Gastrointestinal tract innervation of the mouse: afferent regeneration and meal patterning after vagotomy

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Powley, Terry L., Michael M. Chi, Elizabeth A. Baronowsky, and Robert J. Phillips. Gastrointestinal tract innervation of the mouse: afferent regeneration and meal patterning after vagotomy. *Am J Physiol Regul Integr Comp Physiol* 289: R563–R574, 2005. First published April 14, 2005; doi:10.1152/ajpregu.00167.2005.—Mice, with the variety of genotypes they provide, should be particularly useful for studies of growth factors and gene products in regeneration of autonomic pathways such as the vagus nerve. To provide a foundation for examinations of mouse vagal reorganization, two experiments assessed the rate, extent, and accuracy of afferent reinnervation of the stomach after vagotomy and related these patterns to feeding behavior. In experiment 1, the pattern of afferent regrowth into the gut after unilateral truncal vagotomy was characterized by labeling of these afferents with wheat germ agglutinin-horseradish peroxidase and Micro-Ruby. Regenerating neurites had reached and, in some cases, already reinnervated the stomach by 4 wk after axotomy. By 8 wk, regrowth was more extensive, and many fibers had redifferentiated terminals in the smooth muscle. By 16 wk, vagal projections had reached or exceeded normal density in the corpus, density in the forestomach was still reduced, and regrowth in the antrum was minimal. At all time points, not only appropriate terminals, but also aberrant endings, were observed. In experiment 2, meal patterns of vagotomized mice were evaluated using a solid diet over the period of regeneration; cholecystokinin suppression of a liquid meal after unilateral and bilateral truncal vagotomies was also evaluated. Unilaterally, as well as bilaterally, vagotomized animals ate smaller and more frequent meals. These disturbed patterns became more pronounced in the first 8 wk after vagotomy, during regeneration. Cholecystokinin inhibition of intake was attenuated by bilateral, but not unilateral, vagotomy. Overall, the spatial and temporal patterns of structural and functional changes observed during regeneration verify that the mouse provides a useful preparation for examining the control of vagal plasticity.

satiety; stomach; vagus; visceral afferents; cholecystokinin

**EXPERIMENT 1: REGENERATION OF THE MOUSE ABDOMINAL VAGUS**

For the rat, detailed information about the vagal innervation of the GI tract has made feasible a series of analyses on vagal regeneration. In particular, the characteristic pattern of normal vagal innervation provides two particularly useful features for observations on plasticity. First, because vagal axons in the stomach fan out circularly in a characteristic pattern from the lower esophageal sphincter and because these fibers end in two differentiated types of terminals within the smooth muscle wall, each with a characteristic regional distribution (1, 25), it is practical to evaluate the rate, extent, and accuracy of vagal fiber regeneration. Second, the vagal innervation of the rat stomach is almost completely lateralized. This has the practical application that one wall of the stomach can be left innervated while the other is denervated, and the animal is then monitored for regeneration. Such a unilateral vagotomy strategy offers the advantage that the regeneration period is essentially unaffected by the nutritional and other physiological disturbances that often accompany bilateral vagotomy.

Observations with such a preparation demonstrate that rat vagal afferents have considerable capacity for regeneration. When the animal sustains a unilateral truncal vagotomy above the highest abdominal branch (i.e., hepatic), afferents regenerate into the stomach and duodenal smooth muscle walls (16, 17). These afferents continue to extend and ramify more and more distally within the stomach and duodenum over ≥45 wk after vagotomy. In this progressive regeneration, many afferents display growth cone profiles and eventually reestablish endings that appear normal in appearance and location; other fibers produce endings that are disordered in structure and/or location. Even at 45 wk after vagotomy, vagal regeneration in the rat appears incomplete and partially disordered (16).

Because it has been established that the normal patterns of vagal innervation of the mouse GI tract are similar to those of the rat, in terms of projections and regional distributions and in terms of lateralized innervation patterns (9), the present experiment was designed to evaluate the patterns of afferent vagal regeneration in the mouse GI tract. We hypothesized that the spatial pattern of regrowth would be similar to that in the rat.
but that the time course would be considerably shorter and, thus, possibly more practical for future experimental evaluations of intervention strategies.

MATERIALS AND METHODS

Animals. Male C57BL/6 mice (6–8 wk old, 20–25 g body wt at arrival, n = 75; Harlan Industries, Indianapolis, IN) were housed individually, maintained on a 12:12-h light-dark schedule (lights on at 0600) at 23°C, and given ad libitum access to tap water and chow (Laboratory Rodent Diet 5001, PMI Feeds, Brentwood, MO). At 2 wk before surgery, all mice were transitioned to a nutritionally complete diet of 20 mg of dustless precision pellets (product no. F0071, Bio-Serv, Frenchtown, NJ). The caloric densities of the diets were as follows: 28.0% protein, 12.2% fat, and 59.8% carbohydrate for chow and 21.8% protein, 12.0% fat, and 66.2% carbohydrate for pellets. The mice were maintained on the 20-mg pellets for the duration of the study to facilitate accurate measurement of intake and direct comparisons with experiment 2. On each day at 1400, body weight and 24-h food consumption were recorded. Data from any mouse that did not completely consume its assigned food (e.g., died during surgery or during tracer injection) were dropped from analyses. All protocols were conducted in accordance with the Principles of Laboratory Animal Care (NIH Publ. No. 86-23, revised 1985) and the guidelines of the American Association for Accreditation of Laboratory Animal Care.

Nerve cuts. Mice were anesthetized with a mixture of ketamine hydrochloride (Ketaset, Miles, Elkhart, IN; 75 mg/kg ip) and xylazine (Rompun, Fort Dodge Laboratories, Fort Dodge, IA; 50 mg/kg ip). Each mouse was then laparotomized. In some animals, which served as sham controls, the laparotomy was immediately closed. The mice assigned to selective vagotomy groups received unilateral ventral trunk vagotomies designed to be similar to the selective vagotomy in the rat in similar experiments on vagal plasticity (16, 17). Briefly, the ventral branch of the vagus nerve was isolated from the esophagus by blunt dissection, supported by a microdissecting hook, and cut with fine iris scissors above the point where the hepatic branch bifurcated from the main bundle. Additional mice served as nonsurgical controls.

Survival protocol. Before surgery, the mice were randomly assigned to one of three groups on the basis of postsurgery survival times: 4 wk [vagotomy (n = 15) and sham (n = 5)], 8 wk [vagotomy (n = 14) and sham (n = 5)], and 16 wk [vagotomy (n = 16) and sham (n = 5)]. An additional group of vagotomized mice (n = 5) was examined histologically 4 days after vagotomy to verify the completeness of the surgery. Other C57BL/6 mice, which served as nonsurgical controls, were killed at ages that encompassed the range of survival times.

Tracer injections into the nodose ganglion. Individual mice were injected with one of two complementary tracers to label selectively the vagal sensory innervation of the ventral stomach muscle wall. Wheat germ agglutinin-horseradish peroxidase (WGA-HRP; WGA-HRP; Vector Laboratories, Burlingame, CA) was used to label the vagal afferent projections to the gut in its entirety (9, 25). Micro-Ruby (MR; lysine-fixable dextran-tetramethylrhodamine-biotin, 3,000 mol wt; Molecular Probes, Eugene, OR) was used to obtain complete Golgi-like fills of individual vagal sensory terminals (9). Briefly, mice were anesthetized with an intraperitoneal injection of ketamine-xylazine, and the left nodose ganglion was exposed. Then 4% WGA-HRP or 7% MR was pressure injected (~1.0 μl) through a glass micropipette into the ganglion.

Tissue preparation. Each mouse was deeply anesthetized with a lethal dose of methohexital sodium (Brevital, Lilly Lilly, Indianapolis, IN; 100 mg/kg ip) 1 day (WGA-HRP) or 7 days (MR) after injection into the nodose ganglion. When the animal was completely unresponsive to noxious stimuli, the left ventricle of the heart was injected with a mixture of heparin (0.2 ml, 1,000 U/ml; Elkins-Sinn, Cherry Hill, NJ) and propanolol (0.1 ml; Ayerst Laboratories, Philadelphia, PA) and then perfused transcardially with 0.9% saline (~150 ml at 40°C). Next, the stomach was expanded with physiological saline, and the tissues were perfused with an additional 500 ml of 3% paraformaldehyde-0.75% glutaraldehyde (for WGA-HRP) or 4% paraformaldehyde (for MR).

Stomachs were harvested and prepared as whole mounts. Briefly, the organs were rinsed in cold tap water and separated along the greater and lesser curvatures into dorsal and ventral halves. Finally, the gastric mucosal and submucosal layers were separated from the smooth muscle layers. Smooth muscle whole mounts labeled with WGA-HRP were then processed with tetramethylbenzidine as the chromagen, as described by Mesulam (14), mounted, and cleared in xylene, and coverslips were applied with Cytoseal XYL (Richard-Allen Scientific, Kalambazoo, MI). Smooth muscle whole mounts labeled with MR were processed using the Vectastain Elite ABC kit (PK-6100, Vector Laboratories) with 3,3'-diaminobenzidine tetrahydrochloride as the chromagen, as described by Fox et al. (8, 9), and counterstained with cuproline blue (11), a pan-neuronal marker for myenteric neurons. The procedure for MR whole mounts was similar to that for WGA-HRP whole mounts; however, they were run through an ascending series of graded alcohols before clearing. Whole mounts processed for WGA-HRP were examined with dark-field illumination, whereas MR-processed tissue was examined using conventional bright-field microscopy and Nomarski differential interference contrast optics.

Statistical analysis and display of data. Statistica software (version 6.0, Statsoft, Tulsa, OK) was used to analyze body weight and daily food intake. Data were analyzed using separate one-way ANOVAs, with surgical group (4, 8, and 16 wk) as the independent variable and food intake or body weight as the dependent variable. Significance was considered achieved for analyses with P < 0.05. GraphPad Prism (version 3.0, GraphPad Software, San Diego, CA) was used for graphical representation of the data. Digital images were acquired with a Spot RT Slider cooled charge-coupled device digital camera (Diagnostic Instruments, Sterling Heights, MI). Photoshop software (version 6.0, Adobe Systems, Mountain View, CA) was used to 1) apply scale bars and text; 2) adjust brightness, contrast, color balance, and sharpness; and 3) organize the final layouts for printing.

RESULTS

In intact control mice, vagal afferent fibers coursed through the connectives and ganglia of the myenteric plexus to terminate as intramuscular arrays (IMAs) in association with smooth muscle fibers of the muscularis externa (Fig. 1, A and B) or as intraganglionic laminar endings (IGLEs) in association with individual ganglia (Fig. 1, A and C), as previously described (9). IMAs consisted of ensembles of rectilinear neurites running parallel to smooth muscle fibers (and associated interstitial cells of Cajal) in either muscle layer (Fig. 1B), whereas IGLEs consisted of distinctive plates of puncta applied to the superficial or deep side of myenteric ganglia (Fig. 1C).

When viewed at a somewhat lower power, the dense IGLE plates and the bundles of axons of all the afferents coursing in the connectives delineated the myenteric plexus in control animals (Fig. 2A).

In striking contrast, in all animals examined 4 days after ventral trunk vagotomy, the entire ventral or anterior wall of the stomach was almost completely devoid of vagal afferent innervation (Fig. 2B). An exception to this pattern of complete-deervation is a small amount of residual innervation of the contralateral or dorsal corpus, presumably supplied by a fascicle of the left vagal trunk that crossed to join the right vagal trunk at some point above the level of the unilateral abdominal vagotomy (Fig. 2C).
In terms of food intake (Fig. 3A) and body weight (Fig. 3B), over the full course of the experiment, unilaterally vagotomized animals were indistinguishable from controls. Repeated-measures ANOVAs indicated no significant group differences for food intake or body weight over the postsurgery period. Both groups exhibited transient dips in intake and body weight in conjunction with surgery, but unilateral vagotomy was no more debilitating than sham vagotomy.

The mice killed at 4 wk after the surgery exhibited considerable regeneration. Vagal afferents had reached the stomach and were observed to once again course through the connectives of the myenteric plexus (Fig. 4). In some of these afferents, redifferentiated terminal endings aggregated on myenteric ganglia and appeared to be IGLEs (Fig. 4, A and B). One conspicuous feature suggesting that 4 wk after vagotomy...
was early in the period of regeneration was the substantial
difference in the amount of reinnervation in different animals
at that time. In the corpus, the region receiving the earliest
regrowth, reinnervation was just sparse and had only begun to
redelineate connectives and ganglia in some animals (Fig. 4A),
reinnervation was sufficient to clearly distinguish the myen-
teric plexus in some animals (Fig. 4B), and in other mice,
patterns of corpus innervation were as dense as in control
animals (Fig. 4C).

The progression of the reinnervation that occurred over the
course of the experiment can be appreciated in a series taken
from the same region, the corpus, of different mice at different
times. The afferent innervation of a sham-vagotomized control
animal is illustrated in Fig. 5A, and representative examples of
the afferent patterns at 4 days, 4 wk, 8 wk, and 16 wk are
shown in Fig. 5, B–E. The animal-to-animal variability in
extent of regeneration that was conspicuous 4 wk after vagot-
omy tended to diminish substantially by the later time points,
suggesting that regeneration processes may have decelerated
and approached a relatively asymptotic condition by 3–4 mo
after axotomy.

Two features of the reinnervation pattern of mice were
striking and appeared to be unique to the mouse, insofar as they
have not been observed in the rat. First, as suggested by a

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

Fig. 3. Cutting the left subdiaphragmatic branch of the vagus nerve, which
provides the majority of extrinsic sensory and motor innervation to the ventral
stomach wall and the first few centimeters of the small intestine, had no effect
on food intake (A) or body weight (B).

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

Fig. 4. There was a large between-subject variability in density of regeneration
4 wk after vagotomy. A–C: images from the ventral corpus of 3 separate mice.
Scale bar, 200 μm.

comparison of Fig. 5A (controls) with Fig. 5E (16 wk of
regeneration), by ~4 mo after surgery, vagal afferents ap-
peared to innervate the myenteric plexus of the corpus even
more densely than normally might be expected in a sham
animal. At the same time that regenerating vagal afferents
appeared to hyperinnervate the corpus, as illustrated by the
tracing of the pattern of innervation from a stomach whole
mount of an animal at 16 wk (Fig. 6), the afferents made much
less progress in regrowing into the forestomach and were
largely unsuccessful in regrowing into the antrum.
By other indexes as well, the patterns of reinnervation in mice were only partially successful. Inspection of individual afferent terminals at higher power with the tracer MR established that, as has been noted for the rat, although many fibers had differentiated into endings such as those illustrated in Fig. 1, other afferents had produced ectopic and highly aberrant terminals of different sorts that are not typically observed in intact animals (Fig. 7). Aberrant endings with some of the features of IGLEs (Fig. 7A) and dystrophic terminals with some of the characteristics of IMAs (Fig. 7B) were observed. Ectopic terminals were variously found in myenteric ganglia (Fig. 7, D–F), smooth muscle (Fig. 7, B and C), and serosal ganglia (Fig. 7G). Finally, it appeared that afferents more rapidly reestablished IGLE profiles that associated with myenteric ganglia and less readily reestablished processes that were comparable to IMAs in the smooth muscle sheets.

EXPERIMENT 2: PATTERNS OF MOUSE FEEDING BEHAVIOR PRODUCED BY VAGOTOMY AND THE SUBSEQUENT REGENERATION

Experiment 1 established the pattern and time course of vagal afferent regeneration for the C57BL/6 mouse and also indicated that the unilateral vagotomy employed to trace the pattern and course of regeneration had no obvious effects on total daily food intake and body weight.
Experiment 2 pursued three strategies to identify and evaluate feeding end points that might be sensitive to unilateral vagotomy and that might be used to track resolution or complications of axotomy effects over the course of regeneration. First, experiment 2 employed microstructural meal pattern analyses of vagotomized and sham-operated mice maintained on the same pelleted diet during the immediate pre- and postvagotomy periods as well as over the interval during which afferent regeneration occurs, as established in experiment 1. Meal pattern analysis has proved sensitive and able to detect differences in feeding patterns of mouse genotypes that have distorted (4), reduced (8), or exaggerated (3) phenotypic patterns of vagal innervation of the GI tract. Thus it seemed possible that meal pattern analysis might detect altered patterns of ingestion in unilaterally vagotomized mice such as those examined in experiment 1.

Second, experiment 2 also included not only a group with ventral trunk vagotomy (comparable to those in experiment 1) but also a group with bilateral truncal vagotomy that would presumably display the feeding and body weight disorders typically associated with complete subdiaphragmatic vagotomy.

Finally, because the participation of the different primary branches of the rat abdominal vagus in cholecystokinin (CCK) inhibition of food intake has been evaluated (21, 23), additional mice with the same selective vagotomies employed for the meal-patterning analyses were also examined for their responses to CCK. The available observations with rats would predict that a unilateral truncal vagotomy would not block CCK suppression, whereas a complete bilateral vagotomy would block the hormone’s effect. Such an outcome would verify another parallel between rat and mouse vagal function. Alternatively, in the event of the less likely outcome, effects of unilateral vagotomy on CCK suppression tests might provide another assay for tracking regeneration.

MATERIALS AND METHODS

Animals. Thirty-nine C57BL/6 mice of the same gender (male), age (7–8 wk of age), and body weight (20–25 g on arrival) as in experiment 1 were obtained from the same supplier (Harlan Industries). Also, as in experiment 1, mice were housed in individual plastic shoebox cages and maintained on a 12:12-h light-dark cycle, with lights on at 0600 and off at 1800. The animals had ad libitum access to tap water and were weighed on a daily basis. The colony was maintained at 22°C, with 40% humidity.

Experimental groups and surgery. Mice were randomly assigned to one of three experimental groups [sham vagotomy (n = 12), ventral trunk vagotomy (n = 6), and bilateral vagotomy (n = 9)], and the appropriate surgeries were performed as described for experiment 1. After recovery, mice were returned to their home cages with attached food magazines for further testing. Mice were tested in these feeding-monitoring cages for ≥12 wk after surgery.

Meal analysis. For 1 wk, at the beginning of the experiment, mice were gradually adapted to the test pellets (Bio-Serv, Frenchtown, NJ). The animals were then moved to similar individual plastic cages that had been adapted to contain a computer-controlled food magazine. Software (Graphic State 2.0, Coulbourn Instruments, Allentown, PA) maintained a single pellet in the magazine by delivering a new pellet whenever a pellet was withdrawn. The program also recorded concomitantly the patterns of ingestion, as previously described in full (4). Animals had ad libitum access to the food magazine for 19 h/day, from 1430 to 0930 the following morning. For 7 days before surgery, the meal patterns of each animal were recorded to obtain baseline values. On the day immediately before surgery, mice were given pellets until 1700 and then fasted overnight.

Meal onset was defined as the consumption of three of the 20-mg precision pellets within a 7-min period, and the end of a meal was considered to be the start of a 20-min period with no pellet consumption. Validation of these criteria and other details of the definitions of meal patterning parameters are described by Chi and Powley (4). Values were averaged for each mouse in 7-day blocks. Group data were then obtained by averaging the values for each mouse within a group. Because the majority of their feeding is in the dark, values for the 12-h dark cycle are presented, although the total 19-h daily test was also examined.

Daily food intake/body weight. Mice were weighed daily between 0930 and 1000, and total food intake was calculated from the number of 20-mg pellets eaten. Values for each mouse were averaged over 7-day blocks.

CCK tests. Additional naïve 7- to 8-wk-old male C57BL/6 mice (Harlan; n = 31, 20–25 g body wt on arrival) were obtained and housed in a manner identical to that described in Meal analysis.

For ≥1 wk, mice were presented with 12.5% glucose for 30 min at 0900 until consistent intakes were achieved. Mice were then randomly assigned to one of the three experimental groups (8 sham, 8 ventral, and 8 bilateral), and surgeries were performed as described for the mice in experiment 1. After 1 wk of recovery, testing began.

On the day before a test, the mice were fasted at 1700. On test days, the mice were injected intraperitoneal with saline or 2 μg/kg CCK-8 (catalog no. H-2080, Bachem). All injections were performed between 0900 and 0930. At 5 min after injections, the mice were presented with the 12.5% glucose solution for 30 min. On completion of the 30-min intake test, the mice were allowed ad libitum access to their maintenance diet. Tests were performed ≥2–3 days apart, so that the mice were never fasted or injected on consecutive nights. Also, at least one saline injection was included between CCK trials. On nontest days, mice were presented with 12.5% glucose at 0900 for 30 min. For analysis, the intakes for each mouse after saline injection and after CCK injection were averaged. Percent suppression for each mouse was calculated as a function of intake after CCK injection compared with intake after saline injection.

Statistical analysis and display of data. Statistical analyses were performed using Statistica 6.0 (StatSoft). Differences on the various measures between groups were analyzed using one-way ANOVAs, with or without repeated measures as appropriate, with group as the independent variable and the different parameters as the dependent variables. In all cases, Tukey’s post hoc tests were performed to examine which groups were significantly different from each other. Values were considered significant for P < 0.05.

All graphs were constructed using GraphPad Prism 3.0 (Graphpad Software).
RESULTS

As illustrated in Fig. 8, overall daily food intake and body weight measures for the ventral vagal trunk vagotomy group and the sham-vagotomized group paralleled the patterns observed for the comparable groups in experiment 1. Unilateral vagotomy did not significantly alter daily food intake or body weight over the 3 mo after surgery. In contrast, however, and as might be expected from earlier vagotomy experiments with rats, bilateral vagotomy produced a substantial and chronic reduction in food intake and a corresponding reduction in body weight for the duration of the experiment.

Microstructural analysis of meal patterns provided more sensitive indexes than simple daily totals and revealed significant and chronic effects of unilateral ventral trunk vagotomy as well as bilateral vagotomy. In the main, the effects of ventral trunk vagotomy were in the same direction and statistically significant but less dramatic than those of bilateral vagotomy. When meal taking was examined for the 12-h dark cycle, during which almost all eating occurred, several key parameters reflected the effect of vagotomy.

Specifically, as illustrated in Fig. 9, when meal pattern parameters for the entire postsurgery period were combined, sham-vagotomized animals ate on average <10 meals per

![Fig. 8. Daily food intake (A) and body weight (B) over a 12-wk period after unilateral truncal vagotomy were almost identical to shams. Bilateral vagotomy resulted in more dramatic changes, e.g., larger decreases in intake, which led to a corresponding decrease in body weight that persisted for the remainder of the experiment.

![Fig. 9. Average meal parameters for the 12-h dark cycle during the entire postvagotomy period. Bilateral vagotomy resulted in an increase in meal frequency (A) to account for the decreases in meal duration (B) and meal size (C). Animals subjected to ventral trunk vagotomy more closely resembled sham animals, although they ate significantly more meals. Bilateral vagotomy also resulted in a significant increase in satiety ratio (D), indicating that similar intakes resulted in longer intermeal intervals.](http://www.ajpregu.org)
night, whereas bilaterally vagotomized mice averaged ∼12 meals per night ($P < 0.001$). The unilateral ventral trunk vagotomy group ate significantly more meals per night than the sham group ($P < 0.05$) and almost as many as the bilateral vagotomy group (difference not significant). In the case of meal size measured by the number of pellets consumed and often reflected in meal duration, bilaterally vagotomized animals ate significantly smaller average meals than sham animals [number of pellets ($P < 0.01$) and duration ($P < 0.01$)], whereas on these same meal size measures, the unilaterally vagotomized animals tended to eat meals that were intermediate between those of controls and those of bilaterally vagotomized mice (for number of pellets and duration: controls > unilateral > bilateral) but not significantly different from control or bilaterally vagotomized animals. By another index, satiety ratio (the ratio of meal size to the succeeding intermeal interval), bilaterally vagotomized mice were significantly different from sham-operated animals ($P < 0.001$), whereas the animals subjected to ventral trunk vagotomy were not different from sham animals. [Because the light phase was interrupted by the maintenance period and because mice are primarily nocturnal feeders, we have summarized the meal patterning for the 12-h night phase; however, similar patterns were observed when the data from the light phase were combined with the data for the dark phase only (19-h access data not shown).]

Analyses of the same microstructural parameters for the later postsurgical weeks indicate that some of the alterations in meal patterning became more pronounced over time. The altered ingestive patterns did not resolve with time, as might be expected if regeneration were able to restore normal function. Bilaterally vagotomized mice increased gradually their number of meals per night, reaching a plateau at ∼6 or 7 wk after surgery (Fig. 10). The unilateral ventral trunk vagotomy group exhibited a similar pattern, plateauing at nearly the same number of meals per night at ∼8 or 9 wk after surgery (Fig. 10). ANOVA of meal number for the last 2 mo (weeks 5–12) was significant ($P < 0.001$), and Tukey’s post hoc honestly significant difference tests indicated that animals with ventral trunk vagotomy ate more meals than sham controls ($P < 0.05$) and bilaterally vagotomized animals ate more meals than sham controls ($P < 0.001$) but the same number of meals as the unilaterally vagotomized animals (not significant). Similar patterns were also seen for the respective groups on the measures of meal size. Bilaterally vagotomized animals took progressively shorter meals of fewer pellets and appeared to stabilize their meal size average at 6 or 7 wk after surgery (Fig. 10), whereas the unilaterally vagotomized mice even more gradually reduced their average meal duration and number of pellets per meal. Repeated-measures ANOVAs for the last 2 mo indicated group differences in meal duration ($P < 0.001$)}
and size \((P < 0.01)\); Tukey’s post hoc analyses indicated that the intermediate meal durations of unilaterally vagotomized animals were not significantly different from meal durations for sham or bilaterally vagotomized mice, whereas bilaterally vagotomized animals ate significantly shorter meals than sham animals \((P < 0.001)\). Similarly, the intermediate meal sizes of the unilaterally vagotomized animals were not significantly different from those of sham or bilaterally vagotomized animals, whereas bilaterally axotomized animals ate significantly smaller meals than sham animals \((P < 0.001)\). Finally, the bilaterally vagotomized group also evidenced a gradually progressive change in the satiety ratio over weeks, whereas, as suggested by their overall means (cf. Fig. 9), neither the unilaterally vagotomized group nor the sham-operated group display much change over time in the satiety ratio.

The CCK suppression test results (Fig. 11) were consistent with the pattern that has been described for the rat. Specifically, the moderate dose of CCK suppressed intake by \(-30\%\) in controls, and the effect of the peptide was not reduced by the partial or unilateral ventral trunk vagotomy, whereas it was attenuated after bilateral truncal vagotomy. The suppressed intake of the unilaterally vagotomized group after CCK injection was not significantly different from that of sham controls, whereas the intake of the bilaterally vagotomized group was significantly less than that of the unilaterally vagotomized or sham group after CCK injection \((P < 0.05 \text{ and } P < 0.01\text{, respectively})\).

**GENERAL DISCUSSION**

One of the major objectives of the present experiments was to investigate whether the pattern of abdominal vagal regeneration previously observed for the rat (16, 17) would generalize to the mouse and, thus, whether the mouse might be an advantageous preparation for examining the impact of particular growth factors and other gene products on vagal plasticity. A second major objective was to examine a set of ingestive end points that might serve as indexes of impairment and as measures of potential changes that might evolve over the course of an extended postsurgical interval. The results can be considered in terms of these two objectives.

**Pattern of mouse abdominal vagal regeneration.** The results of *experiment 1* established that mice, similar to rats (cf. Refs. 16, 17), display substantial regeneration of vagal afferents that reinnervate the GI tract after a truncal vagotomy. In both species, these afferents are partially successful in reestablishing axonal courses in the connectives of the myenteric plexus and redifferentiating some afferent terminals as IGLEs and IMAs that appear morphologically normal. In addition, the regenerating vagus of the mouse, as previously observed for the regenerating vagus of the rat (16, 17), produces a number of anomalous and aberrant terminals, in many cases in ectopic locations.

The parallels between vagal regeneration of the mouse and rat are not complete, however, and temporal and spatial differences in their patterns of plasticity might be of consequence in designing future analyses of the mouse vagus: the temporal difference is that, as anticipated, the reinnervation is much faster for the mouse than for the rat stomach. Specifically, there were a number of equivalences between the 16-wk regeneration group of the mouse and the previously described 45-wk regeneration group of the rat (16). In all likelihood, the differences in animal size, distances of axon regrowth, and proximity to the denervated site and, thus, to any diffusible growth factors that the denervated organ produced were major determinants of the accelerated reinnervation we observed in the mouse. Some of the compressed time scale observed in the mouse, however, may also be attributable to the fact that the mouse vagus was sectioned with microsurgical scissors in this experiment, whereas the rat vagotomies were performed with a surgical cautery.\(^1\) This truncated regeneration interval, regard-

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\(^1\) In the present experiments, vagotomies were performed by sectioning the nerve without cauteration (which we have routinely used in rat vagotomy experiments). Given the size of mice, we elected not to use cautery because of the risk of inadvertent tissue damage with even the finest microsurgical cutters. Not cautering, however, did make verification of the vagotomies more problematic. When cautering is practical, as in rats, a retrograde tracer strategy with true blue or fluoro-gold provides a particularly versatile and powerful verification (18). Without cautering, more preganglionic axons remain viable and establish aberrant terminals in the neuroma or scar, and these axons are still able to take up tracer. The pattern of retrograde labeling is then more spotty, with a small randomly distributed subset of neurons exhibiting reduced, but nonetheless clear, label.

For the present experiments, we instituted several criteria and checks to ensure that the vagotomies were effective: 1) At surgery, we eliminated any animals for which the exposure or surgery was at all problematic. 2) In *experiment 1*, the distinctive and unique reinnervation patterns defined with anterograde tracer verified the appropriateness of our surgeries. (In none of our vagotomized animals, did we observe the conventional, normal topography of afferents. Conversely, in none of the sham animals, did we see the regrowth pattern.) 3) The vagotomies produced functional defects, specifically as studied in *experiment 2*, that would be
less of its causes, might make the mouse particularly practical for exploring therapeutic manipulations of vagal regeneration.

Some of the details of the spatial pattern of regeneration observed for the mouse are unlike those previously seen in the rat. Specifically, in the mouse stomach, regional differences, i.e., strong regeneration, perhaps even hyperinnervation, in the corpus region, relatively limited regeneration in the forestomach, and minimal regeneration in the antrum, stand in contrast to the comparable densities of reinnervation of the gastric regions in the rat. The explanation for the pattern seen in the mouse is unclear. It could be argued that the time scale for effective regeneration in the mouse is >3–4 mo and that eventually the forestomach and antrum would receive considerably greater amounts of reinnervation, but such an argument does not explain the very rapid and apparently even excessive plasticity in the corpus.

Although attributing the contrasts in regeneration patterns to species differences is reasonable and perhaps parsimonious, it should also be noted that the distinct regional distributions of vagal afferent regeneration that we have observed may not be so much species differences as they are simply differences between two genotypes. If surveys were done on strains other than Sprague-Dawley rats, which were employed in the plasticity studies of Phillips and colleagues (16, 17), it is conceivable that the spatial distribution of some or all of these other rat strains might actually better parallel that observed in the C57BL/6 mouse. Conversely, other mouse strains may have patterns of afferent regeneration similar to those that have been observed for the Sprague-Dawley rat. Such issues can only be addressed experimentally, but the differences between the two species that have been examined suggest the need for genetic or other experimental analyses of growth factors or regeneration therapies to determine whether different rodent species and/or background strains may have different patterns of ingrowth of regenerating fibers.

**Functional end points and vagal afferent regeneration.** The present experiments examined several end points related to the regulation of energy balance to identify measures that might be sensitive to vagotomy and that might also track the evolution of any functional adjustments through the period in which vagal afferents were regenerating.

In our experiments, we monitored body weight and total daily food intake after unilateral vagotomy and did not detect any impairments (although both measures were affected by bilateral vagotomy). As predicted, however, the microstructural analyses of mouse feeding patterns in experiment 2 identified changes in meal number and meal size after unilateral vagotomy (as well as somewhat larger changes after bilateral surgeries). These results, taken together with the changes in meal patterns that have been observed in several mouse models with altered phenotypic patterns of vagal inner-

predicted from the rat vagotomy literature and that would not be explained by sparing the vagus. 4) In a subset of animals (n = 5 each, unilaterally and bilaterally vagotomized) from experiment 2, we did evaluate the use of a retrograde tracer verification. In all these trials, the animals appeared to have received the appropriate vagotomy, although in all cases, as anticipated from the lack of cauterization, more vagal preganglionic neurons than would have been expected after a cauterization did accumulate some tracer.

Given the promise of using mice for regeneration analyses, as demonstrated in the present experiments, it would seem worthwhile to find or develop a safe and efficient cautery scaled for the mouse.
CCK trials established another parallel between vagal afferent functions in mice and rats, and this test should provide a particularly instructive follow-up for future vagal regeneration experiments. Although we did not try to follow the evolution of any potential recovery of CCK sensitivity over months (in part because the initial loss of sensitivity proved to require bilateral vagotomy and we had not characterized the spatial or temporal patterns of recovery after bilateral axotomy in experiment 1), it might well be that vagally mediated CCK suppression of feeding, which does not require vagal efferent innervation, would be a particularly appropriate assay for assessing functional afferent recovery. These predictions, too, will need to be addressed experimentally.

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GRANTS

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