2013

Analysis of NaCl-LiCl Taste Discrimination Using Electrophysiological and Behavioral Methods

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THE FLORIDA STATE UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

ANALYSIS OF NaCl-LiCl TASTE DISCRIMINATION USING ELECTROPHYSIOLOGICAL AND BEHAVIORAL METHODS

By

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A Thesis submitted to the
Department of Psychology
in partial fulfillment of the
requirements for the degree of
Master of Science

Degree Awarded:
Summer Semester, 2013

Joseph A. Kostansek IV defended this dissertation on June 26, 2013

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ACKNOWLEDGMENTS

I would like to thank my major professor, Dr. Robert J. Contreras for giving me the opportunity to pursue scientific goals and acquire new and exciting knowledge and skills. I will forever appreciate his knowledge, support, and patience. Thanks go out to the members of my thesis committee, Dr. Thomas A. Houpt and Dr. Jon Maner. All of my work could never have been done without some amazing teachers who devoted their time and patience to train me. I would like to thank Dr. Joe Breza, Dr. Thomas A. Mast, Dr. Anne-Marie Torregrossa, and Brennan Paedae for their efforts in providing guidance in their areas of expertise and rescuing me when things went wrong.

Behind every successful endeavor is an elaborate support group in place to aid in the goal's completion. To this end, special thanks to Fred Fletcher and Dr. Te Tang for their contributions to making sure all of the equipment was functional. I appreciate the help, support, and advice provided by: Dr. Lisa Lyons, Dr. Bo Lu, Dr. Jim Smith, Cherie Miller, Dr. Ellen Berler, Dr. Orenda Johnson, Michelle Bales, Lyonna Alcantara, David Brown, John Corthell, Claudia Lieberwirth and all those in the Department of Psychology and the Program in Neuroscience.

Last but not least, many thanks go out to my friends and family for their undying support and encouragement. I know sometimes the completion of this goal was harder on you than it should have been, but I could never have made it without any of you. My love goes out to Amanda Gan, my future wife and rock for handling this process so well and keeping me afloat when I was more than willing to sink.

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ABSTRACT

The detection of salty taste stimuli depends on two salt-sensing transduction pathways that involve membrane channels on the surface of fungiform taste bud cells located on the anterior tongue in rats. These two pathways are the transcellular, amiloride-sensitive pathway and paracellular, amiloride-insensitive pathway. The transcellular is specific to NaCl and LiCl. Past studies have shown that LiCl and NaCl generate specific responses from taste nerves and that they are indistinguishable in behavioral tests. To this end, we generated the first dose-response curve comparing various concentrations of NaCl and LiCl and performed brief-access behavioral tests in order to determine if there is differential firing of the chorda tympani nerve to NaCl and LiCl stimulation and whether rats can discriminate between the two salts. We found that CT responses are higher for LiCl and NaCl for most concentrations used. This same trend was found when the salts were adulterated with amiloride. Measurements of the phasic portion of the CT response showed a larger amiloride-sensitive portion to the CT nerve response to NaCl than to LiCl. This difference may be sufficient to mediate LiCl/NaCl discrimination. We also found that rats were able to distinguish 30 mM LiCl from 30 mM NaCl, 30 mM LiCl from water, and 75 mM LiCl from water. These results hint to the possibility that with particular concentrations and particular tests, rats should be able to discriminate between NaCl and LiCl. Our results are similar to past research showing that LiCl responses are greater than NaCl responses. Our findings also suggest that there is differential signaling between NaCl and LiCl at the level of the whole nerve.

INTRODUCTION

The detection of salty taste stimuli depends on two salt-sensing transduction pathways that involve membrane channels on the surface of fungiform taste bud cells located on the anterior tongue in rats. One of the two pathways involves the amiloride-sensitive epithelial sodium channel (ENaC) that is selective for sodium and lithium cations. This pathway, known as the transcellular pathway is essential for sodium discrimination [1-5]. The other, known as the paracellular pathway, is amiloride insensitive and cannot discriminate between sodium, potassium or ammonium cations [6]. These two salt detection pathways in fungiform taste buds communicate with two different groups of axons in the chorda tympani (CT) nerve. The cell bodies of the two groups of axons reside in the geniculate nucleus of cranial nerve VII. One group consists of narrowly-tuned NaCl-specialist neurons that selectively respond to sodium and lithium salts with high spike rates and display little to no responses to other salts and other taste stimuli. The second group consists of broadly-tuned Acid-generalist neurons; these neurons respond best to acid solutions, but also respond with high spike rates to sodium chloride (NaCl), potassium chloride (KCl) and ammonium chloride (NH_4Cl) as well as to other taste stimuli [7,8]. ENaC antagonists such as amiloride attenuate NaCl responses of NaCl-specialist neurons but not the NaCl responses of the Acid-generalists [9-11]. Thus, Acid-generalists detect salt through an amiloride-insensitive transduction mechanism, the nature of which has yet to be elucidated.

Interestingly, lithium is the only other cation, other than sodium, for which ENaCs are specific. Most studies in the taste field that use lithium are conditioned taste aversion studies. Lithium chloride (LiCl) is used in these studies as the toxic substance to which the taste stimulus is paired. The effects of lithium on physiological systems are well documented in the study of salt and water balance, due to its similarity to sodium, and mood disorders [12, 13]. In the realm

of salt taste, lithium is most similar to sodium in terms of eliciting a "pure" salty sensation. This similarity between these two salts has been known for quite some time, even resulting in lithium being used as a salt substitute in the 1940s. Lithium was subsequently pulled as a salt substitute following the discovery of its toxic effects [14].

Toxic substances are usually not palatable and are usually aversive, so why does lithium – a toxic substance - taste similar to sodium, a necessary mineral for organism function? Perhaps because of lithium's toxic effect, there is a paucity of electrophysiological and behavioral studies investigating lithium taste. An early whole nerve study by Beidler [15] and a single-fiber study by Fishman [16] from the chorda tympani, respectively, found that responses to NaCl were the same or a less than the responses to LiCl – suggesting some differential firing. Electrophysiological recordings from the geniculate ganglion show that responses of salt-sensitive neurons responded best to LiCl and NaCl and that these salts have responses most similar to each other in comparison to other salts. Recording from Acid-generalist neurons in the geniculate ganglion also found that LiCl and NaCl responses were most similar to each other and different from other salts [17, 18]. Studies have also shown that LiCl and NaCl elicit the most appreciable responses from amiloride-sensitive chorda tympani fibers and that amiloride attenuates responses to NaCl and LiCl application [10, 11] to similar levels, implicating that both lithium and sodium cations pass through ENaCs. To our knowledge, there have been no studies to determine and evaluate dose response curves for both NaCl and LiCl in rodents.

There are strong connections between the physiology and behavior in the rodent in regards to the sodium-selective pathway and sodium ingestion [7]. The physiology of the system matches up in a predictable manner with the behavior. However, salt intake studies involving LiCl are scarce. Most behavioral studies involving LiCl come in the form of conditioned taste

aversion studies. Past studies comparing NaCl and LiCl [22,23] have shown that in brief access tests, consumption levels of NaCl, LiCl and water were indistinguishable from each other. However, rats learned to avoid LiCl in a prolonged (10 minute) intake test. This avoidance was due to a learned association between the taste of LiCl and its post-ingestive consequence (sickness). Those studies also revealed that LiCl aversion was readily and strongly generalized to NaCl. To our knowledge, studies have not been performed to discover if rats can learn to discriminate the taste of LiCl from that of NaCl. Brief access behavioral tests should uncover the answer to this question as rats discriminate taste quality and intensity within 600ms of tasting the solution [24, 25].

Advances in technology have allowed us to record from the chorda tympani in a more physiologically relevant setting. For example, past research has shown that temperature influences taste nerve responses to chemical stimulation [23, 24, 25]. Studies in the 1950s [15, 16] were performed under sub-optimal (one concentration, no temperature control, tongue in a pressurized chamber) conditions before the role of temperature on nerve responses was elucidated. We now employ a fluid delivery system that controls stimulus temperature and flow rate that matches the rat's rate of consumption [26]. The introduction [9] of the electrogustogram (EGG) allows us to record the time when the stimulus contacts the surface of the tongue in order to obtain a more accurate measurement of immediate neural responses. The advent of the Davis Rig lickometer has allowed us to perform brief-access, temperature controlled behavioral tests to evaluate the differential consumption of LiCl and NaCl solutions. One purpose of this study was to generate a dose-response curve for NaCl and LiCl solutions across a variety of concentrations in order to locate possible differential firing of the CT. The other purpose of this study was to use behavioral testing to determine if LiCl solutions and NaCl solutions are consumed differently.

METHODS

Experiment 1

Male adult Sprague-Dawley rats (n = 7, Charles River Laboratories) weighing 350-600 g were housed individually with enrichment (bone and pipe tube) in plastic cages in a temperature-controlled (72°F) colony room on a 12:12 light-dark cycle with lights on at 0700 h. All animals had free access to Purina Rat Chow (No. 5001) and deionized water (dH_2O). All the animals were habituated to the animal facility for at least 1 week before chorda tympani recording or behavioral testing. All procedures were approved by the Florida State University Animal Care and Use Committee.

The rats were anesthetized with IP administration of urethane (1.5 g/kg body wt). Supplemental urethane injections were given, if needed, to maintain a deep level of anesthesia without reflex response to foot pinch. The trachea was then cannulated with tubing to aid in breathing and the rat was then secured in a non-traumatic head holder. Using a mandibular approach, the right CT branch of the facial nerve was exposed and transected where it enters the tympanic bulla. The CT was desheathed and placed on a platinum wire electrode (positive polarity) and the entire cavity was then filled with high quality paraffin oil (VWR) to isolate the nerve signal from ground and maintain nerve integrity. An indifferent electrode (negative polarity) was attached to the skin overlying the cranium with a tinned-copper alligator clip. Neural activity was differentially amplified (X10,000; A-M Systems, Sequim WA), observed with an oscilloscope, digitized with waveform hardware and software (Spike 2; Cambridge Electronic Design, Cambridge England), and stored on a computer for off-line analysis.

The tongue was slightly extended and held in place with a small suture on the ventral surface and secured to the grounding table. Solutions were presented to the anterior tongue at a constant flow rate (50 µl/s) and controlled temperature (35°C) by an air-pressurized 32-channel commercial fluid-delivery system and heated perfusion cube (OctaFlow Multi-function Multi-valve Perfusion System, ALA Scientific Instruments, Farmingdale, NY). All solutions were made from reagent-grade chemicals and dissolved in a dilute salt mixture (0.015 M NaCl, 0.022 M KCl, 0.003 M $CaCl_2$, and 0.0006 $MgCl_2$) of artificial saliva. A range of NaCl and LiCl concentrations (10, 20, 30, 75, 150, 300, 600 mM) was followed by the same NaCl and LiCl concentrations mixed with the ENaC blocker amiloride hydrochloride (100 µM; Sigma, St. Louis, MO; NaCl/LiCl + Amiloride); both 300 mM ammonium chloride (NH_4Cl) and 600 mM NaCl were presented at the beginning and end – NH_4Cl between NaCl and LiCl series – to verify the viability of the nerve. If the response to NH_4Cl at the end of the protocol was <85% of the initial NH_4Cl response, the data from the recording were not included in the analysis. CT nerve responses were recorded from 10-s applications of each taste stimulus. Each stimulus was followed by a rinse of artificial saliva (AS) for 60-90 s to ensure that the nerve activity returned to stable baseline levels after each stimulus. Amplified nerve activity was monitored on-line, digitized using Spike 2, and integrated with a root mean square (RMS) calculation with a time constant of 150ms. The average baseline neural activity immediately preceding each chemical stimulus presentation was subtracted from the integrated response resulting from the 10-s stimulus to calculate the area under the curve (AUC). To control for individual differences among preparations, each response was normalized to the response to 300 mM NH_4Cl – a standard stimulus used in this study.

The electrogustogram (EGG) was used as confirmation of stimulus delivery onto the tongue and was recorded in vivo with Ag/AgCl electrodes via saline-agar-filled capillary pipettes (Ø 100µM, 0.15 M NaCl, 0.5% agar). The EGG electrode and the stimulus tube were placed near the CT receptive field area at the tip of the tongue and remained unchanged until the stimulus protocol was completed. All AUC measurements began at the location of EGG deflection.

All data are presented as group means ± SEM. Two-way repeated measures analysis of variance (ANOVA) was used to analyze CT nerve responses to the stimuli as a function of stimulus concentration both within salts and between salts (NaCl and LiCl) as well as concentration/drug interactions. A paired t-test was also used to compare amiloride suppression at each concentration. Amiloride suppression of the CT response to NaCl and LiCl (%) at each concentration was calculated as [(normalized salt response – normalized salt + amiloride response)/normalized salt response] X 100. Comparisons of the differential suppression were done by paired t-tests. ANOVAs were followed by post-hoc pairwise comparisons of statistically significant ($P < 0.05$) main effects or interactions using Fisher's LSD test. Analyses were done using Statistica, Statsoft, Tulsa, OK and GraphPad Prism.

Experiment 2

Male Sprague-Dawley rats, weighing 370 g on average at the start of the study and 445 g by the end of the study were housed individually with enrichment (bone and pipe tube) in plastic cages in a temperature-controlled (72°F) colony room on a 12:12 light-dark cycle with lights on at 0700 h. Animals had ad libitum access to standard laboratory chow throughout the study. Rats were deprived of deionized water for 23 h before each daily test session and received 1 h of water repletion at the conclusion of each session.

In the behavioral experiments, brief-access tests were used in order to minimize possible effects of post-ingestive cues on intake. Rats were tested in a Davis rig (MS-180) which was modified to control the temperature of the test fluids. The Davis rig was made up of a Plexiglas chamber with a wire mesh floor and an aperture that permitted access to 1 of 4 sipper tubes which were alternated by a motorized sliding platform. An automated shutter controlled access to each of the tubes for a pre-programmed length of time. The computer controlled the shutter and the order of stimulus presentation. Each sipper tube was held in an aluminum block containing a Peltier device to control the solution temperatures of the taste stimuli set at 35°C for this study. Additionally, each individual lick on the sipper tube was detected and recorded by a contact lickometer connected to a computer installed with the DavisRig3 collection software (FSU custom software). Data were then analyzed in DavisPro (Dialog Instruments).

Rats (n=8) were placed on 23-h water restriction schedule during the 4 days of water training. On days 1 and 2, the rats were presented with a single stationary tube of water and trained to lick in the apparatus. The session began when the rat licked the spout and lasted 25 min. On days 3 and 4, four sipper tubes (2 with water and 2 with 75mM NaCl) were prepared and presented one at a time in 10 s trials over a 25-min session. NaCl solutions were used to eliminate stimulus novelty bias.

Rats were water restricted for 23 h in preparation for experimental trials. In the testing apparatus rats had 180 s to initiate licking, 10 s once a lick was recorded, and a 10 s total trial time. Rats were tested with two concentrations of LiCl and NaCl (30 and 75mM). Each rat received one 10 s presentation of one salt per day – 4 days for each salt and 4 days for water trials. Salt trials alternated using the following scheme: alternate 30mM NaCl and 30mM LiCl four times, followed by 2 days of water trials, then alternating 75mM NaCl and 75mM LiCl trials

four times, followed by 2 days of water trials. Test solutions were held at a constant temperature of 35°C. Water was used as a test solution to establish baseline activity and was used as a control.

Presented data represent average lick values to each test solution, and average lick amounts across all trials – to detect stimulus generalizations. Data was tested for significance by paired t-tests of planned comparisons.

RESULTS

Experiment 1

Sample traces of electrophysiological CT activity are shown in Fig.1. Fig. 1A is representative of NaCl and NaCl + amiloride (amil) responses. Fig. 1B is representative of LiCl and LiCl + amil responses. Figure 2 shows normalized CT responses (AUC) to NaCl and NaCl + amil and LiCl and LiCl + amil at each concentration (75, 150, 300, 600 mM). A two-way repeated measures ANOVA showed that the responses to NaCl [$F(3, 18) = 67.85$, $P < 0.001$] and LiCl [$F(3, 18) = 26.32$, $P < 0.001$] varied as a function of concentration. There was a concentration x drug interaction for NaCl [$F(3, 18) = 26.32$, $P < 0.001$] and for LiCl [$F(3, 18) = 14.56$, $P < 0.001$]. Shown in Fig. 1, a dose-response curve was generated by the application of the various concentrations of NaCl and LiCl. For both salts, the smallest response was from the 75 mM solution. The largest response was generated by the 600 mM solution, except for LiCl, where 300 mM and 600 mM were similar. This trend repeated in the salt + amil concentration series, with the exception of LiCl + amil, where 600 mM generated the largest response. Post-hoc analyses revealed within salt significant differences (all $p < 0.05$) to all concentrations of NaCl, LiCl, NaCl + amil and LiCl + amil with the exception of 300 and 600 mM LiCl. Figure 3 depicts between salt comparisons of salt alone (3A) and salt + amil (3B). With the exception of 300 mM and 600 mM NaCl and LiCl alone, responses to LiCl are greater than responses to NaCl. Further post-hoc tests revealed significant differences in between salt comparisons. Averaged normalized CT responses to NaCl and LiCl are significantly different at 75, 150, and 600 mM (all $p < 0.05$). The average response to LiCl + amil was significantly higher than that to NaCl + amil at 600 mM ($p < 0.05$). There were no differences in response to the standards, confirming a stable baseline.

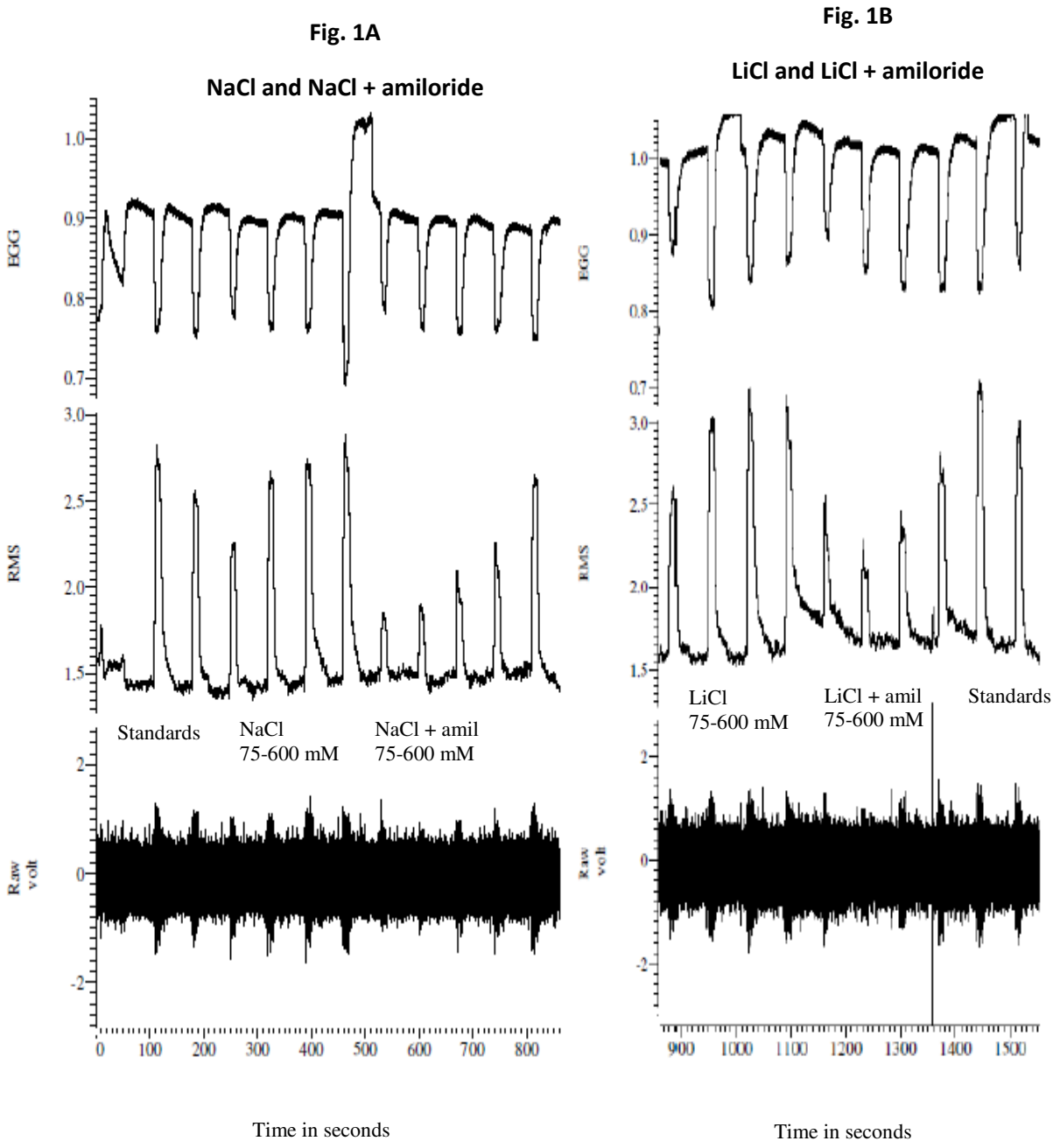


Fig. 1A. Chorda tympani nerve (CT) whole nerve traces showing the raw voltage response (V), the RMS response, and the EGG trace in response to10 s stimulation. Lingual application of 600 mM sodium chloride (NaCl), 300 mM ammonium chloride (NH_4Cl), NaCl (75, 150, 300, 600 mM) and NaCl mixed with amiloride, and lastly 300 mM NH_4Cl.

Fig 1B. CT responses to lithium chloride (LiCl, 75, 150, 300, 600 mM), LiCl mixed with amiloride and lastly 600 mM NaCl and 300 mM NH_4Cl. All stimulations 10 s.

Fig. 2

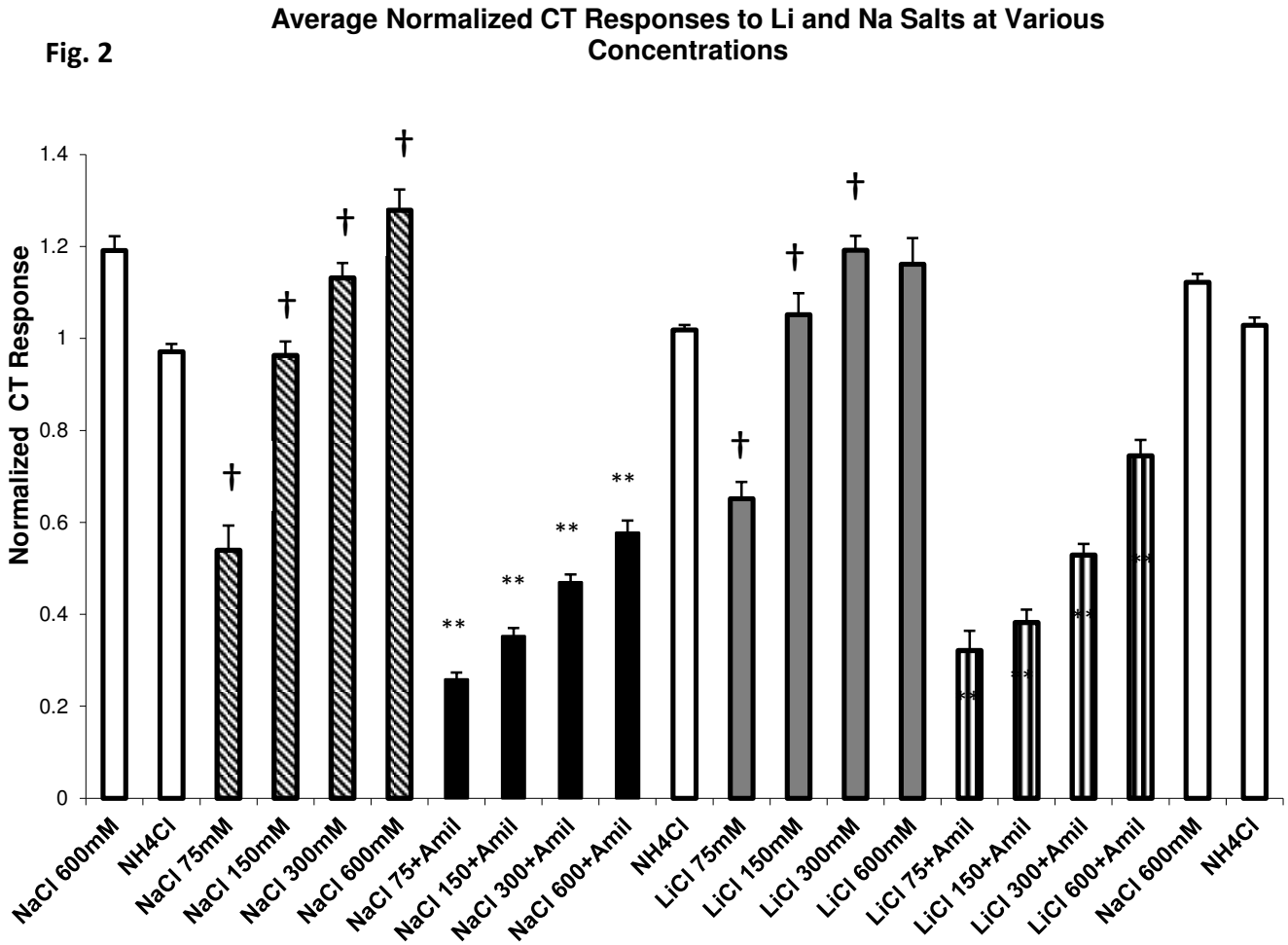


Fig. 2. Normalized CT whole nerve responses (AUC over 10 s) to a range of NaCl, NaCl + amil, LiCl, and LiCl + amil concentrations. At the beginning, middle, and end of the protocol are NaCl and NH_4Cl salts used as standards. All NaCl salt responses were significantly different from themselves and fromNaCl + amil responses. All LiCl salt responses were significantly different from themselves and from LiCl + amil responses with the exception of the comparison of LiCl 300 mM and LiCl 600 mM.

** $p < 0.05$ for all within NaCl + amil and LiCl + amil comparisons
† $p < 0.05$ for all within NaCl alone and LiCl alone comparisons

Fig. 3A

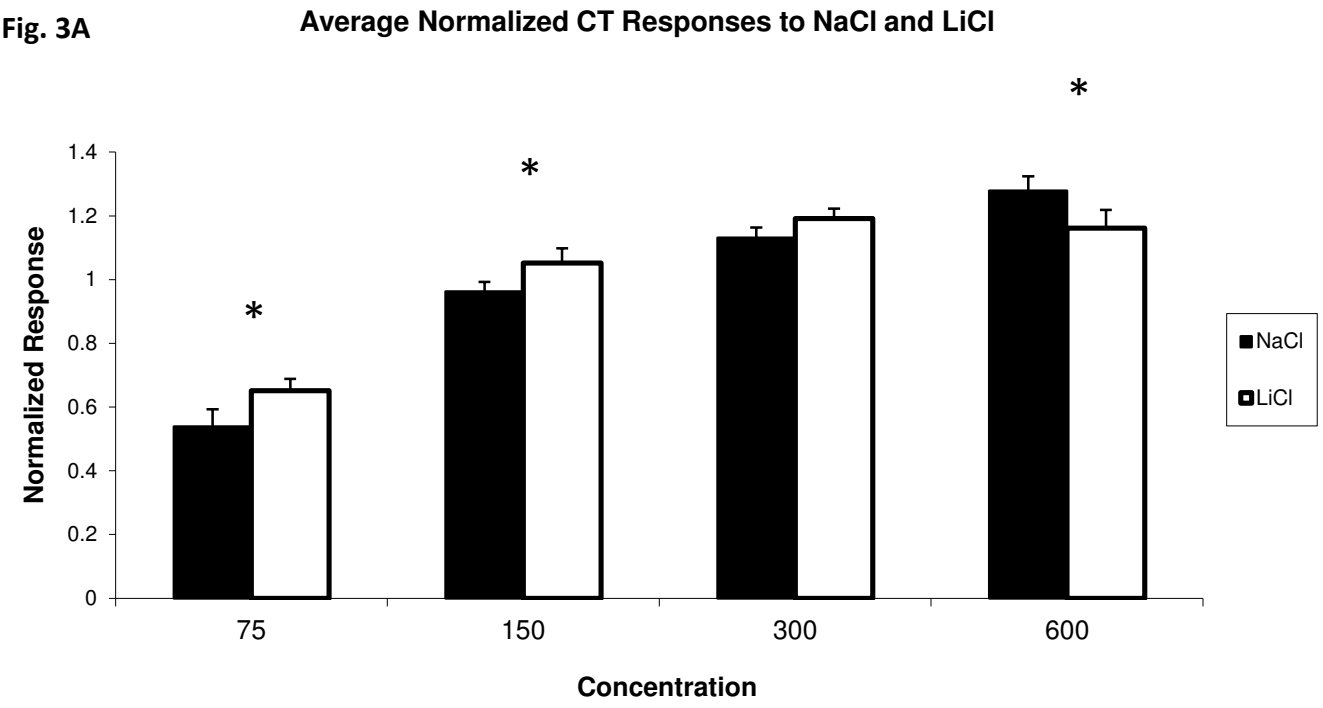


Fig. 3B

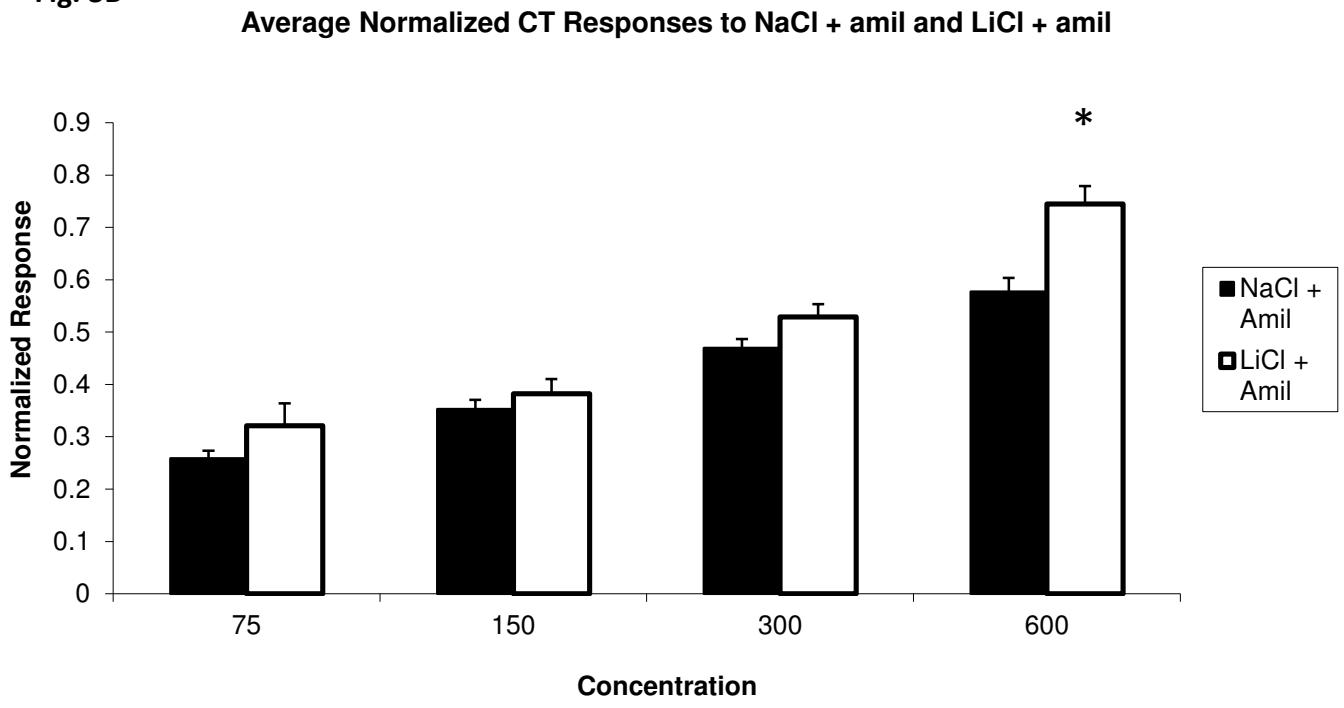


Fig. 3. Average normalized responses (AUC over 10 s) of the CT to NaCl, LiCl (3A). NaCl + amil and LiCl + amil (3B), and their between salt comparisons. * All significant between salt comparisons, $P < 0.05$.

Figure 4 shows the percent suppression of CT responses to NaCl and LiCl by amiloride. There was a significant stimulus effect. Paired t-tests ($t = 7.6510$, $df = 6$, $p < 0.0005$) revealed that amiloride was more effective in suppressing CT responses to 600 mM NaCl, 54.9%, to 600 mM LiCl, 35.6%. Amiloride was equally effective at suppressing 75, 150, and 300 mM concentrations of NaCl and LiCl.

Understanding that rats can discriminate NaCl concentration differences in less than 1 s of tasting the solution [24,25] and that the immediate phasic response rate of the CT nerve is responsible for coding NaCl intensity information [28], the first 1.5 s of the AUC response of the CT for each salt and concentration was analyzed for differences (Fig. 5). Whole nerve responses to NaCl and LiCl over 1.5 s across all concentrations were very similar to one another, and a general trend of a dose-response curve is seen. At lower concentrations (Fig. 5A), 75 and 150 mM, NaCl responses were generally higher than LiCl responses but not different. At higher concentrations, 300 and 600 mM, responses to LiCl were equal or higher than NaCl but not different. Figure 5B shows similar dose-response trends with NaCl and LiCl + amil, with the difference that CT responses to LiCl + amil are greater than NaCl + amil at 150, 300, and 600 mM concentrations. A two-way repeated measures ANOVA on the data as presented in Fig. 5 showed that the CT nerve responses to NaCl [$F(3, 18) = 72.36$, $P < 0.0001$] and LiCl [$F(3, 18) = 18.62$, $P < 0.0001$] within the first 1.5 s of stimulation varied as a function of concentration. There was a drug effect for both NaCl [$F(1, 6) = 48.83$, $P < 0.0004$] and LiCl [$F(1, 6) = 10.04$, $P < 0.0194$]. There was no interaction effect for either salt. Further post-hoc tests (all $p < 0.05$) revealed significant differences only when comparing LiCl + amil and NaCl + amil at 150, 300, and 600 mM concentrations, with LiCl + amil responses being greater than NaCl + amil responses. No other significant differences were found either between or within salts.

Fig. 4

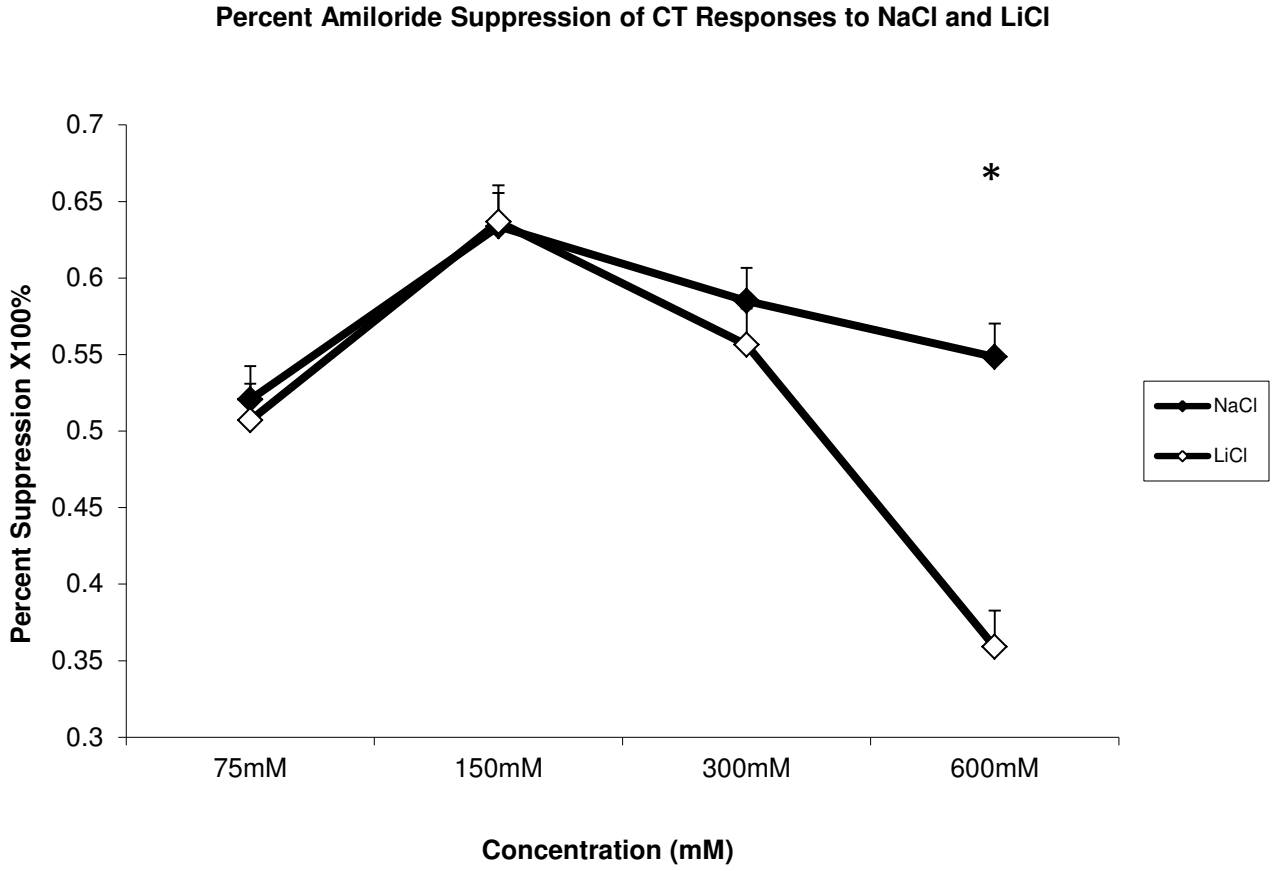


Fig. 4. Percent suppression by amiloride of CT responses to a range of NaCl and LiCl concentrations. Amiloride suppressed CT responses significantly more to NaCl than LiCl at 600 mM. * Significance of suppression at 600 mM at $p < 0.05$

Fig. 5A

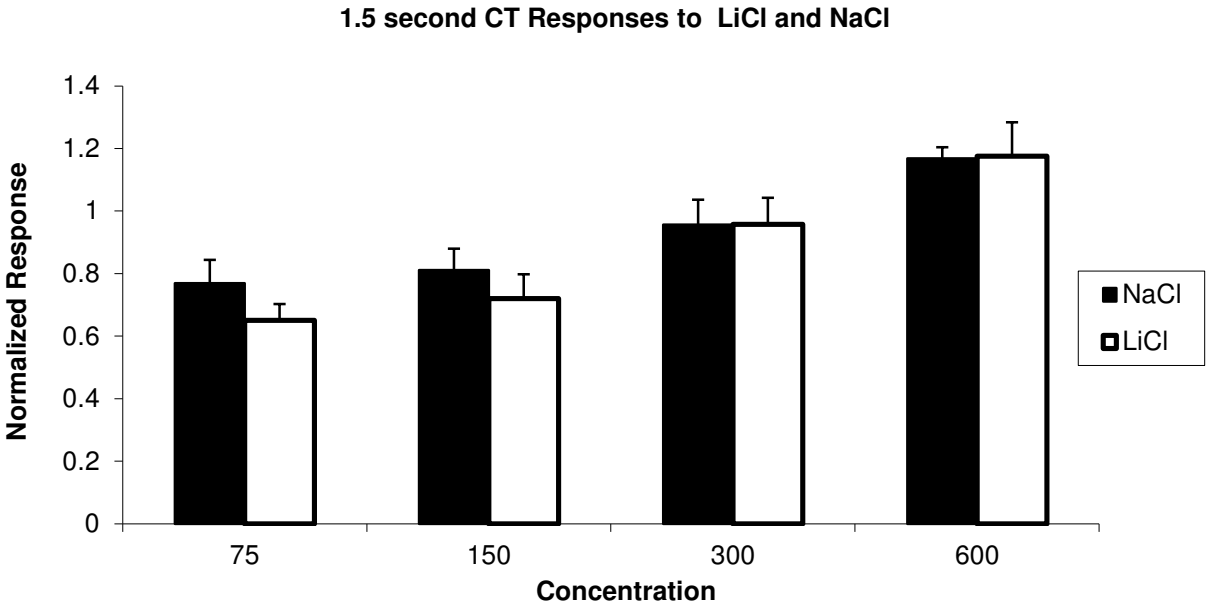


Fig. 5B

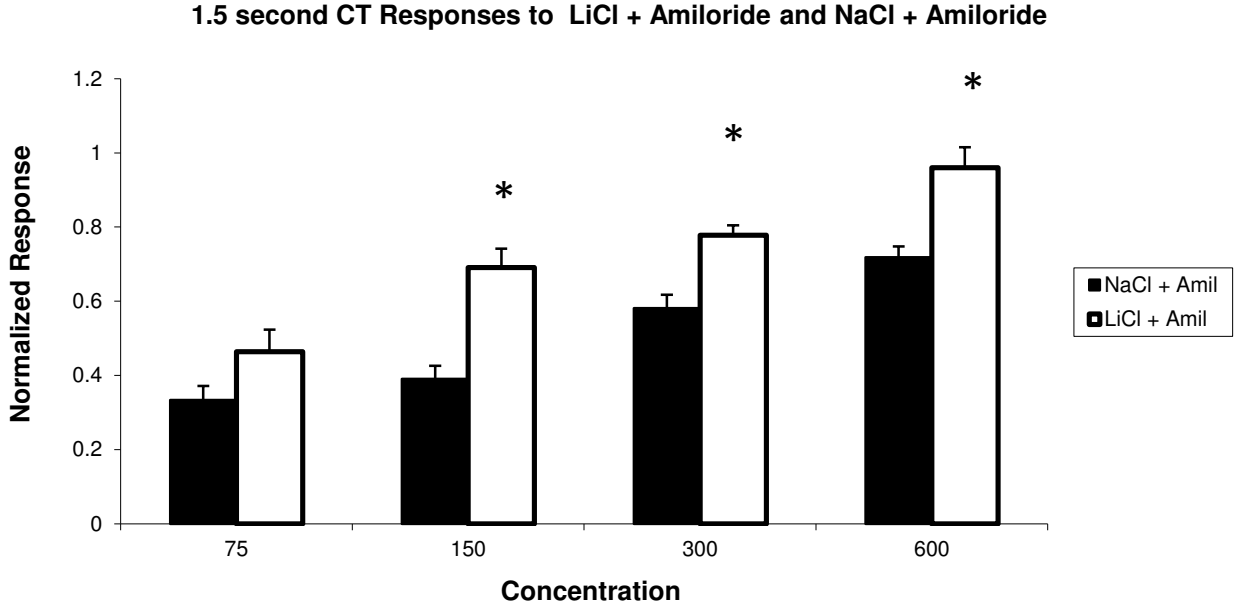


Fig. 5. Normalized CT responses (AUC over first 1.5 s) to NaCl, LiCl and NaCl + amil, LiCl + amil. No significant differences in CT responses to NaCl and LiCl without amiloride. CT responses to LiCl + amil were significantly greater than those to NaCl + amil responses at 150, 300, 600 mM. * All significant between salt comparisons, $P < 0.05$

Experiment 2

Figure 6A shows normalized CT responses (AUC) to NaCl and LiCl at each concentration (10, 20, 30 75 mM). A one-way repeated measures ANOVA showed that the responses to NaCl and LiCl varied as a function of concentration. Shown in the graph, a dose-response curve was generated by the application of the various concentrations of NaCl and LiCl. For both salts, the smallest response was generated from the application of 10 mM solution. The largest response was generated by the application of 75 mM solution. In general, the CT responsed more to LiCl than NaCl at all stimulus concentrations [$F(7, 35) = 99.241$, $P < 0.001$]. Post-hoc analyses revealed within salt significant differences (6B, all $p < 0.05$) to all concentrations of NaCl and LiCl. There were no differences in responses to the standards, confirming a stable baseline. Post-hoc tests evaluating between salt differences showed that there was a significant difference in the comparison of CT responses to 75 mM NaCl and 75 mM LiCl ($p < 0.02$). This difference was also seen in the data shown in figures 2 and 3.

Fig. 6A

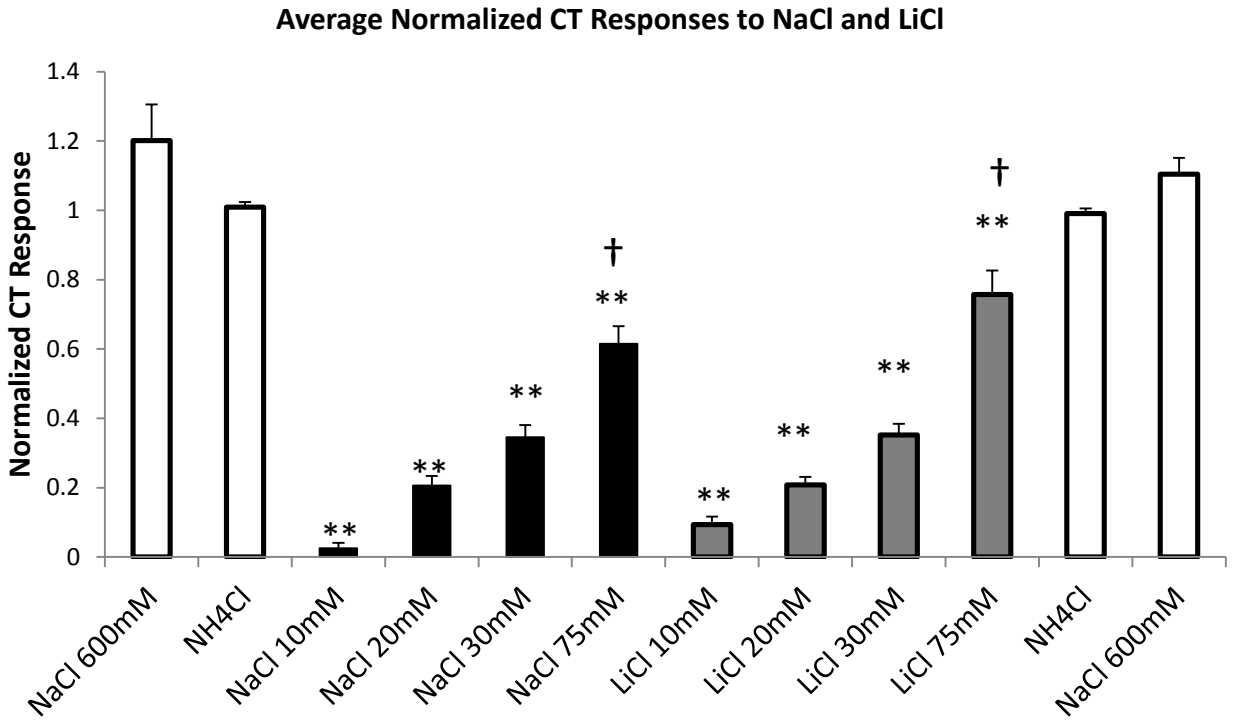


Fig. 6B

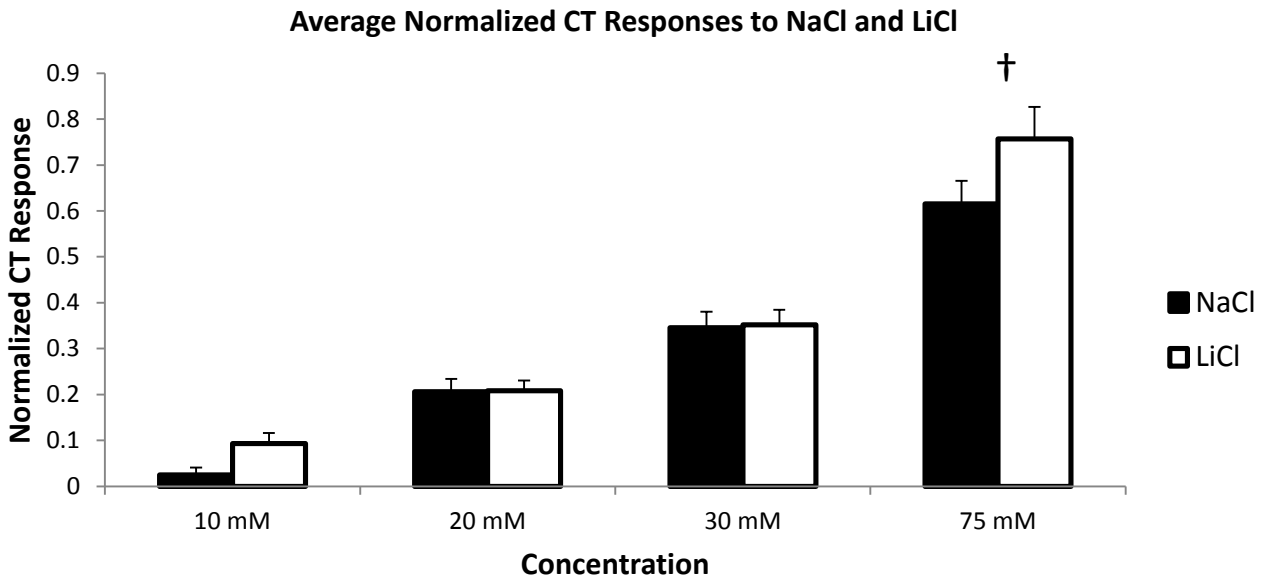


Fig. 6. Normalized CT responses to NaCl, and LiCl (AUC 10 s). All within salt comparisons are significant (** $p < 0.05$). NaCl and LiCl 75 mM are significantly different from each other (6B, $p < 0.02$ †).

Figure 7A shows the average number of licks over 10 s to water (baseline), 75 mM NaCl, 75 mM LiCl, 30 mM NaCl and 30 mM NaCl. Rats licked water the most, followed by 30 mM NaCl, 75 mM LiCl, 75 mM NaCl and 30 mM LiCl – the fewest. Post-hoc paired t-tests revealed significant differences in the average licks of 75 mM LiCl to water ($t = 2.32$, $df = 31$, $SE = 1.789$, $p < 0.03$), 30 mM LiCl to water ($t = 2.54$, $df = 31$, $SE = 3.984$, $p < 0.02$) and 30 mM LiCl to 30 mM NaCl ($t = 2.30$, $df = 31$, $SE = 3.48$, $p < 0.03$). Rats licked significantly more to water than to the two concentrations of NaCl and LiCl. Rats also licked more to 30 mM NaCl than to 30 mM LiCl.There were no significant differences in any other comparison. Figure 7B shows the average lick amounts in response to each salt presented over the course of the 4 day experimental period. At the start of the experiment the average licks on Trial Day 1 were variable and by the end of the experiment, on Trial Day 4 the average licks to each stimulus solution were more similar to each other. Figure 7C tracks the average lick amounts across all trials for all salts during the experimental period. In general, there was a slight decrease in the number of licks from one NaCl trial to the next LiCl trial with a few exceptions.

Fig 7. Mean number of licks over 10 s to water, 30 mM NaCl and LiCl, and 75 mM NaCl and LiCl (7A). Rats licked significantly more to water than to 75 and 30 mM LiCl (**), and more to 30 mM NaCl than to 30 mM LiCl (†). All p-values < 0.03. Fig 7B depicts the average lick amounts across the 4 trial days for all test solutions. Fig. 7C depicts the lick amounts across all trials (time).

Fig. 7A

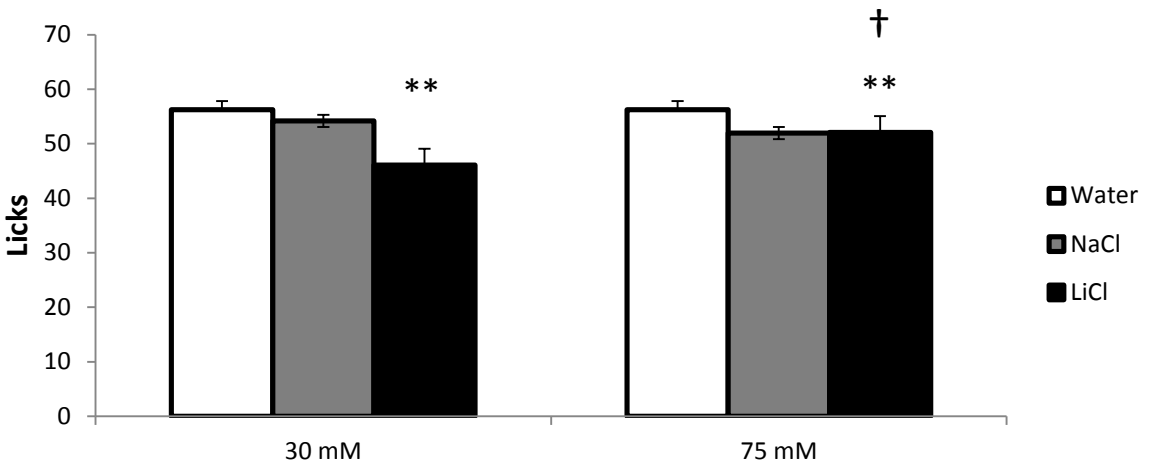


Fig. 7B

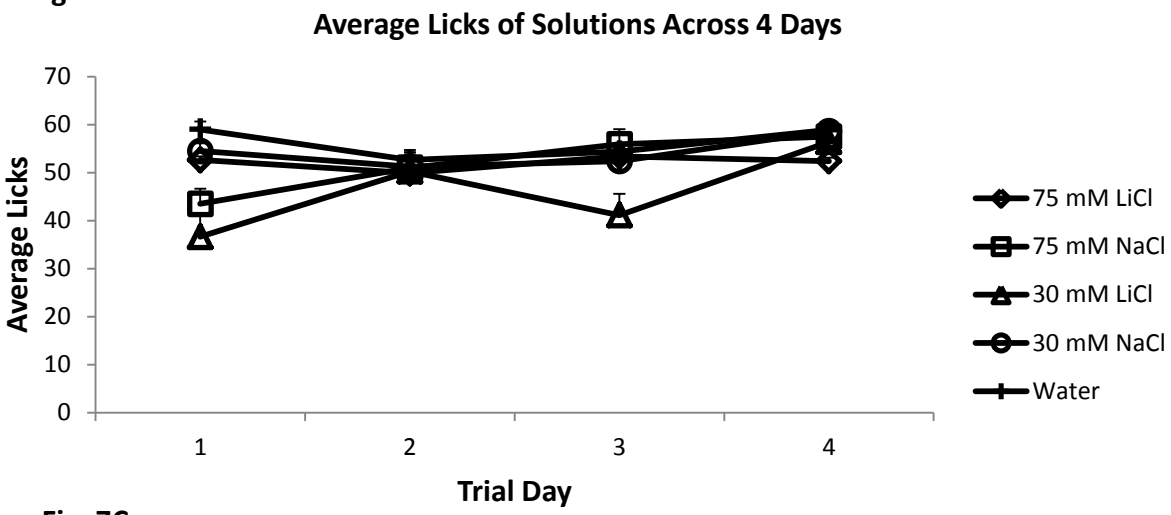
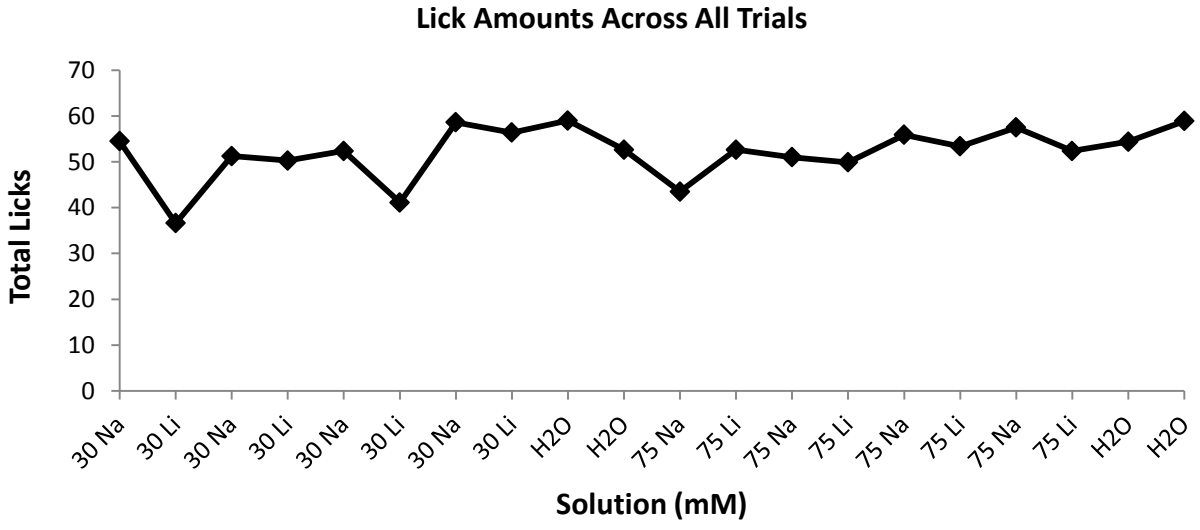


Fig. 7C



DISCUSSION

In Experiment 1, we found that the CT nerve responses to NaCl and LiCl at 4 different concentrations generated a dose- response curve for each salt. The response of the CT nerve (AUC) increased with concentration for both salt solutions with and without amiloride except between 300 to 600 mM LiCl – a possible ceiling effect. Between salt comparisons revealed a significant difference between NaCl and LiCl at all concentrations with the exception of 300 mM. A significant difference between NaCl + amiloride and LiCl + amiloride was revealed only for the 600 mM concentration. In this case, amiloride was able to suppress 55% of the NaCl signal and only 36% of the LiCl signal. During the phasic portion of the CT response, the response to LiCl + amil was significantly greater than NaCl +amil. This means that there is a larger amiloride-sensitive portion to the CT nerve response to NaCl than to LiCl. This difference may be sufficient to mediate LiCl/NaCl discrimination. Studies by Beidler [18] revealed a response ratio of 1.1 for NaCl when compared to1.0 for NaCl for a single concentration – 0.1M. In our study the ratio comparing LiCl and NaCl was 1.20 for 75 mM, 1.09 for 150 mM and 1.05 for 300 mM – all in favor of LiCl. The ratio for 600 mM was 1.1 in favor of NaCl. These results confirm our hypothesis of LiCl responses being similar or greater than NaCl responses [15 - 21] and the effect of amiloride on the transcellular sodium pathway [1-5] and give a mechanism for possible NaCl/LiCl discrimination.

Previous research had only compared CT responses NaCl and LiCl at a single concentration, 0.1 M, instead of performing an entire concentration series. Our research, then, marks a first in evaluating NaCl and LiCl CT responses over a broad concentration series. These results are a reflection of advances in technology that have allowed us to record from the chorda tympani in a more physiologically relevant setting as compared with previous research.

Temperature was controlled at 35°C – body temperature of the rat. Temperature is known to influence taste nerve responses to chemical stimulation [7, 8, 9]. The EGG permitted us to note the exact time when the stimulus contacts the surface of the tongue, which enabled us to have a more accurate measurement of immediate neural responses [12]. Our fluid delivery system controlled stimulus temperature and flow rate that matched the rat's rate of consumption [9]. As stated earlier, we dissolved the test solutions in artificial saliva to make the test solutions. Artificial saliva contains all the major ions normally found in saliva. Saliva is critical in influencing taste sensitivity as well as protecting lingual receptors and enhancing spontaneous activity and neural responsiveness [12, 29]. Due to sodium detection thresholds being at a higher concentration than that found in saliva, artificial saliva was perfect as a rinse and did not confound the experiment. Our updated methods in CT recording, ensures that the data were clear, physiologically relevant, and increases their impact. This physiological relevance is essential in connecting these results to behavioral studies.

The detection of salty taste stimuli depends on two salt-sensing transduction pathways – transcellular and paracellular – which are defined by their amiloride sensitivity or insensitivity. The amiloride sensitive pathway is specific to NaCl and LiCl and the receptor used for transduction is the ENaC. The amiloride-insensitive pathway responds to other salts and taste stimuli [1, 2, 3, 4, 5, 6]. ENaC blockers such as amiloride block only a part of the taste signal, the amiloride-sensitive pathway. The amiloride insensitive pathway generates the rest of the signal. Our study has shown a significantly greater CT response to 600 mM LiCl + amil than 600 mM NaCl + amil. At this concentration, amiloride was better able to suppress the response to NaCl (55%) relative to LiCl (36%) – resulting in a mostly amiloride-insensitive signal for LiCl. For all the other concentrations of LiCl adulterated with amiloride, the LiCl response was also

greater. This raises the issue of the interaction of LiCl with ENaCs when blocked by amiloride. Ninomiya [14] reported that amiloride attenuated both NaCl and LiCl CT fiber responses in to a similar extent. This study used one 0.1M concentration of NaCl and the same 100μM concentration of amiloride as the present study. The previous study was not conducted with the same level of physiological control as the current study. Interestingly, aside from human psychophysical studies done by Schiffman [30], Ninomiya's study was the only one comparing the effects of amiloride on LiCl responses. The LiCl + amiloride results found in this study are the result of either differential transduction of LiCl by both sodium transduction pathways or the ability of the Li^+ to elude the amiloride block of ENaC channels due to the small size of the ion – Na^+ is 1.34 times larger than Li^+. ENaCs are selective to both Na^+ and Li^+ and biochemical studies have been able to switch the affinity from Na^+ to Li^+ [31]. Further studies using benzamil, a more potent ENaC blocker, or higher concentrations of amiloride should be able to investigate the reasons for our effect at the whole nerve level.

The first 1.5 s of the CT response is known as the phasic portion and is enough to code for discrimination and intensity information for salts [28]. The CT responded similarly to NaCl and LiCl without amiloride. However, when mixed with amiloride, the CT nerve responded significantly more to LiCl than NaCl across stimulus concentrations. This means that there is a larger amiloride-sensitive portion to the CT nerve response to NaCl than to LiCl. This difference may be sufficient to mediate LiCl/NaCl discrimination. Since the salt response of NaCl-specialists is mediated by ENaC, while the salt response of acid-generalists is mediated by an amiloride-insensitive mechanism, we would expect future single-cell studies to show that the response of NaCl-specialists is bigger to NaCl while that of acid-generalists would be greater to LiCl.

The behavioral tests in this experiment had a two-fold purpose. The first was to determine whether rats licked solutions of NaCl and LiCl differently from each other and water. Behaviorally, our experiments found a difference in the way the rats licked 30 mM LiCl vs NaCl, 30 mM LiCl and water, and 75 mM LiCl and water. These results are telling, considering that rats will ingest sodium solutions similar to water in concentrations up to 150 mM (isotonic) and then decrease their consumption as the concentration of the salts increase. If these results hold, these results show that rats lick LiCl differently than water and to possibly NaCl, with astounding implications as the concentrations were so small. If these results are anomalous, they provide insight into further tests that can be conducted to discover if these two salts can be discriminated from each other. This experiment was successful in forcing rats to ingest LiCl solutions without getting sick from toxic effects. The results also showed no generalization to any other salts during the test as shown in studies by Nachman [23]. Those studies also found that 0.12M LiCl could be discriminated from water after ingesting for 10 minutes. However, discrimination was only possible after the rat began to suffer the toxic sickness caused by the LiCl solution. This information is useful and makes it possible for further discrimination testing in the gold standard apparatus for such an experiment – the gustometer. Using methods employed in studies by Geran and Spector [25], rats can be tasked with discriminating between the two salts. Sickness due to LiCl toxicity can be avoided by modulating the concentration of the salts, by starting low and working high, and modulating how much LiCl is actually ingested during the tests. Rats can distinguish concentrations between NaCl concentrations in 5 licks [25]. If rats can distinguish between NaCl concentrations in 5 licks and if the behavior matches the electrophysiology, then in a proper test, rats should be able to distinguish between the two salts.

In conclusion, our study was able to generate a concentration curve for LiCl and NaCl from whole-nerve chorda tympani recordings for the first time in published literature. Our results also corroborated previous findings in single-cell and single-fiber recordings that found that NaCl responses were similar or less than LiCl responses. Recordings of various concentrations of these salts, when adulterated with amiloride show less suppression of LiCl signals than NaCl, revealing a possible differential firing profile for LiCl against NaCl. Measurements of the phasic portion of the CT response showed a larger amiloride-sensitive portion to the CT nerve response to NaCl than to LiCl. This difference may be sufficient to mediate LiCl/NaCl discrimination. Brief-access tests were partially successful in determining how rats will lick NaCl and LiCl in relation to water. One benefit of the results of the behavioral tests was that the salts were not generalized and that the rats did not get sick from ingesting the lithium. When performed at higher concentrations and extra care making sure the animals do not get sick from the lithium, our data can be used in more stringent discrimination tasks to determine if rats can discriminate between these salts. Advances in technology allowed these experiments to be more physiologically relevant and therefore more valid and allows these results to contribute more into the investigation as to whether or not LiCl elicits a different CT response profile and whether it can be discriminated from NaCl.

APPENDIX

ACUC APPROVAL LETTER

Animal Care and Use Committee (ACUC)
101 Biomedical Research Facility
P.O. Box 3064341
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Telephone: 644-4262 Fax: 644-5570
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MEMORANDUM

TO: Dr. Robert Contreras
Department of Psychology

FROM: Dr. Paul Q. Trombley, Chair PQT
Animal Care and Use Committee

SUBJECT: Approval of Protocol **#1108**

DATE: March 10, 2011

"YOUR NEW PROTOCOL IS APPROVED"

The Animal Care and Use Committee approved new **Protocol #1108, "Neural Taste and Thermal Coding in the Geniculate Ganglion",** for proposed vertebrate animal use at the **February 23, 2011** ACUC meeting with the request for ***clarifications*** as noted in committee comments. You are approved for the following species and numbers for the proposed protocol approval period.

Species	*Number Animals Approved*	*Protocol Approval Expiration Date*	*Rewrite Due*
Rat, *Sprague-Dawley*	990	February 23, 2014	January 1, 2014
Mice, C57BL/6J & gene targeted variants	450		

Enclosed for your records are:
- ✓ A copy of the **Committee Comments**
- ✓ A copy of the **Protocol and supporting documents**

When you order animals on this protocol, please remember to convey the ACUC number to the LAR at 644-4262. In addition, if you do not currently have animal housing or procedural space assigned or should you need additional animal housing or procedural space, please make a request for space in writing to the Biomedical Advisory Committee (BAC) care of Kristin Auter at kauter@fsu.edu. Animals will not be ordered unless adequate animal housing/procedural space is confirmed by the LAR Facility Manager.

We appreciate your contribution to assuring that animal research at Florida State University complies with federal guidelines and regulations. Let us know if we can be of further assistance.

PQT/kjj
Enclosures

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BIOGRAPHICAL SKETCH

Joseph Anthony Kostansek IV was born in Charleston, South Carolina in 1988, and he grew up in the cities of Jacksonville and Pensacola, Florida. He was a member of the 2006 CAC State Championship Team representing Escambia County. He graduated with an International Baccalaureate Diploma from Pensacola High School in 2006 and began his college career at Florida State University the following Fall.

Education:

2010	B.S., Biological Science (with Chemistry and Psychology minors), The Florida State University, Tallahassee FL
2010-	Master's Candidate, Department of Psychology, The Florida State University, Tallahassee FL

Honors and Awards:

2007 – 2008	Florida State University - Dean's List
2010 – 2012	Florida State University Program in Neuroscience Research Fellowship
2010	Florida State University Department of Biological Science Citizen of the Month – September
2012-2013	Florida State University Nomination for 2013 Outstanding Teaching Assistant Award

University Service:

2009-2010	President of the Cabinet of Florida State University Presbyterian University Center
2012-2013	Member of Florida State University Program in Neuroscience Outreach Committee – Brain Awareness Week Coordinator

Research Experience:

2008 – 2010 Undergraduate Research. Dr. Paul Q. Trombley, Department of Biological Science – Program in Neuroscience, Florida State University

2010 – 2011 Laboratory Rotation. Graduate Student to Lisa Lyons, Ph.D., Department of Biological Science and Program in Neuroscience, Florida State University

2011 - Graduate Student to Robert J. Contreras, Ph.D., Department of Psychology and Program in Neuroscience, Florida State University

Teaching Experience:

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Spring 2013 Sensation and Perception Laboratory (EXP3202C). TA, Department of Psychology, Florida State University

Posters and Presentations:

July 2011 A Tasteful Look At the Chorda Tympani Nerve: Responses to NaCl and LiCl. Summer Seminar, Program in Neuroscience, Florida State University, Tallahassee, FL

October 2012 Mast TG, Kostansek JA, Kelly SM, Contreras RJ. (2012). Water restriction enhances chorda tympani responses to NaCl and KCl in rats. 42nd Annual Meeting of the Society for Neuroscience, New Orleans, LA.