ARTICLE
Pedagogical Activities for Assessing Human and Rat Taste-Related Behavioral Responses to *Gymnema sylvestre*

Elisa S. Na[1] and Michael J. Morris[1, 2]

[1]Department of Psychology and Philosophy, Texas Woman's University, Denton, TX 76204. [2]Previous address: Department of Natural Sciences, University of Michigan – Dearborn, Dearborn, MI, 48198.

Extracts of the plant *Gymnema sylvestre* are known to block sweet receptors on the tongue and have been previously used to demonstrate principles of taste sensation and perception in undergraduate neuroscience courses. We have combined demonstrations of altered perception of sweet and bitter in student participants with a laboratory taste reactivity protocol in which rat orofacial responses to intraoral infusion of a 2% sucrose solution before and after exposure to *Gymnema sylvestre* were quantified. As expected, after experiencing *Gymnema sylvestre* students reported dramatic changes in perception of sweet tastes and a tendency to perceive sugary tastants as increasingly bitter. In rats, infusion of *Gymnema sylvestre* for 1 minute led to an increase in total number of aversive responses to 2% sucrose. The results promoted discussion of qualitative similarities and differences in observable behavioral responses between species and served as an effective springboard for lecture coverage of sensory physiology and gustatory transduction. Moreover, these activities in combination may be useful as an introduction to animal models of human phenomena in behavioral neuroscience as well as discussions centered on issues of anthropomorphism and research design.

Key words: Taste reactivity, laboratory exercise, animal model, Gymnema sylvestre

The ability of *Gymnema sylvestre* to decrease the perception of sweet tastes in humans was first described in an empirical report by Edgeworth in 1847 (Edgeworth, 1847). Extracts from the plant suppress sweet transduction presumably by their direct inhibition of sucrose receptors (Diamant et al., 1965; Bartoshuk, 1969). Animal studies assessing taste-related responses to isolates from *Gymnema sylvestre* have relied primarily on electrophysiological recordings from the chorda tympani nerve, which conveys gustatory information to the brainstem, whereas perceptual effects can be assayed more directly in humans (e.g., verbal report) (Bartoshuk, 1969; Hellekant and Gopal, 1976; Hellekant et al., 1985; Imoto et al., 1991; Hellekant et al., 1996; Ninomiya et al., 1999; Aleman et al., 2016). At present, two distinct substances isolated from *Gymnema sylvestre* are known to have species-dependent effects on suppressing responses to sweet tastants. In a series of studies, Hellekant and colleagues have demonstrated that the 35 amino acid peptide Gymnemic acid has differential potency for suppression of chorda tympani nerve responses to sugars depending on species. For example, in chimpanzees, in addition to reducing voluntary preference for saccharin, application of 2 ml of gymnemic acid on the tongue produced a 75% suppression of chorda tympani nerve response to 0.3 M sucrose, complete suppression to 0.0018 M aspartame, and no suppression to quinine (0.001 M), NaCl (0.1 M), ascorbic acid (0.04 M), or citric acid (0.04 M), with somewhat less robust effects observed in hamster, and very little effect in rat, pig, or rabbit (Hellekant and Gopal, 1976; Hellekant et al., 1985; Hellekant et al., 1996). A second, more recently discovered extract, dubbed "gurmarin" (in reference to "Gurmar" which translates to "sugar destroyer" in Hindi) reduced chorda tympani responses to sucrose in rat and mouse (Imoto et al., 1991; Ninomiya et al., 1999) and also selectively suppressed responses to sucrose in neurons in the nucleus of the solitary tract (NTS), a nucleus that receives chorda tympani afferent input (Lemon et al., 2003). Thus, distinct components of the *Gymnema sylvestre* plant alter sweet perception or neurological responses to sugar, and these effects are variably robust between species.

Schroeder and Flannery-Schroeder (2005) have used *Gymnema sylvestre* as a pedagogical tool that provides students with hands-on experiences to accompany lecture material covering taste sensation and perception. Similarly, we have utilized a modified version of their activity to enhance students' appreciation of taste transduction, reinforce the concept that there are distinct modes of receptor-mediated transduction, and to introduce concepts of "across–fiber pattern" processing vs. "labeled line" processing with respect to the gustatory system. "Miracle berries", which lead to the perception of sour tastes being perceived as sweet, have been used in a similar fashion to enhance the teaching of taste sensation and gustatory physiology (Lipatova and Campolattaro, 2016).

In the current report we similarly utilized *Gymnema sylvestre* to enhance student learning of sensation and perception and combined this activity with a novel laboratory activity that used taste reactivity testing to quantify rat orofacial behavioral responses to infused *Gymnema sylvestre*. The taste reactivity paradigm ensures experimenter control over the amount and duration of exposure to tastants and enables quantification of stereotypic orofacial responses by fixation of a cannula in an animal's oral cavity (Grill and Norgren, 1978). The paradigm has been frequently used to assess changes in

reward valence in various physiological states, as well as the dissociable nature of reward and motivational circuits in the brain (Pecina et al., 2006; Na et al., 2012). Typically, both aversive and appetitive behaviors in response to infused tastants are quantified in experimental studies using this method. We chose to limit our analyses to only aversive responses as it was not considered feasible to quantify both aversive and appetitive responses in a single laboratory session, and appetitive responses can be relatively more difficult for an untrained observer to distinguish and quantify accurately. As far as we are aware, few activities in introductory level neuroscience courses provide students with opportunities to draw comparisons between their own perceptual experiences and behavioral events occurring in animal models designed to putatively assess similar phenomena. Beyond enhancing students' basic understanding of gustatory physiology, the activities were intended to elicit discussion of the appropriate use of animal models in behavioral neuroscience and to highlight the difficulties in making behavioral comparisons between species.

LEARNING OBJECTIVES

The primary learning objectives for these activities were as follows:

1. To provide students an opportunity to conduct experiments and quantify behaviors in animal and human behavioral experiments related to gustatory sensation and perception
2. To assess students' ability to describe and qualitatively analyze animal behavioral data from a taste reactivity experiment and compare the results to their own experiences with *Gymnema sylvestre*.
3. To encourage discussion of the use of animal models in behavioral neuroscience to aid in understanding human physiology and behavior.
4. To increase student understanding of research design and methods

To assess these objectives, students were required to submit laboratory reports (a single report per lab group) within one week of the conclusion of the activity. We encouraged and directed general discussion of these objectives during the lecture portion of the course. In addition, we included an extra credit short answer question on an exam covering sensation and perception.

MATERIALS AND METHODS

Human Subjects

Subjects were 32 undergraduate students in an Introduction to Neurobiology course at the University of Michigan-Dearborn and 42 students in a Physiological Psychology course at Texas Woman's University. The human portion of the study was carried out during a lecture session in these courses and required 30- 40 minutes to complete. *Gymnema sylvestre* (Starwest Botanicals) was brewed within 1-2 h of the demonstration, using ¼ cup of the herb leaf in 1 quart of boiling water for 10 minutes. Based on previous work suggesting differential efficacy, we used the raw leaves, rather than the capsulated form for the activity (Schroeder and Flannery-Schroeder, 2005). The tea can be served at a wide range of temperatures and maintain its efficacy.

Students were read the following prior to initiation of the activity: "We are conducting a research study on tastants to assess how consumption of *Gymnema sylvestre* tea affects your ability to perceive different tastants. As a participant, you will spend approximately 30-40 min in this study. You will be asked to taste and rate your perception of each of the following: salt, Equal, sugar, Skittles and Coca-Cola. You will then swish *Gymnema sylvestre* tea for 20-30 seconds. Next, you will sample each of the tastants again and rate your perception of the tastes. Your participation in this experiment is completely voluntary. You can withdraw at any time from this study. Your decision to participate in this study will not affect your grade or relationship to the instructor. All information regarding this study is confidential and no identifying information will be linked to your participation. You will receive extra credit for this study and these points will be added to your total grade". All procedures were approved by the Institutional Review Board (IRB) prior to being conducted. Per IRB requirements, students that chose not to take part in the demonstration were offered an alternative extra credit assignment. The majority of students in attendance chose to take part in the demonstration (86%). Diabetic students were encouraged to opt for the alternate assignment if not comfortable with a low amount of sugar intake. For the sake of privacy, students that chose not to participate were not required to disclose any reasoning for opting out, nor did these students inform the instructor of their reasoning. The alternate assignment in this case was to submit a summary (2-3 page, single spaced) of a peer-reviewed journal article that used the taste reactivity method.

Students were instructed to sample a "small amount" of sugar, Equal, or salt at the tip of the small plastic spoon (demonstrated by the authors), approximately 1/3rd of a 3 oz disposable cup full of Coca – Cola, and 2-3 Skittles . These substances were chosen based on previous work (Schroeder and Flannery-Schroeder, 2005) as well as personal experience of the authors. Bottled water was provided and students were instructed to rinse and swallow a small amount of water between each sampling throughout the activity. Students then gargled with *Gymnema sylvestre* for 20 - 30 seconds to allow for sufficient coating of the tongue and blockade of taste buds. The tea could be swallowed or expectorated with no obvious qualitative or quantitative differences in the results. Students sampled each substance again in the same order and were asked to rate each on a scale of 0 to 10 for the perceived intensity of "saltiness", "sweetness", and "bitterness" (0 being no intensity and 10 being very intense; students rated the "saltiness" of salt, but not any perceived sweetness, and the "sweetness" of the other 4 tastants; "bitterness" was rated for all 5 tastants before and after exposure to the tea.

Animal Subjects

A total of 22 male Sprague-Dawley rats (Charles River Laboratories) weighing 250–350 g were used for the

animal portion of this activity, which was conducted at University of Michigan-Dearborn. Rats were maintained on a 12-hour light/dark cycle in a temperature- and humidity-controlled room for two weeks prior to testing. Rats were given *ad libitum* access to water and standard rat chow (Teklad Standard Irradiated Diet, Harlan Laboratories). All procedures were approved by the University of Michigan Institutional Animal Care and Use Committee (IACUC) prior to initiation.

Implantation of Intraoral Cannula in Rat

An intraoral (I-O) cannula was created by threading a length (approximately 5 cm) of polyethylene tubing (PE-50) through two washers composed of a 7 mm circular polyethylene stopper to hold the cannula in place in the oral cavity as well as subcutaneously at the exit point. The PE-50 was secured in place by flaring the ends of the tubing. Using a trocar, the tubing was tunneled subcutaneously behind the last maxillary molar and out of an exit point located posterior to the ears, and slightly anterior to the shoulder blade and then secured using 4-0 sterile suture and veterinary surgical glue (Vetbond, University of Michigan Pharmacy). Surgeries were performed by the authors 5-6 days prior to the laboratory exercise. Rats were anesthetized using isoflurane (5% for induction, 1-2% for maintenance). In experienced hands the surgery requires approximately 10 -15 minutes including induction of anesthesia. Rats were allowed to recover for five days during which time they received moistened powdered rat chow and daily flushing of the cannulas with approximately 0.5 ml distilled water to ensure proper functioning and to adapt the rats to the sensation of infusion. Animals with non-functional or occluded cannulas (n=4) were excluded from the experiments. We believe it would be feasible for students to perform these surgeries following proper IACUC training and approval. Our course was relatively large and time did not permit us to train students to perform the surgeries as the entire exercise was limited to a single lab session. An additional reason the authors performed the surgeries as opposed to the students was due to the size of our section (32 students) making it difficult for all to participate, and difficult to accommodate a large number in our surgical space. See Table 1 for a list of materials required for implantation of oral cannula in the rat. For additional description and guidance regarding the intraoral cannula surgeries, please see Grill and Norgren (1978) (King et al., 1999).

Taste Reactivity Test In Rat

The base for the taste reactivity chamber was comprised of an 11 X 11 X 11-inch Plexiglas box (shoppopdisplays.com) cut to hold a mirror that was positioned at an approximately 45-degree angle. A video camera was directed at the mirror to record rat orofacial responses from below. During intraoral infusions students placed the rats in a 4-liter beaker that was cleaned with 70% ETOH and allowed to dry between subjects. The intraoral catheter was connected to an infusion pump (Braintree Scientific) which dispensed solutions at a rate of 0.25 ml per minute. Approximately 3 ft of PE-50 line was needed to connect the infusion pump to the rats I/O cannula. The dead space in the line was cleared prior to connecting the I/O cannula. Rats were allowed to adapt to the apparatus and the sensation of intraoral infusion by a water infusion for 3 minutes the day prior to testing during the laboratory activity. For the experimental group (n=9) testing included the following sequence of infusions: 2% sucrose for 3 minutes, distilled water for 30 seconds, *Gymnema sylvestre* tea for 1 minute, distilled water for 30 seconds, and finally another infusion of 2% sucrose for 3 minutes. The control group (n=9) was exposed to the same sequence, with *Gymnema sylvestre* replaced by distilled water. For both groups 3-minute rest periods were given between each infusion. Sucrose solution was chosen as it is a potent agonist at sweet receptors, is commonly used as a reward in animal behavioral experiments, and is frequently used to assess hedonic state (e.g., anhedonia) in animal models (Morris et al., 2006).

Students, working in groups of 4 or 5, were asked to observe the infusions and the elicited behaviors in real time, and subsequently to analyze the video-recorded behavioral responses on a laptop computer. Each group recorded data from 2-3 rats. The frequency of “gapes”, “full-body shakes”, “face wipes”, and “forelimb flails” were recorded upon completion of all infusions. A “gape” is a rapid and very wide opening of the mouth; during a “face wipe” the rat typically reaches both forepaws behind the ears and rubs them toward the chin; a “full body shake” appears similar to a “wet-dog” shake which involves a rapid whole-body shaking movement; a “flail” is a rapid, symmetrical movement of the forelimbs which are typically in close proximity to the rat’s face when the movement occurs. Appetitive behaviors (e.g., lateral and midline tongue protrusions, paw licking) were not scored during the laboratory exercise as their scoring, in the opinion of the authors, tends to be more time-consuming and can be challenging for untrained observers to quantify relative to aversive responses. For further description of typical responses quantified in taste reactivity tests, including appetitive behaviors, see Grill and Norgren (1978). Given the nature of the activity, scoring restricted to aversive behaviors was deemed appropriate to not only assess the effects of *Gymnema sylvestre* on responses to sucrose, but also to promote discussion of the comparison of the responses in the rat to what the students reported following their own experience. Data were recorded on pre-made score sheets with one data sheet turned in per group. The authors then collated the data for dissemination to the students during lecture, and for presentation in the current report. See Table 1 for a list of materials needed for performing the taste reactivity exercise. Statistical testing was not performed by students in the course as statistics courses were not required as a prerequisite for enrollment in either course in which our study was conducted. For the human portion of the exercise, paired t-tests were used for comparison of subjective ratings of sweetness and bitterness before and after exposure to *Gymnema sylvestre*. A mixed-model ANOVA was utilized to determine if there was a significant interaction effect

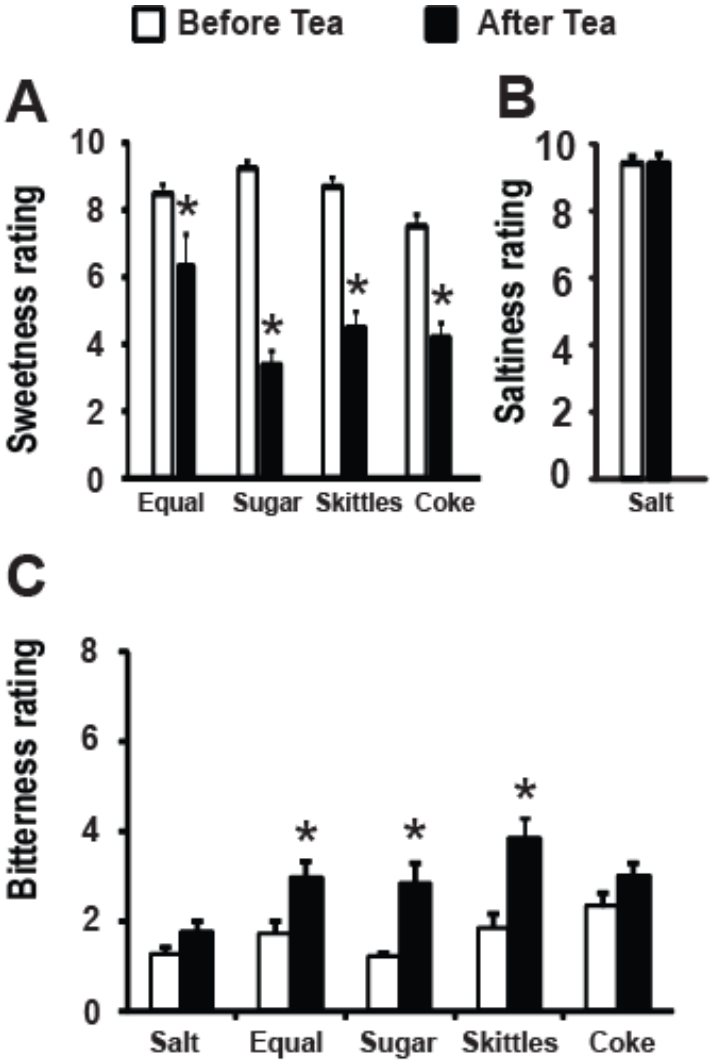

Figure 1. Students' subjective responses to various tastants before and after exposure to *Gymnema sylvestre*. Data are presented as mean + standard error. *A.* Subjective ratings of the sweetness of Equal, sugar, Skittles, and Coca-Cola significantly declined following *Gymnema sylvestre*. *B.* Ratings of saltiness were not altered by *Gymnema sylvestre*. *C.* Bitterness rating significantly increased for Equal, sugar, and Skittles *$p<0.05$ as compared to before tea rating.

between infusion number X group, with paired t-tests for post hoc comparisons following a significant global F ratio. Statistical analyses were performed using SPSS statistical software. Statistical significance was defined as $p \leq 0.05$.

RESULTS

Activity 1: The Effects of *Gymnema sylvestre* on Taste Perception in Undergraduate Students.

This activity was based on previously published work in the Journal of Undergraduate Neuroscience (Schroeder and Flannery-Schroeder, 2005). As reported previously, the effects of *Gymnema sylvestre* on human taste perception were profound. Exposure to the tea dramatically altered the reported perception of sweet tastants (see Figure 1A) with a somewhat less robust effect apparent for aspartame (Equal) which is a more powerful agonist for sweet receptors than are natural sugars. While the perception of sweet was clearly diminished, students reported little if any change in their perception of salty (Figure 1B). As shown before, exposure to *Gymnema sylvestre* led to a shift in typically sweet tastants being perceived as increasingly bitter (Figure 1C). Students were engaged in the demonstration and surprised by the dramatic effects of the tea on their perception of sweet and bitter. Based on conversations between the authors and student participants, as well as previous work (Diamant et al., 1965; Schroeder and Flannery-Schroeder, 2005), the effects of *Gymnema sylvestre* on taste perception are reversible in an hour or less.

Activity 2: The Effects of Intraoral Infusion of *Gymnema Sylvestre* on Taste Reactivity Responses to 2% Sucrose Solution In Sprague-Dawley Rats.

Following a significant global F ratio, $F_{(1,6)} = 4.674$, $p=0.046$, it was determined that rats infused with *Gymnema* tea demonstrated a significant and robust increase in total number of aversive responses to a subsequent infusion of 2% sucrose solution (M$\pm$SD for sucrose infusion 1 = 7.22$\pm$1.27; sucrose infusion 2 = 20.0$\pm$3.53; $p < 0.05$; see Figure 2A, B), whereas total number of aversive responses to the second sucrose infusion in the control group were similar to the total number observed during the first infusion (M$\pm$SD for sucrose infusion 1 = 5.23$\pm$1.49; sucrose infusion 2 = 8.11$\pm$2.21; Figure 2A, B). The most frequently occurring aversive response for the control and experimental groups for both sucrose infusion 1 and infusion 2 were face wipes (M$\pm$SD control infusion 1 = 4.22$\pm$1.28; infusion 2 = 6.66$\pm$1.88; experimental infusion 1 = 2.88$\pm$1.05; infusion 2 = 12.0$\pm$1.83; $p<0.05$ for the experimental group, infusion 2 vs. infusion 1; see Figure 2C for example face wipe). The number of gapes increased in the experimental group during the second sucrose infusion (7.55$\pm$2.05) as compared to the first (2.77$\pm$0.86), which did not occur in the control group (0$\pm$0; 0.11$\pm$0.11; $p<0.05$ for experimental group infusion 2 vs. infusion 1; see Figure 2C for an example of a gape). "Wet-dog shakes" and "flails" occurred infrequently regardless of group or infusion (Figure 2A). Aversive responses by the experimental group to the tea infusion were elevated in comparison to the control group in response to water (control = 1.55$\pm$0.41 experimental = 7.23$\pm$1.52).

DISCUSSION

In the current studies, carried out in undergraduate neuroscience courses, we have combined lecture and laboratory activities designed to assess behavioral and subjective responses to sweet and salty tastants following exposure to the herb *Gymnema sylvestre*. We have repeated a previously published activity with student participants (Schroeder and Flannery-Schroeder, 2005) and introduced cross-species qualitative comparisons by utilizing a taste reactivity laboratory exercise in rats. Students reported dramatic changes in their perceived ratings of sweet for multiple tastants and rated tastants as increasingly bitter. In rats, we observed a large increase in

number of aversive responses to 2% sucrose following exposure to *Gymnema*. Students did not necessarily describe the experience as aversive *per se*, but rather, for example: "weird", "sugar tastes like "sand", "I would never drink Coke if it tasted like that", Skittles taste "gross". In particular, changes in the experience of drinking Coca-Cola seemed to have the greatest subjective impact in terms of demonstrating the sweet blockade of *Gymnema sylvestre*. Many students expressed the observation that rats' behavioral responses to sugar/sweet appeared to be similar to what they experienced in the demonstration. Importantly, the combined activities were successful in eliciting discussion and debate regarding the applicability and appropriate use of animal models to understand complex human behavior, and stoked broader discussions of anthropomorphism in behavioral sciences. Many of these discussions occurred *ad hoc* in the laboratory section of the course, and in speaking with students at the beginning and end of lecture sessions. For future iterations of the exercise, we believe that the addition of a short demonstration with instruction of graphing and statistics, and subsequent requirement that the students graph and analyze human and rat data using excel, will improve the activity. Under these circumstances, two laboratory sessions devoted to the exercise may be more appropriate. In addition, students in our lab sections did not directly handle the rats as they lacked the appropriate training and experience, and in some cases verbally expressed hesitancy in being near the animals. The overall experience would likely be enhanced if IACUC training were provided prior to the exercise allowing students a more direct hands-on experience, if they chose the option to handle the rats.

We conducted this activity prior to lectures on taste and olfaction and believe that the exercise substantially increased engagement in the lecture material. As described previously (Schroeder and Flannery-Schroeder, 2005), the exercise is conducive to enhancing students' appreciation of the molecular events underlying gustatory transduction, and the distinctions between sensation and perception. In our case, students had already been exposed to lectures describing ion channels and distinct receptor types (e.g., ionotropic vs. metabotropic) which facilitated more targeted discussions of mechanisms underlying gustatory transduction specifically.

Taste as a sensory system may be readily amenable to student comparisons between their own subjective experience and the behavioral responding of rats (e.g., responses can be measured in real-time; rat gustatory physiology is in many ways comparable to human, and rat orofacial responses to sweet and bitter are strikingly comparable to motor responses of human infants; Berridge, 2000). We suggest further experiments and manipulations that can be introduced beyond what is reported here. For example, changes in taste reactivity in various need states could be addressed (e.g., following sodium restriction, mild food deprivation or restriction – manipulations that conceivably could be introduced in both human and rat). In rats, these experiments could then be combined with pharmacological treatment(s); for example,

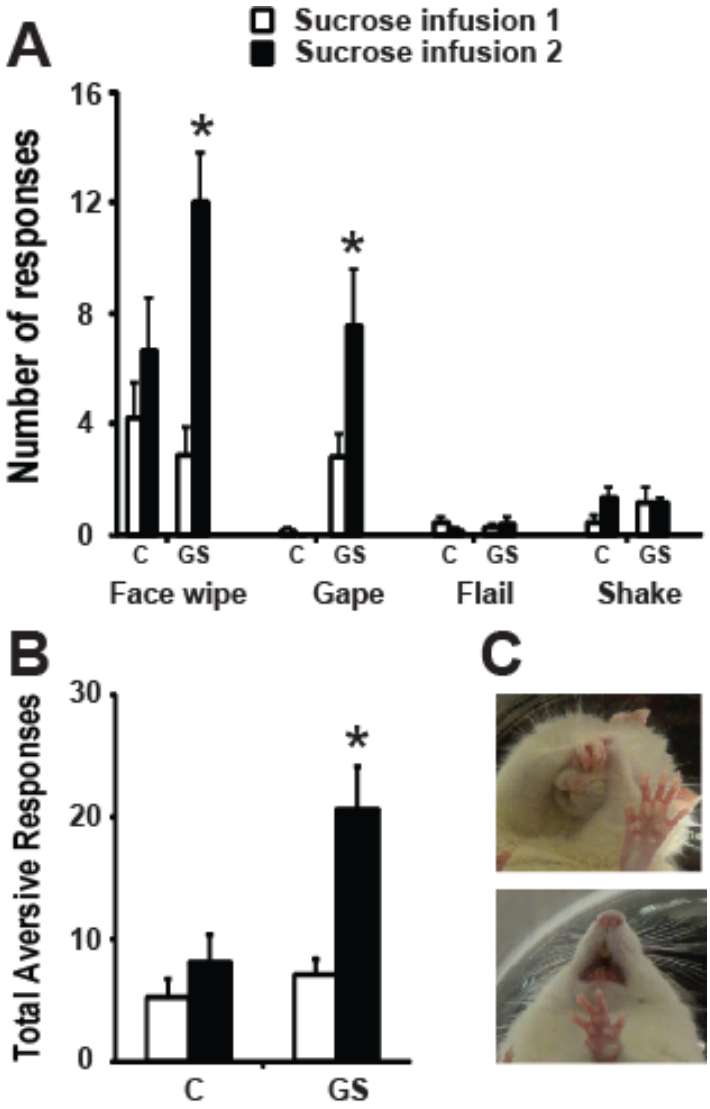

Figure 2. Rat taste reactivity responses to intraoral 2% sucrose before and after exposure to water or *Gymnema sylvestre*. "C" refers to the control group infused with water between sucrose infusions 1 and 2; "GS" refers to the group infused with *Gymnema sylvestre* between sucrose infusions. Data are presented as mean + standard error. *A.* Number of face wipes and gapes increased in response to 2% sucrose after infusion of *Gymnema sylvestre*, an effect not observed in the control group. Number of flails and shakes occurred infrequently across all infusions. *B.* Total number of aversive responses to 2% sucrose were significantly greater following *Gymnema sylvestre* but not significantly increased in response to the second sucrose infusion in the control group. *C.* Example of facewipe (top) and gape (bottom). *$p<0.05$ compared to sucrose infusion 1.

manipulation of central opioid receptors has been shown to impact taste reactivity in states of relative sodium deficit (Na et al., 2012). In courses lacking a laboratory component, videos of rat behavioral responses can be shown in lecture and provide novel activities that encourage students to form hypotheses, analyze data/videos individually or in groups, write-up the data, and create figures. Attempts such as these at drawing comparisons between species can introduce the concept of anthropomorphism, provoke discussion and debate

ITEM	VENDOR	CATALOG #	PRICE
Gymnema sylvestre leaf – 1 lb	Starwest Botanicals-	209346-31	$19.67
Trocar	SAI Infusion Technologies	TRO-10-6	$84.00
PE-50 tubing (30 ft)	Stoelting	51158	$135
Plexiglass box	Shoppopdisplays.com	10222	$80
4 L beaker	Fisher Scientific	02-555-25K	148.10
Sterile gauze pads	Fisher Scientific	19-090-734	$15.75
Betadine scrub	Fisher Scientific	19-027132	$32.00
Meloxicam (5 mg/ml)	Henry Schein Animal Health (now Covetrus)	069021	$11.90
Isoflurane (100 ml)	Henry Schein Animal Health (now Covetrus)	029404	$17.82
Infusion pump	Braintree Scientific	BS-300 120V	$275.00

Table 1. List of materials used for intraoral cannula surgery and taste reactivity testing. Note: this list excludes common lab materials that will also be needed (e.g., conical tubes, laboratory tape, syringes, needles).

regarding the use of animal models to better understand complex physiological and behavioral phenomena in humans, and urge students to consider to what degree inferences about humans can be appropriately drawn from results obtained using animals.

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Address correspondence to: Dr. Michael J Morris, Psychology & Philosophy Department, 304 Administration Dr, Denton, TX 76204. Email: mmorris18@twu.edu