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Prolonged Jaw Opening Promotes Nociception and Enhanced Cytokine Expression

Jordan L. Hawkins, MS and

Senior Research Scientist, Center for Biomedical & Life Sciences, Missouri State University, Springfield, Missouri, USA

Paul L. Durham, PhD

Distinguished Professor, Director, Center for Biomedical & Life Sciences, Missouri State University, Springfield, Missouri, USA

Abstract

Aims—To test the hypothesis that prolonged jaw opening, as can occur during routine dental procedures, increases nociceptive sensitivity of the masseter muscle and increases cytokine expression.

Methods—Sprague-Dawley rats were used to investigate behavioral and cellular changes in response to prolonged jaw opening. A surgical retractor was placed around the maxillary and mandibular incisors, and the jaw was held at near maximal opening for 20 minutes. Head-withdrawal responses to mechanical stimuli applied to the facial skin overlying the left and right masseter muscles were determined following jaw opening. Cytokine levels in the upper cervical spinal cord containing the caudal part of the spinal trigeminal nucleus were evaluated using protein antibody microarrays ($n = 3$). Statistical analysis was performed using a nonparametric Mann-Whitney U test.

Results—Prolonged jaw opening significantly increased nocifensive head withdrawal to mechanical stimuli at 2 hours, and days 3 and 7 postinduction ($P < .05$). The increase in nociceptive response resolved after 14 days. Sustained jaw opening also stimulated differential cytokine expression in the trigeminal ganglion and upper cervical spinal cord that persisted 14 days postprocedure ($P < .05$).

Conclusion—These findings provide evidence that near maximal jaw opening can lead to activation and prolonged sensitization of trigeminal neurons that results in nociceptive behavior evoked by stimulation of the masseter muscle, a physiologic event often associated with temporomandibular disorders (TMD). Results from this study may provide a plausible explanation for why some patients develop TMD after routine dental procedures that involve prolonged jaw opening.

Keywords

central sensitization; nociception; spinal trigeminal nucleus; temporomandibular disorders; trigeminal ganglion

Temporomandibular disorders (TMD) encompass conditions that affect the temporomandibular joint (TMJ), which are known as arthralgia, or affect the muscles of mastication, which are known as myalgia, or affect both TMJ and muscle.^{1,2} The main symptoms of TMD may include pain in the TMJ area, pain in the ear, masticatory muscle tenderness after or during chewing, clicking in the TMJ, and limitation of mandibular function.^{3,4} Reported causes of TMD include trauma, infection, arthritis, and malocclusion.⁵ Ironically, it is not uncommon for patients to experience pain in their TMJ and associated structures following routine dental visits. Treatment approaches such as removal of dental caries, crown procedures, root canal therapy, tooth extractions, and orthodontic procedures sometimes require the patient's mouth to be open for extended periods of time, which can result in inflammation and pain in the TMJ and associated muscles that may last for days after the dental visit. The pain can be dull or sharp, but it usually subsides with time. However, in some cases the person may experience prolonged and severe pain in their TMJ and jaw and neck muscles that may develop into TMD.

The pain associated with TMD involves sensitization and activation of nociceptive neurons in the trigeminal ganglion and spinal trigeminal nucleus located in the medulla and upper cervical spinal cord.⁶⁻⁸ Activation of the neurons in the trigeminal ganglion is initiated by nociceptive inputs from the somatic axons that provide sensory innervation of the TMJ and the muscles, ligaments, and tendons associated with mastication.^{8,9} Upon injury, release of inflammatory agents at the peripheral terminals of the axons induces inflammation in the local tissues and the development of peripheral sensitization resulting in a lower activation threshold of the primary nociceptor. In response to peripheral trigeminal nociceptor activation, calcitonin gene-related peptide (CGRP) is released by the neuronal body within the ganglion, causing activation of satellite glial cells within the ganglion.¹⁰⁻¹³ CGRP-mediated stimulation of satellite glial cells increases the synthesis and release of cytokines, a family of proteins known to promote a prolonged state of neuronal sensitization.¹⁴ In addition, CGRP and other proinflammatory molecules are released from terminals of primary afferent neurons located in the spinal dorsal horn and subnucleus caudalis (also known as the medullary dorsal horn) of the spinal trigeminal nucleus and facilitate sensitization and activation of second-order nociceptive neurons.¹⁵ The release of these proinflammatory molecules stimulates the production and secretion of cytokines from astrocytes and microglia that promote and sustain an excitable or sensitized neuronal environment that is characteristic of central sensitization of second-order nociceptive neurons. Thus, injury to the TMJ or associated masticatory structures that may result in TMD involves both peripheral and central sensitization of trigeminal nociceptive neurons associated with a persistent pain state.

The aim of this study was to test the hypothesis that prolonged jaw opening, as can occur during routine dental procedures, increases nociceptive sensitivity of the masseter muscle

and increases cytokine expression. This would provide evidence that prolonged jaw opening results in a transient increase in mechanical nociception and elevated levels of cytokines implicated in the maintenance of peripheral and central sensitization of trigeminal nociceptive neurons.

Materials and Methods

Animals

A total of 50 adult Sprague-Dawley male rats (30 for the behavioral analysis and 20 for the cytokine analysis) weighing 200 to 300 g (Charles River Laboratories Inc) were allowed to acclimate for 1 week to facility conditions prior to use. Animals were housed individually in clean, standard plastic rat cages (VWR) with unrestricted access to both food and water in a room with 12 hour/light dark cycles. All protocols were approved by Missouri State University's Institutional Animal Care and Use Committee and conducted in compliance with all established guidelines in the Animal Welfare Act of 2007, National Institutes of Health, and ARRIVE Guidelines. Concerted efforts were made to minimize suffering as well as the number of animals used in this study.

Prolonged Jaw Opening

To mechanically induce stress to the TMJ and associated muscles, animals anesthetized through inhalation of 5% isoflurane (Webster Veterinary) were subjected to prolonged jaw opening. A retractor (Fine Scientific Tools) was placed in the animal's mouth, allowing the maxillary and mandibular incisors to rest inside the retractor's loops (Fig 1). The retractor arms were separated by a distance of 22 mm, measured from the gingival line on the lingual surface of the maxillary incisors to the gingival line on the lingual surface of the mandibular incisors by a CaliMax Vernier Caliper (Wiha Tools). Once the retractor was in place, the animal was returned to the chamber and maintained at 3% isoflurane for 20 minutes (n = 20). As controls, some animals were either placed only under 3% isoflurane for 20 minutes (n = 19) while other anesthetized animals were placed in a position similar to the jaw-opening group with the unopened retractor resting gently in their mouth (n = 6). At the completion of the procedure, the retractor was released, removed, and the animal allowed to recover in its original cage.

Nocifensive Response to Mechanical Stimulation

All behavioral assessments were carried out as described in a previous study¹⁶ using the Durham Animal Holder (Ugo Basile, Gemonio). Animals freely entered the holder and were allowed to remain in the device for 5 minutes on 3 consecutive days. A plastic restraining block was inserted behind the animal to secure the animal in the optimal position for testing mechanical sensitivity in the orofacial region and minimizing limb movement. To minimize false responses, animals were conditioned to a mechanical stimulus by gently rubbing the facial hair follicles and epidermis located over the masseter muscle of the face with a pipette tip. Initially, baseline mechanical nocifensive thresholds were determined in response to a series of calibrated von Frey filaments (15, 26, 60, 100, 180, and 300 g; North Coast Medical, Inc) applied in increasing force to the cutaneous area over the right and left masseter. The researcher responsible for directly testing the response to each filament was

blinded to the experimental conditions. A positive response, which was defined by head withdrawal prior to the bending of the filament, was recorded by a second researcher. Each filament was applied five times, and the data are reported as the average number of responses obtained from five applications of each specific calibrated filament. The 100-g force was used for subsequent studies, since the average number of positive head-withdrawal responses to this force was less than one out of five for both right and left masseter muscle stimulation. In addition to baseline values, measurements were taken at 2 hours and at 3, 7, and 14 days postretraction of the jaw. Behavioral data were collected from 16 isoflurane control animals, 8 animals subjected to jaw-opening, and 6 animals for the retractor-only condition for a total of 30 animals.

Cytokine Analysis

Tissues were acquired from naïve animals ($n = 5$), from animals at 2 hours after 20-minute isoflurane exposure ($n = 3$), and from experimental animals at 2 hours and at 3, 7, and 14 days after jaw opening (each $n = 3$). Animals that were used to study changes in cytokine levels were euthanized by CO₂ asphyxiation gradient and decapitation. Right and left trigeminal ganglia, as well as upper cervical spinal cord tissue (obex to -5 mm) containing the caudal component of the spinal trigeminal nucleus were acquired through cranial dissection and tissues immediately frozen with liquid nitrogen. Whole tissue protein lysates were homogenized in 1× RayBio Cell Lysis Buffer (RayBiotech, Inc), and total protein levels were determined prior to cytokine array analysis by the Bradford Assay (BioRad) by using bovine serum albumin as the protein standard.

Cytokine analysis was performed using R&D System Proteome Profiler Rat Cytokine Array Kits (R&D Systems) according to the manufacturer's protocol. To determine relative cytokine levels from each sample, 200 µg of isolated protein was assayed. Detection was accomplished using Pierce ECL Plus detection system (Thermo Scientific) and X-ray film. Exposed film was developed and fixed in preparation for densitometry analysis. Integrated density measurements were acquired from grayscale JPEG images by using Image J software (NIH) with rolling ball background subtraction and dot blot analysis plugins. Integrated density values were normalized to positive control values. Cytokine levels were determined in duplicate and obtained from the five naïve animals and the three animals used for each of the other experimental conditions, for a total of 20 animals.

Statistical Analyses

At each time point, the nocifensive responses to mechanical stimulation were reported as the combined average number of responses $\pm$ standard error of the mean (SEM). To determine the effects of isoflurane on nocifensive behaviors as compared to baseline measurements, statistical analysis was conducted by performing a one-way repeated-measures ANOVA test with dependent t test, post hoc test, and a Bonferroni correction. Effects of an unopened dental retractor as compared to isoflurane controls were determined by performing a Student t test. To determine statistical significance between control and near-maximal jaw opening groups, a nonparametric Mann-Whitney U test was used at each individual time point because collected data were shown to have unequal variance. All analysis was conducted using SPSS 16 software (IBM). Differences were considered significant at $P < .05$. Each

condition was repeated in a minimum of six independent experiments. For the cytokine studies, data for those cytokines statistically different from isoflurane control levels (see Results) were reported as the average-fold change $\pm$ SEM when compared to values obtained from control animals, which was set at equal to 1. Statistical analysis was performed with a nonparametric Mann-Whitney U test using SPSS 16 software, since collected data were shown to have unequal variance. Differences were considered significant at $P < .05$.

Results

Animals subjected to near-maximal jaw opening (22 mm) for 20 minutes on average exhibited a significant increase in the number of head-withdrawal responses to a 100-g-force filament (3.4 ± 0.4 , $P = .001$) applied over the right and left masseter muscles 2 hours after jaw opening when compared to control animal responses (Fig 2a). Similarly, a significant increase in reactivity to application of 100-g force over the masseter muscles was observed on days 3 and 7 after jaw opening (Fig 2a; 3.2 ± 0.6 , $P = .038$ and 3.8 ± 0.5 , $P = .007$, respectively). Mechanical nocifensive sensitivity over the masseter muscles returned to near-baseline levels by day 14 (Fig 2a; 1.75 ± 0.5 , $P = .505$). The average number of nocifensive responses did not change significantly from initial basal values in the animals treated with isoflurane only (Fig 2b; basal, 0.8 ± 0.3 , $P = 1.00$; 2 hours, 0.7 ± 0.2 , $P = .99$; 3 days, 1.4 ± 0.5 , $P = .99$; 7 days, 1.2 ± 0.5 , $P = .99$; 14 days, 1.1 ± 0.6 , $P = .99$). Likewise, control animals subjected to retractor placement did not exhibit increased nocifensive withdrawal responses (Fig 2c; basal, 0.8 ± 0.3 , $P = .835$; 2 hours, 0.4 ± 0.2 , $P = .383$; 1 day, 0.7 ± 0.2 , $P = .278$).

Initially, cytokine levels of animals 2 hours after exposure to 3% isoflurane for 20 minutes ($n = 3$) were compared to cytokine levels of naïve animals ($n = 5$) in the upper cervical spinal cord tissue. The relative levels of cytokines in naïve and 2-hour isoflurane control samples were not significantly different (data not shown). Animals that were subjected to jaw retraction for 20 minutes exhibited a significant increase in cytokine levels for 19 of the 29 cytokines detected by array analysis 2 hours after jaw opening ($n = 3$) as compared to naïve animals ([Table 1] $P < .05$: sICAM-1, CINC-3, IL-3, Thymus Chemokine, GM-CSF, VEGF, Fractalkine, MIP-3 α ; $P < .01$: TNF- α , CINC-2 α/β , CNTF, IL-17, IL-1 β , IL-13, TIMP-1, LIX, L-Selectin, IFN- γ , RNATES). However, at 3 days after jaw retraction ($n = 3$), only 1 cytokine ([Table 1] $P < .05$: CNTF) was found to be significantly upregulated. In contrast, 7 cytokines were significantly upregulated in samples collected 7 days after near-maximal jaw retraction ($n = 3$) when compared to control levels ([Table 1] $P < .05$: TNF- α , L-Selectin; $P < .01$: CNTF, sICAM-1, LIX, Thymus Chemokines, Fractalkine). Analysis of samples collected 14 days after jaw retraction ($n = 3$) identified 15 cytokines significantly upregulated when compared to levels in control samples ([Table 1] $P < .05$: IL-1 β , VEGF, GM-CSF, IL-17, RANTES, IL-1 α ; $P < .01$: CINC-2 α/β , IL-13, TNF- α , IP-10, CINC-3, IL-3, IL-2, TIMP-1, LIX).

Cytokine levels in the trigeminal ganglion were found to be similar in isoflurane-treated and naïve samples (data not shown). Trigeminal ganglia obtained 2 hours after jaw opening had significantly elevated levels of 12 cytokines ([Table 2] $P < .05$: CINC-2 α/β , MIG, TNF- α , LIX, GM-CSF; $P < .01$: L-Selectin, TIMP-1, IL-4, Fractalkine, IL-3, VEGF; CNTF), while

the level of IL-10 was significantly decreased ($P < .01$). Further analysis of trigeminal ganglia samples collected 3 days after jaw opening identified four cytokines with significantly increased levels ([Table 2] $P < .05$: IL-13, CINC-1, IL-1ra; $P < .01$: IL-3). Interestingly, only two cytokines were found to be significantly upregulated in day 7 trigeminal ganglion samples ([Table 2] $P < .05$: GM-CSF; $P < .01$: IL-3). Similar results were also observed in tissues at 14 days after jaw retraction, with only two cytokines being significantly upregulated ([Table 2] $P < .05$: CINC-1, RANTES) while IFN- γ levels were significantly decreased ($P < .05$).

Discussion

The rationale for the experimental design used in this study was that patients subjected to prolonged jaw opening in clinical settings often complain of sensitivity of both the TMJ and associated muscles to mechanical pressure the same day as this event. Recently, data from the Orofacial Pain: Prospective Evaluation and Risk Assessment (OPPERA) study have confirmed a greater occurrence of TMD in individuals who have experienced events that cause injury to the jaw, such as yawning or prolonged opening.⁵ Findings in the present study are consistent with this observation, since animals subjected to near-maximal jaw opening exhibited a significant increase in mechanical sensitivity in the cutaneous tissue directly above the masseter muscle, which was apparent at 2 hours following the procedure and was sustained for 7 days. It is important to note that this model for studying behavioral and cellular changes in response to injury to structures associated with mastication does not involve injection of inflammatory molecules such as capsaicin or complete Freund's adjuvant to promote inflammation and nociception.^{17–21} Rather, the injury and subsequent inflammatory response that promotes prolonged nociceptive behavior is likely in response to mechanical trauma of masticatory structures including the TMJ, masticatory muscles, associated tendons, and ligaments that are innervated by nociceptive trigeminal neurons.²² However, given the placement of the retractor, a possible contribution to the observed behavioral and cellular changes of periodontal inflammation caused by sustained pressure to the gingiva and/or periodontal ligament cannot be excluded. The model used in this study more closely mimics the pathologic mechanisms by which prolonged jaw opening may contribute to the development of persistent mechanical sensitivity of the masseter, a clinical symptom commonly reported by patients with TMD.²

To better understand the underlying mechanisms involved in the development of trigeminal nociceptor sensitivity, changes in the temporal expression of cytokines following prolonged jaw opening were investigated. This study focused on cytokines, since they are a large family of proteins implicated in the initiation and maintenance of both peripheral and central sensitization of nociceptive neurons, including those of the trigeminal system.²³ Both peripheral and central sensitization, which are implicated in the underlying pathology of TMD, promote development of hyperalgesia and allodynia.^{8,24} In response to sustained jaw opening, changes in the expression of multiple cytokines were observed both in the trigeminal ganglion and the upper cervical spinal cord. The increased expression of proinflammatory cytokines within the ganglion and spinal cord would facilitate changes in ion channels and receptor activity associated with lowering the excitation threshold of trigeminal nociceptive neurons that could promote the mechanical sensitivity observed at 2

hours and at days 3 and 7.²⁵ The elevated levels of cytokines in the trigeminal ganglion that could facilitate peripheral sensitization are likely the result of increased neuron-glia signaling between the cell bodies of trigeminal primary afferent nociceptive neurons and the satellite glial cells, which synthesize and release cytokines and other molecules to control the excitability state of the neuron.¹¹ It is likely that the local inflammatory signaling in response to trauma or injury to somatic tissues caused by prolonged jaw opening would mediate inflammation and peripheral sensitization of primary nociceptors. The sustained signaling from primary afferent nociceptive neurons would then promote persistent central sensitization of second-order trigeminal neurons in the upper cervical spinal cord. In support of this notion, significantly elevated levels of numerous cytokines were observed for up to 14 days after jaw opening within the upper cervical spinal cord tissue containing the caudal part of the spinal trigeminal nucleus, which is the site of trigeminal primary afferent projections from mandibular (ie, V3) nociceptive neurons. In response to prolonged trigeminal nociceptor activation, neuropeptides and other inflammatory molecules, including cytokines, are released in the caudal part of the spinal trigeminal nucleus from astrocytes and microglia that help maintain a sustained state of central sensitization.^{8,14,23,26–28} Although glial cells in the caudal part of the spinal trigeminal nucleus are a major site of cytokine synthesis and release, the contribution of cytokine release from other sources such as the solitary tract nucleus and reticular formation may also be involved in cytokine expression and thus regulate neuronal excitability.

An interesting finding of this study was that the levels of cytokines were still elevated 14 days after jaw opening in the ganglion and upper cervical spinal cord even though the animals no longer exhibited nocifensive behavior to mechanical stimulation. It is probable that the cytokines whose levels were significantly elevated in the trigeminal ganglion and upper cervical spinal cord are functioning to maintain a latent sensitized²⁹ or primed state³⁰ of trigeminal nociceptive neurons in response to severe tissue injury or prolonged peripheral tissue inflammation. Sensitization, which refers to the lowering of the stimulus intensity required to trigger an action potential in peripheral or central neurons, provides a mechanism to protect the tissue from further injury. Findings from this study are consistent with other models that report the development of central sensitization and then latent sensitization of spinal nociceptive neurons.^{31,32} In response to prolonged stimulation of nociceptive neurons, there is a reported remission phase in which the neurons have not returned to a normal basal state but rather are in an altered state characterized by the ability to be activated with low-level stimuli.^{33–35} In a latent sensitization state, which can persist for months, there is an absence of hypersensitivity but a long-lasting state of vulnerability that results following severe tissue injury. Thus, there is a prolonged state of greater susceptibility to chemical stimuli that serves a protective function until the tissue is fully healed and homeostasis is restored. Results from this study support the notion that tissues innervated by branches of the trigeminal nerve remain more sensitized in response to prolonged jaw opening, which might occur during a normal dental procedure.

Conclusions

Data from this study provide evidence that prolonged near-maximal jaw opening can lead to activation and prolonged sensitization of the trigeminal system that results in a pathologic

condition similar to what is observed in TMD. Furthermore, findings from this study may provide a plausible explanation for why some patients develop TMD after routine dental procedures that involve prolonged jaw opening.

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Fig. 1.

Prolonged jaw-opening model. **(a)** Mandible being retracted to 22 mm to achieve near maximal jaw opening without subluxation. **(b)** The retractor was placed in a position to allow it to rest in the same horizontal plane to prevent unwanted torque on the joints. **(c)** Close-up image illustrating the position of retractor during jaw-opening procedure. **(d)** Placement of retractor in unopened position in procedural control animals.

Fig. 2.

Effects of near-maximal jaw opening for 20 minutes on nocifensive head-withdrawal responses to 100 g of force applied to the cutaneous area over the right and left masseter muscles, **(a)** Average number of nocifensive withdrawal responses (out of 5) to 100-g force 2 hours, 3 days, 7 days, and 14 days after jaw retraction compared to control levels, **(b)** Average number of nocifensive withdrawal responses (out of 5) after 3% isoflurane for 20 minutes as compared to basal measurements recorded prior to exposure, **(c)** Average number of nocifensive withdrawal responses (out of 5) after a retractor was placed in an unretracted position for 20 minutes under isoflurane as compared to animals exposed to isoflurane only.

* Denotes any time point where $P < .05$.

Table 1

Cytokines in the Upper Cervical Spinal Cord That Were Significantly Regulated as a Result of Prolonged Jaw Opening

Cytokine	Fold ^a ± SEM
2 hours postretraction	
TNF-α	2.8 ± 0.2
CINC-2α/β	2.6 ± 0.4
CNTF	2.6 ± 0.3
IL-17	2.3 ± 0.4
sICAM-1	2.2 ± 0.3
IL-1β	2.0 ± 0.2
IL-13	2.0 ± 0.2
TIMP-1	2.0 ± 0.3
CINC-3	1.9 ± 0.3
IL-3	1.8 ± 0.2
LIX	1.8 ± 0.3
L-Selectin	1.8 ± 0.2
Thymus Chemokines	1.8 ± 0.2
GM-CSF	1.7 ± 0.2
IFN-γ	1.7 ± 0.2
RANTES	1.7 ± 0.2
VEGF	1.6 ± 0.2
Fractalkine	1.5 ± 0.2
MIP-3α	1.5 ± 0.1
3 days postretraction	
CNTF	2.1 ± 0.2
7 days postretraction	
CNTF	3.3 ± 0.1
TNF-α	2.1 ± 0.4
sICAM-1	1.9 ± 0.1
LIX	1.7 ± 0.1
Thymus Chemokines	1.7 ± 0.1
Fractalkine	1.3 ± 0.1
L-Selectin	1.3 ± 0.1
14 days postretraction	
CINC-2α/β	3.3 ± 0.6
IL-13	3.0 ± 0.5
TNF-α	2.8 ± 0.4
IP-10	2.3 ± 0.4

Cytokine	Fold ^a ± SEM
CINC-3	2.1 ± 0.3
IL-1β	2.1 ± 0.3
IL-3	2.1 ± 0.4
IL-2	2.0 ± 0.3
TIMP-1	2.0 ± 0.3
VEGF	2.0 ± 0.3
GM-CSF	1.9 ± 0.3
IL-17	1.9 ± 0.3
RANTES	1.7 ± 0.2
IL-1α	1.6 ± 0.2
LIX	1.6 ± 0.1

^a Average-fold change.

CINC-2α/β = cytokine-induced neutrophil chemoattractant 2 alpha/beta; CINC-3 = cytokine-induced neutrophil chemoattractant 3; CNTF = ciliary neurotrophic factor; GM-CSF = granulocyte macrophage-colony stimulating factor; IFN-γ = interferon gamma; IL-1α = interleukin 1 alpha; IL-1β = interleukin 1 beta; IL-2 = interleukin 2; IL-3 = interleukin 3; IL-10 = interleukin 10; IL-13 = interleukin 13; IL-17 = interleukin 17; IP-10 = interferon gamma-induced protein 10; LIX = lipopolysaccharide-induced CXC chemokine; MIP-1α = macrophage induced protein 1 alpha; RANTES = regulated on activation; sICAM-1 = s-intercellular adhesion molecule 1; TIMP-1 = tissue inhibitor of metalloproteinase 1; TNF-α = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.

Table 2

Cytokines in the Trigeminal Ganglion That Were Significantly Regulated as a Result of Prolonged Jaw Opening

Cytokine	Fold ^a ± SEM
2 hours postretraction	
L-Selectin	4.6 ± 0.8
TIMP-1	2.6 ± 0.3
IL-4	2.4 ± 0.2
Fractalkine	2.3 ± 0.1
IL-3	2.3 ± 0.3
CINC-2α/β	1.9 ± 0.3
MIG	1.9 ± 0.3
VEGF	1.9 ± 0.2
TNF-α	1.8 ± 0.3
LIX	1.6 ± 0.2
CNTF	1.4 ± 0.1
GM-CSF	1.4 ± 0.2
IL-10	0.7 ± 0.1
3 days postretraction	
L-3	2.0 ± 0.3
IL-13	1.7 ± 0.2
CINC-1	1.6 ± 0.2
IL-1ra	1.2 ± 0.1
7 days postretraction	
IL-3	1.9 ± 0.2
GM-CSF	1.4 ± 0.1
14 days postretraction	
CINC-1	1.8 ± 0.3
RANTES	1.4 ± 0.1
IFN-γ	0.6 ± 0.1

^a Average-fold change.

CINC-1 = cytokine-induced neutrophil chemoattractant 1; CINC-2α/β = cytokine-induced neutrophil chemoattractant 2 alpha/beta; CNTF = ciliary neurotrophic factor, GM-CSF = granulocyte macrophage-colony stimulating factor; IFN-γ = interferon gamma; IL-1ra = interleukin 1 receptor; IL-3 = interleukin 3; IL-4 = interleukin 4; IL-13 = interleukin 13; LIX = lipopolysaccharide-induced CXC chemokine; MIG = monokine-induced by gamma interferon; RANTES = regulated on activation; TNF-α = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.