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Glutamate and capsaicin effects on trigeminal nociception I: Activation and peripheral sensitization of deep craniofacial nociceptive afferents

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Abstract

We have examined the effect of the peripheral application of glutamate and capsaicin to deep craniofacial tissues in influencing the activation and peripheral sensitization of deep craniofacial nociceptive afferents. The activity of single trigeminal nociceptive afferents with receptive fields in deep craniofacial tissues were recorded extracellularly in 55 halothane-anesthetized rats. The mechanical activation threshold (MAT) of each afferent was assessed before and after injection of 0.5M glutamate (or vehicle) and 1% capsaicin (or vehicle) into the receptive field. A total of 68 afferents that could be activated by blunt noxious mechanical stimulation of the deep craniofacial tissues (23 masseter, 5 temporalis, 40 temporomandibular joint) were studied. When injected alone, glutamate and capsaicin activated and induced peripheral sensitization reflected as MAT reduction in many afferents. Following glutamate injection, capsaicin-evoked activity was greater than that evoked by capsaicin alone, whereas following capsaicin injection, glutamate-evoked responses were similar to glutamate alone. These findings indicate that peripheral application of glutamate or capsaicin may activate or induce peripheral sensitization in a subpopulation of trigeminal nociceptive afferents innervating deep craniofacial tissues, as reflected in changes in MAT and other afferent response properties. The data further suggest that peripheral glutamate and capsaicin receptor mechanisms may interact to modulate the activation and peripheral sensitization in some deep craniofacial nociceptive afferents.

Keywords

excitatory amino acid; TRPV1; pain; temporomandibular joint

1. INTRODUCTION

There is emerging evidence that both peripheral excitatory amino acid (EAA) and vanilloid (TRPV1) receptor mechanisms may modulate nociceptive processing of input from deep craniofacial tissues. Glutamate, the endogenous agonist for EAA receptors, is a well-documented central excitatory neurotransmitter but a number of studies indicate a role for *peripheral* glutamate receptors in the transduction of nociceptive information (Yu *et al.*

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1996; Lawand *et al.* 2000; McNearney *et al.* 2000, 2004; Carlton 2001; Carlton *et al.* 2003; Lam *et al.* 2005b). We have identified a novel *peripheral* nociceptive role for glutamate in the craniofacial region. Intramuscular (masseter) or temporomandibular joint (TMJ) injection of glutamate reflexly evokes a dose-dependent increase in rat jaw muscle activity (Cairns *et al.* 1998), activates and sensitizes mechanosensitive nociceptive afferents through the activation of peripheral EAA receptors (Cairns *et al.* 2001a,b, 2002, 2003; Dong *et al.* 2006, 2007), and activates and induces central sensitization of brainstem nociceptive neurons in the trigeminal subnucleus caudalis/upper cervical cord (Vc/UCC) (Lam *et al.* 2008). The peripheral EAA receptor, N-methyl-D-aspartate (NMDA), in particular may play an important role in glutamate-induced effects on nociceptive afferents since NMDA receptor antagonists applied locally into masticatory muscles significantly decrease glutamate-evoked afferent activation and sensitization as well as jaw muscle activity (Cairns *et al.* 1998, 2003a, 2007; Dong *et al.* 2006, 2007). Similarly, glutamate injection into the human masseter muscle causes pain that may be attenuated by co-injection of an NMDA receptor antagonist (Cairns *et al.* 2001a, 2003a,b, 2006; Svensson *et al.* 2003, 2005).

Another peripheral receptor involved in craniofacial nociceptive mechanisms is the TRPV1 receptor. This receptor is activated by application to peripheral tissues of the inflammatory irritant capsaicin, protons or noxious heat (Caterina *et al.* 1997; Tominaga *et al.* 1998; Dray 2005). Capsaicin can activate (Liu and Simon 1994, 1996, 2003) and sensitize trigeminal afferents (Strassman *et al.* 1996) and also activate brainstem nociceptive neurons (Carstens *et al.* 1998; Zanotto *et al.* 2007). Furthermore, capsaicin injection into the rat TMJ reflexly evokes a dose-dependent increase in jaw muscle activity (Tang *et al.* 2004; Lam *et al.* 2005a), activates and induces central sensitization of brainstem nociceptive neurons in the Vc/UCC (Lam *et al.* 2008), and in addition produces an inflammatory response within these tissues that can be blocked by TRPV1 receptor antagonists (Hu *et al.* 2005a). Capsaicin injected into human craniofacial regions can also induce secondary hyperalgesia, allodynia and jaw muscle pain associated with changes in jaw motor function (Sohn *et al.* 2000, 2004; Wang *et al.* 2002; Gazerani and Arendt-Nielsen 2005; Gazerani *et al.* 2006).

However, there has been little investigation of possible interactions between peripheral EAA and TRPV1 receptors and their effects on nociceptive processing. There is evidence that EAA receptors may indirectly modulate nociception via interactions with TRPV1 in the central nervous system. Both capsaicin-evoked release of spinal substance P (Afrah *et al.* 2001) and capsaicin-evoked activity at the periaqueductal grey level (Palazzo *et al.* 2002; Xing and Li 2007) may be dependent on glutamate-evoked activation of NMDA receptors. A role for NMDA receptor modulation of capsaicin-induced *c-fos* expression within the rat Vc has also been demonstrated (Mitsikostas *et al.* 1998). Similarly, the presence of EAA and TRPV1 receptors on primary afferents and findings that their agonists have effects when injected into peripheral tissues raise the possibility that the actions of peripherally applied glutamate and capsaicin could involve interactions between EAA and TRPV1 receptors in these tissues. Indeed, jaw muscle activity reflexly evoked by the TMJ application of capsaicin can be attenuated by pre-injection into the TMJ of NMDA receptor antagonists (Lam *et al.* 2005a), suggesting that peripheral EAA receptors may interact with peripheral TRPV1 receptor mechanisms. While it is also well known that capsaicin can sensitize or desensitize nociceptive primary afferents (Baumann *et al.* 1991; Craft and Porreca 1992; LaMotte *et al.* 1992; Liu and Simon 1996; Sikand and Premkumar 2007), it is not known whether glutamate or capsaicin would sensitize or desensitize each other's effects on afferent responses. Thus, the aim of the present study was to examine the effect of the peripheral application of glutamate and capsaicin in deep craniofacial tissues in influencing the activation and peripheral sensitization of deep craniofacial nociceptive primary afferents. The data have been briefly presented in abstract form (Lam *et al.* 2004a,b).

2. RESULTS

NEURONAL PROPERTIES

A total of 68 trigeminal nociceptive primary afferents with receptive fields (RFs) in deep craniofacial tissues were recorded: 54 A δ -fiber and 14 C-fiber afferents. The estimated antidromic (A δ -fiber: mean \pm SE= 10.2 \pm 0.9 m/s, n=48; C-fiber: 1.7 \pm 0.2 m/s, n=12) and orthodromic (A δ -fiber: 9.8 \pm 0.8 m/s, n=54; C-fiber: 1.6 \pm 0.1 m/s, n=14) conduction velocities (CVs) were not statistically different ($p>0.05$, Mann-Whitney U test). The majority of afferents could be activated antidromically and orthodromically (60/68; 48 A δ -fiber, 12 C-fiber); the remainder were activated only orthodromically (8/68; 6 A δ -fiber, 2 C-fiber). A small proportion (10%, 7/68, 6 A δ -fiber, 1 C-fiber) were spontaneously active (0.38 \pm 0.12 spikes/second) prior to injection of receptor agonists. Table 1 displays the baseline mean CV and mechanical activation threshold (MAT) for afferents with RFs in the TMJ (n=40), masseter (n=23) and temporalis (n=5).

There were no differences between the A δ -fiber and C-fiber afferents in baseline MAT ($p>0.05$, t-test) (Table 1) or in responses (R_{mag}, R_{dur}, R_{lat} and P_{freq}) to glutamate and capsaicin ($p>0.05$, Mann-Whitney U test; $p>0.05$, Fisher exact test) (data not shown) and as a result, the data were pooled together for analysis of glutamate and capsaicin-induced activation and sensitization. Examples of typical afferent RF and response properties are shown in Fig. 1A and 1B.

GLUTAMATE EFFECTS

ACTIVATION—Injection of glutamate alone (but not vehicle alone, 0/4) activated 43% (12/28) of the afferents tested (9/23 A δ -fibers, CV= 8.2 \pm 1.5 m/s; 3/5 C-fibers, CV= 1.6 \pm 0.3 m/s) (e.g. Fig. 1B) ($p<0.001$, Fisher exact test), with the activation response properties outlined in Table 2. Glutamate-evoked afferent activation began approximately 6 seconds from injection (R_{lat} = 5.8 [8.2] seconds) and lasted over 2 minutes (R_{dur} = 144 [138] seconds). There was no difference in mean CV between glutamate-sensitive (n=12, CV=6.3 \pm 1.4 m/s) and glutamate-insensitive afferents (n=16, CV=9.4 \pm 1.4 m/s, $p>0.05$, t-test).

MAT REDUCTION—The spontaneous afferent activity returned to baseline level prior to determination of MAT post-injection of glutamate in all 28 afferents tested. Injection of glutamate alone (but not vehicle alone, 0/4) induced a marked incidence of MAT reduction ($\geq 50\%$ threshold reduction from baseline MAT score) at 10–20 minutes post-injection in 46% (13/28) of the afferents (11/23 A δ -fibers; 2/5 C-fibers; $p<0.05$, Fisher exact test). Similarly, for the afferent population tested as a whole, the mean baseline MAT value was 33.2 \pm 3.6 g, and injection of glutamate alone (but not vehicle alone, n=4, $p>0.05$, RM ANOVA-on-ranks) produced a significant reduction in MAT values (19.9 \pm 2.2 g) relative to their pre-injection baseline ($p<0.05$, RM ANOVA-on-ranks, Dunn's method) (Fig. 2a). Many afferents activated by glutamate [50% (6/12)] injection alone did not show MAT reduction, whereas some afferents displaying MAT reduction following glutamate [44% (7/16)] injection alone showed no prior activation by glutamate ($p>0.05$, Fisher exact test).

CAPSAICIN EFFECTS

ACTIVATION—Injection of capsaicin alone (but not vehicle alone, 0/4) activated 24% (6/25) of the afferents (2/18 A δ -fibers, CV= 7.5 \pm 0.3 m/s; 4/7 C-fibers, CV= 1.7 \pm 0.2 m/s) tested ($p<0.01$, Fisher exact test), with the activation response properties outlined in Table 2. Capsaicin-evoked afferent activation began approximately 10 seconds from injection (R_{lat} = 9.8 [20] seconds) and lasted about half a minute (R_{dur} = 30 [50] seconds). The median CV for capsaicin-sensitive (n=6, CV=1.7[0.5] m/s) afferents was significantly smaller than that for

capsaicin-insensitive afferents ($n=19$, $CV=9.0[10.3]$ m/s, $p<0.05$, Mann-Whitney U test) reflecting the difference in the properties of A δ - and C-fibers activated.

The incidence of afferent activation induced by injection of capsaicin alone (24%) was similar to that induced by glutamate alone (43%) ($p>0.05$, Fisher exact test). However, afferent activation response properties (R_{mag} , R_{dur} and P_{freq}) induced by capsaicin alone were significantly less compared with glutamate alone ($p<0.05$, Mann-Whitney U test) (Table 2).

MAT REDUCTION—The spontaneous afferent activity returned to baseline level prior to determination of MAT post-injection of capsaicin in all 22 afferents tested. Injection of capsaicin alone (but not vehicle alone, 0/4) induced a marked incidence of MAT reduction ($\geq 50\%$ threshold reduction from baseline MAT score) at 10–20 minutes post-injection in 41% (9/22) of the afferents (6/15 A δ -fibers; 3/7 C-fibers; $p<0.05$, Fisher exact test). However, for the afferent population tested as a whole, the median baseline MAT value was 20.2[29.7] g, and injection of capsaicin alone (or vehicle alone, $n=4$) did not significantly alter the MAT values (19.2[28.2] g) compared with their pre-injection baseline ($p>0.05$, RM ANOVA-on-ranks) (Fig. 2b). Many afferents activated by capsaicin [83% (5/6)] injection alone did not show MAT reduction, whereas some afferents displaying MAT reduction following capsaicin [36% (8/16)] injection alone showed no prior activation by capsaicin ($p>0.05$, Fisher exact test).

GLUTAMATE AND CAPSAICIN INTERACTIONS

ACTIVATION—There were four types of agonist-responsive deep craniofacial afferents found in the glutamate followed by capsaicin subgroup: (1) glutamate-sensitive and capsaicin-sensitive [15% (4/27)]; (2) glutamate-sensitive and capsaicin-insensitive [30% (8/27)]; (3) glutamate-insensitive and capsaicin-sensitive [18% (5/27)]; and (4) glutamate-insensitive and capsaicin-insensitive [37% (10/27)]. Following glutamate injection, capsaicin (but not vehicle, 0/3) evoked responses in 33% (9/27) of the afferents tested and produced greater increases in R_{mag} and P_{freq} ($p<0.05$, Mann-Whitney U test) but no change in R_{lat} and R_{dur} compared with capsaicin alone ($p>0.05$, Mann-Whitney U test) (Table 2). There was no significant difference in the incidence of capsaicin-induced activation with capsaicin alone, compared with capsaicin following glutamate injection ($p>0.05$, Fisher exact test).

There were also four types of agonist-responsive afferents found in the capsaicin followed by glutamate subgroup: (1) capsaicin-sensitive and glutamate-sensitive [9% (2/22)]; (2) capsaicin-sensitive and glutamate-insensitive [18% (4/22)]; (3) capsaicin-insensitive and glutamate-sensitive [23% (5/22)]; and (4) capsaicin-insensitive and glutamate-insensitive [50% (11/22)]. Following capsaicin injection, glutamate (but not vehicle, 0/4) evoked responses in 32% (7/22) of the afferents tested that were not significantly different in R_{mag} , R_{lat} , R_{dur} and P_{freq} compared to glutamate alone (Table 2). Similarly, there was no significant difference in the incidence of glutamate-induced activation with glutamate alone, compared with glutamate following capsaicin injection ($p>0.05$, Fisher exact test).

MAT REDUCTION—Compared to the incidence of capsaicin-induced MAT reduction with capsaicin alone, capsaicin following glutamate injection induced MAT reduction in only 12% (3/25) of the afferents ($p<0.05$, Fisher exact test). Furthermore, when injected following glutamate, capsaicin (or vehicle, 0/3) produced a non-significant increase in the median MAT from pre-injection baseline, indicating that capsaicin induced no further MAT reduction than that induced by the preceding glutamate injection ($p<0.05$, RM ANOVA-on-ranks, Dunn's method) (Fig. 2a).

There was no significant difference in the incidence of glutamate-induced MAT reduction with glutamate alone compared with glutamate following capsaicin injection [45% (9/20)] ($p>0.05$,

Fisher exact test). Furthermore, when injected following capsaicin, glutamate (but not vehicle, 0/4) produced a significant reduction in MAT values relative to their pre-injection baseline ($p < 0.01$, RM ANOVA-on-ranks, Dunn's method) (Fig. 2b).

3. DISCUSSION

This is the first study to document that a considerable proportion of deep craniofacial nociceptive afferents may be activated or sensitized by the peripheral application of glutamate or capsaicin or both, and that these EAA or TRPV1 receptor agonists may interact to modulate activation as well as peripheral sensitization evoked from deep craniofacial tissues. Glutamate sensitized afferent responses to subsequent noxious stimulation of the deep craniofacial tissues by capsaicin, whereas capsaicin neither sensitized nor desensitized afferent responses to subsequent noxious stimulation by glutamate. These changes in response properties reflect peripheral sensitization as shown in enhanced activation by agonist injection (e.g. increase in Rmag and Pfreq). Taken together, these findings suggest that both peripheral EAA and TRPV1 receptor mechanisms may be involved in the nociceptive processing of deep craniofacial nociceptive afferents and may interact to modulate the activation and peripheral sensitization in some nociceptive afferents supplying deep craniofacial tissues.

Properties of deep craniofacial afferents projecting to Vc/UCC and their CVs are consistent with previous findings (Cairns *et al.* 2001a,b, 2002a, 2003a; Dong *et al.* 2006, 2007). Our findings of glutamate-induced activation and peripheral sensitization in trigeminal nociceptive afferents are consistent with previous evidence of glutamate-evoked dose-dependent increases in jaw muscle activity, activation and sensitization of deep nociceptive afferents, and pain in human masticatory muscles (Cairns *et al.* 1998, 2001a,b, 2002, 2003a,b, 2006, 2007; Svensson *et al.* 2003, 2005; Dong *et al.* 2006, 2007). Likewise, the incidence and discharge pattern of capsaicin-induced trigeminal afferent activation in the present study are consistent with those found in mechanosensitive spinal (Baumann *et al.* 1991; LaMotte *et al.* 1992) and trigeminal (Strassman *et al.* 1996; Ikeda *et al.* 1997) afferents in previous studies. In general, capsaicin tended to primarily activate very slowly conducting afferents and the discharge was irregular and the majority of afferents ceased discharging within the first 60 seconds. Thus, it seems that the majority of trigeminal nociceptive afferents with deep craniofacial RFs in the present study responded too weakly or transiently to be able to account for the magnitude and duration of capsaicin-induced craniofacial pain in humans (Sohn *et al.* 2000, 2004; Wang *et al.* 2002; Gazerani and Arendt-Nielsen 2005; Gazerani *et al.* 2006). When injected alone, the capsaicin-induced MAT reduction was not as robust as that induced by glutamate alone. While the non-significant reduction in MAT for the afferent population as a whole is consistent with previous studies suggesting capsaicin-evoked mechanical sensitization is due to central rather than peripheral sensitization (Baumann *et al.* 1991; LaMotte *et al.* 1992), this non-significant capsaicin-evoked MAT reduction may be due, in part, to the lower afferent responses (Rmag, Rdur and Pfreq) evoked by capsaicin alone compared to glutamate alone. Nevertheless, the significant incidence of MAT reduction induced by capsaicin alone demonstrates that some trigeminal nociceptive afferents can be readily sensitized by the peripheral application of capsaicin and these findings are consistent with our previous evidence of capsaicin-evoked dose-dependent increases in jaw muscle activity (Tang *et al.* 2004). Taken together, the above findings indicate that peripheral TRPV1 receptors in addition to peripheral EAA receptors in deep craniofacial tissues may play an important role in nociceptive processing.

The finding that glutamate or capsaicin may induce MAT reduction without prior afferent activation may be explained by glutamate and capsaicin acting on peripheral non-neuronal cells in addition to neuronal cells and causing them via paracrine activation to release mediators that sensitize the deep craniofacial afferents (Lam *et al.* 2005a,b). There has been recent compelling evidence for the expression and function of glutamate and capsaicin in signaling

processes in several types of non-neuronal cells (Meddings *et al.* 1991; Skerry and Genever 2001; Kato *et al.* 2003; Rizvi and Luqman 2003; Li *et al.* 2005; Xin *et al.* 2005). Thus it may be possible that the activation of glutamate and capsaicin receptors on non-neuronal cells may result in the release of various other sensitizers including bradykinin, amines, prostanoids, growth factors, chemokines, cytokines, protons and ATP (Sikand and Premkumar 2007; Woolf and Ma, 2007) that may sensitize the deep craniofacial afferents.

Glutamate and Capsaicin Receptor Interactions in Deep Craniofacial Tissues

Capsaicin-evoked activation of trigeminal nociceptive afferents following glutamate injection were significantly enhanced compared to capsaicin alone, suggesting that glutamate may sensitize the nociceptive afferents and produce more immediate (e.g. decrease Rlat), larger (e.g. increased Rmag and Pfreq) and more prolonged (e.g. increased Rdur) responses to subsequent noxious stimuli (e.g. to capsaicin). These results contrast with those in a recent rat behavioral model in which masseteric injection of glutamate and capsaicin, in alternating order 10 minutes apart, evoked comparable nocifensive responses regardless of injection sequence (Ro and Capra 2006) but are consistent with findings that pre-injection of NMDA receptor antagonists into the TMJ region attenuates jaw muscle activity evoked by capsaicin (Lam *et al.* 2005a). A protein that is likely to mediate the interactions between peripheral NMDA and TRPV1 receptors is the Ca²⁺-calmodulin-dependent kinase II (CaMKII) which is persistently activated after NMDA receptor stimulation (see Yamakura and Shimoji 1999; Petrenko *et al.* 2003; Paoletti and Neyton 2007) and phosphorylation of TRPV1 by CaMKII is required for its ligand binding (Jung *et al.* 2004; Suh and Oh 2005; Tominaga and Tominaga 2005). Although no studies to date have demonstrated the co-localization of peripheral NMDA and TRPV1 receptors on the same trigeminal primary afferent terminal, nociceptive responses could be enhanced if the same nociceptive afferent expresses both EAA and TRPV1 receptors. Taken together, these findings suggest that the activation and/or sensitization of peripheral EAA receptors may be important in the mechanisms whereby capsaicin via TRPV1 receptors evoke nociceptive trigeminal responses.

In addition to possible interactions between ionotropic receptors, there is evidence of a major coupling between G-protein-coupled receptors and some TRP channels in the membrane such as TRPA1 and TRPV1 (Sikand and Premkumar 2007; Woolf and Ma 2007). For example, TRPA1 may function as a receptor-operated channel for bradykinin by allowing Ca²⁺ influx following activation of the B2 receptor (Bautista *et al.* 2006). Bradykinin can also significantly potentiate TRPV1 activity by activating the Ca²⁺/phospholipid-dependent kinase (PKC) pathway (Sikand and Premkumar 2007). Activation of the PKC pathway has also been shown to lower the heat threshold of TRPV1 below body temperature and sensitize TRPV1 receptor responses to capsaicin (Premkumar and Ahern 2000; Crandall *et al.* 2002). The mechanism behind this effect is thought to involve direct phosphorylation resulting in PKC-dependent insertion of TRPV1 receptors into the neuronal membrane (for review, see Hucho and Levine 2007). This type of coupling may also exist between TRPV1 and other G-protein-coupled receptors (Sikand and Premkumar 2007; Woolf and Ma 2007), such as the metabotropic EAA receptors, and provide an additional means for interactions between peripheral EAA and TRPV1 receptors.

Peripheral glutamate receptor mechanisms may not only modulate capsaicin-evoked activity but they may also modulate capsaicin-induced MAT reduction. Capsaicin neither sensitized nor desensitized glutamate-induced MAT responses since glutamate-induced MAT reduction in the nociceptive afferents following capsaicin injection were unchanged compared to glutamate alone. However, the capsaicin-induced median MAT following glutamate injection was increased and the incidence of MAT reduction was significantly lower compared to capsaicin alone. This finding represents an apparent paradox where glutamate may sensitize a

trigeminal afferent to subsequently produce greater capsaicin-evoked activity yet at the same time, it may also result in an attenuation of capsaicin-induced MAT reduction. One possible explanation for this paradox is that the greater capsaicin-evoked afferent activity following glutamate may cause an excess influx of calcium ions into the afferent and result in desensitization of the trigeminal nociceptive afferent to mechanical stimuli. This finding is consistent with previous studies in which capsaicin may either sensitize or desensitize spinal (Baumann *et al.* 1991; LaMotte *et al.* 1992; Simone *et al.* 1997; Serra *et al.* 2004) or trigeminal (Liu and Simon 1996; Strassman *et al.* 1996) nociceptive afferents to mechanical stimuli (*i.e.* sensitization with low concentrations and desensitization with high concentrations). Another possibility is that the trigeminal nociceptive afferents may be resistant to the subsequent sensitizing effects of capsaicin application due to a possible ceiling effect from the prior glutamate-induced peripheral sensitization. That is, the nociceptive afferents may have reached their maximal reductions in MAT following glutamate injection such that they cannot be sensitized further by capsaicin activation 30 minutes post-glutamate injection.

4. EXPERIMENTAL PROCEDURE

ANIMAL PREPARATION

Adult male ($n=55$, 250–400 grams (g)) Sprague-Dawley rats were prepared for acute *in vivo* recording activity of trigeminal afferents under surgical anesthesia (O_2 : 1 L/min; halothane: 1.5–2.5%; Cairns *et al.* 2001a,b). A tracheal cannula was inserted and artificial ventilation initiated. The rat's head was then placed in a stereotaxic frame and the skin over the dorsal surface of the skull was reflected. A trephination was made on the left side of the skull to allow a recording microelectrode to be lowered through the brain and into the trigeminal ganglion. An incision was also made in the skin overlying the Vc/UCC region, a C1 laminectomy was performed, and the dura was reflected to expose the Vc/UCC and facilitate placement of a stimulating microelectrode in the left Vc/UCC (Cairns *et al.* 2001a,b; Hu *et al.* 2005b).

After completion of all surgical procedures, the halothane level was slowly reduced to a level (1–1.3%) that was just sufficient to produce reflex suppression of the hindlimb to noxious pressure applied to the hindpaw to ensure that an adequate level of anesthesia was maintained for the duration of the experiment. Heart rate and body core temperature were continuously monitored throughout the experiment and kept within the physiological range of 330–430/min and 37–37.5°C, respectively. All procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

RECORDING AND STIMULATING PROCEDURES

Extracellular activity of single trigeminal nociceptive primary afferents with RFs in deep craniofacial tissues (masseter or temporalis muscles, or TMJ) was recorded with an epoxy-resin-coated tungsten microelectrode. One hour after completion of surgery, the microelectrode was slowly lowered into the brain under stereotaxic guidance (anterior 3.5–4 mm, lateral 3–4 mm) until afferent discharges were observed in response to light brush stimuli applied to the craniofacial region; these discharges were usually found 7–8 mm below the cortical surface. A round dental burnisher (1-mm diameter) was then applied as a noxious mechanical search stimulus (~100 g) over the craniofacial cutaneous tissues in an attempt to identify trigeminal afferents with deep craniofacial nociceptive RFs (Cairns *et al.* 2001b). This ~100 g force was found to be noxious to the experimenter when applied to his TMJ region or dorsal surface of his hand.

When an afferent that responded to direct, blunt noxious mechanical stimuli was found, a careful assessment of its RF was made to ascertain that the afferent was responding to deep as

opposed to cutaneous stimulation. The skin overlying the RF was pulled gently away from contact with the deep tissue, and brush, pinch, and pressure stimuli were applied directly to the skin surface. If the afferent did not respond to any of these cutaneous stimuli, then the afferent was considered to have a deep mechanonociceptive RF. The RF and MAT of the afferent was assessed at the time intervals specified under the experimental paradigm (see below). The deep RF of each afferent was determined through the use of a round dental burnisher. The size and location of the afferent's deep RF was also outlined on a life-size drawing of the rat's head. The MAT of the afferent's deep RF was determined with an electronic von Frey device (Model 735, 1.0-mm diameter probe tip, Somedic Sales AB, Sweden) applied to the center of the RF and was defined as the force (g) required to evoke the first spike, or a firing rate of greater than 2 standard deviations above baseline afferent activity when the afferent was spontaneously active, measured at the afferent's deep RF site with a ramp of gradually increasing force. The reproducibility of the electronic von Frey device in determining MAT has been demonstrated previously (Moller *et al.* 1998; Cairns *et al.* 2002).

To test whether an afferent with a deep craniofacial RF projected to the caudal brainstem, electrical stimuli (50 μ s biphasic pulse, range 10–80 μ A, 0.5 Hz) were applied to a stimulating electrode lowered into the left Vc/UCC (0–6 mm caudal to obex). The stimulating electrode was moved mediolaterally (0.1-mm steps) and rostrocaudally (0.5-mm steps) in the Vc/UCC until electrical stimulation evoked antidromic responses as determined by classical criteria for antidromic activation (all-or-none at threshold, invariant latency, high-frequency following (\geq 100 Hz), and collision) (Price *et al.* 1976; Cairns *et al.* 1996, 2001a,b). The initial electrical stimuli were applied 6 mm caudal to the obex. If stimulation at this location did not evoke an antidromic action potential, the stimulating electrode was moved rostrally toward the obex until either an antidromic action potential was evoked or electrical stimuli had been applied unsuccessfully up to the level of the obex. Antidromic action potentials were collided with the orthodromic action potentials evoked by mechanical stimulation of the deep craniofacial RF, to confirm the projection of the deep nociceptive afferent to the caudal brainstem. At the end of the study, the antidromic CV of the afferent was determined by calculating the straight-line distance between the stimulating microelectrode placed in the Vc/UCC and the recording microelectrode, divided by the antidromic latency. The skin overlying the deep craniofacial RF of the afferent was also surgically excised and it was confirmed that mechanical and/or electrical (50–100 μ s biphasic pulse, range 10–80 μ A, 0.5 Hz) stimuli applied directly to the deep tissue could also evoke activity in the afferent. If electrical stimulation applied to the deep tissue RF evoked an orthodromic action potential of invariant latency (<0.2 ms variability) with the ability to follow high-frequency electrical stimuli (\geq 100 Hz), then the conduction distance between the stimulation location and the trigeminal ganglion was estimated and an orthodromic CV calculated. For afferents that could not be demonstrated to project to the Vc/UCC, only orthodromic CVs were determined.

RECEPTOR AGONISTS

Glutamate (0.5M; 10 μ L; Sigma Chemical Company, St. Louis, MO), 1% capsaicin (10% capsaicin in ethanol:Tween-80: sterile normal saline in a 1:1:8 ratio by volume; 10 μ L; Calbiochem, La Jolla, CA) or vehicle (isotonic saline as control for glutamate or ethanol:Tween 80:sterile normal saline in a 1:1:8 ratio by volume as control for capsaicin; 10 μ L) was injected into the deep craniofacial afferent RF at 30 minute intervals in both experimental subgroups of rats. The concentrations of glutamate and capsaicin were chosen on the basis of their efficacy in evoking jaw muscle activity and inflammation and we have previously documented that such injected solutions are localized to the site of injection (Cairns *et al.* 1998, 2001a,b, 2002, 2003a; Tang *et al.* 2004; Hu *et al.* 2005a). All solutions were adjusted to approximate physiologic pH (7.2–7.6).

EXPERIMENTAL PARADIGM

Experimental animals were divided into subgroups according to the sequence of injection of receptor agonists: afferent response properties of rats with glutamate (or vehicle) injection followed by capsaicin (or vehicle) injection in one subgroup of rats were compared with properties of afferents in a second subgroup of rats with capsaicin (or vehicle) injection followed by glutamate (or vehicle) injection. The following experimental paradigm was applied to each of the subgroups: 10 minutes after a nociceptive afferent was classified on the basis of deep RF, antidromic CV and response properties as an A δ (CV \geq 2.5–30 m/s) or C (CV < 2.5 m/s) fiber nociceptive afferent (Price *et al.* 1976; Cairns *et al.* 2001a,b), the baseline MAT (in g) was determined by averaging the threshold for three consecutive mechanical stimuli applied at 1-minute intervals. The needle tip of a catheter (a 27-gauge needle connected by polyethylene tubing to a Hamilton syringe, 100 μ L) was carefully inserted into the deep tissue RF of the afferent. It was observed that insertion of the catheter used to inject the receptor agonist evoked a spike discharge in all nociceptive afferents identified in order to confirm that the injection site was within the afferent RF. Baseline afferent activity was recorded for 10 minutes prior to injection of the first receptor agonist or vehicle control into the deep tissue RF. The receptor agonist or vehicle control was then slowly injected into the deep tissue (over a 5-second period). The following four response properties were assessed over the next 10 minute period: (1) *Response magnitude (Rmag)*: the total number of evoked spikes, or a firing rate of greater than 2 standard deviations above baseline afferent activity when the afferent was spontaneously active, following agonist or vehicle injection, (2) *Response duration (Rdur)*: the total time (seconds) from the first spike following agonist or vehicle injection to the last spike, (3) *Response latency (Rlat)*: the total time (seconds) from agonist or vehicle injection to the first spike following agonist injection, and (4) *Peak frequency (Pfreq)*: the highest firing rate in a one second period (Hz) during the Rdur. The needle was then withdrawn at the end of the 10 minute period and an assessment was made at this 10 minute time point and again at 20 minute after the injection of the receptor agonist or vehicle control to determine if any MAT changes from baseline had occurred. *MAT reduction* was defined as \geq 50% threshold reduction from baseline MAT score measured at the center of the RF site. Raw MAT threshold values measured post-injection were normalized to the initial baseline pre-injection value to illustrate population responses. The same protocol described above was used for injection of the second receptor agonist (or vehicle) 30 minutes post-injection of the first receptor agonist (or vehicle).

In 13 rats, it was possible to examine the effect of injected receptor agonists on more than one afferent on the ipsilateral (left) side because the RFs of the afferents were at different deep tissue sites or opposite ends of the same muscle. In these cases, which helped minimize the total number of animals used in the present study, a minimum of 2 hours elapsed between injections into the RFs. At the end of each experiment, rats were euthanized with the agent T61 (Hoechst, Canada). The brain was removed and it was confirmed that microelectrode tracks were visible on the surface of the trigeminal ganglion.

DATA ANALYSIS

Recorded afferent activity was stored electronically and analyzed off-line. Most of the population data are reported as mean \pm SE. However, if not normally distributed, population data are reported as median values with interquartile ranges indicated in square brackets; median [IQR]. Mann-Whitney U test, t-test, Fisher exact test, and RM ANOVA-on-ranks were used as appropriate ($p < 0.05$ considered to reflect statistical significance).

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REFERENCES

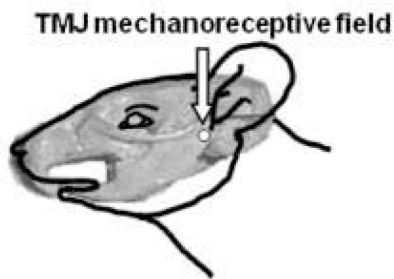
- Afrah AW, Stiller CO, Olgart L, Brodin E, Gustafsson H. Involvement of spinal N-methyl-D-aspartate receptors in capsaicin-induced in vivo release of substance P in the rat dorsal horn. *Neurosci Lett* 2001;316:83–86. [PubMed: 11742721]
- Baumann TK, Simone DA, Shain CN, LaMotte RH. Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. *J Neurophysiol* 1991;66:212–227. [PubMed: 1919668]
- Bautista DM, Jordt SE, Nikai T, Tsuruda PR, Read AJ, Poblete J, Yamoah EN, Basbaum AI, Julius D. TRPA1 mediates the inflammatory actions of environmental irritants and proalgesic agents. *Cell* 2006;124:1269–1282. [PubMed: 16564016]
- Cairns BE, Dong X, Mann MK, Svensson P, Sessle BJ, Arendt-Nielsen L, McErlane KM. Systemic administration of monosodium glutamate elevates intramuscular glutamate levels and sensitizes rat masseter muscle afferent fibers. *Pain* 2007;132:33–41. [PubMed: 17335976]
- Cairns BE, Svensson P, Wang K, Castrillon E, Hupfeld S, Sessle BJ, Arendt-Nielsen L. Ketamine attenuates glutamate-induced mechanical sensitization of the masseter muscle in human males. *Exp Brain Res* 2006;169:467–472. [PubMed: 16292641]
- Cairns BE, Svensson P, Wang K, Hupfeld S, Graven-Nielsen T, Sessle BJ, Berde CB, Arendt-Nielsen L. Activation of peripheral NMDA receptors contributes to human pain and rat afferent discharges evoked by injection of glutamate into the masseter muscle. *J Neurophys* 2003a;90:2098–2105.
- Cairns BE, Wang K, Hu JW, Sessle BJ, Arendt-Nielsen L, Svensson P. The effect of glutamate-evoked masseter muscle pain on the human jaw-stretch reflex differs in men and women. *J Orofac Pain* 2003b;17:317–325. [PubMed: 14737876]
- Cairns BE, Gambarota G, Svensson P, Arendt-Nielsen L, Berde CB. Glutamate-induced sensitization of rat masseter muscle fibers. *Neurosci* 2002;109:389–399.
- Cairns BE, Hu JW, Arendt-Nielsen L, Sessle BJ, Svensson P. Sex-related differences in human pain and rat afferent discharge evoked by injection of glutamate into the masseter muscle. *J Neurophysiol* 2001a;86:782–791. [PubMed: 11495950]
- Cairns BE, Sessle BJ, Hu JW. Characteristics of glutamate-evoked temporomandibular joint afferent activity in the rat. *J Neurophysiol* 2001b;85:2446–2454. [PubMed: 11387390]
- Cairns BE, Sessle BJ, Hu JW. Evidence that excitatory amino acid receptors within the temporomandibular joint region are involved in the reflex activation of the jaw muscles. *J Neurosci* 1998;18:8056–8064. [PubMed: 9742172]
- Cairns BE, Fragoso MC, Soja PJ. Active-sleep-related suppression of feline trigeminal sensory neurons: evidence implicating presynaptic inhibition via a process of primary afferent depolarization. *J Neurophysiol* 1996;75:1152–1162. [PubMed: 8867125]
- Carlton SM, McNearney T, Cairns BE. Peripheral glutamate receptors: Novel targets for Analgesics?. In: Dostrovsky, JO.; Carr, DB.; Koltzenburg, M., editors. *Proceedings of the 10th World Congress on Pain*. Seattle, WA: IASP Press; 2003. p. 125-139.
- Carlton SM. Peripheral excitatory amino acids. *Curr Opin in Pharmacol* 2001;1:52–56.
- Carstens E, Kuenzler N, Handwerker HO. Activation of neurons in rat trigeminal subnucleus caudalis by different irritant chemicals applied to oral or ocular mucosa. *J Neurophysiol* 1998;80:465–492. [PubMed: 9705444]
- Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 1997;389:816–824. [PubMed: 9349813]
- Craft RM, Porreca F. Treatment parameters of desensitization to capsaicin. *Life Sci* 1992;51:1767–1775. [PubMed: 1435085]
- Crandall M, Kwash J, Yu W, White G. Activation of protein kinase C sensitizes human VR1 to capsaicin and to moderate decreases in pH at physiological temperatures in *Xenopus* oocytes. *Pain* 2002;98:109–117. [PubMed: 12098622]
- Dong XD, Mann MK, Kumar U, Svensson P, Arendt-Nielsen L, Hu JW, Sessle BJ, Cairns BE. Sex-related differences in NMDA-evoked rat masseter muscle afferent discharge result from estrogen-mediated modulation of peripheral NMDA receptor activity. *Neuroscience* 2007;146:822–832. [PubMed: 17382479]

- Dong XD, Mann MK, Sessle BJ, Arendt-Nielsen L, Svensson P, Cairns BE. Sensitivity of rat temporalis muscle afferent fibers to peripheral N-methyl-D-aspartate receptor activation. *Neuroscience* 2006;141:939–945. [PubMed: 16716525]
- Dray, A. Pharmacology of inflammatory pain. In: Merskey, H.; Loeser, JD.; Dubner, R., editors. *The Paths of Pain 1975–2005*. Seattle, WA: IASP Press; 2005. p. 177-190.
- Gazerani P, Arendt-Nielsen L. The impact of ethnic differences in response to capsaicin-induced trigeminal sensitization. *Pain* 2005;117:223–229. [PubMed: 16098662]
- Gazerani P, Staahl C, Drewes AM, Arendt-Nielsen L. The effects of Botulinum Toxin type A on capsaicin-evoked pain, flare, and secondary hyperalgesia in an experimental human model of trigeminal sensitization. *Pain* 2006;122:315–325. [PubMed: 16677761]
- Hu JW, Fiorentino PM, Cairns BE, Sessle BJ. TRPV1 Receptor Mechanisms Involved in Capsaicin-Induced Oedema in the Temporomandibular Joint Region. *Oral Bio & Med* 2005a;2:241–248.
- Hu JW, Sun KQ, Vernon H, Sessle BJ. Craniofacial inputs to upper cervical dorsal horn: implications for somatosensory information processing. *Brain Res* 2005b;1044:93–106. [PubMed: 15862794]
- Hucho T, Levine JD. Signaling pathways in sensitization: toward a nociceptor cell biology. *Neuron* 2007;55:365–376. [PubMed: 17678851]
- Ikeda H, Tokita Y, Suda H. Capsaicin-sensitive A delta fibers in cat tooth pulp. *J Dent Res* 1997;76:1341–1349. [PubMed: 9207766]
- Jung J, Shin JS, Lee SY, Hwang SW, Koo J, Cho H, Oh U. Phosphorylation of vanilloid receptor 1 by Ca²⁺/calmodulin-dependent kinase II regulates its vanilloid binding. *J Biol Chem* 2004;279:7048–7054. [PubMed: 14630912]
- Kato S, Aihara E, Nakamura A, Xin H, Matsui H, Kohama K, Takeuchi K. Expression of vanilloid receptors in rat gastric epithelial cells: role in cellular protection. *Biochem Pharmacol* 2003;66:1115–1121. [PubMed: 12963499]
- Lam DK, Sessle BJ, Hu JW. Glutamate and capsaicin effects on trigeminal nociception II: Activation and central sensitization in brainstem neurons with deep craniofacial afferent input. *Brain Res*. Submitted. 2008
- Lam DK, Sessle BJ, Cairns BE, Hu JW. Peripheral NMDA receptor modulation of jaw muscle electromyographic activity induced by capsaicin injection into the temporomandibular joint of rats. *Brain Res* 2005a;1046:68–76. [PubMed: 15927551]
- Lam DK, Sessle BJ, Cairns BE, Hu JW. Neural mechanisms of temporomandibular joint and masticatory muscle pain: a possible role for peripheral glutamate receptor mechanisms. *Pain Res Manag* 2005b;10:145–152. [PubMed: 16175250]
- Lam, DK.; Sessle, BJ.; Hu, JW. International Association for Dental Research. 2004a. Glutamate and capsaicin-evoked activity in deep craniofacial trigeminal nociceptive afferents. Program No. 3817. 2004 Abstract Viewer/Itinerary Planner.
- Lam, DK.; Sessle, BJ.; Hu, JW. Washington, DC: Society for Neuroscience; 2004b. Glutamate and capsaicin-induced activation and peripheral sensitisation in deep craniofacial trigeminal nociceptive primary afferents. Program No. 294.6. 2004 Abstract Viewer/Itinerary Planner. Online.
- LaMotte RH, Lundberg LE, Torebjork HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. *J Physiol* 1992;448:749–764. [PubMed: 1593488]
- Lawand NB, McNearney T, Westlund KN. Amino acid release into the knee joint: key role in nociception and inflammation. *Pain* 2000;86:69–74. [PubMed: 10779662]
- Li T, Ghishan FK, Bai L. Molecular physiology of vesicular glutamate transporters in the digestive system. *World J Gastroenterol* 2005;11:1731–1736. [PubMed: 15793854]
- Liu L, Simon SA. Modulation of IA currents by capsaicin in rat trigeminal ganglion neurons. *J Neurophysiol* 2003;89:1387–1401. [PubMed: 12626618]
- Liu L, Simon SA. Capsaicin-induced currents with distinct desensitization and Ca²⁺ dependence in rat trigeminal ganglion cells. *J Neurophysiol* 1996;75:1503–1514. [PubMed: 8727394]
- Liu L, Simon SA. A rapid capsaicin-activated current in rat trigeminal ganglion neurons. *Proc Natl Acad Sci USA* 1994;91:738–741. [PubMed: 8290592]
- McNearney T, Baethge BA, Cao S, Alam R, Lisse JR, Westlund KN. Excitatory amino acids, TNF- α , and chemokine levels in synovial fluids of patients with active arthropathies. *Clin Exp Immunol* 2004;137:621–627. [PubMed: 15320917]

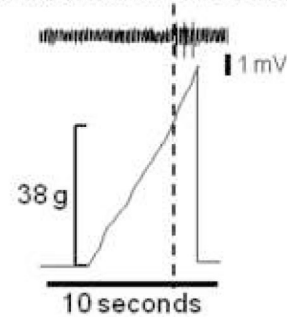
- McNearney T, Speegle D, Lawand N, Lisse J, Westlund KN. Excitatory amino acid profiles of synovial fluid from patients with arthritis. *J Rheumatol* 2000;27:739–745. [PubMed: 10743819]
- Meddings JB, Hogaboam CM, Tran K, Reynolds JD, Wallace JL. Capsaicin effects on non-neuronal plasma membranes. *Biochim Biophys Acta* 1991;1070:43–50. [PubMed: 1751537]
- Mitsikostas DD, Sanchez del Rio M, Waeber C, Moskowitz MA, Cutrer FM. The NMDA receptor antagonist MK-801 reduces capsaicin-induced c-fos expression within rat trigeminal nucleus caudalis. *Pain* 1998;76:239–248. [PubMed: 9696479]
- Möller KA, Johansson B, Berge OG. Assessing mechanical allodynia in the rat paw with a new electronic algometer. *J Neurosci Methods* 1998;84:41–47. [PubMed: 9821632]
- Palazzo E, de Novellis V, Marabese I, Cuomo D, Rossi F, Berrino L, Rossi F, Maione S. Interaction between vanilloid and glutamate receptors in the central modulation of nociception. *Eur J Pharmacol* 2002;439:69–75. [PubMed: 11937094]
- Paoletti P, Neyton J. NMDA receptor subunits: function and pharmacology. *Curr Opin Pharmacol* 2007;7:39–47. [PubMed: 17088105]
- Petrenko AB, Yamakura T, Baba H, Shimoji K. The role of N-methyl-D-aspartate (NMDA) receptors in pain: a review. *Anesth Analg* 2003;97:1108–1116. [PubMed: 14500166]
- Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. *Nature* 2000;408:985–990. [PubMed: 11140687]
- Price DD, Dubner R, Hu JW. Trigeminothalamic neurons in nucleus caudalis responsive to tactile, thermal, and nociceptive stimulation of monkey's face. *J Neurophysiol* 1976;39:936–953. [PubMed: 824411]
- Rizvi SI, Luqman S. Capsaicin-induced activation of erythrocyte membrane sodium/potassium and calcium adenosine triphosphatases. *Cell Mol Biol Lett* 2003;8:919–925. [PubMed: 14668915]
- Ro JY, Capra NF. Assessing mechanical sensitivity of masseter muscle in lightly anesthetized rats: a model for craniofacial muscle hyperalgesia. *Neurosci Res* 2006;56:119–123. [PubMed: 16857282]
- Serra J, Campero M, Bostock H, Ochoa J. Two types of C nociceptors in human skin and their behaviour in areas of capsaicin-induced secondary hyperalgesia. *J Neurophysiol* 2004;91:2770–2781. [PubMed: 14762154]
- Sikand P, Premkumar LS. Potentiation of glutamatergic synaptic transmission by protein kinase C-mediated sensitization of TRPV1 at the first sensory synapse. *J Physiol* 2007;581:631–647. [PubMed: 17363391]
- Simone DA, Marchettini P, Ochoa JL. Primary afferent nerve fibers that contribute to muscle pain sensation in human. *Pain Forum* 1997;6:207–212.
- Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. *Trends Pharmacol Sci* 2001;22:174–181. [PubMed: 11282417]
- Sohn MK, Graven-Nielsen T, Arendt-Nielsen L, Svensson P. Effects of experimental muscle pain on mechanical properties of single motor units in human masseter. *Clin Neurophysiol* 2004;115:76–84. [PubMed: 14706472]
- Sohn MK, Graven-Nielsen T, Arendt-Nielsen L, Svensson P. Inhibition of motor unit firing during experimental muscle pain in humans. *Muscle Nerve* 2000;23:1219–1226. [PubMed: 10918259]
- Strassman AM, Raymond SA, Burstein R. Sensitization of meningeal sensory neurons and the origin of headaches. *Nature* 1996;384:560–564. [PubMed: 8955268]
- Suh YG, Oh U. Activation and activators of TRPV1 and their pharmaceutical implication. *Curr Pharm Des* 2005;11:2687–2698. [PubMed: 16101449]
- Svensson P, Wang K, Arendt-Nielsen L, Cairns BE, Sessle BJ. Pain effects of glutamate injections into human jaw or neck muscles. *J Orofac Pain* 2005;19:109–118. [PubMed: 15895833]
- Svensson P, Cairns BE, Wang K, Hu JW, Graven-Nielsen T, Arendt-Nielsen L, Sessle BJ. Glutamate-evoked pain and mechanical allodynia in the human masseter muscle. *Pain* 2003;101:221–227. [PubMed: 12583864]
- Tang ML, Haas DA, Hu JW. Capsaicin-induced joint inflammation is not blocked by local anesthesia. *Anesth Prog* 2004;51:2–9. [PubMed: 15106683]
- Tominaga M, Tominaga T. Structure and function of TRPV1. *Pflugers Arch* 2005;451:143–150. [PubMed: 15971082]

- Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. The cloned capsaicin receptor integrates multiple pain-modulating stimuli. *Neuron* 1998;21:531–543. [PubMed: 9768840]
- Wang K, Arendt-Nielsen L, Svensson P. Capsaicin-induced muscle pain alters the excitability of the human jaw-stretch reflex. *J Dent Res* 2002;81:650–654. [PubMed: 12202650]
- Witting N, Svensson P, Arendt-Nielsen L, Jensen TS. Repetitive intradermal capsaicin: differential effect on pain and areas of allodynia and punctate hyperalgesia. *Somatosens Mot Res* 2000a;17:5–12. [PubMed: 10833080]
- Witting N, Svensson P, Gottrup H, Arendt-Nielsen L, Jensen TS. Intramuscular and intradermal injection of capsaicin: a comparison of local and referred pain. *Pain* 2000b;84:407–412. [PubMed: 10666547]
- Woolf CJ, Ma Q. Nociceptors--noxious stimulus detectors. *Neuron* 2007;55:353–364. [PubMed: 17678850]
- Xin H, Tanaka H, Yamaguchi M, Takemori S, Nakamura A, Kohama K. Vanilloid receptor expressed in the sarcoplasmic reticulum of rat skeletal muscle. *Biochem Biophys Res Commun* 2005;332:756–762. [PubMed: 15907794]
- Xing J, Li J. TRPV1 receptor mediates glutamatergic synaptic input to dorsolateral periaqueductal gray (dl-PAG) neurons. *J Neurophysiol* 2006;97:503–511. [PubMed: 17065246]
- Yamakura T, Shimoji K. Subunit- and site-specific pharmacology of the NMDA receptor channel. *Prog Neurobiol* 1999;59:279–298. [PubMed: 10465381]
- Yu XM, Sessle BJ, Haas DA, Izzo A, Vernon H, Hu JW. Involvement of NMDA receptor mechanisms in jaw electromyographic activity and plasma extravasation induced by inflammatory irritant application to temporomandibular joint region of rats. *Pain* 1996;68:169–178. [PubMed: 9252012]
- Zanotto K, Merrill AW, Carstens MI, Carstens E. Neurons in superficial trigeminal subnucleus caudalis responsive to oral cooling, menthol and other irritant stimuli. *J Neurophysiol* 2007;97:966–978. [PubMed: 17151223]

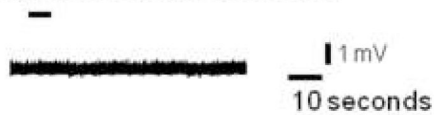
(a) Deep mechanoreceptive field



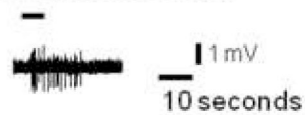
(b) Mechanical activation threshold (TMJ)



(c) Glutamate vehicle control
Glutamate vehicle injection



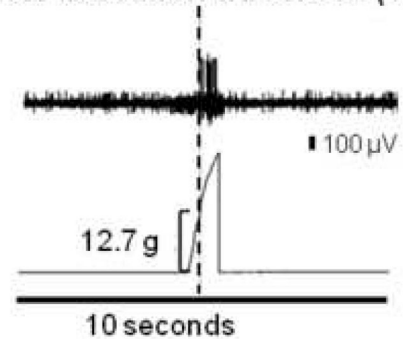
(d) Capsaicin-evoked spikes post-Glutamate vehicle
Capsaicin injection



(a) Deep mechanoreceptive field



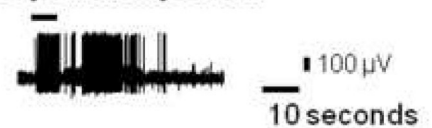
(b) Mechanical activation threshold (TMJ)



(c) Glutamate-evoked spikes
Glutamate injection



(d) Capsaicin-evoked spikes post-Glutamate
Capsaicin injection



(e) High-Frequency Following



(f) Collision

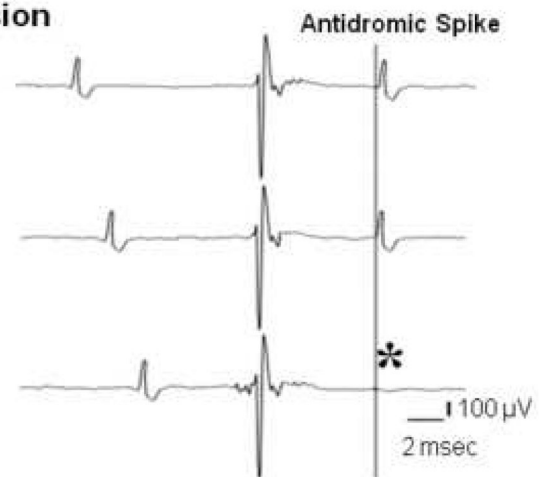
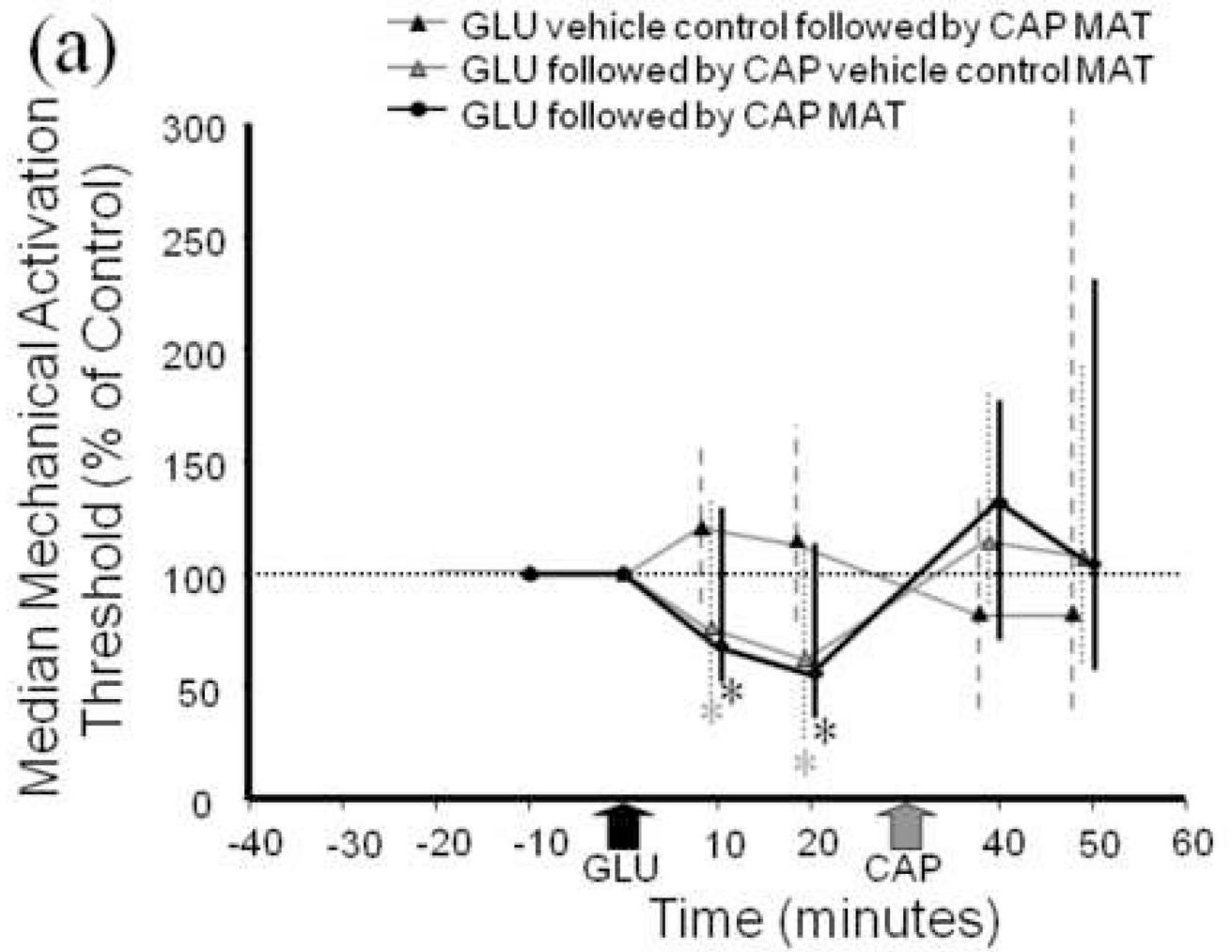


Fig. 1.

Fig. 1A. Example of typical mechanoreceptive field and response properties of deep craniofacial nociceptive afferent to injection of glutamate vehicle followed by capsaicin (CV=2.8m/s, MAT=38 g, A δ -fiber TMJ afferent). (a) Deep mechanoreceptive field (white circle) of nociceptive afferent involving the TMJ region indicated by arrow, (b) Afferent mechanical activation threshold determined with von Frey device, (c) No response in this afferent was evoked by injection of glutamate vehicle into the TMJ, (d) Afferent response evoked by injection of capsaicin following glutamate vehicle into the TMJ.

Fig. 1B. Example of typical mechanoreceptive field and response properties of deep craniofacial nociceptive afferent to injection of glutamate followed by capsaicin (CV=2.0m/s, MAT=12.7 g, C-fiber TMJ afferent). (a) Deep mechanoreceptive field (white circle) of nociceptive afferent involving the TMJ region indicated by arrow, (b) Afferent mechanical activation threshold determined with von Frey device, (c) Afferent response evoked by injection of glutamate into the TMJ, (d) Afferent response evoked by injection of capsaicin following glutamate into the TMJ, (e) Stimulation of Vc/UCC (50 μ s, 50 μ A, 100 Hz) evoked an antidromic action potential (latency: 6.0ms). By measuring the distance between the recording electrode and the stimulating electrode in Vc/UCC and dividing by the antidromic latency, the CV of this afferent was estimated to be 2.0 m/s, (f) Blunt mechanical stimulation of the TMJ tissue was used to evoke orthodromic spikes that served as a trigger for electrical stimulation of Vc/UCC (antidromic spike). Shortening the delay between the orthodromically evoked spike and the electrical stimulus applied to Vc/UCC resulted in a collision, as evidenced by the disappearance of the antidromic spike.



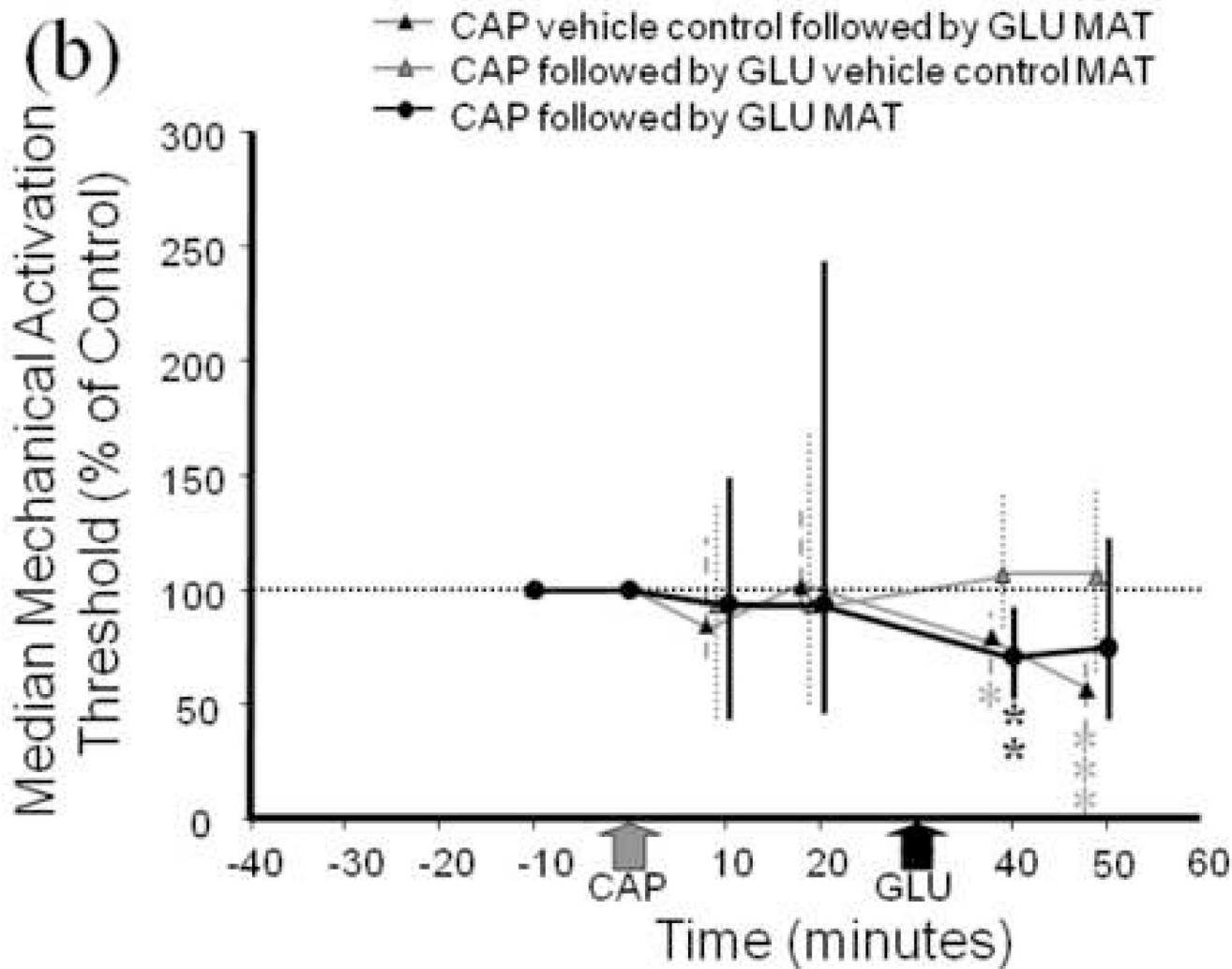


Fig. 2. The time courses of glutamate (GLU) and capsaicin (CAP)-induced mechanical activation threshold (MAT) in deep craniofacial afferents. Arrow indicates time point for injection of GLU (black) and CAP (grey) into deep craniofacial tissues. Circles indicate median normalized MAT following injection of GLU and CAP. Triangles indicate GLU or CAP-induced median normalized MAT before or after injection of vehicle controls for GLU or CAP. Raw MAT threshold values were normalized to the initial baseline pre-injection value of the first agonist. Lines: interquartile range. Note that injection of (a) GLU alone and (b) GLU following CAP into deep craniofacial tissues significantly reduced the MAT (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; RM ANOVA-on-ranks, Dunn's Method).

Mechanoreceptive field (RF) and baseline mean pooled orthodromic conduction velocity (CV) and mechanical activation threshold (MAT) of deep craniofacial nociceptive afferents.

Table 1

| RF location | A δ -fiber afferent Mean \pm SE | | | C-fiber afferent Mean \pm SE | | |
|---------------|---|-------------------------------|--------------------------------|-----------------------------------|-------------------------------|--------------------------------|
| | n | Mean CV (m/s) | Mean MAT (g) | n | Mean CV (m/s) | Mean MAT (g) |
| TMJ | 31 | 9.8 \pm 1.1 | 29.6 \pm 3.5 | 9 | 1.6 \pm 0.1 | 30.0 \pm 8.8 |
| Masseter | 19 | 8.8 \pm 1.2 | 27.6 \pm 5.3 | 4 | 1.7 \pm 0.3 | 21.1 \pm 17.1 |
| Temporalis | 4 | 16.9 \pm 1.9 | 24.7 \pm 9.9 | 1 | 1.7 | 4.9 |
| Pooled | 54 | 9.8\pm0.8 | 28.5\pm2.8 | 14 | 1.6\pm0.1 | 24.9\pm7.2 |

Table 2

Response properties of trigeminal nociceptive afferents to injection of glutamate (or vehicle) and capsaicin (or vehicle) into deep craniofacial tissues

| Response Property | Glutamate \rightleftarrows Capsaicin Median [interquartile range] | | Capsaicin \rightleftarrows Glutamate Median [interquartile range] | |
|-------------------|--|--|--|--|
| | Glutamate alone (n=28) | Capsaicin post-Glutamate (n=27) | Capsaicin alone (n=25) | Glutamate post-Capsaicin (n=22) |
| Rmag (spikes) | 168 [377] ^{††} | 170 [642] [‡] | 14 [24] | 431 [487] |
| Rlat (sec) | 5.8 [8.2] | 7.5 [6.7] | 9.8 [20] | 5.6 [5.3] |
| Rdur (sec) | 144 [138] [†] | 70 [272] | 30 [50] | 149 [236] |
| Pfreq (Hz) | 19 [25] ^{††} | 24 [36] ^{‡‡} | 3.0 [3.0] | 19 [22] |
| Response Property | Glutamate vehicle (n=4) | Capsaicin post-Glutamate vehicle (n=4) | Capsaicin vehicle (n=4) | Glutamate post-Capsaicin vehicle (n=4) |
| Rmag (spikes) | 0 ^{***} | 14 [31] | 0 ^{**} | 149 [27] |
| Rlat (sec) | 0 ^{***} | 6.0 [20] | 0 ^{**} | 9.4 [2.5] |
| Rdur (sec) | 0 ^{***} | 28 [32] | 0 ^{**} | 131 [139] |
| Pfreq (Hz) | 0 ^{***} | 4.0 [4.2] | 0 ^{**} | 18 [14] |
| Response Property | Glutamate alone (n=3) | Capsaicin vehicle post-Glutamate (n=3) | Capsaicin alone (n=4) | Glutamate vehicle post-Capsaicin (n=4) |
| Rmag (spikes) | 168 [550] ^{††} | 0 ^{***} | 14 [31] | 0 ^{***} |
| Rlat (sec) | 3.2 [3.2] | 0 ^{***} | 6.0 [20] | 0 ^{***} |
| Rdur (sec) | 144 [148] [†] | 0 ^{***} | 28 [32] | 0 ^{***} |
| Pfreq (Hz) | 20 [27] ^{††} | 0 ^{***} | 4.0 [4.2] | 0 ^{***} |

*** p<0.001, Glutamate vehicle vs. Glutamate alone or Capsaicin vehicle post-Glutamate vs. Capsaicin post-Glutamate or Glutamate vehicle post-Capsaicin vs. Glutamate post-Capsaicin

** p<0.01, Capsaicin vehicle vs. Capsaicin alone

[†] p<0.05

^{††} p<0.01, Glutamate alone vs. Capsaicin alone

[‡] p<0.05

^{‡‡} p<0.01, Capsaicin alone vs. Capsaicin post-Glutamate; Mann-Whitney U test