

# The Time Course of Taste Bud Regeneration after Glossopharyngeal or Greater Superficial Petrosal Nerve Transection in Rats

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## Abstract

We previously have published data detailing the time course of taste bud regeneration in the anterior tongue following transection of the chorda tympani (CT) nerve in the rat. This study extends the prior work by determining the time course of taste bud regeneration in the vallate papilla, soft palate and nasoincisor ducts (NID) following transection of either the glossopharyngeal (GL) or greater superficial petrosal (GSP) nerve. Following GL transection in rats ( $n = 6$  per time point), taste buds reappeared in the vallate papilla between 15 and 28 days after surgery, and returned to 80.3% of control levels ( $n = 12$ ) of taste buds by 70 days postsurgery. The first appearance and the final percentage of the normal complement of regenerated vallate taste buds after GL transection resembled that seen previously in the anterior tongue after CT transection. However, in the latter case, regenerated taste buds reached asymptotic levels by 42 days after surgery, whereas within the time frame of the present study, a clear asymptotic return of vallate taste buds was not observed. In contrast to the posterior (and anterior) tongue, only 25% of the normal complement of palatal taste buds regenerated by 112 days and 224 days after GSP transection ( $n = 9$ ). The difference in regenerative capacity might relate to the surgical approach used to transect the GSP. These experiments provide useful parametric data for investigators studying the functional consequences of gustatory nerve transection and regeneration.

## Introduction

Taste buds are distributed in several distinct receptor fields in the oral cavity of the rat (Miller, 1977). The chorda tympani branch (CT) of cranial nerve VII innervates the taste buds of the anterior two-thirds of the tongue. These taste buds are housed in specialized protrusions on the lingual surface called fungiform papillae. In the rat, each papilla usually houses a single taste bud (Miller and Preslar, 1975). The lingual-tonsillar branch of the glossopharyngeal nerve (GL; cranial nerve IX) innervates the taste buds of the posterior tongue. These taste buds are found primarily in the vallate and foliate papillae. In the rat, the vallate papilla is a single crescent-shaped trench positioned along the midline of the posterior dorsal surface of the tongue. The foliate papillae are a series of several trenches on the lateral margins of the posterior tongue. Taste buds in the vallate and foliate papillae are found lining the walls of the trenches. A few taste buds in the anterior trench of the foliate are innervated by the CT.

The palatal taste buds are distributed in three fields. The most anterior field is found in the incisive papilla associated with the nasoincisor ducts (NID) in the hard palate just behind the upper incisors. Taste buds can be found in the medial wall of the ducts near their opening in the oral cavity.

The second field consists of a strip of taste buds at the border of the hard and soft palates referred to as the 'Geschmacksstreifen' or palatal taste stripe. The third field of taste buds is found in the soft palate proper and is referred to as the posterior palatine field. Virtually all of the palatal taste buds are innervated by the greater superficial petrosal branch (GSP) of cranial nerve VII, but there is evidence that a few may be innervated by another nerve (Cleaton-Jones, 1976; Miller and Spangler, 1982). The superior laryngeal branch of cranial nerve X innervates the taste buds of the laryngeal epithelium. The exact numbers and proportions of taste buds in the various fields vary across subjects, species and studies (Miller, 1977; Miller and Smith, 1984; Travers and Nicklas, 1990).

In the rat, as is the case in other species, the taste buds are trophically dependent on the innervating nerve (von Vintschgau and Honigschmied, 1876; Whiteside, 1927; Guth, 1957; Cleaton-Jones, 1976; Cheal and Oakley, 1977; Miller, 1977; Miller and Spangler, 1982; Ganchrow and Ganchrow, 1989a; Hard af Segerstad *et al.*, 1989; Barry and Frank, 1992; Oakley *et al.*, 1993; St. John *et al.*, 1995; Ninomiya, 1998). Thus when gustatory nerves are transected, the taste buds in their respective receptor fields

degenerate. One partial exception to this rule, which is more noteworthy in the hamster than in the rat, involves the taste buds of the anterior tongue, some of which seem to degenerate incompletely following resection of the CT (Whitehead *et al.*, 1987; Hard af Segerstad *et al.*, 1989; Oakley *et al.*, 1990, 1993; Parks and Whitehead, 1998; St. John *et al.*, 1995). However, even in this receptor field, these buds are readily discriminated in cross-section by their smaller size and atrophic appearance; in addition, surface stains like methylene blue readily discriminate innervated and denervated buds because denervation reliably alters the structure of the taste pore in taste buds of the fungiform papillae (Parks and Whitehead, 1998).

When damaged or transected in rats, the CT and GL readily regenerate to reinnervate their appropriate receptor fields causing the regeneration of taste buds. Regenerated nerves display relatively normal electrophysiological response profiles to sapid stimuli placed on the tongue (Cain *et al.*, 1996; Cheal *et al.*, 1977). There is also no evidence of intact gustatory nerve fibers sprouting to invade denervated taste receptor fields normally supplied by a different nerve. Therefore, the reappearance of taste buds or taste pores following gustatory nerve transection can be taken as evidence for reinnervation by the transected nerve (Cheal and Oakley, 1977).

In previous work in rats, we have shown that following bilateral CT transection in the middle ear, taste buds begin to reappear on the anterior tongue between 14 and 28 days and reach an asymptotic ~70% return at 42 days. In an exhaustive series of parametric studies of the development and regeneration of the rat vallate papillae conducted by Bruce Oakley and colleagues, the time course of *unilateral* regeneration of a *crushed* GL was documented; the contralateral GL was permanently transected (Hosley and Oakley, 1987; Hosley *et al.*, 1987a,b; Oakley, 1993). We are unaware of any published reports on the time course of regeneration of the GSP or the superior laryngeal branch of X.

We sought to extend the prior work on the time course of GL regeneration by examining the return of taste buds after *bilateral* GL transection. The taste buds of the vallate papilla receive bilateral innervation from the GL. Moreover, most studies of the behavioral effects of GL transection involve bilateral manipulations. A second purpose of this study was to detail the regeneration of palatal taste buds after GSP transection. Collectively, this information is potentially useful not only for investigation of regeneration phenomena, but also for examination of the functional consequences of gustatory nerve transection. The rapidity with which gustatory nerves can regenerate in rodents [for example, the CT (Cheal and Oakley, 1977; Hard af Segerstad *et al.*, 1989; St. John *et al.*, 1995)] sets a limit on the duration of postsurgical assessment in nerve-transected animals, an approach that has yielded a great deal of information about the organization of the peripheral gustatory system. Time-course data allow the investigator to design

postsurgical assessments of gustatory function before the regeneration of the lost taste buds. In other instances, it may be desirable to relate behavioral performance with the number of returning taste buds (St. John *et al.*, 1995). Portions of this work have appeared in abstract form (St. John *et al.*, 2002).

## Materials and methods

### Subjects

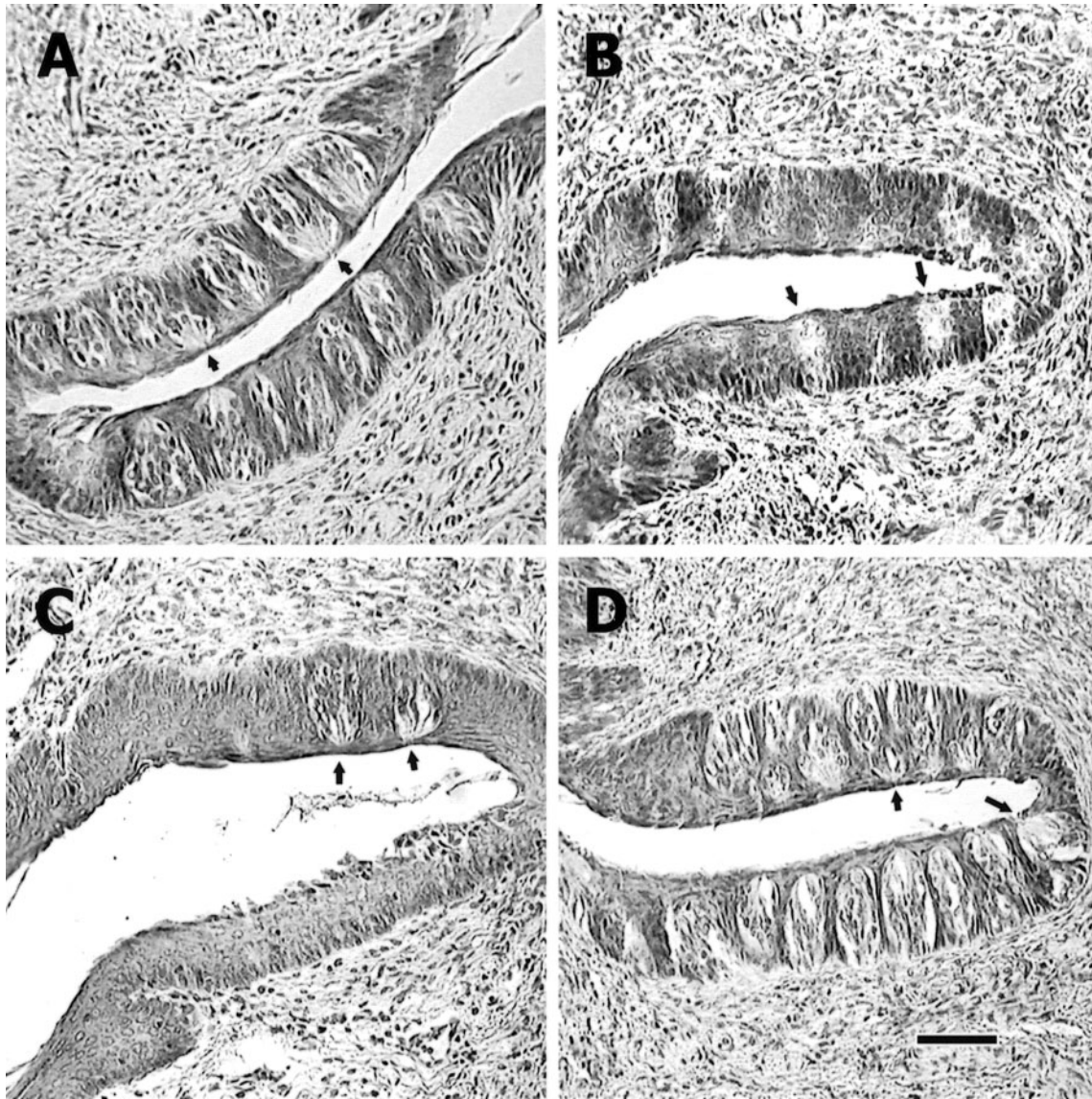
Seventy-nine, adult, male Sprague–Dawley rats (CD stock; Charles River Laboratories, Wilmington, MA) served as subjects. The rats were housed individually in hanging, wire mesh cages where food (Laboratory Chow 5001; Purina Mills Inc., St Louis, MO) and tap water were available *ad libitum*. Temperature, humidity and lighting (12:12 h light–dark cycle) were controlled automatically; all surgical manipulations were performed during the lights-on phase. Each subject participated in only one of two experiments designed to measure the time course of GL regeneration (Experiment 1) or the time course of GSP regeneration (Experiment 2). Subjects were maintained in NIH-approved housing and all procedures were approved by the Institutional Animal Care and Use Committee of the University of Florida.

### Experiment 1

Forty-two rats (336–603 g at the start of the experiment) were divided into seven experimental groups ( $n = 6$  per group). Rats in five groups received bilateral transection of the GL (GLX) and rats in the other two groups served as surgical controls (CON). Subjects were anesthetized with a mixture of ketamine hydrochloride (86 mg/kg body mass, i.p.) and xylazine hydrochloride (13 mg/kg body mass, i.p.). All rats also received a prophylactic injection of 30 000 U penicillin (i.m.) on the day prior to surgery.

Rats were placed supine in a customized headholder and an incision was made in the ventral skin of the neck along the midline. The GL was visualized close to its exit from the posterior lacerated foramen in the auditory bulla and inferior to the hypoglossal nerve following retraction of the sublingual and submaxillary salivary glands, the sternohyoid, omohyoid, and posterior belly of the digastric muscles. For GLX, the GL was dissected free of the surrounding fascia, held with a no. 7 microforceps, and cut with microscissors. The nerve was not damaged beyond this clean cut, and no explicit attempt was made to approximate the cut ends of the nerve or to form an anastomosis of the cut ends. For CON, the nerve was visualized but was left undisturbed. The incision was sutured closed. Because the CON rats also served as controls in a previously published study of CT regeneration (St. John *et al.*, 1995), these rats also had the tympanic membrane punctured with a no. 7 microforceps. This procedure did not involve removing the rat from the same headholder; by merely repositioning the





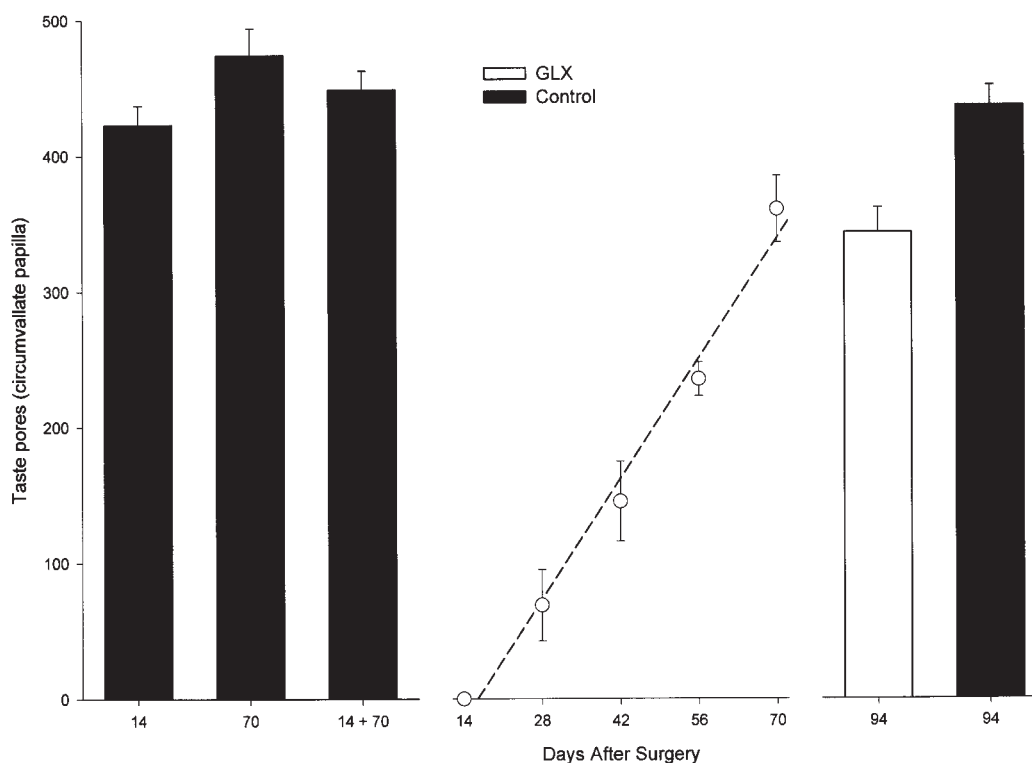
**Figure 1** Photomicrographs of hematoxylin- and eosin-stained 10 µm sections through the vallate papilla. **(A)** A representative section of a control rat showing numerous taste buds in one trench of the papilla. Two of several visible taste pores are indicated by arrows. **(B)** A section through the papilla of a rat perfused 14 days after GLX. No taste buds are present. Two 'ghost buds', paler-staining regions of gustatory epithelium, are indicated by arrows. Although these regions do not stain well and may represent the location of degenerated taste buds, the ghost buds lack elongated, mucosally oriented cells characteristic of taste receptor cells within taste buds [see also von Vintschgau and Honigsmied (von Vintschgau and Honigsmied, 1876)]. **(C)** A section from a rat perfused 42 days after GLX. The trench is mostly devoid of taste buds or ghost buds, but two normal-looking taste buds are clearly indicated by the arrows. **(D)** A section through the papilla in a rat perfused 70 days after GLX. The trench appears as densely packed with taste buds as in the control section (compare with A); the arrows point to two of several taste pores visible in the section. GLX = bilateral transection of the GL nerve. Scale bar in D = 50 µm and applies to all panels.

rat, the ear canal could be widened with five blunted and curved hypodermic needles for visualization of the tympanic membrane.

Rats of the GLX groups were perfused in five groups (14, 28, 42, 56 or 70 days after surgery) and surgical controls in two groups (14 or 70 days). Body weight was monitored after surgery. In two cases, rats were treated with additional penicillin during the survival period. One of these rats also received a liquid nutritional supplement (Precision Diet,

Research Diets Inc., New Brunswick, NJ) for 8 days to promote feeding.

For tissue collection, the rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with isotonic saline followed by 10% buffered formalin. The tongue was removed carefully and stored in formalin for at least 5 days. The vallate papilla was embedded in paraffin and sectioned on a rotary microtome (10 µm). The sections were mounted on glass slides and stained with hematoxylin



**Figure 2** **Left**, the mean ( $\pm$ SE) number of taste pores counted in the vallate papilla of control rats killed 14 ( $n = 6$ ) or 70 days ( $n = 6$ ) after sham surgery, and the combined mean of all control rats (14 + 70). **Middle**, the mean ( $\pm$ SE) number of taste pores counted in the vallate papilla of rats killed various times after transection of the GL nerve (open circles). The dashed line represents the linear best fit ( $r^2 = 0.986$ );  $n = 6$  per group. **Right**, the mean ( $\pm$ SE) number of taste pores counted in the vallate papilla of control rats (filled bar) and rats killed 94 days after nerve transection (open bar) in a previously published study (King *et al.*, 2000). This comparison is offered to assess whether more taste buds would have appeared if a survival time longer than 70 days were used or whether the number of regenerated vallate taste buds might remain asymptotically short of the control value. Both studies used the same procedure to transect the GL nerve, the same histological protocol, the same criterion for counting taste pores, and the same experimenter quantifying the tissue (M.G.).

and eosin and the number of taste buds was quantified throughout the entirety of the vallate papilla (see below).

## Experiment 2

Thirty-seven rats (318–470 g at the start of the experiment) were divided into nine experimental groups (14 day surgical controls,  $n = 3$ ; 224 day surgical controls,  $n = 5$ ; 224 day GSP transection,  $n = 5$ ; all other groups,  $n = 4$ ). Rats in six of the groups received bilateral transections of the GSP in the middle ear (GSPX); using this surgical approach the CT was usually also sectioned. In some cases, an attempt to spare the CT was made, but these experiments are not the primary focus of this report. Rats in the remaining three groups served as surgical controls (CON).

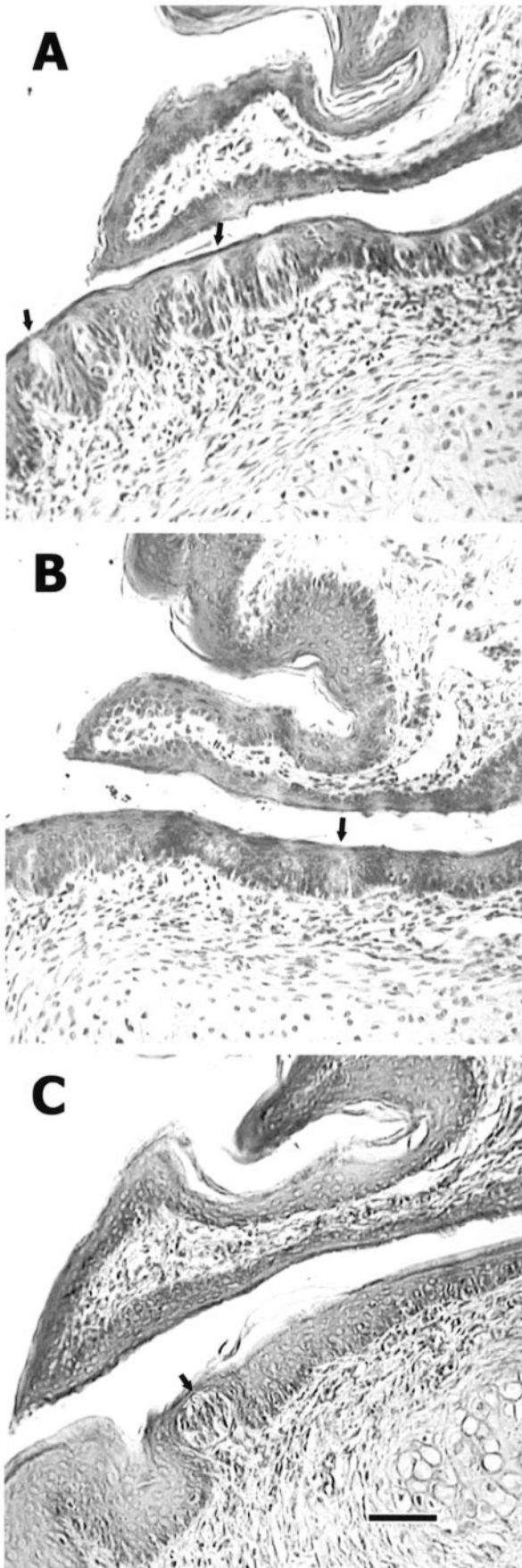
Subjects were anesthetized with a mixture of ketamine hydrochloride (125 mg/kg body mass, i.m.) and xylazine hydrochloride (5 mg/kg body mass, i.m.), and were treated with 30 000 U penicillin (s.c.) the day of and for 3 days after surgery. The modification of our anesthetic protocol was motivated by intervening observations that i.m. injection of ketamine–xylazine was a safer alternative to achieving

sustained surgical levels of anesthesia in Sprague–Dawley rats.

The rats were then fixed in a custom headholder with the head tilted  $80^\circ$  away from the surgeon. For GSPX, an incision was made around the external ear and the pinna retracted. The ear canal was then punctured and widened by careful dissection of the fascia and retraction of the surrounding musculature. The bony meatus was enlarged with a drill. The tympanic membrane, ossicles, tensor tympani muscle, and a small piece of temporal bone were removed to expose the GSP, which was cut with micro-forceps. In general it was expected that the distal and proximal ends of the cut nerve remained in approximation, but no explicit attempt was made to form an anastomosis of the cut ends of the nerve. The incision was closed with wound clips. For CON, the pinna was retracted and the soft tissue of the ear canal was widened as described above, and the tympanic membrane punctured, but the internal structures of the middle ear were undisturbed.

For 3 days after surgery, rats were offered a highly palatable, high-calorie diet of Purina 5001 powder chow mixed with  $\sim 2.5$  g Nutrical (IGI EVSCO Pharmaceutical, Buena,





**Figure 3** Photomicrographs of hematoxylin and eosin stained 10 µm sections of the incisive papilla and the NID. **(A)** A section through the papilla of a control rat showing numerous taste buds lining the NID. The arrows indicate visible taste pores. **(B)** A section through the papilla of a rat perfused 14 days after GSPX. There are no taste buds present. The arrow indicates a taste bud shaped region of pale staining, or 'ghost bud', which may be the remnant of a degenerating taste bud. This region lacks the elongated, mucosally oriented cells characteristic of taste receptor cells within taste buds, and is clearly composed of epithelial tissue. **(C)** A section through the papilla of a rat perfused 224 days after GSPX. The arrow points to the taste pore of the single, normal-looking taste bud visible in this section. Even after this lengthy recovery time, few taste buds were found to regenerate after GSPX. GSPX = bilateral transection of the greater superficial petrosal nerve. Scale bar in C = 50 µm and applies to all panels.

NJ) and a sweetened condensed milk diet (diluted 50% with vitamins added). The rats were supplied with wet mash until body weight was restored to the presurgical value. Rats after GSPX are often hypophagic for weeks (St. John *et al.*, 1994), and thus, several different procedures were used to maintain the health of these rats in the postoperative interval. Milk diet was continued for some rats beyond the first 3 days, and for others, occasional injections of penicillin were administered when the possibility of an infection was suspected.

Rats of the GSPX groups were perfused (as described in Experiment 1) at six different time periods after nerve transection (14, 28, 42, 56, 112 or 224 days after surgery) and CON rats at three different time periods after sham surgery (14, 56 or 224 days after surgery). The soft palate and incisive papilla surrounding NID were removed and stored in 10% buffered formalin. They were later embedded in paraffin and cut, mounted and stained (as described in Experiment 1 for the vallate papilla).

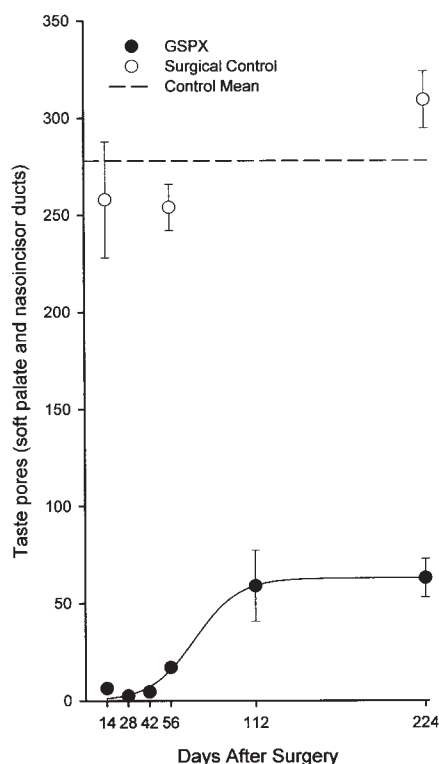
#### Quantification of taste bud number

In innervated intact taste buds, the apical portions of the taste receptor cells converge and then protrude into the taste pore; this morphology is not observed in the atrophic or remnant taste buds sometimes observed in denervated tissue (Oakley *et al.*, 1993). This convergence is generally recognizable in just one 10 µm section and is usually accompanied by visualization of a taste pore. Any taste bud displaying this structural characteristic was counted as morphologically intact. This counting method helped minimize the possibility that a single bud would be counted twice. The number of intact taste buds was quantified for each rat in the relevant taste bud receptor fields.

## Results

### Experiment 1

The mean number of taste buds was somewhat higher in the CON/70 day than in the CON/14 day (473.83 versus 422.83) group, but this difference was not statistically significant [ $t(10) = -2.099$ ,  $P = 0.062$ ]. The groups were combined in subsequent analyses.



**Figure 4** The mean ( $\pm$ SE) number of taste pores counted in the palate (soft palate and NID) of rats killed at various times after sham surgery (open circles) or transection of the GSP nerve (filled circles). The horizontal dashed line represents the mean of all sham rats. The curve is the sigmoidal (three-parameter) best fit to the data from nerve-transected rats.  $n = 4$  per group for all nerve cut time points, and  $n = 3, 4$  and  $5$  for the 14, 56 and 224 day controls, respectively. In some cases, the error bars are obscured by the symbol.

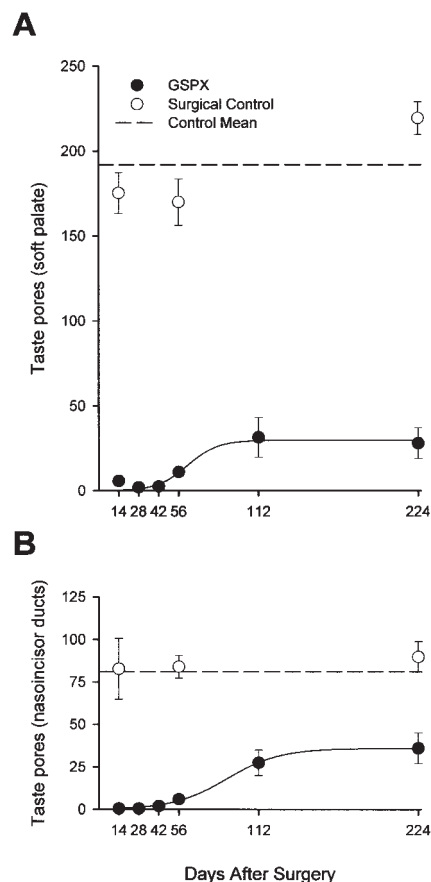
Taste buds in the vallate papillae returned as a near-linear function of time since GL transection (Figures 1 and 2). In fact, a linear model accounted for 98.4% of the variance, with a slope of 6.33 taste buds/day. No taste buds were seen at 14 days postsurgery, but if a linear model accurately describes the rate of taste bud regeneration, the first buds must have appeared 16 days after GLX (i.e. the x-intercept was 16.15 days). At the very least, taste buds begin to reappear after GLX between 14 and 28 days after surgery.

According to a one-way analysis of variance, the groups differed reliably in taste bud number [ $F(5,36) = 89.7$ ,  $P < 0.001$ ]. A Tukey–HSD *post hoc* test indicated that all groups had fewer taste buds than the CON (all  $P$ -values  $< 0.017$ ). The number of taste buds seen 70 days after GLX was 80.3% that of CON.

## Experiment 2

The three control groups did not differ in the total number of palatal taste buds [ $F(2,9) = 3.31$ ,  $P = 0.08$ ].

The total number of palatal taste buds seen even 224 days after GSPX was considerably less than that seen in controls. The five cases in the GSPX/224 day group had 40, 54, 78, 84



**Figure 5** The mean ( $\pm$ SE) number of taste pores counted in the soft palate (A) and NID (B) of rats killed at various times after sham surgery (open circles) or transection of the GSP nerve (filled circles). The horizontal dashed line represents the mean of all sham rats. The curve is the sigmoidal (three-parameter) best fit to the data from nerve-transected rats.  $n = 4$  per group for all nerve cut time points, and  $n = 3, 4$  and  $5$  for the 14, 56 and 224 day controls, respectively. In some cases, the error bars are obscured by the symbol.

and 216 taste buds. The latter case did have a remarkable number of taste buds relative to the control average (278.0); however, several observations strongly suggest that this animal had an unsuccessful nerve transection on one side. First, the number of taste buds in this rat was more than 2.5 times greater than that seen in the rat with the second highest number of taste buds returning and was over 7 standard deviations from the mean of the other four rats in the GSPX/224 day group. Second, the distribution of taste buds on the palate (though not the NID) was strikingly unilateral, with one half of the palate devoid of taste buds except for a few near the midline. Although this pattern of denervation has not been described in earlier reports of unilateral GSPX (Cleaton-Jones, 1976; Miller and Spangler, 1982), it is difficult to find an alternative explanation than unilateral innervation given that intact animals always display a relatively even bilateral coverage of the palatine field in our own observations and in those reported by others

(Cleaton-Jones, 1976; Miller and Spangler, 1982). Finally, as described in the Materials and methods, following GSPX, rats in our procedure reliably fail to recover their body weight until weeks after surgery, whereas the case with 216 taste buds recovered body weight at a rate comparable to controls, achieving its presurgical body weight on the eighth day after surgery. For comparison, this animal's four cohorts in the GSPX/224 day group reached their presurgical body weight 21–41 days after surgery, and CON/224 day rats did so 3–7 days after surgery. Based on this profile of results strongly suggesting that the transection was incomplete on one side, this outlying case was not included in the formal statistical analysis.

We found that, in contrast to the facility of lingual taste bud regeneration after transection of the GL (Experiment 1) or CT (St. John *et al.*, 1995), palatal taste buds regenerated after GSP transection at a very slow rate (Figures 3 and 4). At 56 days, when the other major gustatory nerves had induced considerable taste bud regeneration in their respective receptor fields, an average of just 17 total taste buds had returned to the palatal fields innervated by the GSP (6.1% relative to all controls). Furthermore, although there were considerably more taste buds at 112 days (59 taste buds representing 21.2% relative to controls), the rate of returning taste buds seemed to asymptote at this value because by 224 days there was only an average of 64 buds (23.0% of controls). Apparently, for whatever reason, the surgical method outlined here permanently prevents the regeneration of over three-quarters the typical number of palatal taste buds.

When the soft palate and NID were examined separately, taste buds returned at roughly a similar rate in both receptor fields (Figure 5). Because the soft palate has far more taste buds than the NID, the percentage of returning taste buds is higher in the latter. By 224 days, the soft palate had an average of 28 taste buds (14.6% of the number seen in all controls), whereas the NID had 36 taste buds (41.8% of controls). In both fields, regeneration reached asymptotic levels by 112 days. Of the eight rats that had apparently achieved asymptotic regeneration (i.e. those at 112 and 224 days), four had more taste buds in the soft palate than the NID, three had more in the NID than the soft palate, and one had an equal number in each field.

Interestingly, the control groups did differ in the number of taste buds in the soft palate [ $F(2,9) = 5.91$ ,  $P = 0.02$ ] but not in the NID [ $F(2,9) = 0.13$ ,  $P = 0.88$ ]. This finding underscores the poor regeneration seen in the 224 day group and suggests that there is no drop in taste bud number over this age span and perhaps even a small increase, at least in some receptor fields.

## Discussion

It is clear from this study and the work of others that the lingual gustatory nerves regenerate rapidly in the rat, pro-

moting the reformation of taste buds in the appropriate receptor field. There is evidence that this regeneration is also accompanied by functional recovery as assessed behaviorally, electrophysiologically, and by the immunohistochemical staining of taste stimulus induced expression of immediate early genes in the rostral nucleus of the solitary tract (Cheal *et al.*, 1977; Barry *et al.*, 1993; Zuniga *et al.*, 1994, 1997; St. John *et al.*, 1995; Cain *et al.*, 1996; Ninomiya, 1998; Barry, 1999; King *et al.*, 2000; Kopka *et al.*, 2000; Saito *et al.*, 2000; Kopka and Spector, 2001; Saito *et al.*, 2001a,b). Apparently, the GSP does not have the same proclivity to regenerate as does the CT and GL, at least not with our surgical approach.

In recent years, the functional consequences of gustatory nerve transection have led to hypotheses concerning the organization of gustatory system. Because of the ease of the lingual taste nerves to regenerate, and the potential for the extent of regeneration to influence functional competence, histological analysis should always accompany such reports, particularly when postsurgical testing regimens necessitate long survival times. The present report provides important empirical information to guide the design of experiments studying the functional competence of animals with transected and regenerated gustatory nerves.

## Source of innervation of regenerated taste buds

It is true that the present report provides no explicit verification that the taste buds reappearing after surgery were induced by the regeneration of the transected nerve as opposed to sprouting from another nerve. We made no attempt to verify functional connectivity by recording from the transected nerve during taste stimulation of the relevant receptor field. Indeed, there are some reports that the lingual nerve proper can support the maintenance and regeneration of some morphologically normal fungiform taste buds (Kinnman and Aldskogius, 1988; Hard af Segerstad *et al.*, 1989). Nevertheless, there is ample evidence in the rodent taste system demonstrating that sprouting from other gustatory nerves does not occur naturally, although experimentally cross-anastomosed nerves can induce taste bud formation in their non-native field (Oakley, 1967, 1970; Nejad and Beidler, 1987; Ninomiya, 1998; Smith *et al.*, 1999). Moreover, taste buds that reappear have been shown to be dependent on the regeneration of the transected nerve (Cheal *et al.*, 1977; Barry *et al.*, 1993; Cain *et al.*, 1996; Montavon *et al.*, 1996). In support of this, when the CT or GL are prevented from regenerating, there is no significant increase in the number of taste buds appearing in the denervated field over time (King *et al.*, 2000; Kopka *et al.*, 2000; Kopka and Spector, 2001).

## Regeneration of the CT

Previously, only the time course of taste bud regeneration in the anterior tongue after bilateral CT transection had been examined parametrically (Cheal and Oakley, 1977; St. John



*et al.*, 1995), although a partial time course for the other nerves can in some cases be inferred by combining results from various studies in the literature. St. John, Markison and Spector (St. John *et al.*, 1995) found that after transection of the CT in the middle ear, taste buds began reappearing between 2 and 4 weeks after nerve transection, with asymptotic levels of two-thirds the control number of taste buds seen by 6 weeks. Although, in some cases, others have found a somewhat greater number of taste buds returning (relative to controls) after long survival times [e.g. ~80% (Kopka *et al.*, 2000)], there nevertheless may be a limit imposed on regeneration by the number of intact, healthy fungiform papillae that remain. St. John *et al.* (St. John *et al.*, 1995) found that the number of anterior tongue taste buds increased, but the number of normal fungiform papillae decreased with time since surgery. This time-dependent decrease in papilla number is supported by data collected at single time points across studies reported in the literature (Ganchrow and Ganchrow, 1989a,b; Robinson and Winkles, 1991; Smith *et al.*, 1999; Kopka *et al.*, 2000). Other investigators have demonstrated that either the CT or the taste buds themselves appear to maintain the normal structure of the fungiform papillae; for example, in the absence of the innervating nerve many fungiform papillae develop ectopic filiform spines (Ganchrow and Ganchrow, 1989a; Hard af Segerstad *et al.*, 1989; Oakley *et al.*, 1990; Robinson and Winkles, 1991; Oakley *et al.*, 1993; St. John *et al.*, 1995), and even in species where the denervated papilla maintains an atrophied taste bud (the hamster), there are considerable morphological changes in the pore region (Parks and Whitehead, 1998). The degeneration of fungiform papillae is even more pronounced with concomitant removal of the lingual branch of the trigeminal nerve (Hard af Segerstad *et al.*, 1989) or if the CT is transected in the early postnatal period (Sollars and Bernstein, 2000), and this is associated with proportionately fewer regenerated taste buds after these manipulations.

### Regeneration of the GL

Despite the considerable differences in the morphology of papillae that the GL and CT innervate and the surgical method used to transect these two nerves, the time-course data for the regeneration of taste buds innervated by these nerves is remarkably comparable in some ways (St. John *et al.*, 1995). With both nerves there was no evidence of reinnervation at 14 days, but in both cases taste buds began to return by 28 days. The first appearance of taste buds after GL transection in our study is similar to that seen in the rabbit, 25 days, reported by Fugimoto and Murray (Fugimoto and Murray, 1970) and the rat by Iwayama and Nada (Iwayama and Nada, 1969). One difference between the time course of regeneration between the CT and GL is that in the former case the return of taste buds on the anterior tongue reached an apparent asymptote by 42 days postsurgery, whereas return of vallate taste buds is linear

through 70 days. On the other hand, the number of taste buds seen 70 days after GLX was 80.3% that of our sham-operated controls, which is virtually the same extent of regeneration seen in anterior tongue taste buds after CT transection after a long postsurgical survival time (>84 days) in another study (Kopka *et al.*, 2000).

As discussed above, there is correlational evidence that CT transection-induced degenerative changes in fungiform papillae might contribute to the asymptotic levels of taste bud reformation seen 42 days after nerve transection. Like the fungiform papillae, the vallate papilla undergoes morphological changes following denervation (Guth, 1957, 1963; Kennedy, 1972; State, 1977). Whereas Kennedy found a thickening of the epithelium formerly containing taste buds (Kennedy, 1972), Guth (Guth, 1957, 1963) and State (State, 1977) found a pronounced atrophy. The reason for this difference is unclear. Whether the degenerative changes represent a progressive process awaits further anatomical scrutiny. Thus, whereas there is a reasonable relationship between the time course of fungiform papillae degeneration and the compromised ability of the regenerated CT nerve to re-form taste buds (fueling speculation of a causal relationship), it is premature at this time to posit a similar relationship between degenerative changes in the vallate papilla and the extent of taste bud reformation after GL transection.

In the current study, the rate of taste bud regeneration was strikingly linear (Figure 2), but, at the same time, only 80.3% of the control number of buds were seen 70 days postsurgery. If the number of buds were to continue to return in linear fashion, the vallate papilla would regain normal numbers of taste buds 87.3 days after surgery. Alternatively, it is possible that, like the fungiform receptor field, the vallate papillae does not return to preexisting numbers of taste buds. In a previously published study using identical surgical and histological methods, in which the strain of rat, diet and housing conditions were consistent with the present study, and in which taste pores were quantified by a common experimenter (M.G.), the number of taste buds in the vallate papilla was significantly less than in GL-intact rats 94 days after transection [data from King *et al.* (King *et al.*, 2000), replotted in our Figure 2]. Thus, had the present study been carried out to another data point, evidence for an asymptote may have been obtained.

The rate and extent of regeneration in our rats appears to be quite consistent with a parametric analysis of a group reported by Hosley *et al.* (Hosley *et al.*, 1987a). In their AV75/CR75 group, 75-day-old rats (i.e. adults similar in age to those of the present report) received avulsion of the right GL in a manner that prevented regeneration, and a crushing of the left GL in a manner that promoted regeneration. In their study, vallate taste buds reappeared in a more or less linear fashion over the next 75 days (at virtually the same rate as in our rats, six or seven buds per day), achieving 66.4% of the normal complement of their 'normal' rats (610



buds, somewhat higher than our CON group). Because a unilateral GL can maintain greater than 80% of the rat vallate taste buds (Guth, 1963) due to the nerve's bilateral distribution in the papilla (Whiteside, 1927; Oakley, 1974; Hosley *et al.*, 1987b), the time course data from the AV75/CR75 group in the Hosley *et al.* (Hosley and Oakley, 1987; Hosley *et al.*, 1987a,b) experiment would appear to be quite consistent with our own bilateral transections.

Consistent with the conclusions of studies on the development of the GL-vallate system (Hosley *et al.*, 1987a,b; Hosley and Oakley, 1987; Oakley, 1993), our data suggest that the time course of regeneration in adult rats is somewhat different from initial formation of taste buds during development. Although the overall time course over which taste buds appear is quite similar to that during development [i.e. 75–90 days (Hosley and Oakley, 1987)], during development the appearance of taste buds is not approximately linear, but rather follows a third-order function characterized by an early accelerated accumulation of buds (~13 taste buds per day over postnatal days 3–33), and a later deceleration in the accumulation of buds (just two taste buds per day over postnatal days 60–90). To what extent this difference reflects the different states of the developing and adult-transected papilla, differences in the rate that axons reenter the papilla, or other factors cannot be discerned from our data.

### Regeneration of the GSP

In contrast to the CT and GL, transection of the GSP in the middle ear caused a pronounced and seemingly permanent decrease in the taste bud count in its receptor field. After 224 days, only 25% of the normal complement of palatal taste buds had regenerated, a number not significantly different from that seen at 112 days. Thus, this may represent the maximal amount of regeneration to be expected from our surgical approach. The case with the most taste buds at either 112 or 224 days had just 38.8% that seen in the average of all 12 controls.

What accounts for the poor percentage of regenerated taste buds after GSPX relative to the nearly complete regeneration after transection of the other major gustatory nerves? The target organ of the GSP (the palate) is different from that of the other gustatory nerves (the tongue). It is possible that denervation in adulthood renders these epithelia nonconductive to the formation of taste buds as is the case with neonatal avulsion of the other gustatory nerves (Hosley *et al.*, 1987a,b; Sollars and Bernstein, 2000). We did not make morphometric analyses of the palatal epithelium in the current study, and, to our knowledge, epithelial changes after GSPX have not been examined as they have been for the other gustatory nerves.

A more relevant factor, however, may be the nature of the surgical approach necessary to transect the GSP. Whereas transection of the CT and GL can both be relatively benign with regard to nearby structures, our exposure of the GSP

involved considerable disruption of the tissue surrounding the nerve (see Materials and methods). Perhaps the approach used to transect the GSP produced scars that impeded regenerating fibers. In support of this, Kopka *et al.* (Kopka *et al.*, 2000) successfully prevented regeneration of the CT by cauterizing structures of the external and middle ear to stimulate the secretion of cerumin which fills the cavity of the bulla.

It should be noted that others have apparently been able to achieve regeneration of palatal taste buds after a cross-anastomosis of the proximal stump of the transected CT and the distal stump of the transected GSP (Nejad and Beidler, 1987). Likewise, these same investigators were able to form a successful cross-regeneration of anterior tongue taste buds by bridging the proximal stump of the transected GSP with the distal stump of the transected CT. Although the presence of taste buds was not quantified, these investigators were able to record electrophysiological responses in the cross-regenerated nerves to taste stimuli applied to the appropriate receptor field. Thus, it would appear that the GSP, under some circumstances, does have the capacity to regenerate. Likewise the palate, under some circumstances, appears to be able to support the reformation of new taste buds following a period of denervation.

The GSP normally innervates three receptor fields: the posterior palatine field and geschmacksstreifen (both on the soft palate), and the NID of the hard palate (Cleaton-Jones, 1976; Miller, 1977). Soft and hard palate taste buds returned at the same rate, and if anything, the NID were preferentially innervated by the returning GSP fibers. Whether this means that certain axons within the GSP are 'targeted' to one specific receptor field (e.g. the NID) rather than accepting the first available denervated epithelial tissue remains to be determined.

### Concluding remarks

Histological analysis following gustatory nerve transection is critical for the interpretation of behavioral studies, because rodent gustatory nerves are noteworthy for their propensity to regenerate rapidly. This is especially important in cases where behavioral tasks require long-term testing following surgery [e.g. detection threshold testing (Spector *et al.*, 1990)]. These data also provide a framework for experiments designed to examine functional or anatomical consequences of completely regenerated gustatory structures, or attempting to relate the extent of functional recovery in incompletely regenerated structures. The current study was an attempt to fill gaps in the literature on the time course of regeneration of taste buds after bilateral transection of the major gustatory nerves. While specific details will of course vary with surgical approach [e.g. crushed gustatory nerves regenerate more rapidly than transected nerves (Cheal and Oakley, 1977)], we have nonetheless uncovered some useful parameters enabling the investigator

to temporally predict the extent of taste bud regeneration following gustatory nerve transection.

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