Chemotherapy-induced kaolin intake is increased by lesion of the lateral parabrachial nucleus of the rat

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CANCER CHEMOTHERAPY AGENTS, such as cisplatin, stimulate nausea, vomiting, anorexia, and other behaviors indicative of malaise (for a review, see Refs. 2, 21, 27, 51). Several species, including dogs, cats, ferrets, pigs, and shrews, have been used to study emesis after treatment with chemotherapy agents (16, 18, 31, 33, 58). In contrast, laboratory rats and mice appear to lack a vomiting response (see Ref. 2 for review). For these species, kaolin intake has been used as a proxy for emesis (60, 64). A wide variety of stimuli, including cisplatin, induce kaolin consumption in rats (36, 53, 59, 60, 65). Like emesis, geophagia appears to be a defensive response. Clay may serve to bind or dilute a toxin in the gastrointestinal (GI) tract, thus reducing its adverse effects (45, 46). After injection with cisplatin, rats consuming kaolin show less body weight loss and a smaller reduction in food intake than rats without kaolin access (12). The consumption of clay by humans is also recognized as a potential detoxification strategy (48).

Despite the use of kaolin intake as an index of malaise in rats, very little is known about the neural systems that generate this behavior. Similar to the action of cisplatin on emesis (1, 7, 50), cisplatin-induced kaolin consumption in the rat is largely dependent on an intact subdiaphragmatic vagus (10) and is inhibited by common antiemetic drugs, such as 5-HT3 receptor antagonists, as well as by corticosteroids (29, 49, 60). Cisplatin also activates GI vagal afferent fibers in the rat and ferret, a response that is blocked by antagonism of 5-HT3 receptors (15, 24). Furthermore, cisplatin stimulates c-Fos expression in brain areas receiving vagal afferent input—the nucleus of the solitary tract (NTS) and area postrema (AP)—and the extended amygdala of the rat (22, 23) and musk shrew (11), suggesting that a distributed neural system is involved in the behavioral effects of this drug.

Here, we investigated the role of the lateral parabrachial nucleus (IPBN) in cisplatin-induced kaolin consumption because this pontine area serves as an important connection between hindbrain and forebrain components of the gut-brain axis. The PBN receives projections from the NTS and AP and has outputs to the extended amygdala, including the central amygdala (CeA) and bed nucleus of the stria terminalis (BNST) (25, 28, 39, 41, 62). In earlier studies, we reported brain c-Fos expression after cisplatin treatment, but we did not analyze the pons and midbrain (22, 23). Therefore, in the current report, we conducted an experiment to assess c-Fos expression in the IPBN after cisplatin injection. We also made bilateral ibotenic acid lesions that included the IPBN areas in which c-Fos was concentrated after cisplatin and compared the behavioral responses of lesioned and control animals after identical drug treatment. We assessed the impact of IPBN lesions on cisplatin-induced kaolin intake, anorexia, water consumption, and body weight. We also compared the behavior of IPBN-lesioned animals with controls after apomorphine injections because this emetic drug and cisplatin apparently stimulate kaolin intake by different routes, blood-born and vagal afferent activation, respectively, as suggested by our previous work (10). On the basis of the position of the IPBN to connect hindbrain and forebrain viscerosensory pathways, we hypothesized that bilateral lesion of the IPBN would reduce both cisplatin- and apomorphine-induced kaolin intake.
MATERIALS AND METHODS

Subjects

Forty-five male Sprague Dawley rats (Charles River; Kingston, NY) were housed individually in mesh-floored stainless-steel hanging cages (25 × 18 × 19 cm) and maintained in a temperature-controlled vivarium (~23°C), with a 12:12-h light-dark cycle (lights on at 0600). All rats had ad libitum access to water. Animals also had free access to standard rat chow during testing in experiments 1 and 2 (LabDiet 5001, PMI Nutrition) and prior to and several days after surgery in experiment 2 (Rodent Diet-W 8604; Harlan Teklad, Madison, WI). The protocol was approved by the Institutional Animal Care and Use Committees at the Monell Chemical Senses Center and The Pennsylvania State University College of Medicine.

Experiment 1: Cisplatin-Induced c-Fos Expression

Thirteen rats were used for testing c-Fos expression after injection of saline (0.15 M NaCl; n = 7) or cisplatin (6 mg/kg; n = 6). The animals received an intraperitoneal injection of 2 ml/kg body wt. To adapt the animals to handling and injection, rats received a mock injection for the 2 days prior to testing, in which a syringe needle was inserted but no fluid was injected. On the test day, the animals were injected with saline or cisplatin between 0900 and 1145. Food cups were removed 2 h prior to euthanization to eliminate the possibility of increased brain c-Fos expression by eating behaviors. At the time of euthanization, rats weighed 441 ± 13 g (means ± SE) in the saline group and 445 ± 11 g in the cisplatin group.

Perfusion and fixation. Twenty-four hours after treatment with saline or cisplatin, the rats were deeply anesthetized with sodium pentobarbital (50 mg ip, total per animal). The thoracic cavity was opened and, to assure a thorough fixation of the brain, rats were given 0.3 ml of heparin (1000 IU/ml) intracardially. This was followed by transcardial perfusion (~20 to 50 ml/min) with 300 ml of 0.2 M PBS (pH 7.4) and then 500 ml of 2% acrolein-4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The animals were then perfused with an additional 150 ml of PBS to remove excess fixative. The brains were removed and placed in 10% sucrose/PBS followed by 20% and 30% sucrose/PBS, each for 24 h. After cryoprotection in sucrose, the brains were blocked, frozen on dry ice, and cut coronally in three series at 50 μm using a freezing microtome. After perfusion, stomachs were also removed and weighed because reduced gastric emptying is an indicator of the efficacy of the cisplatin treatment (29).

c-Fos immunohistochemistry. Immunohistochemistry was conducted as previously described (38). Briefly, after rinses in 0.1 M PBS, the sections were treated with 0.5% NaBH4 (Sigma-Aldrich, St. Louis, MO) in 0.1 M PBS (pH 7.3–7.4), rinsed in PBS several times, then put in 5% goat serum (catalog no. G-6767; Sigma Chemical) in 0.4% Triton X-100/PBS for 1 h. They were then incubated for 24 h at room temperature in primary polyclonal IgG rabbit c-Fos antibody (1:3,000; catalog no. SC-52, Lot#D1508 Santa Cruz Biotechnology, Santa Cruz, CA) along with goat serum [5.0 μM/ml] in 0.4% Triton X-100/PBS. After further rinses with PBS, the sections were incubated for 2 h in secondary biotinylated goat anti-rabbit IgG and 0.4% Triton X-100/PBS (1:500; catalog no. 62-6140; Zymed Laboratories, San Francisco, CA), and mixed with goat serum [10.0 μM/ml]. After three more PBS rinses, the sections were exposed for 1 h to conjugated avidin-biotin complex (330 μM/ml) of each of solutions “A” and “B” from a Vectastain Elite kit (#PK-6100; Vector Laboratories, Burlingame, CA) in 0.4% Triton X-100/PBS. Following three more PBS rinses, a visible immunoreactive product was produced after 5 min of incubation in a 0.175 M sodium acetate (CH3COONa) solution containing 3.3-diaminobenzidine tetrahydrochloride [DAB; 0.35 mg/ml], nickel ammonium sulfate [NiSO4(NH4)2 6H2O, 0.01 mg/ml] and 30% H2O2 [0.3 μM/ml] (Sigma-Aldrich). The tissue then went through final PBS rinses and was mounted onto slides. The c-Fos immunohistochemical reaction product appears black in the nuclei of neurons. Fos distribution and quantification. The sections were examined with a light microscope (Nikon Optiphot, Tokyo, Japan), equipped with a digital video camera (Diagnostic Instruments, Sterling Heights, MI). With the objective focused on the upper surface of the section, specific regions were captured with video-imaging software (Spot for Windows, version 4.0.4) and subsequently analyzed with Optimas (Bioscan, Edmonds, WA). c-Fos cell staining was counted in the NTS and subareas of the PBN. The NTS was used as a positive control since we know that cisplatin consistently induces c-Fos expression in this area (22–23). Within these areas, the c-Fos immunoreactivity profiles were counted by Optimas based on a grayscale reference. Whenever possible, the same grayscale threshold was maintained for all areas within each brain. In any event, counting was not automatic. The program outlines each counted area, and this permitted the investigator to inspect it both in the digitized image and through the microscope. This reduces counting artifacts and undercounting-labeled nuclei that are stacked on one another. Most areas required counting over multiple sections, but the same number of sections was counted for each area in all brains. The results are presented as the average number of labeled cells per area. See Mungangme et al. for further details (38).

On the basis of visual inspection, three areas were identified for counting c-Fos expression, one in the caudal nucleus of the solitary tract (NTS; Fig. 1A, arrow) and two in the IPBN (Fig. 1, C and D, arrow). In both the NTS and IPBN, the area in which c-Fos profiles were counted was determined by a computer-generated circle. The size of circle was held constant for each comparable section across all the experimental and control brains. The placement and size of the circle varied slightly from one level to the next because the relative size and position of the NTS and IPBN changes in the anterior-posterior plane. Relative to the clusters of c-Fos-positive neurons, the size of the circles was made large enough that slight differences in their placement did not materially affect the counts.

Experiment 2: Cisplatin-Induced Pica in Lateral PBN-Lesioned Rats

Excitotoxic brain lesions. Before surgery, animals assigned to the groups weighed 329.6 ± 14.7 g (means ± SE), unoperated controls; 324.5 ± 15.3 g, operated controls; and 320.8 ± 27.1 g, IPBN lesioned animals. The lesions were produced following a standard protocol and, thus, are abbreviated here (for details see Ref. 17). After being anesthetized with pentobarbital sodium (Nembutal, 50 mg/kg ip), the rat was mounted in a stereotaxic instrument, and the cerebellum was exposed via bilateral burr holes in the interparietal bone. Gustatory responses were identified in the pontine parabrachial nuclei (PBN) using glass insulated tungsten electrodes (~1.0 MΩ), conventional amplification, and 0.1 M NaCl as a sapid stimulus. Once taste responses were identified bilaterally, the recording electrode was replaced with a 1.0-μl microsyringe (Hamilton) with a sturdy glass micropipette glued to the end. This assemblage was filled with ibotenic acid (IBO, 20 μg/μl in PBS, pH 7.4) and repositioned 0.5 mm anterior, lateral, and deep to the taste coordinates to approximate the center of the lateral PBN. With the pipette in place, 160–170 nl of IBO was infused over 10 min. The pipette was then left in place for an additional 10 min. The entire procedure was then repeated on the contralateral side. Bilateral IPBN lesions (IPBNx) were completed in 14 rats. Two of these died before the experiment was completed, so the experimental group had an n of 12. In addition, there were two control groups. The surgical controls (n = 10) were treated identically to the IPBNx group except that the recording electrode penetrated only into the cerebellum and no pipette (or injection) was used. The remaining 10 rats were naïve controls. After surgery, the rats had a minimum of 1 wk to recover before transport from Hershey to Philadelphia, PA. Once there, an additional recovery week was given before testing began.
Behavioral testing. At the beginning of behavioral testing, the animals weighed 491.2 ± 10.1 g (means ± SE), unoperated controls; 494.8 ± 15.7 g, operated controls; and 491.3 ± 11.9 g, lPBN lesioned. Rats were given access to kaolin pellets (~50 g), placed in a food hopper hung from the back of the animal cage. Standard powdered rat chow (~100 g) was available in an open-topped glass jar attached to the front panel of the animal cage; water was provided ad libitum. Body weight, water bottles, food cups, and kaolin hoppers, as well as spillage from the latter two, were weighed daily at ~0900. Chow and kaolin spillage was easily separated because the former is brown, the latter white. After a week of adaptation to these circumstances, all animals received an injection of apomorphine (10 mg·kg⁻¹·ml⁻¹ ip). Four days later, they received an injection of saline (2 ml/kg ip). After three more days, they received an injection of cisplatin (6 mg·kg⁻¹·2 ml⁻¹). The timing and order of treatments was based on our previous studies showing no effects of the control injections on kaolin intake, no lasting effect of apomorphine treatment, and a significant long-term effect of cisplatin injection on food intake and body weight (10, 12). Three days after the cisplatin injection, the animals were anesthetized (pentobarbital sodium, 50 mg ip, total per animal) and then euthanized by exsanguination and perfused using the method described for experiment 1.

Histology. As in experiment 1, the brains were blocked, frozen, and cut at 50 μm in 3 coronal series. Different series of sections were stained for NeuN, a neuron-specific protein (38), and with cresyl violet. The NeuN series was of poor quality, low contrast, and variable staining, and thus were of limited use in determining the extent of the lesions. The adequacy of the lesions was judged from the cresyl violet series by comparing the areas without neurons with comparable areas in the brains of the PBS-injected controls. The PBN was defined from neuroanatomical criteria (39, 42, 52).

Statistical analysis. c-Fos cell counts, body weight, food, water, and kaolin intake data are expressed as means ± SE. c-Fos cell counts were compared using independent sample Student’s t-tests. For behavioral measures and body weight data, three-way (2 × 3 × 4) ANOVA were conducted using treatment (saline or drug), surgical condition (unoperated control, operated control, or lPBN lesioned), and time (4 days; 1 day prior and 3 days after injection) as factors. Body weight measures were transformed to percent baseline body weight (baseline = body weight at 2 days prior to an injection). Planned comparisons were performed using the Benjamini-Hochberg procedure to control Type I error rate (6). Differences were considered statistically significant if P < 0.05 for ANOVA or P < 0.025 to 0.001 for Benjamini-Hochberg tests. Statistical analyses were computed with Statistica (ver. 8.0, StatSoft, Tulsa, OK) and Excel (Microsoft).

RESULTS

Experiment 1: Cisplatin-Induced c-Fos Expression

c-Fos expression. In the NTS, c-Fos expression was concentrated in areas adjacent to the AP (Fig. 1A). Within the NTS counting area, the rats injected with cisplatin showed significantly more c-Fos expression than saline-treated controls [t(11) = 4.6, P < 0.001; Fig. 2, NTS]. In the lPBN, two areas were counted, one ventrolateral (Fig. 1C, arrow), the other dorsal (Fig. 1D, arrow). In the ventrolateral area, cisplatin-treated rats displayed consistently strong c-Fos label compared with saline controls [t(11) = 6.0, P < 0.001; Fig. 2, lPBNv]. The more dorsal area in lPBN also had consistent, if less dense, c-Fos label, but it did not differ between treatment groups [t(11) = 0.80, P = 0.4; Fig. 2, lPBNd].

Stomach weights. As an additional test for the potency of cisplatin, we also measured the weight of the excised stomach and its contents at the time of euthanization. Animals injected with cisplatin had significantly greater stomach weights than saline-treated rats [12.5 ± 1.9 vs. 8.0 ± 1.4 g; t(11) = 1.9, P < 0.05, one-tailed].
PARABRACHIAL LESIONS ENHANCE CISPLATIN-INDUCED PICA

Experiment 2: Cisplatin- and Apomorphine-Induced Pica in IPBN Lesioned Animals

Lesion verification. All 12 of the experimental animals had bilateral damage in the IPBN on both sides. Histology also was done on 6 of the 10 surgical controls, and none of them showed signs of neural damage in the PBN or elsewhere in the brain stem. The brain of one rat with lesions was poorly preserved. In this case, it was possible to determine that damage existed in the lateral PBN on both sides but not the extent of the damage. The behavioral data from this animal were consistent with that of the other rats with lesions, so they were included in the analysis. In these brains, the NeuN stain failed to work consistently, so the evaluation of the lesions relied primarily on the cresyl violet series (Fig. 1, E and F).

Eight of the twelve lesioned rats had sparing of the gustatory or medial PBN on at least one side, four unilaterally and four bilaterally. This sparing often included the most dorsomedial aspect of IPBN. This demonstrated that the damage to the medial PBN and the dorsomedial IPBN was not necessary to alter the effects of cisplatin. In all of the other brains, the lesions spread into the supratrigeminal area (between the PBN and the trigeminal motor nucleus), the Kolliker-Fuse nucleus (rostroventral to PBN), and in some cases the dorsal (intraoral) aspect of the principal trigeminal nucleus. Thus, it is difficult to rule out these structures as contributing to the effect. Nevertheless, one rat had lateral PBN damage confined to that subnucleus on one side (Fig. 1E). This animal consumed 24.4 g and 34.9 g of kaolin on the two test days; perfectly representative amounts for the PBNx group. Although only a single example, this militates against these adjacent structures as mediating the effect on pica. In fact, despite the damage in and near the central trigeminal apparatus, during the control period, the group with PBN lesions ate and drank amounts virtually identical to that of both control groups.

Cisplatin treatment. There were significant three-way interaction effects of surgical condition with drug injection and test day on kaolin intake, food intake, water intake, and percent body weight [kaolin, F(6,87) = 12.4, P < 0.000001; food, F(6,87) = 3.9, P < 0.005; water, F(6,84) = 2.9, P < 0.05; body weight, F(6,87) = 12.3, P < 0.000001].

Figure 3 shows mean comparisons between saline and cisplatin at each time point. Compared with saline injections, cisplatin induced kaolin intake on days 1 and 2 in operated control and IPBN-lesioned rats and on day 1 in unoperated controls (Fig. 3; Benjamini-Hochberg tests). After cisplatin injection, food intake was significantly suppressed in all groups on days 1–3 (Fig. 3; Benjamini-Hochberg tests). On day 3, in IPBN-lesioned animals, cisplatin treatment reduced water intake (Fig. 3; Benjamini-Hochberg test). Percent baseline body weight was reduced on days 1–3 after cisplatin treatment in unoperated and operated control animals (Fig. 3; Benjamini-Hochberg tests). In comparison, IPBN-lesioned animals showed a significant increase in body weight from baseline on day 1 after cisplatin injection, but this declined by day 3 (Fig. 3; Benjamini-Hochberg tests).

Mean comparisons between only cisplatin-treated IPBN-lesioned and control animals were also conducted. After cisplatin treatment, rats with IPBN lesions ingested significantly more kaolin than unoperated controls on days 1–3 and operated controls on days 1 and 2 (P ≤ 0.01, Benjamini-Hochberg tests). There were no significant differences between water and food intake measures across surgical conditions. Percent body weight also increased significantly from baseline in IPBN-lesioned rats compared with unoperated controls on days 1–3 and operated controls on days 1 and 2 (P ≤ 0.01, Benjamini-Hochberg tests).

Apomorphine treatment. There were no significant effects of surgical condition on apomorphine-induced kaolin intake, food intake, water intake and body weight [all ANOVAs for interaction or main effects of surgical condition were nonsignificant]. For example, unoperated controls, operated controls, and IPBN-lesioned animals consumed 25.0 ± 3.1, 24.2 ± 3.0, and 23.1 ± 2.9 g of food, respectively, for the 24 h after injection. There were significant effects of apomorphine treatment on kaolin intake, food intake, and percent body weight over test days [kaolin, F(3,87) = 91.2, P < 0.000001; food, F(3,87) = 15.2, P < 0.000001; body weight, F(3,87) = 5.6, P < 0.005, injection by day interaction effect]. Apomorphine stimulated kaolin consumption on days 1 and 2 postinjection (Fig. 4; Benjamini-Hochberg tests) in all surgical conditions. The only significant effect of apomorphine on a mean comparison of food intake occurred in the operated control animals on day 1 postinjection (data not plotted; saline, 30.3 ± 0.9 vs. apomorphine, 24.2 ± 1.0, Benjamini-Hochberg test, P < 0.001). Although not statistically significant, there was also a trend in the other groups for apomorphine to reduce food intake on day 1 postinjection (unoperated control, saline, 30.0 ± 1.4 vs. apomorphine, 25.0 ± 1.0; IPBN-lesioned, saline, 26.8 ± 1.2 vs. apomorphine, 23.1 ± 1.1; nonsignificant Benjamini-Hochberg test). Although planned comparisons revealed no significant effects of apomorphine on body weight, there was a small tendency for apomorphine to reduce percent body weight on day 1 postinjection (unoperated control, saline, 101.4 ± 0.4 vs. apomorphine, 100.5 ± 0.4; operated control, saline, 101.2 ± 0.2 vs. apomorphine, 100.6 ± 0.7; PBN lesioned, saline, 100.9 ± 0.2 vs. apomorphine, 99.8 ± 0.5; nonsignificant Benjamini-Hochberg tests). There were no statistically significant effects of apomorphine on water intake using ANOVA or planned comparisons.
DISCUSSION

The current data reveal both behavioral and immunohistochemical evidence that the IPBN may function in the expression and modulation of chemotherapy-induced malaise. c-Fos expression results show that the IPBN (ventrolateral or external area) is activated by systemic injection of cisplatin. Furthermore, bilateral ibotenic acid lesions of the IPBN produced a dramatic increase in cisplatin-induced kaolin intake. Cisplatin treatment stimulated kaolin consumption in all groups, but lesioned animals showed a greater than 600% increase in kaolin intake by 24 h after injection compared with controls. Food intake was reduced to a similar degree by cisplatin treatment in lesioned and control animals, and all animals showed a similar level of kaolin intake after apomorphine, an emetic agent believed to act directly on the hindbrain (3, 19, 26).

After an injection of cisplatin, c-Fos expression in the NTS was present in areas that receive afferent input from the GI tract (Fig. 1A) (40). In turn, neurons from this region of the NTS project to the ventrolateral (external) IPBN (20), where c-Fos-positive cells were concentrated after cisplatin treatment in the current study. The lack of differential c-Fos label between the cisplatin and saline groups in the dorsal IPBN demonstrates that the effect of the chemotherapeutic treatment did not reflect generalized neural activation of the PBN. The parabrachial nuclei project monosynaptically to the limbic system, particularly to the hypothalamus, CeA and BNST (39), and these areas exhibit increases in c-Fos-positive neurons after intraperitoneal cisplatin injections (22, 23).

This leads to the hypothesis that cisplatin treatment activates a pathway beginning at the level of GI vagal afferent fibers that extends through the NTS, PBN, and forebrain areas to produce c-Fos expression and perhaps pica. Our recent work demonstrated that ablation of the subdiaphragmatic vagus (SDX) blunted the effects of cisplatin treatment on c-Fos expression in the NTS and CeA (22), but the effects in the PBN were not assessed. Furthermore, we showed that cisplatin-induced pica is reduced by cutting the common hepatic branch (CHB) but not by section of the celiac, accessory celiac, or ventral gastric branches (10). These branches are sub-branches of the SDX, with the CHB innervating the duodenum and liver, the celiacs innervating the distal small intestine, and the ventral gastric innervating the stomach (44, 63).
These residual effects on c-Fos expression and pica after vagal section could be mediated via afferent neurons in the splanchnic nerves that synapse first in the spinal cord. At least some of the second-order neurons in this so-called sympathetic afferent system terminate in the most caudal aspect of the NTS (54), but cisplatin does not significantly increase c-Fos expression in the spinal cord (22). Another possible route of action could be via blood-born cisplatin, or another humoral factor released by cisplatin, acting directly in the hindbrain, perhaps in the AP. In this case, the subsequent rostral projections to the PBN, and other areas, would closely parallel or overlap those from the subjacent NTS. Indeed, cisplatin produces a strong c-Fos response in the AP (22, 23), and it is known that AP ablation can disrupt cisplatin-induced emesis in the cat (34); however, caution should be used in interpreting AP lesion experiments because the AP also receives vagal afferent input (40, 57).

If the presumed pathway through the PBN is important for the expression of cisplatin-induced pica, then lesion of this area should produce a reduction in this response. Sectioning only the CHB blunts the pica response (10) so, logically, if this afferent vagal activity is interrupted anywhere along its central course, the ingestion of clay should also be diminished. As is evident in experiment 2, this is far from the case.

In fact, IPBN lesions vastly increased cisplatin-induced pica. Indeed, regardless of the stimulus—a wide range of toxins and motion—the norm is kaolin consumption up to 6 g in 24 h (e.g., 36, 53, 59, 60, 65). We are not aware of any stimulus that produces the excessive kaolin intake reported here. IPBN-lesioned animals, when injected with 6 mg/kg cisplatin, ingested nearly 30 g of kaolin in 24 h. The body weight increase in IPBN-lesioned rats is likely the result of this excessive consumption of clay (Fig. 3). In lesioned animals, there was a close relationship between the amount of ingested clay, ~30 g, on day 1 postinjection, and an increase in body weight, ~5% (or ~25 g), which suggests that much of the kaolin remained in the GI tract. Although kaolin intake is used as a metric for malaise, it appears unlikely that IPBN-lesioned animals experienced more malaise than control rats because they had similar levels of basal and reduced food intake after cisplatin treatment. Another possibility is that IPBN damage blocks the inhibition of intake normally signaled by the GI tract. Rats with large PBN lesions typically overconsume normally preferred fluids, such as sucrose, while exhibiting more or less normal rejection of aversive stimuli such as quinine (56). Also, in short-term tests, overconsumption is common after PBN lesions (see Fig. 4 in Ref. 37 for both mPBNx and IPBNx groups; CS and water intakes are almost double that of controls); the normal feeding behavior of the same rats does not vary significantly from controls. The IPBN contains neurons that respond to gastric stretch, and it is possible that destruction of these cells removed this inhibitory pathway (4, 5). Necropsies of IPBN-lesioned animals, at the end of day 3 postinjection, revealed that the stomach was exceptionally full with kaolin. Cisplatin also produces gastric stasis in normal control animals (e.g., see RESULTS and Ref. 29), which might enhance inhibitory feedback related to gastric fill. A lack of inhibition or gating might result from ablation to lateral parabrachial input to the ventrolateral medulla (52), which has an ascending projection to the CeA (43). Cisplatin treatment produces a robust increase in c-Fos expression in the CeA (11, 22, 23), but we have not examined the potential for facilitation of this response in IPBN-lesioned animals.

Cisplatin is well known for producing nephrotoxicity (66), and this can lead to mineral loss, such as excess secretion of sodium and magnesium (9, 30, 32). Reports indicate that lesion or antagonism of serotonin receptors in the IPBN produces enhanced drinking, including salt solution intake, in rats in response to hypovolemia or injection of angiotensin (13–14). Although kaolin is aluminum silicate and would likely not reduce mineral deficiencies produced by cisplatin treatment, it is possible that IPBN-lesioned animals overconsume kaolin because of an enhanced mineral appetite. Other measures of ingestion appear to be unaffected by combination of IPBN ablation and cisplatin injection. Water and food intakes after injection with cisplatin were nearly identical in both surgical groups, with less food intake and more disruption of normal water intake compared with unoperated control animals. There appears to be a small effect of the surgery on these measures (see Fig. 3). Body weight differences were difficult to evaluate because they were confounded by the amount of kaolin in the GI tract of IPBN-lesioned animals.

The present results show that an intact IPBN is not necessary for pica induced by apomorphine or cisplatin treatments. In species with a vomiting response, such as the ferret, apomorphine induces emesis by action on the hindbrain, possibly by targeting dopamine type 2 receptors, a standard emetic trigger (3, 19, 26). We have also reported that vagal lesions in the rat have no effect on apomorphine-induced kaolin intake (10), suggesting that apomorphine-induced pica is also the result of direct action of this drug on the hindbrain. Apomorphine treatment also induces c-Fos expression in the ventrolateral IPBN of the rat (8). Apomorphine produced the same level of kaolin intake in IPBN-lesioned and control animals, which indicates that the IPBN is not necessary for this response either. Although this is not compatible with the idea that IPBN-lesioned rats have a lack of inhibitory feedback related to gastric stretch, it is possible that the level of stimulation might be important, for example, IPBN-lesioned animals might ingest more kaolin relative to controls with a higher dose of apomor-
phine. This would provide further support for the hypothesis that IPBN lesions decrease the signals related to stretch of the GI tract. However, this could be difficult to test since apomorphine, a dopamine agonist, also produces a potent increase in locomotion (47), and it is possible that higher doses would produce additional movements that could interfere with ingestive behavior.

Pica, emesis, and conditioned taste aversion (or avoidance) are responses that are often used to assess malaise in experimental animals (2). A common role for the PBN in these behaviors associated with malaise is not readily apparent. Although it is clear that the caudal hindbrain contains the motor circuitry sufficient for the vomiting response (35, 55), the role of the PBN in emesis has not been investigated. Furthermore, conditioned flavor aversion (or avoidance) learning is also a common method for determining the malaise produced by toxins. The PBN receives taste and visceral input required for this effect, and lesions of this area eliminate the acquisition of conditioned taste aversion (or avoidance) (e.g., 17).

**Perspectives and Significance**

The present work is the first to show the effects of an IPBN lesion on pica. A prior study demonstrated that ablation of more rostral sites, such as the amygdala or hippocampus, can reduce or enhance kaolin intake in rats exposed to motion (61). Although the prediction that IPBN lesion would block or decrease cisplatin- or apomorphine-induced kaolin intake was not supported by the current data, these results suggest that the IPBN might play an important role in modulating the amount of kaolin intake, at least when rats are injected with a highly toxic drug like cisplatin. The current data are important because they contribute to insight into the neural pathways that mediate pica, a common index of malaise. Unfortunately, animal models that are routinely used for biomedical research, such as laboratory strains of mice, rats, and rabbits, do not cause they contribute to insight into the neural pathways that mediate pica, a common index of malaise. Unfortunately, animal models that are routinely used for biomedical research, such as laboratory strains of mice, rats, and rabbits, do not possess a vomiting response (2), which makes it difficult to determine the presence of visceral sickness. By investigating the role of the PBN and other brain regions in pica, we can more fully understand the neural pathways engaged in this behavioral test, particularly in research involving toxic drugs, such as cancer chemotherapy agents.

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