Intraoral cheek fistulae: a refined technique

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Summary

Taste reactivity testing (TRT), which entails infusing a solution into the oral cavity of subjects, is used across a wide range of studies. For laboratories inexperienced in the conventional technique of implanting cheek fistulae, the surgery can be problematic for both the subjects and the experimenter. We have proposed a refined method for fistulae implantation that is less invasive, thereby reducing the pain and distress of the animals. Using this refined technique, we were able to replicate the findings of previous TRT studies, namely that a high dose of lithium chloride produces an increase in aversive and a decrease in ingestive orofacial and somatic responses. Using indices of health, we demonstrate that unlike animals with the conventional method of fistulae implantation, subjects that receive the refined technique regain their pre-surgery body weights rapidly and show no physical signs of discomfort. Additional advantages of the refined technique are discussed.

Keywords Taste reactivity testing; cheek fistulae; orofacial responses; conditioned taste aversions; rat

Intraoral infusion of a tastant is frequently used in a wide range of experimental situations, including research on ingestive behaviours such as taste palatability (Ganchrow *et al*. 1986), satiety (Grill & Berridge 1985, Kaplan *et al*. 1994) and sodium depletion (Berridge *et al*. 1984). This type of procedure is referred to as taste reactivity testing (TRT). TRT also has been used in learning and memory-related studies using consummatory behaviour and classical conditioning (Grill & Norgren 1978, Berridge *et al*. 1981). However, this procedure is most often used in conditioned taste aversion (CTA) paradigms. A CTA is formed when consumption of a novel substance, typically presented in a bottle or delivered directly into the mouth, is followed by administration of a chemical agent that produces physiological changes indicative of malaise. Consequently, animals will reduce their consumption of the substance during subsequent encounters. Using the TRT procedure in such an experimental design allows for the hedonic evaluation of a novel solution after it has been paired with the avoidance-inducing agent (Grill & Norgren 1978, Brining *et al*. 1991, Parker & MacLeod 1991, Parker 1995). Palatability of a tastant is determined by the animal's reaction to the infusion of the solution into the mouth. Taste reactivity can be ingestive (e.g. tongue protrusions, lateral tongue protrusions and paw licks), ambiguous (e.g. mouth movements) or aversive (e.g. gapes, chin rubs, head shakes, passive drips and forelimb flailing, Spector *et al*. 1988). For example, intraoral infusions of an innately palatable sucrose solution increases the number of paw licks, while infusions of innately unpalatable quinine solution produces head shakes and chin rubs (Grill & Norgren 1978). Learned shifts in hedonic evaluation of solutions occur as a

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consequence of pairing sucrose with a high dose (1.2–3.0 mEq/kg) of lithium chloride (LiCl). Orofacial responses shift from ingestive to aversive upon re-exposure to the sucrose solution after the pairing (Berridge *et al*. 1981, Meachum & Bernstein 1990, Parker & MacLeod 1991, Breslin *et al*. 1992, Parker 1995).

The TRT has many advantages over the traditional bottle test used in conditioned taste avoidance studies. First, the TRT abolishes the need to fluid-deprive the animals when tests of very short duration (seconds to a few minutes) are required since the solution is infused directly into the mouth of the subjects. As such, the subjects are spared the distress created by the deprivation process and more importantly they are not physiologically compromised. Second, the TRT can be more informative compared to a bottle test in a conditioned taste aversion paradigm. Although some agents, such as drugs of abuse, can produce a decrease in the amount consumed in a bottle test after they have been paired with a taste solution, they do not cause a shift from positive to negative orofacial reactions, suggesting that decreases caused by these agents are qualitatively different than those caused by toxins such as LiCl (for a review, see Parker 2003). Third, with the TRT, a researcher is able to assess different taste qualities within the same subject, thereby reducing the number of animals needed in a study.

The traditional method used to study taste reactivity is to implant a cannula that extends from the oral cavity to the top of the animal's head (see Figures 1A and 1B, Grill & Berridge 1985). Except for laboratories with a strong history in using this surgical technique, this procedure can result in many complications, including infection and extreme swelling of the affected side of the face, severe weight loss, and in some cases death of the animal. The ethical use of animals in research dictates that whenever possible, investigators should refine a technique to reduce discomfort and suffering by the animals (Russell & Burch 1959).

The aim of this study was two-fold. The first was to develop a less invasive alternative method for cannulating the oral cavity that would minimize the discomfort experienced by the animals, while simultaneously reducing the time and surgical expertise required to perform the procedure. The second aim was to demonstrate that when using a refined technique in a conditioned taste aversion paradigm, our results would replicate the

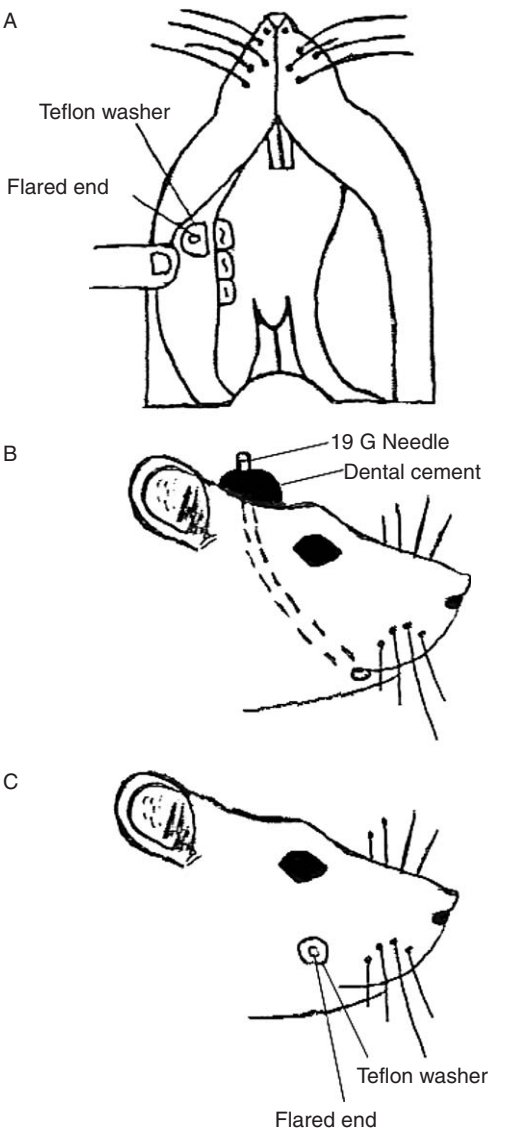

Figure 1 Conventional and refined methods of fistulae implantation. (A) Ventral view of implantation technique. (B) Lateral view of conventional method. (C) Lateral view of refined method

findings of previous TRT studies, namely that a high dose of LiCl paired with a sucrose solution produces aversive orofacial responses to the palatable solution (Berridge *et al*. 1981, Parker 1984, Meachum & Bernstein 1990, Parker & MacLeod 1991, Breslin *et al*. 1992, Parker 1995).

Materials and methods

Subjects

The subjects were 23 male Sprague–Dawley rats (Simonsen Laboratory, Gilroy, CA, USA) that were 64 days old and weighed approximately 250 g at the beginning of the experiment. They were housed in pairs in a room that was temperature (21–22°C), humidity (51%), and light controlled (12 h light:12 h dark cycle with lights on at 10:00 h and off at 22:00 h). Each cage measured 58 × 38 cm and had a solid bottom that was covered with wood chips. The rats were allowed at least one week to adapt to their living conditions before surgery. Rats had *ad libitum* access to rat chow (Rodent Blox, Harlan Teklad, San Diego, CA, USA; protein 24%, fat 4%, fibre 4.5%; Harlan, The Netherlands) and tap water.

On the day of surgery, the animals were anaesthetized using intraperitoneal injections of 5:1 mixture of ketamine hydrochloride (Ketaject: Phoenix Scientific Inc, St Joseph, MO, USA) and xylazine hydrochloride (AnaSed: Ben Venue Laboratories, Bedford, OH, USA) in the dose of 0.15 ml/100 g of body weight. The analgesic, buprenorphine hydrochloride (Buprenex™: Reckitt & Colman Products: Hull, UK; 0.017 mg/kg subcutaneously) and the antibiotic, penicillin benzathine (Dual-Cillin™: G C Hanford Manufacturing Co, Syracuse, NY, USA; 43,000 U/kg subcutaneously) were administered, respectively, to each rat on the day of surgery. The antibiotic was given in the same amount the day before surgery to minimize the susceptibility of the animals to infections due to opportunistic bacteria. The same dose of buprenorphine was also given the day following surgery in order to alleviate any persisting discomfort. Additional postoperative care consisted of weighing and general inspection of the animals (i.e. porphyrin staining around the eyes and nose, fur, mobility) and flushing of the cannulae with sterile water. The experiments were conducted according to the standards set by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (DHEW Publication 80–23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, MD, USA) and the institutional guidelines of the University of Southern California.

Experimental design

Two separate experiments were conducted. In the first experiment, the weights of nine males were measured before and after surgical implantation using the conventional method. In the second experiment, the weights of 14 males were measured before and after surgical implantation using the refined method. In addition, these males were subjected to conditioned taste aversion testing.

Intraoral cannula construction and surgical implantation

For both the conventional and refined methods, the intraoral cannula consisted of polyethylene (PE) 100 tubing (Clay Adams, Franklin Lakes, NJ, USA), with one end flared using a high-temperature cautery blade (Aaron Medical Industries, St Petersburg, FL, USA; see Figure 2). A 19-gauge needle (2.5 cm long with hub removed) was inserted into the lumen of the other end and this end of the tubing was bevelled around the needle. The tubing was threaded through a Teflon washer (outer diameter: 0.7 cm; thickness: 0.07 cm: lumen diameter: 0.1 cm; Small Parts, Inc, Roanoke, VA, USA) so that the washer rested against the flared end of the tubing. To minimize discomfort to the animals, the Teflon washer was cut so that the straight edge would rest on the alveolar ridge of the lower jaw.

Conventional method This procedure included subcutaneous placement of tubing that served as a cannula and extended from

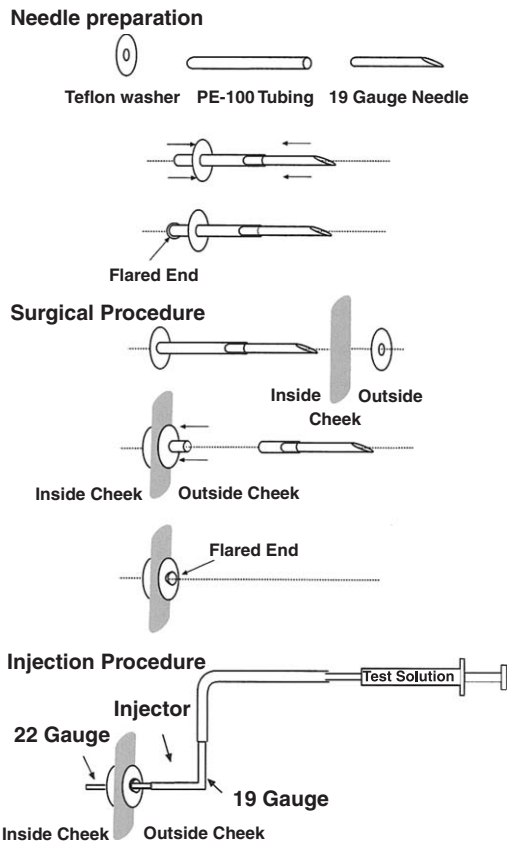

Figure 2 Refined cheek fistula

the cheek to the top of the skull (for illustration, see Figures 1A and 1B, Grill & Berridge 1985). Specifically, each cannula was inserted into the cheek slightly rostral to the first maxillary molar without puncturing the outer skin. The needle was guided subcutaneously along the skull, rostral to the ear and along the zygomatic arch. At the top of the skull, the cannula was adjusted so that the Teflon washer rested against the inside of the animal's cheek. The needle was removed from the cannula and replaced with 19-gauge stainless steel tubing, approximately 0.7 cm long. The cannula with inserted steel tubing was then anchored to the skull using two machine screws and self-curing dental cement.

Refined method In contrast, the refined method that we employed only involved placement of the tubing through the cheek (for illustration, see Figure 1C). The intraoral cannula was approximately 3–4 cm in length (see Figure 2). The needle attached to the tubing was inserted through the cheek just ventral to the second maxillary molar and the tubing was inserted such that the washer was situated flush against the inside of the cheek (see Figure 1A). The cannula was secured on the outside of the cheek by threading another Teflon washer onto the tubing. The tubing on the outside of the cheek was cut approximately 3–4 mm from the washer and heat flared.

Infusion procedure

Conventional method Fluid injections were delivered into the oral cavity through a 19-gauge stainless steel tubing connected to Silastic tubing (45 cm length, 1.6 mm inner diameter, 0.86 mm outer diameter, Dow Corning, Auburn, MI, USA) via a 21-gauge stainless steel 'bridge' (3.8 cm length). The 19-gauge tubing was soldered to the 21-gauge tubing. The other end of the 21-gauge tubing was fitted into the lumen of the Silastic tubing. The tubing was then connected to a syringe filled with the test solution. The test solution was delivered for 30 s at the rate of 1 mL/min using a syringe pump (CMA/100 Microinjection Pump, Carnegic Medicin AB, Stockholm, Sweden).

Refined method An intraoral injector was attached to the tubing on the outside of the cheek only during the time of intraoral infusion. It consisted of a 19-gauge stainless steel tubing, approximately 1 cm long, bent into a 90° angle at a point that was 3/4 of the length from one end (see Figure 2). The long arm was attached to PE 100 tubing, while the short end was soldered to a 22-gauge needle approximately 1 cm in length. The very tip of the needle was removed, leaving a blunted, yet bevelled end. Test solutions were drawn into syringes and connected to the PE 100 tubing via a 21-gauge needle. The solution was delivered for 30 s at a constant rate of 1 mL/min using a syringe pump.

Behaviour testing procedure

Behavioural testing for the animals in the refined experiment was initiated

approximately one week after surgery. The procedure was divided into four periods: preconditioning (days 1–7), acquisition testing (days 8 and 10), post-acquisition recovery (days 9 and 11) and post-acquisition testing (day 12). Prior to each test, the rats were placed in a testing apparatus for 5 min. During preconditioning tests, water was infused into the oral cavity in order to habituate the subjects to the infusion procedure. During acquisition tests, a 10% sucrose solution was infused for 30 s. Immediately afterward, some of the rats ($n = 7$) received an injection of LiCl (0.15 M, 1.5 mEq/kg) while the rest ($n = 8$) received normal saline. This procedure was repeated two days later, resulting in a total of two acquisition tests. A post-acquisition test was given two days after the second acquisition test. This test was conducted in the same manner as the acquisition tests except that LiCl was not administered after the oral infusion. Ingestive (tongue protrusions, lateral tongue protrusions and paw licks) and aversive (gapes, chin rubs, head shakes, passive drips and forelimb flailing), somatic and orofacial responses were recorded during each 30 s test.

Statistical analyses

Differences in postoperative recovery of animals used with the refined technique in the present study and those given the conventional method of implantation in a different study conducted in our laboratory were analysed by comparing the percent weight change (pre- to post-surgery) across post-surgery day 1 and post-surgery day 5 for each of the groups.

For the refined experiment, changes in the combined frequencies of ingestive responses (tongue protrusions, lateral tongue protrusions and paw licks) and aversive responses (gapes, passive drips, head shakes, chin rubs and forelimb flailing) were analysed across the three behavioural tests. In addition, group comparisons were made for each test.

Due to the non-normality and heteroscedasticity of our data, more robust statistical methodologies were used in order to attain reliable results (Wilcox 1992, Wilcox 2003, Wilcox 2005, Hintiryan *et al*. 2005). A percentile bootstrap with 20% trimmed means, which is not predicated on the assumptions of normality or equal variances, was performed to analyse these data. This refined statistical method has been shown to increase power, thus enabling researchers to use fewer subjects per group (Wu 2002, Wilcox 2003). It controls the family wise error rate using adjusted P values (Wilcox & Keselman 2003). In general, this method randomly samples with replacement from the actual data to produce a data-set called a bootstrap sample. Next, the trimmed mean is computed. In the two-sample case, the value of P is computed by repeatedly generating a specified number of bootstrap samples (in this case, bootstrap $n = 1000$). The P value is the probability that a bootstrap trimmed mean from the first group is less than the bootstrap trimmed mean of the second. Finally, the P value is 2 min (P, $1-P$). This procedure can be generalized to a repeated measures design (Wilcox 2003).

The family wise error rate was set at 0.05. For each comparison, a critical significance level is reported along with a significance level. A significant result is obtained when the significance level is lower than the critical significance value. All figures represent trimmed means without error bars (Wilcox 2003).

Results

Body weights

Animals with the refined cheek implant recovered their pre-surgery body weights five days following surgery, while the animals with the traditional cheek implant did not. On post-surgery day 5, the weights of animals with the refined technique did not differ from their pre-surgery body weights (critical significance = 0.05, significance = 0.163; see Figure 3). On the other hand, post-surgery day 5 weights of the animals in the conventional group were significantly lower than their pre-surgery body weights (critical significance = 0.05,

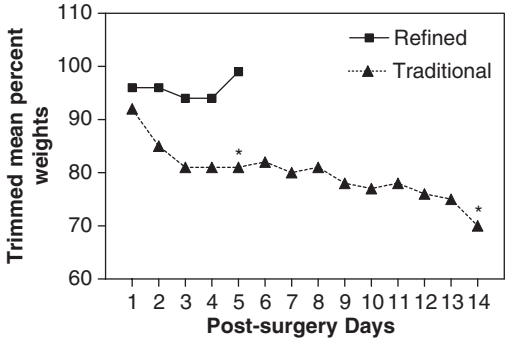

Figure 3 Percent weight change across post-surgery days. Trimmed mean percent weight change (pre-surgery to post-surgery) across five post-surgery recovery days. *Significantly lower percent body weight compared to pre-surgery day weight

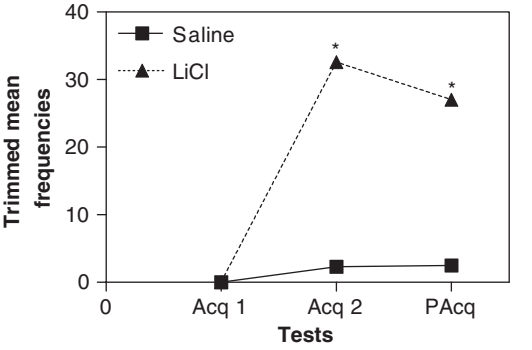

Figure 4 Aversive responses to sucrose across behavioural test days. Trimmed mean frequency of the combined aversive (gapes, chin rubs, head shakes, passive drips and forelimb flailing) responses during acquisition (Acq) and post-acquisition (PAcq) testing. *Significantly higher than the Acq 1 score and the saline group

significance = 0). Furthermore, two weeks following implantation, these rats still had not regained their pre-surgery body weights (see Figure 3). On post-surgery day 14, the animals' weights remained significantly lower compared to their pre-surgery body weights (critical significance = 0.05, significance = 0).

Orofacial responses

One pairing of LiCl with sucrose resulted in an increase in aversive and a decrease in ingestive orofacial and somatic responses. The combined aversive scores for the LiCl group, but not the saline group, were higher during the second acquisition and the post-acquisition test than during the first acquisition test, and the scores for these two groups differed significantly during the second acquisition test and the post-acquisition test (critical significance = 0.02–0.03, significance <0.01 in each case, see Figure 4). On the contrary, the combined ingestive scores for the saline group, but not the LiCl group, were higher during the second acquisition test and the post-acquisition test than during the first acquisition test, and the scores for these two groups differed significantly during the second acquisition test and the post-acquisition test (critical significance = 0.02–0.03, significance <0.01 in each case, see Figure 5).

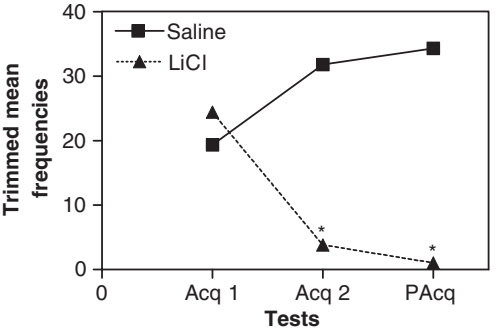

Figure 5 Ingestive responses to sucrose across behavioural test days. Trimmed mean frequency of the combined ingestive (tongue protrusions, lateral tongue protrusions and paw licks) responses during acquisition (Acq) and post-acquisition (PAcq) testing. *Significantly lower than the Acq 1 score and the saline group

Discussion

The refined method for surgical implantation of an intraoral cannula is less invasive than the conventional method, involving a simple cheek puncture as opposed to subcutaneous implantation extending from the cheek to the top of the skull. Animals that received this refined

implantation showed no porphyrin staining or encrustation (i.e. chromodacryorrhoea) around the eyes or nose, both of which are signs of pain and distress in rats (Harkness & Ridgway 1980). Additionally, there were no infections around the implants and none of the animals appeared unkempt. By post-surgery day 5, animals had recovered their pre-surgery body weights. Furthermore, the ability to produce data similar to those obtained when the conventional method is used suggests that data are not compromised with this less invasive and distressing procedure (Grill & Norgren 1978, Berridge *et al*. 1981, Meachum & Bernstein 1990, Parker & MacLeod 1991, Breslin *et al*. 1992, Parker 1995).

Following proficient training in both the refined and conventional techniques, our laboratory compared the recovery of body weights of the animals in each of the studies. The comparison revealed that animals that received the refined technique of implantation had recovered their pre-surgery body weights by post-surgery day 5, while the weights of those subjected to the conventional method remained significantly lower than the pre-surgery body weights 14 days following surgery.

It is acknowledged that the animals were in two separate studies and that direct experimental comparison of the two methods would have been more elegant. However, after refining the technique, it was deemed unethical to subject the animals to the more invasive procedure for the sole purpose of experimentally comparing the surgical outcome of the two procedures. Since weight, which is an objective, sensitive and reliable indicator of rodent health (Morton & Griffiths 1985, Morton 1997, Hawkins 2002) was available for both groups, it was used as the measure of comparison. As illustrated in Figure 3, the difference in the recovery of body weights for each of the groups is striking and provides sufficient evidence for the less invasive nature of the refined technique. It is irrefutable that bringing animals within their normal homeostatic range is in the interest of the animals, but it is also consistent with the objectives of many studies; non-experimental variation may confound the interpretation of the data and would prove to be a misuse of both animals and valuable resources. This is especially true when conducting TRT studies. Because the purpose of these studies entails testing for shifts in hedonic valence of a particular food due to the administration of an illness-inducing agent, it is imperative that animals be completely recovered from surgery and show no signs of sickness prior to behaviour testing.

The inability of animals to recover their body weights in a timely fashion following surgery may pose additional problems depending upon the nature of the experiment. For instance, it has been consistently and reliably demonstrated across various species that oestradiol produces reductions in body weight (Gilbert & Gillman 1956, Ter Harr 1972, Czaja & Goy 1975, Roy & Wade 1977, Butera & Czaja 1984, Gong *et al*. 1989, Geary 2001). One of the aims of our study using the conventional method was to examine the effects of oestradiol on consumption of and taste reactivity to a sucrose solution. However, previous research has demonstrated that if body weight is reduced prior to oestradiol administration, the oestradiol-induced reduction in food consumption is no longer expressed (Blaustein & Wade 1976). Thus, it is imperative that animals regain their pre-surgery weight before the effects of oestradiol on food intake can be examined. Even after two weeks, there was no indication of a return to pre-surgery weights using the conventional method.

Aside from a faster recovery, there are additional advantages to using this refined method. First, the time required for the completion of the refined surgery (approximately 8 min) is shorter than the time required for the conventional method (approximately 25 min). This serves as an advantage for the subjects by minimizing the time they have to be anaesthetized, thus reducing the possible complications associated with using aesthetics for an extended period of time (i.e. hypothermia and respiratory distress). Second, because there are minimal surgery-related

complications and extremely low attrition rate, studies do not require excessive numbers of animals to ensure a desired number of animals per cell for reliable statistical results. Third, due to the simple nature of the surgery, fewer pilot animals are needed to train the surgeons. Finally, with the refined technique, social animals like rats can continue to be housed together without jeopardizing the integrity of the implant. The cannulae implanted using the refined method lasted until the end of the study (approximately 3 weeks after surgery) with the animals being isolated only on post-surgery day 1. To minimize damage to the implant caused by their cage-mates, which would cause further pain and distress, animals used in the conventional method were kept separated for the duration of the experiment.

When using this procedure, five caveats can improve success of the outcome. First, it is important that an optimal length is used for the cannula. A cannula that is too long can easily be damaged (e.g. chewing, tearing) by the subject or by its cage-mates. On the other hand, a length that is too short can cause either absorption of the cannula (e.g. washer) into the cheek or cause the washers to slip off the flared end(s) of the cannula. Second, it is important to practise flaring the end of the PE tubing. Excessive flaring will cause the lumen of the cannula to close, while insufficient flaring can result in the washers slipping off the ends. Third, depending on the size of the animal, it may be necessary to adjust the size of the washer. This can be accomplished by either cutting washers with a smaller outer diameter or cutting opposite sides of the originally described washers. Fourth, the pointed tip of the injector should be somewhat blunted, but not completely removed. This shape decreases the chances of puncturing the tubing, which could cause the cannula to leak. Another benefit of the slightly blunted shape of the injector is that the animals are discouraged from blocking the flow of the injector with their tongues. The animals could prevent the flow of the undesirable fluid into their mouths by occluding the fistulae opening with their tongue. Fifth, the cannulae should be flushed periodically to prevent blockage of the lumen caused by food. With such routine postoperative care, the animals also become habituated to handling and other experimental manipulations.

In conclusion, this simple, yet viable, procedure for intraoral cannulation is a refinement of the conventional method that reduces pain and discomfort in animals. Reducing pain and distress for animals and minimizing non-experimental variation (due to discomfort of subjects) are complimentary objectives for animal research. Therefore, returning animals to physiological homeostasis as soon as possible is in the best interest of both the research subjects and the experimenter.

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References

Berridge KC, Flynn FW, Schulkin J, Grill HJ (1984) Sodium depletion enhances salt palatability in rats. *Behavioral Neuroscience* **98**, 652–60

Berridge K, Grill HJ, Norgren R (1981) Relation of consummatory responses and preabsorptive insulin release to palatability and learned taste aversions. *Journal of Comparative and Physiological Psychology* **95**, 363–82

Blaustein JD, Wade GN (1976) Ovarian influences on the meal patterns of female rats. *Physiology and Behavior* **17**, 201–8

Breslin PAS, Spector AC, Grill HJ (1992) A quantitative comparison of taste reactivity behaviors to sucrose before and after lithium chloride pairings: a unidimensional account of palatability. *Behavioral Neuroscience* **106**, 820–36

Brining SK, Belecky TL, Smith DV (1991) Taste reactivity in the hamster. *Physiology and Behavior* **49**, 1265–72

Butera PC, Czaja JA (1984) Intracranial estradiol in ovariectomized guinea pigs: effects on ingestive behaviors and body weight. *Brain Research* **322**, 41–8

Czaja JA, Goy RW (1975) Ovarian hormones and food intake in female guinea pigs and rhesus monkeys. *Hormones and Behavior* **6**, 329–49

Ganchrow JR, Steiner JE, Canetto S (1986) Behavioral displays to gustatory stimuli in newborn rat pups. *Developmental Psychobiology* **19**, 163–74

Geary N (2001) Estradiol, CCK and satiation. *Peptides* **22**, 1251–63

Gilbert C, Gillman J (1956) The changing pattern of food intake and appetite during the menstrual cycle of baboon (*Papio ursinus*) with consideration of some of the controlling endocrine factors. *South African Journal of Medical Sciences* **21**, 75–88

Gong EJ, Garrel D, Calloway DH (1989) Menstrual cycle and voluntary food intake. *American Journal of Clinical Nutrition* **49**, 252–8

Grill HJ, Berridge KC (1985) Taste reactivity as a measure of the neural control of palatability. *Progress in Psychobiology and Physiological Psychology* **11**, 1–61

Grill HJ, Norgren R (1978) Chronically decerebrate rats demonstrate satiation but not bait shyness. *Science* **201**, 267–9

Harkness JE, Ridgway MD (1980) Chromodacryorrhea in laboratory rats (*Rattus norvegicus*): etiologic considerations. *Laboratory Animal Science* **30**, 841–4

Hawkins P (2002) Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Laboratory Animals* **36**, 378–95

Hintiryan H, Hayes UL, Chambers KC (2005) The role of histamine in estradiol-induced conditioned consumption reductions. *Physiology and Behavior* **84**, 117–28

Kaplan JM, Siemers W, Grill HJ (1994) Ingestion, gastric fill, and gastric emptying before and after withdrawal of gastric contents. *American Journal of Physiology* **267**, 1257–65

Meachum CL, Bernstein IL (1990) Conditioned responses to a taste conditioned stimulus paired with lithium chloride administration. *Behavioral Neuroscience* **104**, 711–15

Morton DB (1997) A scheme for the recognition and assessment of adverse effects in animals. In: *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Bamms M, eds). Amsterdam: Elsevier Science BV, 235–40

Morton DB, Griffiths PHM (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and a hypothesis for assessment. *Veterinary Record* **116**, 431–6

Parker LA (1984) Behavioral conditioned responses across multiple conditioning/testing trials elicited by lithium- and amphetamine-paired flavors. *Behavioral and Neural Biology* **41**, 190–9

Parker LA (1995) Rewarding drugs produce taste avoidance, but not taste aversion. *Neuroscience and Biobehavioral Reviews* **19**, 143–51

Parker LA (2003) Taste avoidance and taste aversion: evidence for two different processes. *Learning and Behavior: A Psychonomic Society Publication* **31**, 165–72

Parker LA, MacLeod KB (1991) Chin rub CRs reflect conditioned sickness elicited by a lithium-paired sucrose solution. *Pharmacology Biochemistry and Behavior* **40**, 983–6

Roy EJ, Wade GN (1977) Role of food intake in estradiol-induced body weight changes in female rats. *Hormones and Behavior* **8**, 265–74

Russell WMS, Burch RL (1959) *The Principles of Humane Experimental Technique*. London: Methuen

Spector AC, Breslin P, Grill HJ (1988) Taste reactivity as a dependent measure of the rapid formation of conditioned taste aversion: a tool for the neural analysis of taste-visceral associations. *Behavioral Neuroscience* **102**, 942–52

Ter Harr MB (1972) Circadian and estrous rhythms in food intake in the rat. *Hormones and Behavior* **3**, 213–19

Wilcox RR (1992) Why can methods for comparing means have relatively low power, and what can you do to correct the problem? *Current Directions in Psychological Science* **1**, 101–5

Wilcox RR (2003) *Fundamentals of Modern Statistical Methods: Substantially Improving Power and Accuracy*. New York: Springer-Verlag

Wilcox RR (2005) New methods for comparing groups: strategies for increasing the probability of detecting true differences. *Current Directions in Psychological Science* **14**, 272–5

Wilcox RR, Keselman HJ (2003) Modern robust data analysis methods: measures of central tendency. *Psychological Methods* **8**, 254–74

Wu PC (2002) *Central Limit Theorem Comparing Means, Trimmed Means, One-Step M-Estimators and Modified One-Step M-Estimators under Non-Normality*. Unpublished Dissertation, Los Angeles: University of Southern California