. Data from these training exposures were not included in the results. Before the actual cookie tests, the pellet food was removed from the animals' cages. During both training exposures and actual tests, cookies were presented in the absence of pelleted rat chow, and intake was monitored for 2 h. Cookie intakes by lesioned and control rats were compared using appropriate analysis of variance followed by t tests when significant interactions were indicated.

The AP has been implicated in the formation of taste aversions conditioned by some circulating chemicals (1, 5, 23). Recently, Dey et al. (5) demonstrated that subpulmonotoxic doses of paraquat-conditioned taste aversions that required an intact AP. To examine the effects of AP and parabrachial lesions on the conditioned taste aversion by a stimulus known to depend on an intact AP, we injected lesioned and sham-lesioned rats with paraquat (40 μmol/kg ip) immediately after exposure to a novel food (chocolate cookies). On the conditioning day, the rats were given access to the cookies for 30 min, and intake was measured (preconditioning intake). Immediately after this 30-min cookie exposure, rats were injected with paraquat. Ninety-six hours later the rats were again given access to chocolate cookies, and the amount ingested was measured (postconditioning intake).

After behavioral testing was complete, all rats were perfused transcardially with physiological saline followed by 10% Formalin. The brains were removed and placed in a 10% Formalin-20% sucrose solution for postfixation. After at least 48 h postfixation, the brains were frozen in a cryostat, and 40-μm sections were cut. The sections were mounted on gelatin-coated slides, stained with cresyl violet, cleared, and a cover slip was placed on top. The sections were then examined under a light microscope, and the lesions were photographed. The photomicrographs from the lesioned rats were compared with photomicrographs of a typical intact rat brain for reference. Destruction of various specific structures was verified using the atlas of Pellegrino et al. (20), and damage to the subnuclei of the IPBN, as described by Fulwiler and Saper (10), was also noted. The lesions were subsequently reconstructed on schematic maps of the pons using the atlas of Pellegrino et al. (20).

RESULTS

Although the lesions were centered in the ventromedial portion of the IPBN, all IPBN lesions that were effective in blocking or reversing the increased ingestion of cookies after AP lesions (n = 16) produced significant damage.
throughout the majority of the IPBN. The area destroyed included the central lateral, internal lateral, dorsal lateral, and ventral lateral subnuclei, according to the terminology of Fulwiler and Saper (10) (Fig. 1). The external lateral subnuclei sustained some damage in eight cases. The brachium of the superior cerebellar peduncle was damaged by all effective lesions. There was little or no damage to the cerebellum overlying the PBN or to midbrain structures such as the cuneiform nucleus, periaqueductal grey, and central nucleus of the inferior colliculus. The medial PBN and Kolliker-Fuse nucleus were also damaged in some cases (medial PBN, 8 cases; Kolliker-Fuse, 5 cases; as was the mesencephalic nucleus of the trigeminal nerve, 6 cases). Figure 2 illustrates the area of common overlap for the parabrachial lesions that prevented or reversed overingestion in AP-lesioned rats. Lesions that did not block the overingestive effects of AP lesions failed to include the entire rostrocaudal extent of the IPBN, bilaterally. In one case, the lesion damaged the overlying cerebellum with little damage to the underlying IPBN.

Figure 3 shows photomicrographs of coronal 30-μm sections of the dorsal hindbrain of an intact and an AP-lesioned rat. The sections shown are ~0.4 mm rostral to obex. All lesions included the entire AP with additional damage to the immediately adjacent NST in all cases. Damage to the adjacent NST was variable, and the most extensive lesion included a major portion of the medial and commissural subnuclei. Additionally, there was damage to the dorsal motor nucleus of the vagus nerve (DMV) in most cases, but this damage was variable and the behavior of these animals was not different from lesioned rats in which the DMV was not damaged.

Body weights and 24-h intakes of pelleted food and water, which were only measured in the second of two replications of this experiment, are shown in Table 1. Body weights and 24-h food intakes were significantly less for rats with AP lesions, and the coexistence of a parabrachial lesion did not attenuate the reduction in weights or food intake.
Lesions consumed significantly more cookies in 120-min tests than sham-operated control rats [Fig. 3; APO (n = 10), 12.3 ± 0.9 g vs. SH (n = 17), 5.1 ± 0.5 g, P < 0.001].

On the other hand, rats that received AP lesions after a previous IPBN lesion failed to consume increased amounts of cookie compared with sham-operated control rats [Fig. 3; PAP (n = 15), 5.9 ± 0.7 g vs. SH (n = 17), 5.1 ± 0.5 g, P > 0.2] and compared with their previous intake (before AP lesion, 5.0 ± 0.9 g vs. after AP lesion, 5.9 ± 0.7 g, P > 0.1). Additionally, AP-lesioned rats, with an established overingestion of cookie, failed to consume increased amounts of cookie diet subsequent to IPBN lesions compared with sham-operated control rats [Fig. 4; APB (n = 4), 6.7 ± 1.0 g vs. SH (n = 17), 5.1 ± 0.5 g, P > 0.1]. Also, compared with their cookie intake before the IPBN lesion, the AP-lesioned-IPBN-lesioned rats consume significantly less (Fig. 5; before IPBN lesion, 12.2 ± 1.7 g vs. after IPBN lesion, 6.7 ± 1.0 g, P < 0.05).

Consumption of cookies by rats with IPBN lesions alone was not different from that of sham-operated control rats [Fig. 3; PBO (n = 18), 5.3 ± 0.8 g vs. SH (n = 17), 5.1 ± 0.5 g, P > 0.2].

Both sham-operated rats (SH, n = 6) and rats with only parabrachial lesions (PBO, n = 4) avoided chocolate cookies 96 h, after a conditioning exposure paired with injection of paraquat (P < 0.01, relative to preconditioning intake). On the other hand, rats with AP lesions alone (APO, n = 6) or AP lesions and parabrachial lesions (PAP, n = 5; APB, n = 1; total n = 6) did not avoid chocolate cookies after conditioning with paraquat (P > 0.1, relative to preconditioning intake). The postconditioning intakes, expressed as a percentage of preconditioning intakes, were significantly greater for the PAP-APB and APO groups than for the SH and PBO groups (P < 0.01). Postconditioning intakes of SH and PBO groups did not differ significantly (P > 0.1). Also,

### Table 1. Body weight and 24-h food and water consumption by lesioned and control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Body Wt, g</th>
<th>24-h Food, g</th>
<th>24-h Water, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP</td>
<td>5</td>
<td>387±26*</td>
<td>19.5±0.9*</td>
<td>30±6</td>
</tr>
<tr>
<td>PBO</td>
<td>4</td>
<td>478±56</td>
<td>23.1±0.9</td>
<td>31±6</td>
</tr>
<tr>
<td>APO</td>
<td>6</td>
<td>372±15*</td>
<td>18.5±0.8*</td>
<td>44±4</td>
</tr>
<tr>
<td>SH</td>
<td>8</td>
<td>488±18</td>
<td>23.2±0.8</td>
<td>38±3</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Significantly less than SH, P < 0.02.

There were no statistical differences in cookie intake between groups when rats had received only an AP lesion or an AP lesion and a sham IPBN lesion (P > 0.1). Therefore data from all groups that had only an AP lesion (APO) were combined for statistical analysis. Likewise, data from all rats with only IPBN lesions (PBO) were combined, as there were no significant differences between cookie intakes between these groups (P > 0.2).

Consistent with previous observations, rats with AP

![Figure 3](http://ajpregu.physiology.org/)

**FIG. 3.** Representative photomicrographs (original magnification ×2) of histology from a sham-operated rat (A) and an area postrema (AP) lesioned rat (B) at approximately same level of brain stem near obex. Note that lesion includes entire AP as well as a portion of adjacent medial and commissural, nucleus of the solitary tract (NST). In this rat, dorsal motor nucleus of vagus was also damaged. Further detail concerning extent of AP lesions can be found in text. Lesions in this study were similar to those we have previously described. DMV, dorsal motor nucleus of vagus nerve; GR, nucleus gracilis; TS, solitary tract; XII, hypoglossal nucleus.

![Figure 4](http://ajpregu.physiology.org/)

**FIG. 4.** Vanilla wafer cookie intake by PAP, APB, PBO, APO, and SH-lesioned rats. Rats with only AP lesions (APB) consumed significantly more than all others (P < 0.001). Also, there was no significant increase in cookie intake over SH intake by rats with only IPBN lesions (PBO) (P > 0.2). Note particularly that rats with both AP lesions and IPBN lesions (PAP and APB) do not consume significantly more than sham-operated control rats (SH) (P > 0.1).
Lesions that destroy the AP and damage the adjacent NST cause rats to overconsume palatable foods (6–8, 21–23). Our results demonstrate that bilateral lesions of the IPBN prevent overingestion by rats that subsequently receive AP lesions. Moreover, our results indicate that AP-lesioned rats, which already overconsume palatable foods, no longer exhibit overconsumption of palatable food after placement of lesions in the IPBN. These data suggest that IPBN neurons that receive projections from the AP or adjacent NST may mediate overingestion of palatable foods after AP lesions.

Reconstruction of our IPBN lesions indicated that 80% of IPBN tissue was ablated. The IPBN, however, has been subdivided into at least 10 subnuclei based on cytoarchitectural considerations (10). An overlap analysis of our lesions indicated that our effective lesions all completely destroyed the central lateral, ventral lateral, dorsal lateral, and internal lateral subnuclei of the IPBN (see Ref. 10 for description of subnuclei). These IPBN subnuclei appear to be the principal areas of termination for a projection from the AP, as described by Shapiro and Miselis (27). When the region of ablation was rostral, dorsal, or medial to these subnuclei, the lesion had no effect on AP lesion-induced overingestion of palatable food. Therefore the placement of our effective lesions correlates well with the location of IPBN cells that receive afferent input from the AP and adjacent NST.

The possibility that removal of AP or NST projections to the IPBN causes overingestion of palatable foods is appealing because anatomic studies indicate that efferents from both the AP and caudal NST terminate in the IPBN (16–19, 27, 28). There is also anatomic evidence for a light projection of IPBN efferent fibers directly to the AP and NST (27), and electrophysiological studies indicate that stimulation of the IPBN can alter activity of neurons in the caudal NST (9). In addition, the AP and adjacent NST also project directly to some structures that also receive IPBN efferents (10, 19, 29). Therefore it is possible that IPBN and AP-NST efferents might normally exert mutually antagonistic effects on ingestion, mediated via projections to unlesioned portions of the NST or other CNS structures with input from both the AP-adjacent NST and the IPBN. However, one would expect that under such an arrangement the IPBN itself would exert a direct stimulatory effect on palatable food intake. Consequently, IPBN lesions alone should decrease consumption of palatable food. However, IPBN lesions, by themselves, neither enhanced nor diminished consumption of palatable food, making this interpretation improbable. Therefore we believe that the effect of IPBN lesions on AP lesion-induced overconsumption results from the destruction of IPBN sites that normally receive afferents from the AP and/or NST.

The fact that IPBN lesions alone did not change the response to palatable foods also argues against the possibility that these lesions produce a general debilitation or malaise that causes reduced ingestion. Additional evidence against this interpretation is also provided by the fact that a 24-h intake of the maintenance diet by IPBN-lesioned rats was indistinguishable from that of intact rats (see Table 1). Finally, we have previously reported that IPBN lesions do not impair increased ingestion in response to glucoprivation (22). Therefore it seems most likely that abolition of AP lesion-induced overingestion by IPBN lesions results from direct interference with AP or NST projections involved in the modulation of ingestion and is not due to nonspecific malaise.

Although much ascending gustatory information from the rostrolateral NST synapses in the medial parabrachial lesions may mediate overingestion of palatable foods.
Parietal nucleus, portions of the IPBN also receive gustatory input (10, 19). Therefore it is possible that IPBN lesions antagonize the effect of AP lesions by impairing gustatory function. Our results do not rule out this possibility. Nevertheless, preliminary studies from our laboratory do indicate that IPBN lesions do not prevent rats from discriminating between two similar palatable foods. For example, rats with only IPBN lesions rejected a novel food after it was paired with an injection of paraquat to condition a taste aversion (see Fig. 6). Thus the lesioned rats did not appear to be agusic and could discriminate between similar foods with different tastes.

The nature of the normal interaction between the AP, adjacent NST, and the IPBN is not revealed by our study. Nevertheless, the subnuclei damaged by our lesions project directly to the central nucleus of the amygdala and to several hypothalamic nuclei (10, 25, 29), which have been implicated in the control of ingestion and gustation (24, 26). Therefore it is possible that neurons destroyed by the AP lesion normally modulate the activity of IPBN neurons that integrate and then relay to the forebrain general visceral sensory, gustatory, and chemosensory information.

Although vagal sensory pathways project to the IPBN via the AP and NST (11, 19), our previous work indicates that overingestion of palatable foods by AP-lesioned rats cannot be attributed to simple removal or reduction of visceral sensory cues that normally inhibit feeding (7). Therefore it seems unlikely that IPBN lesions attenuate AP lesion-induced overingestion simply by damaging the pontine relay for vagal afferent information. It seems more probable that overingestion by AP-lesioned rats is the result of altered orosensory responsiveness or perhaps a learned preference shift.

The IPBN may be necessary for an AP lesion-induced change in oral responsiveness or for learned changes in taste preference. In fact, Kenney and co-workers (14) have suggested that AP lesions may provide an unconditioned stimulus for the formulation of an aversion to the familiar food. Overingestion of a novel or palatable food would represent a response to a food not previously associated with the conditioning stimulus provided by lesion placement. This would constitute a lesion-induced learned preference shift. The effects of IPBN lesions on AP lesion-induced overingestion, however, are difficult to reconcile with this hypothesis because IPBN lesions made after AP lesion-induced overingestion was already established appeared to reverse the AP lesion-induced overingestion. An additional point is that if AP lesioning serves as the conditioning stimulus for a taste aversion-induced preference shift, then one might expect that a lesion that attenuated AP lesion-induced overingestion would also attenuate taste aversion conditioning. This does not seem to be the case because paraquat, a toxin that conditions taste aversions by an AP-dependent mechanism, appears to produce profound taste aversions in rats with only IPBN lesions (see Fig. 6). Furthermore, AP lesions prevent conditioning of taste aversions by paraquat in rats with either intact or lesioned IPBN.

The possibility that AP lesions remove afferents that modulate gustatory influences in the IPBN is interesting but speculative. Nonetheless, electrophysiological data suggest that cells in the IPBN do receive both gustatory input and input from the caudal NST and vagal afferents (11). Thus it may be that the IPBN is involved in modulation of gustatory influences that are relayed to the limbic forebrain. AP lesions may eliminate an influence that normally dampens the animal's ingestive response to certain orosensory cues. Lesions of the IPBN may reinstate the appearance of a normal ingestive response by destroying a pontine substrate that has been deprived of balanced afferent supply. Obviously, additional work is necessary to determine whether this scenario fits the actual relationship between the AP, adjacent NST, and the IPBN.

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