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The Functional Consequences of Gustatory Nerve Regeneration as Assessed Behaviorally in a Rat Model

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When gustatory nerves are transected, the taste buds they normally innervate degenerate and specific deficits in taste-related behavior result depending on the nerves transected, the taste compounds used, and the nature of the behavioral test (see Spector, 2003). In rodents, the chorda tympani nerve (CT) which innervates the anterior tongue, and the glossopharyngeal nerve (GL) which innervates the posterior tongue, both have a great proclivity to regenerate after injury and reinnervate their native receptor fields. It is clear that there are some seemingly permanent anatomical consequences of CT regeneration including reductions in taste bud number and volume, decreases in the number of myelinated axons, decrease in the density of terminal projections, and a decline in the volume of the terminal field in the rostral nucleus of the solitary tract (NST) (Shuler *et al.*, 2004; for review, see Spector, 2003). These regeneration induced changes in the anatomy of the system raise the issue of whether taste function would be altered in some way. Accordingly, my laboratory has been using behavioral procedures to examine whether functions that are disrupted by neurotomy recover upon regeneration of the nerve.

The CT, which innervates ~13% of the total taste buds in the rat, is exceptionally responsive to NaCl. Although for many years the input of the CT was thought to be unnecessary to maintain sensibility to NaCl based on two-bottle preference tests, when more psychophysically rigorous tasks were applied to assess responsiveness to this salt, very severe and unequivocal behavioral deficits were revealed in rats that had the nerve transected. Thus, the ability of the regenerated CT to support taste-guided performance in tasks involving NaCl was a conceptually promising way to begin to assess the functional consequences of gustatory nerve regeneration.

For many of our behavioral assays, we use a specially designed gustometer that allows for the delivery of small volumes of taste solutions and measurement of immediate responses increasing the confidence that behavior is guided by the chemical features of the stimulus. To assess function in the sensory-discriminative domain we use a two-response operant taste discrimination procedure in which thirsty rats are trained to sample a fluid stimulus and to press one of the two levers to indicate whether the stimulus is A or B; correct responses are rewarded with water.

When the CT is transected, NaCl detection thresholds increase by well over an order of magnitude; GL transection is without effect. If the CT regenerates, however, NaCl sensitivity returns to normal despite that only about three-quarters of the normal complement of taste buds returns. Moreover, adulteration of the stimuli with amiloride, a tasteless epithelial sodium channel blocker which raises NaCl detection threshold by about one log unit in intact rats, has no effect in CT-transected rats, but causes normal shifts in sensitivity in rats with regenerated CTs (Kopka and Spector, 2001).

The prior results shows that sensitivity to NaCl recovers upon CT regeneration, but what about salt discriminability? Transection of the CT leads to significant impairments in the ability of rats to discriminate sodium from nonsodium salts on the basis of taste; GL transection is without effect. This includes performance on an NaCl versus KCl operant taste discrimination task using the procedure described above. When the CT regenerates, however, discrimination performance returns to normal and amiloride adulteration of the stimuli completely disrupts the behavior as it does in intact rats (Kopka *et al.*, 2000).

These results demonstrate that sensitivity to and discriminabilty of NaCl, as well as the performance-disrupting effects of amiloride treatment, are restored upon CT nerve regeneration in rats. These findings are consistent with other behavioral and electrophysiological findings in other rodent species (e.g. Barry *et al.*, 1993; Cain *et al.*, 1996; Ninomiya, 1998; Yasumatsu *et al.*, 2003).

Studying the functional consequences of GL regeneration is complicated by that fact that performance on a variety of taste-related tasks is impervious to the transection of this nerve, despite that the GL innervates close to 60% of the taste buds in the rat (see Spector, 2003). One behavior, however, that is severely impaired by GL transection in rats is the unconditioned elicitation of gapes by quinine. The gape is a hallmark oromotor rejection response. Quinine is an especially potent stimulus for eliciting this response in rodents. Typically, in these experiments, the stimulus is infused directly through an indwelling cannula into the oral cavity for a short period of time and the animal's oromotor responses are videotaped. The tape is analyzed and the number of gapes are counted.

As Travers *et al.* (1987) and others (Grill *et al.*, 1991; King *et al.*, 2000) have shown, transection of the GL in rats markedly reduces the number of gapes elicited by quinine, whereas CT transection has marginal, if any, effects. King *et al.* (2000) demonstrated that quinine-elicited gaping is completely restored upon regeneration of the GL despite that only ~three-fourths of the circumvallate taste buds reappear.

Travers and her colleagues (Harrer and Travers, 1996) discovered that intraoral quinine stimulation generates a pattern of neuronal Fos-like immunoreactivity (FLI) in the rostral NST of the rat that can be distinguished from the patterns produced by other fluid stimuli. King *et al.* (1999) later showed that GL transection changes the FLI pattern that quinine produces to one that resembles water stimulation in intact rats. Transection of the CT causes an intermediate decline in the total number of neurons expressing FLI in response to quinine, but does not change their topographic distribution. Interestingly, when the GL regenerates, not only do the number of gapes elicted by quinine return to normal, but so does the pattern of FLI in the rostral NST (King *et al.*, 2000). Moreover, the quinine-stimulated FLI in the waist area of the parabrachial nucleus which is reduced to levels similar to water stimulation by GL transection, also returns to normal upon regeneration of the nerve (King *et al.*, 2003).

Given the effect of GL transection on quinine-elicted gaping, it is perhaps surprising that GL transection alone (or CT transection alone) does not significantly alter lick avoidance of quinine in a brief-access test when rats are tested before and after surgery (St. John *et al.*, 1994, but see Markison *et al.*, 1999). Combined transection of the GL and CT, however, results in substantial blunting of quinine avoidance in rats. Recently, Geran *et al.* (2004) demonstrated that after such massive gustatory deafferentation of the tongue, if

both nerves regenerate or even if only the GL regenerates, the quinine concentration-avoidance function returns basically to its presurgical form with some minor deviations. If only the CT is allowed to regenerate, however, the quinine-concentration avoidance function remains substantially shifted to the right. This is odd given that St. John *et al.* (1994) found that GL transection alone was without effect in this task, at least when animals were tested in a similar design (cf. Markison *et al.*, 1999). Indeed, the failure of the regenerated CT to support a taste-related behavior that the intact CT can, in the absence of the GL, is as yet the only published account to date of a regenerated gustatory nerve failing to restore function.

In summary, when transection of a single gustatory nerve impairs a taste-guided behavior, the available evidence to date suggests that full recovery occurs upon regeneration of the nerve, despite the reported alterations in the peripheral and central anatomy. Neurotomy-induced disruptions in patterns of taste-stimulated neuronal activity, at least as assessed by FLI, also return to normal. If a taste-guided behavior is disturbed only by combined transection of two nerves, function returns to normal provided both nerves regenerate. If, however, only one nerve is allowed to regenerate in the absence of the other, the ability of the regenerated nerve to support normal behavior may or may not emulate that of the nerve when it is intact. Of course, these conclusions are based on a very limited data set. More experiments must be conducted with a broader array of taste stimuli and a wider variety of behavioral assays focusing on different aspects of taste function to test the generality of these principles. It is nonetheless clear that the gustatory system has a tremendous capacity to functionally recover from sometimes massive insult to the peripheral nerves, provided nerve regeneration takes place.

We are currently exploiting the neurotomy-specific deficits in taste function described above by examining the behavioral consequences of cross-regeneration of the CT and GL. We hope that these new experiments will help reveal the functional principles underlying the architecture of the intact gustatory system and define the boundaries of plasticity following the experimental reorganization of peripheral afferent signals.

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