Characteristics of Glutamate-Evoked Temporomandibular Joint Afferent Activity in the Rat

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Cairns, Brian E., Barry J. Sessle, and James W. Hu. Characteristics of glutamate-evoked temporomandibular joint afferent activity in the rat. J Neurophysiol 85: 2446–2454, 2001. Injection of glutamate into the rat temporomandibular joint (TMJ) capsule can reflexly induce a prolonged increase in the electromyographic (EMG) activity of the jaw muscles, however, the characteristics of TMJ afferents activated by glutamate have not been investigated. In the present study, we examined the effect of glutamate injection into the TMJ capsule on jaw muscle EMG activity and the extracellularly recorded activity of single trigeminal afferents that had receptive fields in the TMJ tissue and antidromically identified projections to the brain stem subnucleus caudalis (Vc) in rats of both sexes. Glutamate (0.05–1.0 M, 10 μl) injection into the TMJ capsule evoked EMG activity in a dose-related manner; however, at concentrations of 0.5 and 1.0 M, glutamate-evoked digastric muscle responses were greater in female than in male rats. In experiments where jaw muscle EMG and afferent activity were recorded simultaneously, glutamate (0.5 M, 10 μl) injection into the TMJ capsule evoked activity in the jaw muscles as well as in 27 (26 Aδ and 1 C-fiber afferent) of 34 trigeminal afferents that could be activated by blunt mechanical stimulation of the TMJ tissue. In these experiments, glutamate-evoked jaw muscle activity was significantly increased for 6 min after the glutamate injection, whereas afferent activity was significantly increased only during the first minute after the glutamate injection. The glutamate-evoked afferent activity was inversely related to conduction velocity and, in afferents with conduction velocities <10 m/s, was significantly greater in female (n = 6) than in male (n = 10) rats. These results suggest that glutamate excites putative nociceptive afferents within the TMJ to a greater degree in female than in male rats. This sex-related difference in afferent discharge may, in part, underlie sex-related differences in glutamate-evoked jaw muscle EMG activity.

INTRODUCTION

We have previously suggested that glutamate may play a role in peripheral mechanisms of nociception within the temporomandibular joint (TMJ) (Cairns et al. 1998; Yu et al. 1996). Injection of the inflammatory irritant and small-fiber excitant mustard oil into the rat TMJ capsule results in a characteristic reflexly induced increase in the electromyographic (EMG) activity of both the digastric (jaw opener) and masseter (jaw closer) muscles (Bakke et al. 1998; Yu et al. 1994, 1995). Mustard oil-evoked EMG activity can be attenuated by peripheral application of the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Yu et al. 1996). Injection of glutamate into the rat TMJ capsule induces a similar prolonged co-activation of the jaw muscles through activation of peripheral NMDA and non-NMDA receptors (Cairns et al. 1998). In contrast, injection of the amino acid neurotransmitters γ-aminobutyric acid (GABA) or glycine into the TMJ capsule does not evoke activity in the jaw muscles (Cai et al. 1999; Cairns et al. 1999b).

The rat TMJ is innervated by small myelinated and unmyelinated afferents that project, via the trigeminal ganglion, to the trigeminal brain stem sensory nuclear complex (Casatti et al. 1999; Chen and Turner 1992; Kido et al. 1995; Widenfalk and Wiberg 1990; Yoshino et al. 1998). The subnucleus caudalis (Vc) appears to be an important target for these afferents, since inactivation of the Vc attenuates jaw muscle activity reflexly evoked by injection of mustard oil or glutamate into the TMJ capsule (Cairns et al. 1998; Hu et al. 1997; Tsai et al. 1999). Injection of either mustard oil or glutamate into the TMJ capsule also evokes activity in Vc neurons, which are thought to relay nociceptive information from the craniofacial region to higher centers (Broton et al. 1988; Hathaway et al. 1995; Kishimoto et al. 1999; Kojima 1990; Sessle 1999; Sessle and Hu 1991; Zhou et al. 1999). These findings are consistent with anatomical evidence that primary afferents from the TMJ, like those from other deep craniofacial tissues, project to the Vc (Capra 1987; Capra and Wax 1989; Nishimori et al. 1986; Shigenaga et al. 1988). However, the characteristics of afferents activated by injection of glutamate into the TMJ capsule of rats have not been investigated.

Recently, we have reported that male and female rats differ in their response to injection of glutamate into the TMJ capsule (Cairns et al. 1999b). Specifically, at concentrations of >0.25 M, injection of glutamate into the TMJ capsule reflexly evokes greater jaw muscle responses in female as compared with male rats. However, since glutamate-evoked reflex jaw muscle activity reflects the integration of primary afferent drives with the activity of central neurons intercalated in the TMJ reflex pathway, it remains to be determined whether there is a sex-related difference in glutamate-evoked TMJ afferent discharge.

In the present study we have employed a method that allows the activity of trigeminal primary afferents with TMJ mechanoreceptive fields and projections to the Vc to be recorded in intact, lightly anesthetized rats. This methodology also allows the simultaneous recording of jaw muscle EMG activity. We have used this methodology to investigate the type and dis-
charge characteristics of TMJ afferents excited by glutamate injection into the TMJ capsule and compared these features with the glutamate-evoked EMG activity. Further, we have explored the possibility that there is a sex-related difference in the glutamate-evoked activity of TMJ afferents.

A portion of this data has been previously presented in abstract form (Cairns et al. 1999b, 2000).

METHODS

Surgical preparation

To construct dose-response graphs, adult male (n = 36) and female (n = 72) Sprague-Dawley rats were prepared for acute, in vivo recording of digastric muscle EMG activity (Cairns et al. 1998). To investigate TMJ afferent activity, additional adult male (n = 13) and female (n = 14) Sprague-Dawley rats were prepared for acute in vivo recording of trigeminal primary afferent activity and EMG activity under surgical anesthesia (O2: 0.3–0.4 l/min; N2O: 0.6–0.7 l/min; halothane: 1.5–2%) (Cairns et al. 1998, 1999a). In all experiments, a tracheal cannula was inserted and artificial ventilation initiated. Bipolar electrodes fashioned out of 40-gauge teflon-coated single strand stainless steel wire were inserted into the ipsilateral digastric and in the trigeminal afferent experiments, also in the ipsilateral masseter, and were used to monitor jaw muscle EMG activity. The rat’s head was then placed in a stereotaxic frame, and the skin over the dorsal surface of the skull was reflected. For the EMG experiments, two screws were inserted into the parietal bone and attached to a vertical support bar with dental acrylic as a support for the head.

For the trigeminal afferent experiments, a trephination was made on the left side of the skull to allow a microelectrode to be lowered through the brain and into the trigeminal ganglion. A second incision was made, and the skin and muscle overlying the brain stem and upper cervical spinal cord were removed; a C1 laminectomy was performed, and the dura overlying the brain stem/cervical spinal cord was removed to facilitate placement of a stimulating electrode in contact with the caudal brain stem (Vc or dorsal horn of the upper cervical spinal cord).

In female rats, a vaginal lavage was performed, and the epidermal cells were examined under a microscope (Frye et al. 1992; Martinez-Gomez et al. 1994). This examination revealed that in the EMG experiments, 11 were in estrus, 25 in metestrus, and 36 in diestrus. Of the female rats used for the afferent recording experiments, two were in estrus, seven in metestrus, and four in diestrus.

After completion of all surgical procedures, the halothane level was slowly reduced (1–1.3%) until noxious pressure applied to the hind paw could induce a weak flexion reflex of the hind limb 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 whole experiment and kept within the physiological range of 330–430/min and 37–37.5°C, respectively. All surgeries and procedures were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada).

Stimulation and recording techniques

EMG activity was recorded from the ipsilateral digastric and masseter muscles (Cairns et al. 1998, 1999a). To permit simultaneous recordings of single trigeminal primary afferent unit activity, a parylene-coated tungsten microelectrode (2 MΩ, A-M Systems, Carlsborg, WA) was slowly lowered into the brain under stereotaxic control (3.5–4 mm anterior to the interaural line, 3–4 mm lateral to the midline) until unit discharge was observed in response to light brush stimuli applied to the craniofacial region (Fig. 1). Trigeminal afferent units were usually found 7–8 mm below the cortical surface, and postmortem examination revealed electrode tract marks on the surface of the trigeminal ganglion. Mechanical search stimuli were then applied via a blunt probe (1 mm diam) over the TMJ at an intensity (~100 g) sufficient to evoke jaw muscle EMG activity while slowly lowering the electrode in an attempt to identify trigeminal afferents with deep receptive fields. When a unit was found that appeared to respond to this blunt mechanical stimulus of the TMJ that was considered to be noxious, the skin overlying the mechanoreceptive field was pulled gently away from contact with the TMJ, and brush, pinch, and pressure stimuli were applied directly to the skin surface. If the unit did not respond to any of these cutaneous stimuli, then the mechanoreceptive field was considered to lie within the TMJ. The mandible was then moved once left, right, up, down, forward, and backward through its complete range of motion to investigate whether the units were activated by jaw movement.

FIG. 1. The diagram illustrates the experimental setup employed in the present study. A needle catheter attached to a 25-µL Hamilton syringe was inserted into the temporomandibular joint (TMJ) capsule and used to inject glutamate (0.5 M). A tungsten recording electrode was introduced into the trigeminal ganglion while a second stimulating electrode was lowered into subnucleus caudalis. Primary afferent and jaw muscle activity was amplified, displayed on an oscilloscope, and simultaneously fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2; Cambridge Electronics) for later analysis. Dotted line: exact pathway unknown.
In initial experiments, electrical stimuli (1–10 mA, 0.5 ms, 0.33 Hz) were then applied to the TMJ tissues (articular disk, capsule, and associated ligaments) in an attempt to determine conduction time, and thus allow for estimation of conduction velocity. However, this approach proved unreliable. Since there is physiological and anatomical evidence for the projection of small-diameter TMJ primary afferents to the Vc (Broton et al. 1988; Capra 1987; Hathaway et al. 1995; Hu et al. 1997; Sessle and Hu 1991), constant-current electrical stimuli (50–μs biphasic pulse, range 10–80 μA, 0.5 Hz) were applied to a stimulating electrode (2 MΩ, parylene-coated tungsten electrode, A-M Systems) lowered into the caudal brain stem (1–1.5 mm lateral to the midline, 0–5 mm caudal to the obex, depth 0–5 mm below the brain stem surface; Fig. 1). For each recorded TMJ afferent, the stimulating electrode was moved mediolaterally (0.1-mm steps) and rostrocaudally (0.5-mm steps) in the caudal brain stem to determine whether electrical stimulation could evoke an antidromic action potential with an invariant latency (<0.2 ms variability) and the ability to follow high-frequency electrical stimuli (≥100 Hz) (Cairns et al. 1996; Dostrovsky et al. 1981; Hu and Sessle 1988; Hu et al. 1978). The initial electrical stimuli were applied 5 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 orthodromic action potentials evoked by mechanical stimulation of the TMJ tissue, to confirm the projection of the TMJ afferent to the caudal brain stem. At the end of the experiment, the distance between the stimulating and recording electrodes was measured with a ruler, and divided by the latency of the antidromically evoked response of an afferent to give an estimation of conduction velocity (CV) of the recorded afferent.

After the above characterization of each afferent was completed, the tip of a catheter, consisting of a 27-gauge needle connected by polyethylene tubing to a Hamilton syringe (50 μL), was carefully inserted into the TMJ joint space and was used to inject drug solutions. It was observed that insertion of the catheter needle into the TMJ capsule evoked a spike discharge in all TMJ afferents under study.

In all experiments involving simultaneous recordings of afferent and EMG activity, baseline primary afferent and EMG activity was recorded for 10 min prior to injection of any substance into the TMJ capsule. Glutamate (0.5 M, 10 μL, pH 7.0) was then injected slowly (over 5 s) into the TMJ capsule, and the resulting primary afferent and jaw muscle EMG activity was monitored for 30 min after the injection. Units that did not respond to application of glutamate were excluded from further analysis.

We have previously found that a repeated injection of glutamate into the TMJ capsule at 30-min intervals evokes jaw muscle EMG activity of similar magnitude (Cairns et al. 1998, 1999a). To investigate the effect of repeated glutamate injection on TMJ afferent activity, in three experiments a second injection of glutamate was made 30 min after the initial injection of glutamate into the TMJ capsule. When compared with glutamate, injection of GABA (0.5 M) into the TMJ capsule does not evoke significant jaw muscle EMG activity (Cairns et al. 1999a). To compare the effect of GABA and glutamate on TMJ afferent activity, in four experiments GABA (0.5 M, 10 μL, pH 7.0) was injected 30 min after the initial injection of glutamate into the TMJ capsule. As a positive control for the GABA experiments, in a single experiment the nonselective excitant KCl (2.0 M, 10 μL, pH 7.0) was injected into the TMJ capsule 30 min after glutamate.

At the end of each experiment, rats were killed with the agent T61 (Hoechst, Regina, Canada). Electrolytic lesions were first made in the brain stem of some rats by applying a monopolar, monophasic current pulse of 20 μA for 20 s. The brain and brain stem were removed, and it was also confirmed that microelectrode tracts were visible on the surface of the trigeminal ganglion. Thin sections of the brain stem and upper cervical spinal cord (100 μm) were cut with a vibratome and viewed under a microscope.

Data analysis

To construct EMG dose-response curves, EMG activity was recorded from the ipsilateral digastric muscle, amplified (gain: ×500; bandwidth 30–1,000 Hz), and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2; Cambridge Electronics) (Cairns et al. 1998, 1999a). Recorded EMG activity was stored electronically and analyzed off-line. Saline (165 mM, 10 μL) or one of five doses of glutamate (0.05 M, 0.1 M, 0.25 M, 0.5 M, or 1.0 M, 10 μL saline, pH ~7; Research Biochemicals International, Natick, MA) was injected into the TMJ capsule to determine the dose-response relationship in male (n = 6 per dose) and female (n = 12 per dose) rats.

The activity of identified primary afferents and jaw muscles was amplified (afferent gain: ×100; EMG activity gain: ×500; bandwidth 30–1,000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike 2; Cambridge Electronics). Peristimulus time histograms (PSTH; 1-min bins) were constructed from the recorded primary afferent discharge. Mean baseline afferent discharge was calculated by averaging the first 10 bins prior to injection of glutamate. Mean baseline afferent activity was subtracted from each bin of the PSTH to yield residual afferent discharge. The area under the glutamate-evoked response curve (AUC; spike/min) was calculated by the summation of the residual afferent discharge after glutamate injection.

Recorded EMG activity data were rectified off-line and EMG activity area (1-min bins) calculated. Mean baseline EMG activity was calculated by averaging the first 10 bins prior to injection of glutamate. Mean baseline EMG activity was subtracted from each EMG activity area bin to yield residual area bins. The AUC (μV/min) was calculated by the summation of the residual EMG activity area bins (Cairns et al. 1998, 1999b).

Statistical analysis

Most of the collected data were not normally distributed, and thus all data are reported as a median with the interquartile range indicated in square brackets. Paired comparisons were made with the Mann-Whitney rank sum test; multiple comparisons were made with a Friedman repeated measures ANOVA on Ranks and post hoc Dunnett’s method as appropriate.

Results

Glutamate-evoked EMG dose-response relationship

To determine whether male and female rats differ in their response to glutamate injected into the TMJ capsule, dose-response curves of glutamate-evoked digastic muscle activity were constructed for male (n = 36) and female (n = 72) rats. The median AUC evoked by injection of saline to the TMJ region was similar in male (0 [0–51] μV/min) and female rats (0 [0–100] μV/min; P > 0.05, Mann-Whitney rank sum test; Fig. 2). In both sexes, injection of glutamate into the TMJ capsule in concentrations of 0.25, 0.5, and 1.0 M evoked muscle activity that was significantly greater than that evoked by saline (P < 0.05, ANOVA on ranks, Dunn’s method). The AUC in female rats was greater than the AUC of male rats when 0.5 or 1.0 M glutamate was injected into the TMJ capsule. The 0.5-M concentration was subsequently used for the TMJ afferent recording experiments.
Afferent characteristics

Single-unit recording experiments were undertaken to examine how TMJ afferents respond to glutamate injections and to investigate whether sex differences in glutamate-evoked EMG activity might be due, in part, to a peripheral mechanism. A total of 34 trigeminal afferents responded to mechanical stimuli applied via a blunt probe to the TMJ tissues at an intensity sufficient to evoke jaw muscle EMG activity (Fig. 3). None of these afferents responded to mechanical stimulation (brush, pinch, pressure) of the skin overlying the TMJ. Ten of these afferents also responded to movements of the mandible. Insertion of the catheter needle used to inject glutamate into the TMJ capsule evoked a spike discharge in all afferents.

Injection of glutamate into the TMJ capsule evoked jaw muscle EMG activity in all rats and activity in 27 of 34 afferents. Only five of these glutamate-sensitive afferents also responded to movements of the mandible. The majority of glutamate-sensitive afferents had CVs in the Aδ afferent range (n = 26; CV: 6.5 [4.5–11.8] m/s), although one of the afferents had a CV in the C-fiber afferent range (CV: 1.3 m/s; Fig. 4A). The median CV of the 7 afferents that did not respond to glutamate (14 [11.3–17.3] m/s) was greater than the median CV of the 27 glutamate-sensitive afferents (5.9 [4.2–11.0] m/s, P < 0.05 Mann-Whitney test). Of the seven glutamate-insensitive afferents, five responded to movements of the mandible. Based on the distance from the obex where electrical stimuli were applied, in concert with histological reconstructions of selected stimulation sites, all glutamate-sensitive afferents were found to project to either the caudal Vc or the dorsal horn of the cervical spinal cord (Fig. 4B). The majority of glutamate-sensitive afferents were antidromically activated by electrical stimuli applied 2–3 mm caudal to the obex, a region that includes the caudal Vc, the Vc/C1 transition area, and C1 dorsal horn.

In afferents with CVs of 2.5–10 m/s (slow Aδ afferents, n = 16), injection of glutamate into the TMJ capsule generally evoked a prolonged (30–1,800 s) discharge of action potentials (Fig. 5A). In contrast, the glutamate-evoked activity of afferents with CVs >10 m/s (fast Aδ afferents, n = 10) consisted of only brief (5–20 s) action potential discharges (Fig. 5B). Overall, there was an inverse relationship between the CV and the AUC of these Aδ afferents (Fig. 5C). The median glutamate-evoked AUC calculated for slow Aδ afferents (103 [20.0–450.5] spikes/min) was significantly greater than that

![Fig. 2](https://example.com/fig2.png) Dose-response relationship for activity evoked in the digastric muscle by injection of glutamate to the TMJ capsule of male and female rats. Each data point on the dose-response curves represents a median area under the glutamate-evoked response curve (AUC) value for males (●, n = 6) and females (○, n = 12). Note that the magnitude of jaw muscle activity evoked by injection of 0.25, 0.5, and 1 M glutamate into the TMJ capsule was significantly greater than that evoked by saline. Lines: interquartile range. * P < 0.05, ANOVA on ranks, Dunn’s method.

![Fig. 3](https://example.com/fig3.png) Methodology employed in the identification of TMJ afferents. A: mechanical distension of the TMJ tissue evoked action potentials in a TMJ afferent and also reflex jaw muscle electromyographic (EMG) activity. Note that jaw muscle EMG activity, but not the afferent’s activity, outlasted the period of mechanical stimulation, and the EMG activity started before the onset of the afferent’s activity; this suggests that this afferent was contributing not to the onset but to part of the maintained phase of the reflexly evoked EMG activity. B: stimulation of subnucleus caudalis (30 μA, 50 μs, 100 Hz) evoked an antidromic action potential (latency: 4.3 ms). By measuring the distance between the recording electrode in the ganglion and the stimulating electrode in subnucleus caudalis (see Fig. 1) and dividing by the latency, the conduction velocity of this afferent was estimated to be 2.8 m/s. C: blunt mechanical stimulation of the TMJ tissue was used to evoke orthodromic spikes that served as a trigger for electrical stimulation of subnucleus caudalis (antidromic spike). Shortening the delay between the orthodromically evoked spike and the electrical stimulus applied to subnucleus caudalis resulted in a collision, as evidenced by the disappearance of the antidromic spike.
calculated for fast Aδ afferents (10.0 [4.0–50.0] spikes/min, \(P < 0.05\), Mann-Whitney test).

### Relationship to glutamate-evoked EMG activity

The median glutamate-evoked action potential spike discharge of the different afferent subgroups, as well as the associated glutamate-evoked jaw muscle EMG activity, is illustrated in Fig. 6. A significant increase in the activity of fast and slow Aδ afferents, relative to baseline, occurred only within the first minute after injection of glutamate to the TMJ capsule (repeated measures ANOVA on Ranks, Dunnett’s method, \(P < 0.05\)). In the examples of fast and slow Aδ afferents shown in the insets of Fig. 5, it can be seen that the action potential discharge evoked by glutamate preceded increases in jaw muscle EMG activity by \(\approx 4\) s.

In all experiments, glutamate-evoked TMJ afferent activity began before glutamate-evoked EMG activity. In contrast to the short-duration of glutamate-evoked Aδ afferent activity (1 min), EMG activity in the jaw muscles was significantly increased relative to baseline for a total of 6 min (ANOVA on Ranks, Dunnett’s method, \(P < 0.05\)). Interestingly, injection of glutamate into the TMJ capsule evoked a burst-pause action potential discharge pattern in the single C-fiber afferent that outlasted the EMG response (Fig. 6).

### Comparison of male and female rats

Of the 27 glutamate-sensitive afferents, 13 (10 slow, 3 fast) Aδ afferents were recorded in male rats, and 13 (6 slow, 7 fast) Aδ afferents as well as the single C-fiber afferent were recorded in female rats. The median weight of the male rats (335 [320–360] g) was significantly greater than the median weight of female rats (280 [265–325] g; \(P < 0.05\) rank sum test). However, there was no significant relationship between weight and glutamate-evoked afferent response in either males \((r = 0.02, P > 0.05,\) Spearman rank order correlation) or females \((r = 0.062; P > 0.05,\) Spearman rank order correlation). The median AUC of slow, but not fast, Aδ afferents was significantly greater in female than male rats (Table 1). This differ-

![Fig. 4](http://jn.physiology.org/figure/14/4/2450_Fig4b.png)

**FIG. 4.** The histograms in A and B illustrate the distribution of conduction velocities (CVs) and antidromic stimulation locations for glutamate (0.5 M)-sensitive afferents. A: the CVs of all but one of afferents were in the Aδ range (2.5–35 m/s). The distribution of the CVs was polymodal, with local peaks at 4–6, 10–12, and 16–18 m/s. B: the majority of glutamate-sensitive afferents had projections to the subnucleus caudalis (Vc) region between 2 and 3 mm caudal to the obex. Bin widths for A: 2 ms; and for B: 0.5 mm.

### FIG. 5. The relationship between the magnitude of glutamate-evoked activity and afferent CV.

The peristimulus histogram (1-s binwidth) and electromyogram traces illustrate glutamate-evoked TMJ afferent and jaw muscle activity. An expanded time scale of the initial injection period is shown in the inset (dotted box). In A, injection of glutamate (0.5 M) into the TMJ capsule (open arrow) evoked prolonged discharge in this afferent (CV 5.3 m/s) as well as jaw muscle activity after a latency of several seconds. In B, injection of glutamate (0.5 M) into the TMJ capsule evoked only a brief discharge in this afferent (CV 11.0 m/s). Note that in both A and B, increased afferent activity preceded increased jaw muscle activity (inset). The bar graph in C illustrates the relationship between CV and median response magnitude. Note that TMJ afferents with CVs <10 m/s had significantly greater responses to glutamate (0.5 M) than TMJ afferents with CVs >10 m/s \((P < 0.05\) Mann-Whitney rank sum test). Vertical calibration bars: 20 spikes (afferents), 250 \(\mu\)V (electromyogram).
**FIG. 6.** Population characteristics of glutamate-evoked primary afferent discharge and their relationship to glutamate-evoked jaw muscle activity. Peristimulus histograms (bin width 1 min) show the median action potential discharge of a population of fast (>10 m/s; n = 10) and slow (<10 m/s; n = 16) Aδ afferents as well as a single C-fiber afferent before, during, and after injection of glutamate (0.5 M) into the TMJ capsule (△), while line graphs indicate the median amplitude of the associated jaw muscle activity (n = 25). Note that the afferent discharge was significantly elevated above baseline in both slow and fast Aδ fibers only during the 1st minute after glutamate injection. A pattern of burst-pause action potential discharge was also observed in the single C-fiber afferent. In contrast, median digastric and masseter muscle activity was significantly elevated above baseline for 6 min after injection of glutamate. *P < 0.05 ANOVA, Dunnett’s method. Bars: interquartile range. Vertical calibration bars: 5 spikes (afferents), 50 μV (electromyogram).

**Comparison of glutamate, GABA, and KCl**

In eight Aδ afferents, a second injection of glutamate (n = 3), GABA (n = 4), or KCl (n = 1) was made 30 min after the initial injection of glutamate into the TMJ capsule. The AUC evoked by a second injection of glutamate into the TMJ capsule (33 [26.0–532.5] spikes/min) was comparable to the AUC evoked by the initial injection of glutamate (41 [25.0–482.5] spikes/min; Fig. 8). In contrast, the AUC evoked by GABA (0.5 [0–2.0] spikes/min) was less than that evoked by glutamate (81 [5.3–281.0] spikes/min). In a single experiment, the AUC evoked by KCl (293 spikes/min) was roughly half that evoked by the initial injection of glutamate (581 spikes/min).

**TABLE 1.** Comparison of the magnitude of glutamate-evoked Aδ afferent activity in male and female rats

<table>
<thead>
<tr>
<th>Type</th>
<th>Male Rats</th>
<th></th>
<th></th>
<th>Female Rats</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Median CV, m/s</td>
<td>Median AUC, spikes/min</td>
<td>No.</td>
<td>Median CV, m/s</td>
<td>Median AUC, spikes/min</td>
</tr>
<tr>
<td>Fast Aδ</td>
<td>3</td>
<td>17.6 [17.0–17.8]</td>
<td>1 [1.0–330.0]</td>
<td>7</td>
<td>11.5 [11–15]</td>
<td>4 [0–12.0]</td>
</tr>
<tr>
<td>Slow Aδ</td>
<td>10</td>
<td>6.9 [4.5–7.5]</td>
<td>29 [13.0–174.0]</td>
<td>6</td>
<td>3.9 [3.6–4.6]</td>
<td>381.5* [103.0–845.0]</td>
</tr>
</tbody>
</table>

Ranges are in brackets. Note that the median response of slow Aδ afferents, but not fast Aδ afferents, in female rats was significantly greater than in male rats. CV, conduction velocity; AUC, area under the glutamate-evoked response curve. *P < 0.05 Mann-Whitney test.

**DISCUSSION**

Injection of glutamate into the TMJ capsule evoked activity in ~80% of TMJ afferents. Since all TMJ afferents were, by definition, mechanoreceptors, it could be argued that the glutamate-evoked afferent activity observed in the present study occurred indirectly as a result of glutamate-evoked jaw muscle activity. However, this is unlikely for the following reasons: 1) not all afferents responded to glutamate injection into the TMJ capsule even though this always evoked jaw muscle activity; 2) when glutamate did evoke afferent activity, this preceded reflex jaw muscle EMG activity by several seconds; and 3) there was a marked difference in the duration of glutamate-evoked afferent activity as compared with jaw muscle EMG activity. The possibility that glutamate-evoked responses may have been due solely to distention of the joint or to the osmotic strength of the solution appears unlikely, since injection of the same concentration of GABA evoked much less TMJ afferent activity.
activity than glutamate (Fig. 8). This finding is consistent with our previous results that injection of GABA into the TMJ capsule evokes significantly less jaw muscle activity than glutamate (Cairns et al. 1999a). Therefore we conclude that the observed increases in afferent activity were due to a direct action of glutamate on the TMJ afferents.

In the present study, the rat TMJ was left intact (except for the insertion of a catheter) to avoid extensive injury to the TMJ tissues, which in our experience often prevents jaw muscle activity from being evoked by injection of algic substances into the TMJ capsule. However, this methodology does not allow for direct, electrical stimulation of TMJ tissues. Instead, mechanical search stimuli were applied to the TMJ tissues to identify TMJ afferents, which greatly limited the number and type of TMJ afferents that could be identified in the present study. As a result, the majority of glutamate-sensitive TMJ afferents identified by this method were Aβ afferents, although one glutamate-sensitive C-fiber afferent was also identified. All these glutamate-sensitive TMJ afferents project to the Vc, where anatomical evidence has indicated that there is a selective projection of small-diameter afferents from deep craniofacial tissues (Capra 1987; Capra and Wax 1989; Nishimori et al. 1986; Shigenaga et al. 1988). Moreover, trigeminal brain stem neurons activated by noxious TMJ tissue stimulation have previously been identified in the Vc (Broton et al. 1988; Hathaway et al. 1995; Sessle and Hu 1991), and chemical or surgical disruption of the Vc eliminates the TMJ-jaw muscle nociceptive reflex (Cairns et al. 1998; Hu et al. 1997; Tsai et al. 1999). Thus these findings point to the possibility that glutamate injection into the TMJ capsule may activate putative nociceptive afferents.

The lack of Aβ afferents identified in the present study appears consistent with anatomical evidence that the rat TMJ is principally innervated by small-diameter myelinated and unmyelinated afferents (Kido et al. 1995). With regard to the paucity of C-fiber afferents identified in this study, we propose that our use of mechanical search stimuli on the intact TMJ tissues may have limited our ability to sample TMJ C-fiber afferents. In the knee joint, a subpopulation of C-fiber afferents that do not innervate the TMJ have been recently described; however, the methodology employed in this study would have failed to identify many of the C-fiber afferents innervating the TMJ.

The results of the present study indicate that the glutamate-evoked activity of Aβ afferents precedes but has a markedly shorter duration than glutamate-evoked jaw muscle EMG activity. In vitro investigations of the effects of glutamate on primary afferent neurons in spinal dorsal root, trigeminal mesencephalic nucleus, and trigeminal ganglion neurons as well as corneal afferents have found that glutamate-evoked depolarizing responses are transient, lasting only seconds, and are followed by a variable period of desensitization (Jones et al. 1997; Lovinger and Weight 1988; MacIver and Tanelian 1993; Pelkey and Marshall 1998; Puil and Spigelman 1988). One possible explanation for the disparity between the duration of glutamate-evoked afferent and EMG responses in the present study may be that a brief activation of slowly conducting TMJ afferents by glutamate is sufficient to induce a period of prolonged increase in the excitability of brain stem neurons intercalated in the TMJ-jaw muscle reflex pathway. For example, a prolonged enhancement in the excitability of Vc neurons occurs after acute or chronic inflammation of deep craniofacial tissues, including the TMJ tissues (Chiang et al. 1998; Hu et al. 1992; Iwata et al. 1999; Ren and Dubner 1999; Yu et al. 1993). Further, the duration of activity evoked in Vc nociceptive neurons and the jaw muscles by application of algic chemicals to the TMJ tissues are similar (Broton and Sessle 1988; Broton et al. 1988; Hu et al. 1992; Yu et al. 1995). It is therefore possible that the difference in the duration of afferent and EMG activity evoked by glutamate may reflect, at least in part, a brain stem process of central sensitization (Hu et al. 1992, 1997; Sessle 1999; Sessle and Hu 1991) induced by the brief glutamate-evoked TMJ afferent barrage.

The results of the present study indicate that injection of glutamate into the TMJ capsule produces a dose-dependent reflex increase in jaw muscle EMG activity in both sexes but evokes greater muscle activity in female rats than in male rats. Further, glutamate evoked greater activity in slow (<10 m/s) Aβ afferents in female than in male rats. These results suggest that sex-related differences in glutamate-evoked TMJ afferent activity may, in part, underlie the observation of enhanced jaw muscle responses evoked in female rats by injection of glutamate into the TMJ capsule. Previous research has also indicated that the activation threshold of slowly conducting TMJ afferents to noxious mechanical rotation of the jaw was lower in female than male goats; however, this difference was attrib-
uated to sex-related differences in the biomechanical properties of the TMJ tissues (Loughner et al. 1997). Importantly, activation of peripheral excitatory amino acid receptors, rather than joint distention, was found to be responsible for glutamate-evoked jaw muscle activity (Cairns et al. 1998). Further, the present study, observed increases in afferent activity appear due to a direct action of glutamate on the TMJ afferents. Therefore it is conceivable that differences between males and females in the sensitivity of peripheral excitatory amino acid receptors could underlie the observed sex-related differences in glutamate-evoked jaw muscle activity. A possible mechanism for sex-related differences in glutamate-evoked afferent activity could involve either increased expression of excitatory amino acid receptors or enhancement of receptor function, both of which have been shown to occur secondary to increased levels of the female sex hormone estrogen (Bi et al. 2000; Gazzaley et al. 1996). Further, such peripherally based sex-related differences might also contribute to the differences in glutamate-evoked masseter muscle pain between men and women (B. E. Cairns, J. W. Hu, L. Arendt-Nielsen, B. J. Sessle, and P. Svensson, unpublished data) and to the well-documented gender differences in many types of craniofacial pain conditions (Carlsson and LeResche 1995; Dao and LeResche 2000).

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