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An experimental quantitative sensory testing study in healthy subjects

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Abstract:	<p>Background: In burning mouth patients, analgesia after oral administration of clonazepam may result from modulation of peripheral γ-aminobutyric acid (GABA) receptors.</p> <p>Methods: The effect of oral administration of test solutions (water, 0.5M or 0.05M GABA, 1% lidocaine) was investigated for the amelioration of pain and sensitivity induced by application of capsaicin (1%, 2 minutes) to the tongue of thirty healthy male and female subjects in this 4-session, randomized, placebo-controlled, double-blinded, cross-over study. Intraoral quantitative sensory testing was used to assess cold (CDT), warm (WDT) and mechanical (MDT) detection thresholds as well as mechanical (MPT) and heat (HPT) pain thresholds. Capsaicin-induced pain intensity was continuously rated on a 0-10 electronic visual analogue scale (VAS). The area under the VAS curve (VASAUC) after rinsing was calculated for each solution.</p> <p>Results: Capsaicin application on the tongue evoked burning pain with a peak of 4.8/10, and significantly increased CDT and MDT while significantly decreasing WDT, HPT, and MPT. The VASAUC was significantly smaller after oral rinse with 0.05M GABA, 0.5M GABA, and 1% lidocaine than after oral rinse with water. Rinse with 0.5M or 0.05M GABA were similarly effective in decreasing VASAUC. Rinsing with either 1% lidocaine, 0.5M or 0.05M GABA also significantly attenuated the effects of capsaicin on WDT and HPT in a treatment independent manner. There were no sex-related differences in these effects of GABA.</p> <p>Conclusions Capsaicin-induced burning tongue pain and decreases in WDT and HPT can be ameliorated by rinsing the mouth with lidocaine and GABA solutions.</p>

γ -aminobutyric acid (GABA) oral rinse reduces capsaicin-induced burning mouth pain sensation: An experimental quantitative sensory testing study in healthy subjects

Running head: GABA inhibits burning mouth pain

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Abstract (242/250)

Background: In burning mouth patients, analgesia after oral administration of clonazepam may result from modulation of peripheral γ -aminobutyric acid (GABA) receptors.

Methods: The effect of oral administration of test solutions (water, 0.5M or 0.05M GABA, 1% lidocaine) was investigated for the amelioration of pain and sensitivity induced by application of capsaicin (1%, 2 minutes) to the tongue of thirty healthy male and female subjects in this 4-session, randomized, placebo-controlled, double-blinded, cross-over study. Intraoral quantitative sensory testing was used to assess cold (CDT), warm (WDT) and mechanical (MDT) detection thresholds as well as mechanical (MPT) and heat (HPT) pain thresholds. Capsaicin-induced pain intensity was continuously rated on a 0-10 electronic visual analogue scale (VAS). The area under the VAS curve (VASAUC) after rinsing was calculated for each solution.

Results: Capsaicin application on the tongue evoked burning pain with a peak of 4.8/10, and significantly increased CDT and MDT while significantly decreasing WDT, HPT, and MPT. The VASAUC was significantly smaller after oral rinse with 0.05M GABA, 0.5M GABA, and 1% lidocaine than after oral rinse with water. Rinse with 0.5M or 0.05M GABA were similarly effective in decreasing VASAUC. Rinsing with either 1% lidocaine, 0.5M or 0.05M GABA also significantly attenuated the effects of capsaicin on WDT and HPT in a treatment independent manner. There were no sex-related differences in these effects of GABA.

Conclusions Capsaicin-induced burning tongue pain and decreases in WDT and HPT can be ameliorated by rinsing the mouth with lidocaine and GABA solutions.

Significance: (40 words): Rinsing the mouth with an oral GABA containing solution ameliorated burning pain and increased heat sensitivity produced by application of capsaicin to the tongue. This finding suggests that GABA can act as a local analgesic agent in the oral cavity.

Keywords: Analgesia; Burning mouth syndrome; Orofacial; GABA; Tongue; Quantitative sensory testing,

Introduction

Burning mouth syndrome (BMS) is a chronic intraoral pain condition characterized by symptoms of “burning-like” pain most commonly experienced on the anterior of the tongue (Grushka 1987; Svensson et al., 1993; Tammiala-Salonen et al., 1993; Vanderploeg et al., 1987). It has been proposed that BMS is a type of peripheral deafferentation neuropathy (Forsell et al., 2002). On examination, many BMS patients report a loss of thermal detection sensitivity over the affected area in the tongue and oral mucosa, as well as altered taste sensations (Kolkka-Palomaa et al., 2015). Tongue biopsies from BMS patients when compared with healthy controls have a significantly lower density of epithelial nerve fibers (Lauria et al., 2005). Nerve fiber changes suggestive of axonal degeneration and injury, such as increased levels of nerve growth factor and expression of TRPV₁ and P₂X₃ receptors, appear to be associated with the decreased density of tongue epithelial nerve fibers in these patients (Kolkka-Palomaa et al., 2015; Yilmaz et al., 2007). The source of nerve injury in BMS is not known, but one speculated cause is repeated thermal injury to the mouth from hot food and beverages (Kolkka-Palomaa et al., 2015).

Human experimental pain studies have applied capsaicin to the tongue or gingiva to produce burning pain in the oral cavity (Albin et al., 2008; Baad-Hansen et al., 2007; Baad-Hansen et al., 2003; Boudreau et al., 2009; Mo et al., 2015; Ngom et al., 2001). Application of 1-5% capsaicin causes burning pain which peaks after a few minutes, and then declines (Baad-Hansen et al., 2007; Baad-Hansen et al., 2003; Lu et al., 2013). In addition to burning pain, capsaicin application increases heat pain

sensitivity, but reduces sensitivity to innocuous and noxious mechanical stimuli as well as mechanical windup induced pain (Baad-Hansen et al., 2007; Baad-Hansen et al., 2003; Lu et al., 2013). Thus, topical application of capsaicin to the tongue can replicate some of the pain and pain sensitivity features of BMS.

Conservative medical treatment aimed at symptom resolution is the normal therapeutic approach for BMS. Unfortunately, BMS symptoms have proven quite resistant to pharmacotherapy. Some BMS patients report a significant symptomatic improvement in their pain after sucking, without swallowing, a 1 mg tablet of the benzodiazepine clonazepam three times a day (Gremeau-Richard et al., 2004). Benzodiazepines are anxiolytic drugs which increase the ability of γ -amino-butyric acid (GABA) to open the GABA_A receptor. In some pre-clinical pain models, activation of peripheral GABA_A receptors in orofacial tissues appears to exert anti-nociceptive actions (Cai et al., 2001; Cairns et al., 2001; Cairns et al., 1999). These findings suggest that topical application of benzodiazepines to the oral mucosa may be effective in decreasing pain in BMS through a local action on peripheral GABA_A receptors to decrease the excitability of nociceptors. The present study was conducted to determine if oral administration of GABA containing solutions could reduce pain and sensitivity induced by local application of capsaicin to the tongue of healthy human subjects.

Methods

Subjects

Thirty healthy subjects (19 men and 11 women, mean age 26 ± 3) participated in this study conducted at Aalborg University. Subjects were recruited between June and December, 2016. All subjects were free from ongoing pain. Exclusion criteria included pregnancy, regular use of psychiatric, analgesic or other medications that might influence pain response, previous neurologic, musculoskeletal or mental illnesses, or a lack of ability to cooperate. The study was approved by the Scientific Ethics Committee for the North Jutland Region of Denmark (reference no. N-20160037). Written informed consent was obtained from all participants. This trial was registered at ClinicalTrials.gov (NCT02928328).

Experimental protocol

This study employed a randomized, placebo-controlled, double-blinded, cross-over study design. A computerized randomization table was used to assign treatments (KW), and the examiner (YZ) who undertook the intraoral quantitative sensory testing (QST) was unaware of the treatment subjects received. Each subject was enrolled by either KW or YZ, and participated in 4 experimental sessions (Figure 1). Sessions were a minimum of 3 days apart, and subjects were requested avoid eating chili peppers or garlic for at least 24 hours prior to the experimental session.

At the beginning of each session, baseline intraoral QST on the tip of the tongue was carried out (Base). Then, the subjects received topical application of 1%

capsaicin cream (Aalborg Hospital Pharmacy, Aalborg Denmark) to the tongue dorsum for 2 minutes (Figure 1)(Boudreau et al., 2009). A standardised tongue-shaped piece of plastic film with a circular cut out of it that was approximately 2 cm² was then placed on the surface of tongue (~1.5 cm from the tip) to permit uniform application of capsaicin to the tongue. After application of capsaicin cream (1%, 1 g, Aalborg hospital pharmacy), another piece of tongue-shaped plastic film was placed on top the capsaicin to avoid its spreading. Upon application of the capsaicin cream, subjects were instructed to rate their oral pain on an electronic 0-10 cm Visual Analogue Scale (VAS, "0" indicating no pain, and "10" indicating the worst pain imaginable; Figure 1. At the end of the 2-minute application, capsaicin was removed with a cotton ball, and intraoral QST was performed again (CAP; Figure 1). Subjects were then instructed to rinse their mouths with 20 ml of solution containing one of 2 concentrations of GABA (0.05 or 0.5 M), 1 % lidocaine (positive control) or distilled water (vehicle control) for 2 minutes, after which they spit the solution out. The clear solutions containing the treatments, labelled A, B, C or D, were stored in the fridge prior to use and given based the order in the randomization table. After spitting the solution out, oral QST was assessed (Rinse), and then reassessed after a 10-minute break (Post).

Intraoral QST

Tests included tongue cold and warm detection thresholds, cold and heat pain thresholds, mechanical detection thresholds and mechanical pain thresholds.

Thermal tests were performed using a computerized thermal stimulator (MEDOC TSA-2001 apparatus, Medoc Ltd, Ramat-Yishai, Israel). The contact area of the intra-oral thermode was 36 mm². Cold and warm detection thresholds (CDT, WDT) were measured first, and were followed by cold and heat pain thresholds (HPT). The mean threshold of 3 consecutive measurements was calculated for each time period. During each thermal assessment, the temperature of the thermode was initially 37°C and was decreased or increased at a rate of 1°C/s to a lower or upper limit of 0°C and 50°, respectively. The participants were instructed to press a button on a computer mouse as soon as they detected the appropriate thermal sensation (Mo et al., 2015; Svensson et al., 2011).

MDT and MPT were measured using a “method of limits” technique (Baad-Hansen et al., 2003). Standardized Semmes-Weinstein monofilaments (North Coast Medical, Canada) with 20 different diameters were used to measure MDT. To prevent filament slippage, intra-oral examination sites were dried with gauze before testing [14,15].

To detect the mechanical pain threshold (MPT), weighted pinprick stimuli delivered with a custom-made set of seven pinprick stimulators (Aalborg University, Denmark) were used (Mo et al., 2015; Suzuki et al., 2016). Each stimulator had a flat contact surface of 0.2 mm that exerted forces of 8-512 mN. All pinprick tests were made with the stimulator perpendicular to the examination site and in a vertical position with a contact time of 1 second. Five threshold measurements were made, applying a series of ascending and descending stimulus intensities. Threshold was

determined by calculating the geometric mean of these five measurements.

Oral rinse solutions

GABA powder (Now Foods, Sparks, Nevada, USA) was dissolved in deionized water, and adjusted to a concentration of either 51.5 mg/ml (0.5 M), or 5.2 mg/ml (0.05 M).

Injectable lidocaine (1% , Lidocaine, Copenhagen, Denmark) solution was used as the positive control, as it has been shown to significantly reduce capsaicin induced burning pain on the tongue (Gottrup et al., 2000; Ngom et al., 2001). Deionized water was used as a vehicle control.

Data analysis

The sample size was estimated based on a minimal relevant difference in VAS between treatments of 25% (primary outcome measurement), a conservative estimate of the intra-individual variation in the VAS of 30% and a risk of type I and type II errors of 5% and 20%, respectively. This estimation indicated that 24 subjects would be required. However, to compensate for a possible 20% drop out rate, we recruited 30 subjects for the study.

The area under the VAS curve was calculated (VASAUC) for i) CAP (0-10 min), and ii) Rinse (12-30 min) (Figure 1B). A one-way repeated measures ANOVA on ranks was used to determine if there were significant intersessional differences in the response to capsaicin and to assess whether there were significant differences between the treatments. Tukey tests were used for post-hoc analysis. Sex-related

differences for each treatment were assessed with Mann-Whitney rank sum tests. Two way repeated measures ANOVAs (factors: time, treatment) were also used to assess for significant differences in the QST parameters (secondary outcome measures). Where QST data was not normally distributed, log transformation was applied. Student-Neuman-Keuls tests were employed for post-hoc comparisons. A P value <0.05 was considered significant.

Results

Capsaicin-evoked pain

All 30 subjects completed the study. Capsaicin application to the tongue elicited a mild burning pain with a peak of 4.8 ± 0.3 that was not significantly different in men and women. A significant intersessional difference in the VASAUC for capsaicin was found. Post-hoc testing indicated that the VASAUC for capsaicin alone was significantly greater in the lidocaine session than in the water session (Figure 2A). To compensate, the VASAUC for the post rinse period was normalized to the VASAUC for capsaicin in each session (Figure 2B). Normalized VASAUC was significantly decreased, compared to water, when either concentration of GABA or lidocaine 1% were used in the rinse. Lidocaine reduced the normalized VASAUC to a significantly greater amount than either concentration of GABA. There was no significant concentration-related difference in the analgesic effect of GABA. There were no significant sex-related differences in response to any of the treatments.

Thermal Parameters

CDT was significantly lowered (less sensitive) by capsaicin treatment. There was no significant effect of treatment on capsaicin-induced lowering of the CDT (Figure 3A). Mean baseline CDT (women: 31.8 ± 0.7 C, men: 30.7 ± 0.3 C) was not significantly different between men and women.

Although cold pain threshold was also assessed, most subjects did not report pain from cold stimulation of the tongue and thus these data could not be assessed further.

WDT was significantly decreased (more sensitive) by CAP in all sessions and significantly increased (less sensitive) by rinsing the mouth in all sessions. Rinsing with GABA (0.05 or 0.5M) or lidocaine 1% resulted in a significantly greater increase in WDT compared to rinsing with water (Figure 3B). Mean baseline WDT (women: 41.5 ± 0.8 C, men: 42.3 ± 0.4 C) was not significantly different between men and women.

HPT was significantly decreased (more sensitive) by capsaicin in all sessions and significantly increased (less sensitive) by rinsing the mouth in all sessions. Rinsing with GABA (0.05 or 0.5M) or lidocaine 1% resulted in a significantly greater increase in HPT compared to rinsing with water. Mean baseline HPT (women: 44.4 ± 0.8 C, men: 46.7 ± 0.4 C) was not significantly different between men and women.

Mechanical Parameters

MDT was significantly increased (less sensitive) by capsaicin treatment in all sessions. There was no significant effect of treatment on capsaicin-induced increase of the MDT (Figure 4A). Mean baseline MDT (women: 0.59 ± 0.28 g, men: 0.44 ± 0.09 g) was not significantly different between men and women, and there were no sex-related differences in the effect of the treatments.

MPT was significantly decreased (more sensitive) by capsaicin treatment in all sessions, and this effect was significantly reversed by rinsing the mouth with all of the treatment solutions. There was no difference between the treatments (Figure 4B). Mean baseline MPT (women: 81 ± 8 g, men: 82 ± 8 g) was not significantly different between men and women.

There was no significant difference between men and women in any of the QST parameters.

Blinding

At the end of each session, subjects were asked to indicate which of the 4 possible treatments they thought they had received. For water, GABA 0.05M and GABA 0.5M, 47%, 30% and 27% of subjects guessed correctly. This is not a significantly different frequency of response than would have been expected simply by chance (Fisher exact test). However, when given lidocaine, 80% of subjects guessed correctly what was in the oral rinse. This result suggests that most subjects, not surprisingly, were

able to detect the effects of this local anesthetic when it was administered in the rinse solution.

Adverse Effects

Potential adverse effects of orally consumed GABA may include drowsiness as well as a short term increase in heart rate and shortness of breath. Sensations of tingling, itching, tickling in the neck, face and limbs have also been reported. Subjects in the present study did not report any of these effects after rinsing their mouths with GABA solutions.

Discussion and Conclusions

The present study found that rinsing with either GABA 0.05 M or GABA 0.5 M solution was significantly more effective than water in reducing the burning pain produced by topical application of capsaicin to the tongue. In addition to burning pain, capsaicin application to the tongue increased mechanical detection threshold (less sensitive), but reduced mechanical pain as well as warm detection and heat pain thresholds (more sensitive). Compared to water, GABA containing rinse solutions were also significantly more effective in ameliorating the lowered warmth detection and heat pain thresholds produced by topical capsaicin. These results suggest that GABA containing solutions exert a peripheral localised analgesic effect in this model of burning mouth pain

Capsaicin induced burning mouth pain

A number of studies have previously applied capsaicin to the tongue or gingiva to produce burning pain for a period of up to 25 minutes (Albin et al., 2008; Baad-Hansen et al., 2007; Baad-Hansen et al., 2003; Boudreau et al., 2009; Mo et al., 2015; Ngom et al., 2001). In addition to pain, capsaicin application was reported to increase heat pain sensitivity, but reduced sensitivity to innocuous and noxious mechanical stimuli as well as mechanical windup induced pain (Albin et al., 2008; Baad-Hansen et al., 2007; Baad-Hansen et al., 2003; Lu et al., 2013). The effect of capsaicin on cold pain is variable (Albin et al., 2008). Thus, consistent with our findings, topical application of capsaicin to the oral cavity appears to increase sensitivity to heat and decrease sensitivity to innocuous mechanical stimulation. In contrast, BMS patients often exhibit a loss of cold and heat pain detection sensitivity on the tongue without change in mechanical pain detection thresholds controls (Mo et al., 2015). Thus, while capsaicin application to the tongue can replicate the burning pain reported by BMS patients, it does not produce similar effects on thermal and mechanical sensitivity.

GABA acts on peripheral GABA_A receptors

Injection of GABA into the rat temporomandibular joint has previously been shown to exert analgesic-like actions through activation of the peripheral GABA_A receptor (Cai et al., 2001; Cairns et al., 1999). More recently, GABA_A receptors have been shown to be expressed by a majority of putative nociceptors that innervate the

epithelium of the rat tongue (Tan et al., 2014). Application of 0.5 M GABA or the selective GABA_A receptor agonist muscimol to the rat tongue attenuated the mechanical sensitization of polymodal nociceptors that occurred after application of hot (60°C) water to the tongue (Tan et al., 2014). In the present study, the same concentration of GABA was able to attenuate the sensitizing effect of capsaicin on tongue warmth detection and heat pain thresholds. Taken together, these findings suggest that topical application of benzodiazepines to the oral mucosa, which has been shown to reduce pain in patients with BMS [10-12], could be efficacious due to a local action on peripheral GABA_A receptors.

Treatment of burning mouth pain

Available evidence supports the long term treatment effectiveness of topical clonazepam and low concentration capsaicin oral rinses for BMS (Kisely et al., 2016; Liu et al., 2017; McMillan et al., 2016). Unfortunately, many patients receiving capsaicin oral rinses develop dyspepsia that causes them to withdraw from treatment, which limits the usefulness of this approach (Kisely et al., 2016). Orally restricted administration clonazepam has few reported side effects (Kisely et al., 2016; Liu et al., 2017; McMillan et al., 2016). Systemically administered clonazepam, which does cause some level of drowsiness or sedation, may also not be as effective for pain in BMS as topical clonazepam (Kisely et al., 2016; McMillan et al., 2016). However, in studies that investigated local anesthetics, a minimal and clinically negligible effect on pain in BMS patients was found (Kisely et al., 2016; Liu et al., 2017). This contrasts

with our finding that lidocaine rinse significantly reduced acute capsaicin-induced burning mouth pain, and was in fact significantly more effective than GABA containing solutions in this effect. Lidocaine is a local anesthetic that has been previously shown to effectively block the burning pain induced by capsaicin (Gottrup et al., 2000; Ngom et al., 2001). However, it is important to point out that most subjects given lidocaine rinse were able to correctly identify this treatment due to its recognizable numbing effect, and that this knowledge may have biased their pain ratings. Nevertheless differences in the response characteristics of healthy individuals given capsaicin to induce burning mouth pain in this study and patients diagnosed with BMS to local application of lidocaine indicate that caution should be exercised in attempting a direct translation of the results into clinical treatment.

GABA is currently sold as a health food supplement in the European Union and North America. Although GABA is an inhibitory amino acid, it does not appear to effectively cross the adult human blood brain barrier, and thus little, if any, orally ingested GABA likely makes it into the brain (Boonstra et al., 2015). There are no reports of major side effects after daily consumption of oral GABA up to 3 g (Goldberg 2010; Powers et al., 2008). Nevertheless, to minimize the potential for healthy subjects in the present study to have significant systemic exposure to GABA, they were further required to expectorate oral solutions after rinsing. Subjects receiving GABA in the current study did not report feeling symptoms, such as drowsiness or dizziness, suggestive of a systemic effect of a central nervous system depressant. Further, unlike lidocaine, subjects did not report believing that they had received

GABA more often than expected by chance in the present study. This is interpreted as further evidence that oral rinses with GABA containing solutions act locally within the oral cavity to exert their analgesic effect.

Limitations

A potential limitation of this study was that stimuli were only applied to the tip of the tongue. However, thermal thresholds (WDT, HPT, CDT) from the middle anterior dorsal surface of the tongue are comparable with those of the present study, despite differences in innervation density (Kaplan et al., 2011). Nevertheless, it is unclear whether the local analgesic effects of GABA would extend to the rest of the tongue or oral cavity.

As discussed previously, capsaicin-induced burning tongue pain does not replicate all of the symptoms of burning mouth syndrome. In addition to those mentioned, burning mouth syndrome can be associated with metabolic changes (e.g. diabetes) and mood alterations (e.g. anxiety, depression) in a subset of patients, which cannot be replicated by acute administration of capsaicin (Arap et al., 2010; de Souza et al., 2012; Moore et al., 2007; Penza et al., 2010). Indeed, the acute nature of the burning pain makes this model very different from the chronic ongoing pain that is characteristic of many burning mouth sufferers.

Conclusion

It is concluded that rinsing the mouth with GABA solution resulted in concentration independent decreases capsaicin-induced burning pain in the mouth and some of its associated sensory changes on the tongue in healthy subjects, which suggests that GABA can act as a local analgesic agent in the oral cavity. Future research is required to determine whether use of GABA oral solutions might be effective in the treatment of BMS-related burning pain.

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Figure Legends:

Figure 1: The drawing illustrates the methodology employed in the present study. After baseline quantitative sensory testing (QST) was completed, capsaicin 1% topical cream was applied to the tongue to induce burning pain. Two minutes after the application of capsaicin, the capsaicin was removed and the mouth was rinsed with either GABA (0.5 M, or 0.05M), 1% lidocaine or water for 2 minutes.

Figure 2: **A.** The median intensity of capsaicin evoked burning pain is shown in the line drawing. Rinsing with any solution reduced capsaicin evoked pain, however, compared with water pain resolved more rapidly after administration of GABA or lidocaine. **B.** The bar graph illustrates the normalized median area under the VAS curve (VASAUC) post rinse for all 4 treatments for all 30 subjects. Lines indicate the interquartile range. All treatments were significantly better than water in reducing

capsaicin induced pain. There was an apparent dose-related for response for GABA, whereby the higher concentration of GABA was somewhat better at reducing VASAUC than the lower concentration. *: $P < 0.05$, repeated measures ANOVA and Dunnett's method compared with water

Figure 3: The line and scatter plots illustrate the effect of capsaicin as well as the effect of the rinse solutions on **A.** cold detection threshold (CDT), **B.** warm detection threshold (WDT, and **C.** heat pain threshold (HPT). Application of capsaicin significantly increased CDT and decreased WDT as well as HPT on the tongue. In **A**, none of the treatments significantly altered the lowering of the cold detection threshold by capsaicin. In **B**, rinsing with either GABA solution or lidocaine significantly reversed the capsaicin-induced reduction in warm detection. Similarly, in **C**, rinsing with either GABA solution or lidocaine significantly reversed the capsaicin induced reduction in heat pain threshold. Each circle is the mean from 30 subjects. Error bars indicate standard error of the mean (SEM). #: $P < 0.05$ repeated measures ANOVA and Student-Neuman Keuls method for time. *: $P < 0.05$ repeated measures ANOVA and Student-Neuman Keuls method for treatment compared to water.

Figure 4: The line and scatter plots illustrate the effect of capsaicin as well as the effect of the rinse solutions on **A.** MDT and **B.** MPT. Application of capsaicin significantly increased MDT and significantly lowered MPT. In **A**, none of the treatments significantly altered the increase in MDT by capsaicin. In **B**, all the

treatments significantly reversed the capsaicin induced reduction in MPT. Each circle is the mean from 30 subjects. Error bars indicate standard error of the mean (SEM). #: $P < 0.05$ repeated measures ANOVA and Student-Neuman Keuls method for time.

Figure 1

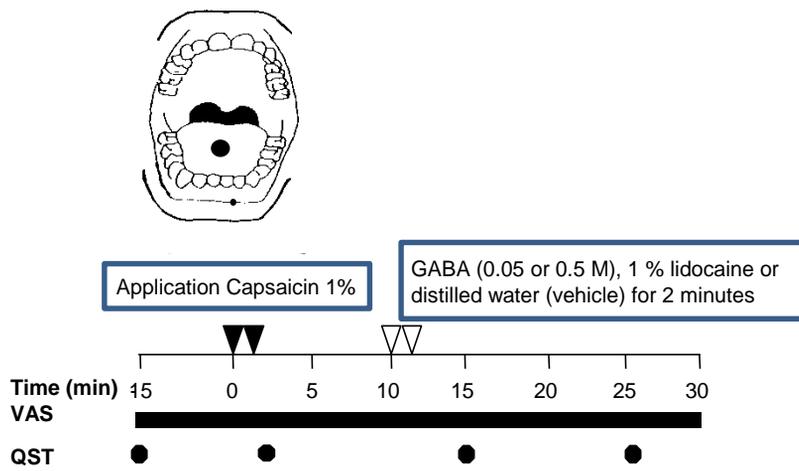
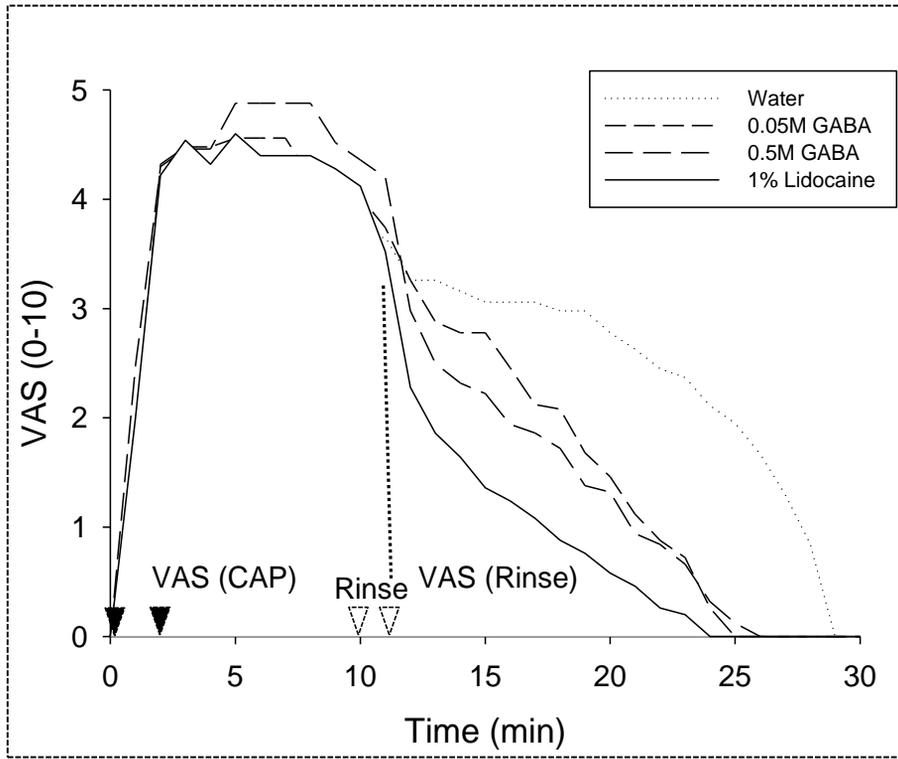


Figure 2

A



B

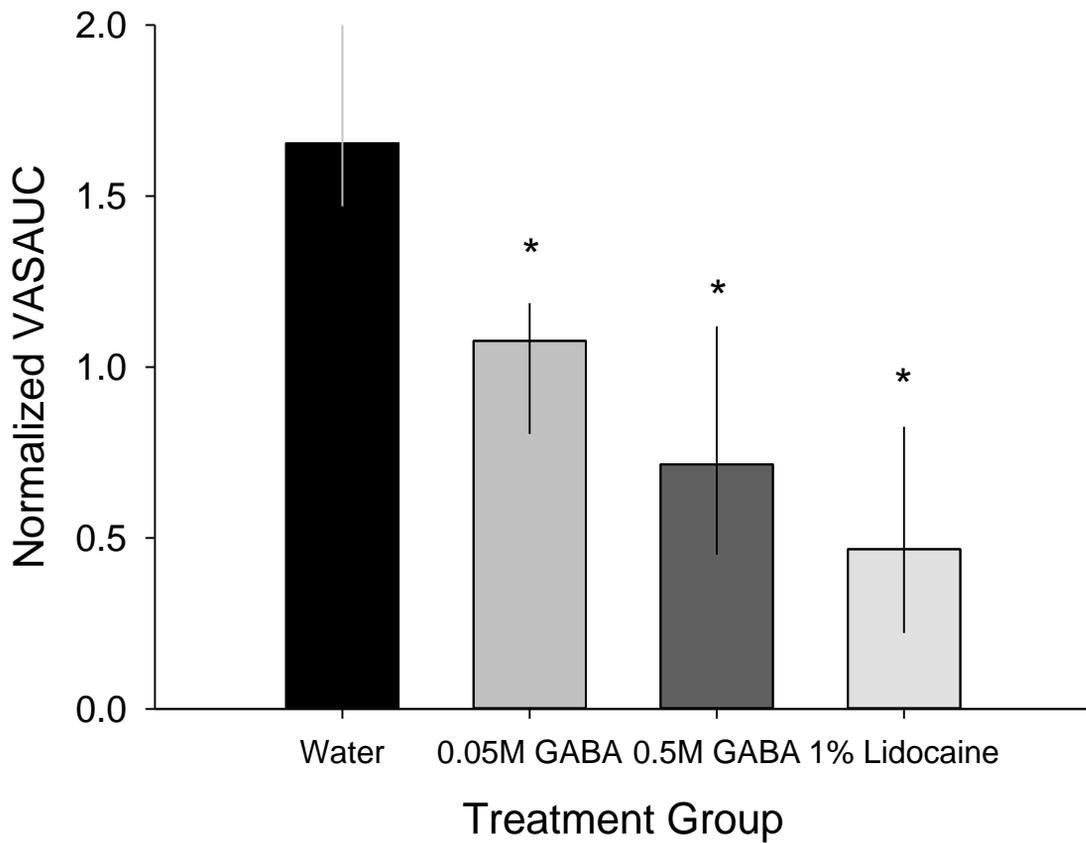
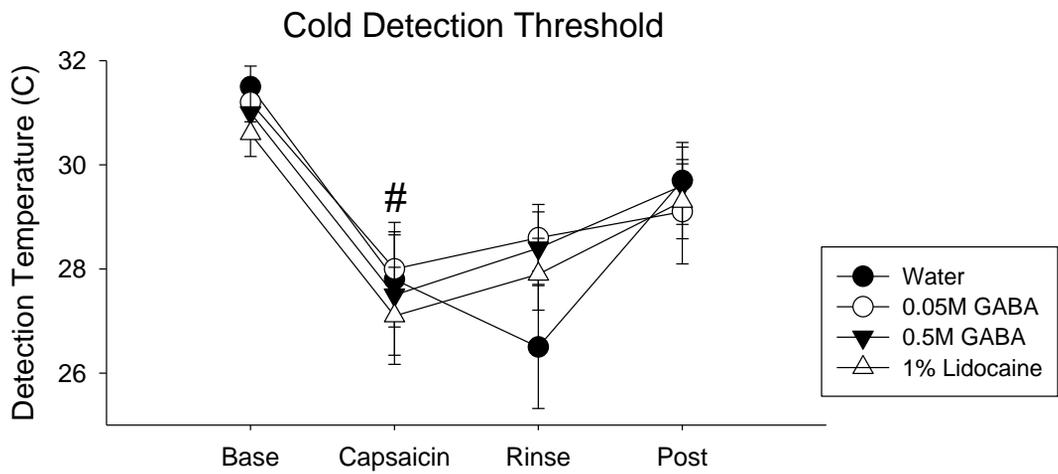
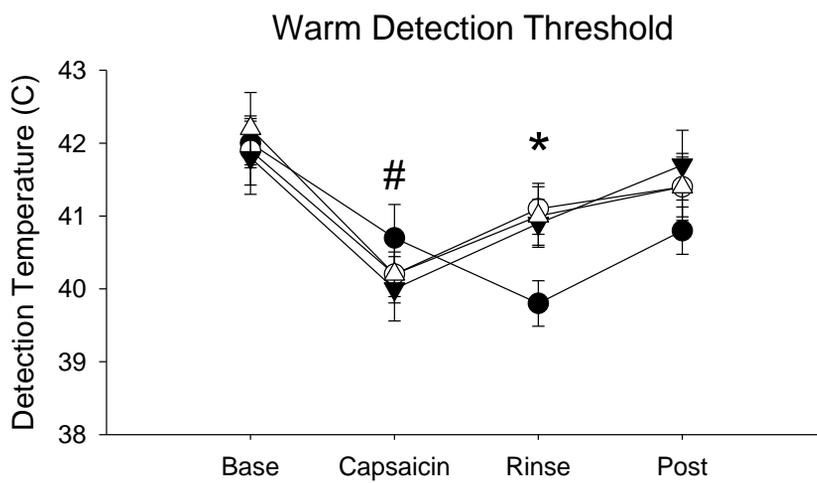


Figure 3

A



B



C

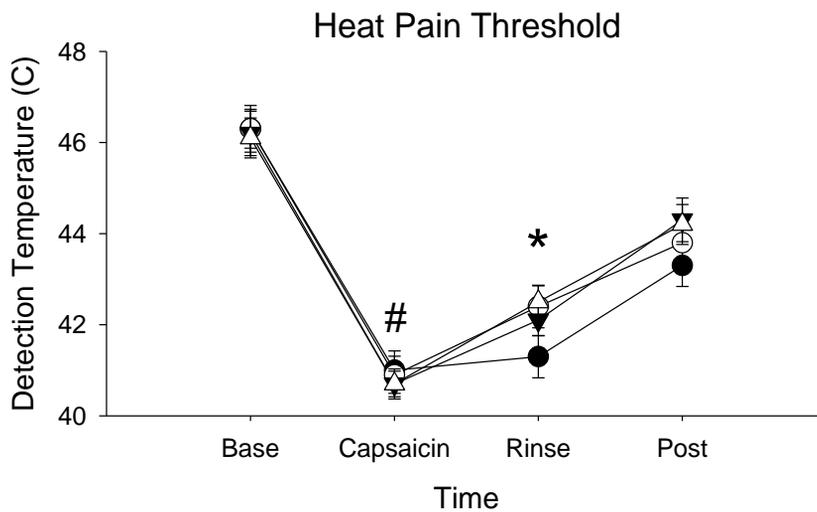
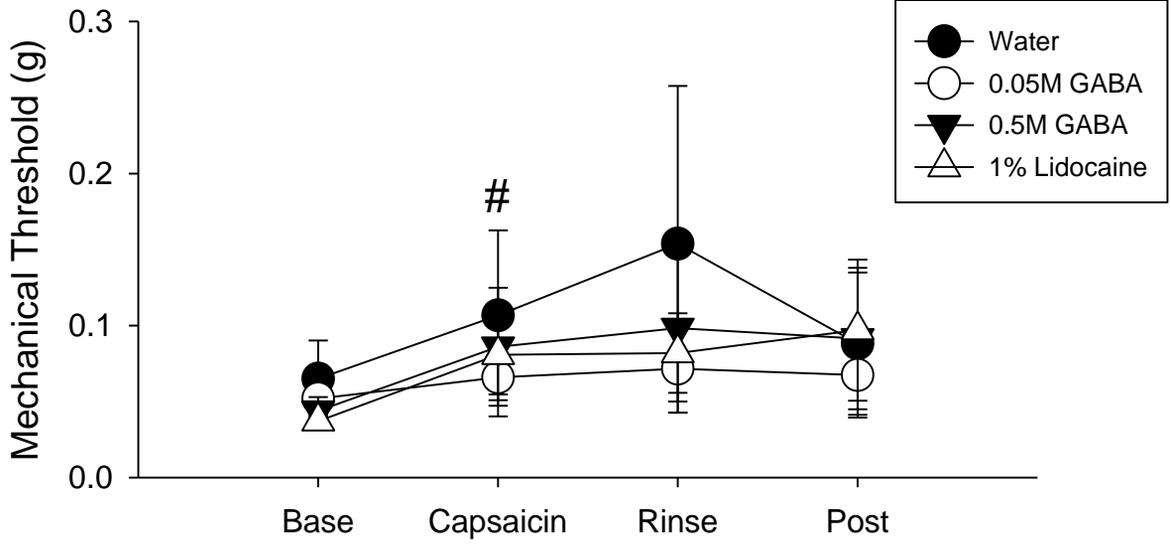


Figure 4

A

Mechanical Detection Threshold



B

Mechanical Pain Threshold

