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DIFFERENTIAL ASCENDING PROJECTIONS OF TEMPOROMANDIBULAR JOINT-RESPONSIVE BRAINSTEM NEURONS TO PERIAQUEDUCTAL GRAY AND POSTERIOR THALAMUS OF MALE AND FEMALE RATS

Z. CHANG, K. OKAMOTO AND D. A. BEREITER*

Department of Diagnostic and Biological Sciences, University of Minnesota School of Dentistry, 18-214 Moos Tower, 515 Delaware Street Southeast, Minneapolis, MN 55455, USA

Abstract—Several craniofacial pain conditions, including temporomandibular joint disorders (TMJDs), are more prevalent in women than men. The basis for sex differences in deep craniofacial pain is not known. The present study compared the magnitude of ascending projections from temporomandibular joint (TMJ)-responsive neurons in trigeminal brainstem with the ventrolateral periaqueductal gray (vlPAG) or posterior nucleus of the thalamus (Po) in males and female rats. Fluorogold (FG) was injected into vlPAG or Po, and TMJ-responsive neurons were identified by Fos-like immunoreactivity (Fos-LI) after mustard oil injection. TMJ-evoked Fos-LI was similar in males and females; however, significant differences in cell counts were seen for FG single-labeled and Fos/FG double-labeled neurons in trigeminal brainstem. After vlPAG injections, the number of FG-labeled neurons in trigeminal subnucleus interpolaris (Vi), ventral interpolaris/caudalis transition (vl-Vi/Vc), and dorsal paratrigeminal region (dPa5) was greater in females than males. The percentage of Fos/FG double-labeled neurons in vl-Vi/Vc and dPa5 after vlPAG injection also was greater in females than males. In contrast, after Po injections, males displayed a greater number of FG-labeled neurons in superficial laminae (Lam I/II) of trigeminal subnucleus caudalis (Vc) and upper cervical spinal cord (C_{1-2}) and deeper laminae (Lam III/V) at C_{1-2} than females. The percentage of Fos/FG double-labeled neurons in Lam I/II of Vc after Po injection also was greater in males than females. These data revealed significant sex differences in ascending projections from TMJ-responsive neurons in trigeminal brainstem. Such differences may influence the ability of males and females to recruit autonomic reflexes and endogenous pain control circuits relevant for TMJ nociception.

Key words: sex difference, temporomandibular joint, trigeminal brainstem, periaqueductal gray, posterior thalamus.

*Corresponding author. Tel: +1-612-625-4541; fax: +1-612-626-2651.
E-mail address: bereiter@umn.edu (D. A. Bereiter).

Abbreviations: CVLM, caudal ventrolateral medulla; C_{1-2}, upper cervical spinal cord; dPa5, dorsal paratrigeminal region; E2, estradiol; FG, fluorogold; Fos-LI, Fos-like immunoreactivity; Lam I/II, superficial laminae; Lam III/V, deep laminae; MO, mustard oil (allyl isothiocyanate); NTS, nucleus tractus solitarii; OvX, ovariectomized; PAG, periaqueductal gray; PBS, phosphate-buffered saline; Po, posterior thalamic nucleus; RVM, rostroventromedial medulla; TBNC, trigeminal brainstem nuclear complex; TMJ, temporomandibular joint; TMJD, temporomandibular joint disorder; Vc, trigeminal subnucleus caudalis; Vi, trigeminal subnucleus interpolaris; Vi/Vc, trigeminal interpolaris/caudalis transition region; vlPAG, ventrolateral periaqueductal gray.

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Temporomandibular joint disorders (TMJDs) represent a heterogeneous family of conditions that present with pain in the jaw joint and muscles of mastication. The signs and symptoms of TMJD suggest a central neural dysfunction resulting in pain amplification; however, the central neural pathways that underlie TMJD pain are not well defined (Diatchenko et al., 2006; Maixner, 2009). Although women are more likely to develop TMJD than men (Bereiter and Okamoto, 2011), little is known about brain pathways that process noxious sensory information from deep craniofacial tissues in males and females. Sensory afferent nerves that supply the temporomandibular joint (TMJ) and muscles of mastication terminate at multiple rostrocaudal levels of the trigeminal brainstem nuclear complex (TBNC) and upper cervical dorsal horn (Shigenaga et al., 1988; Dessem et al., 2007). C-fos immunohistochemical studies have confirmed that select regions of the TBNC that receive direct input from the TMJ, for example, dorsal paratrigeminal region (dPa5) and superficial laminae of trigeminal subnucleus caudalis (Vc) and upper cervical dorsal horn (C_{1-2}), encode the intensity of an intra-TMJ stimulus in an estrogen-dependent manner (Bereiter, 2001; Okamoto et al., 2008). Anatomical tract-tracing combined with c-fos methods has been used to report that TBNC neurons responsive to jaw muscle (Ikeda et al., 2003; Sugiyo et al., 2005) or TMJ injury (Yamazaki et al., 2008) projected to higher brain centers associated with nociceptive processing; however, only male animals were included in these studies.

The aim of the present study was to determine if ascending projections from TMJ-responsive neurons in the TBNC displayed sex differences. Cell counts were made after fluorogold (FG) was injected into the ventrolateral periaqueductal gray (vlPAG) or posterior thalamic nucleus (Po) in adult male and female rats. The periaqueductal gray (PAG)-rostroventromedial medulla (RVM) complex plays a pivotal role in pain modulation (Tracey and Mantyh, 2007), including pain evoked by stimulation of jaw muscles (Kupers et al., 2004) and facial skin (Mainero et al., 2007). The vlPAG preferentially integrates noxious sensory input from deep somatic and visceral tissues (Clement et al., 2000), and, more recently, the anatomical and functional organization of vlPAG-RVM pathways was shown to display sex-related differences that may underlie sexually dimorphic responses to opioid analgesics in spinal pain models (Loyd and Murphy, 2006). The Po receives significant direct input from the TBNC (Iwata et al., 1992; Gau-

riau and Bernard, 2004a). Spinothalamic input to Po derives mainly from nociceptive neurons in superficial and deep laminae (Zhang and Giesler, 2005), while Po neurons that encode noxious sensory information project strongly to cortical regions that integrate affective and sensory aspects of pain (Gauriau and Bernard, 2004b). Stimulation of specialized craniofacial tissues, such as tooth pulp (Zhang et al., 2006) and dura (Burstein et al., 2010), activates Po neurons, while cornea-responsive (Hirata et al., 2000) and TMJ-responsive neurons (Takeshita et al., 2001) recorded from superficial laminae of Vc are driven antidromically by stimulation sites in the Po, but not from sites in the ventral posterior medial thalamic nucleus (VPM). Although considerable evidence suggests that the vlPAG and Po contribute to the proposed "TMJ pain matrix" in the brain (Bereiter and Okamoto, 2011), it is not known if ascending projections from TMJ-responsive neurons in the lower brainstem to these regions are similar in males and females.

EXPERIMENTAL PROCEDURES

The animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota and conformed to the established guidelines set by the National Institutes of Health guide for the care and use of laboratory animals (PHS Law 99–158, revised 2002). Every effort was made to minimize the number of animals used and their suffering.

Estradiol treatment in females

Ovariectomized (OvX) female rats were injected daily with 17β-estradiol-3-benzoate (Sigma, St. Louis, MO, USA), (estradiol, E2; 3 μg/kg) dissolved in 200 μl sesame oil for 2 days before the day of perfusion. Our rationale for comparing males with low E2-treated females was 2-fold. Firstly, we were interested in sex differences in projections of TMJ-responsive neurons rather than the effect of estrogen status per se. Females under low estrogen conditions and males produced similar Fos-like immunoreactivity (Fos-LI) responses in TBNC regions after TMJ stimulation (Bereiter, 2001). Secondly, E2 was given as a low-maintenance dose to normalize the elevation of hypothalamic and pituitary hormones re-

Table 1. Total cell counts for Fos/FG double-labeled, Fos-positive, and FG-positive neurons in rats that received fluorogold injection into ventrolateral PAG and TMJ stimulation

	Vi	dm-Vi/Vc	vl-Vi/Vc	dPa5
Male-ipsi				
Fos/FG	6.2±2.6	2.1±1.0	8.0±1.7	2.8±1.3
Fos	356±93.1	200.3±35.3	193±47.8	254±56.8
FG	472±51.8	105.7±11.5	69.3±6.0	9.33±3.0
Female-ipsi				
Fos/FG	8.0±2.8	1.1±0.6	19.0±5.5[a]	12.0±4.3[b]
Fos	199±116.5	117±18.7	277±18.7	290±56.7
FG	764±50.3[b]	131±10.4	93.0±10.7[a]	41.0±14.2[a]
Male-contra				
Fos/FG	0.0±0.0	0.8±1.0	3.1±2.2	0.0±0.0
Fos	172±65.2**	94.1±21.4**	74.3±17.4*	156±37.3
FG	370±70.6	79.3±16.2	48.7±3.0*	5.3±1.7
Female-contra				
Fos/FG	1.3±1.0	0.7±0.5	5.1±1.3**	3.1±1.9**
Fos	64.0±32.1	92.5±42.1	65.5±5.7**	179±23.6
FG	355±81.4**	92.0±36.7*	58.5±10.8**	27.0±10.3*
	Vc I–II	Vc III–V	C_{1-2} I–II	C_{1-2} III–V
Male-ipsi				
Fos/FG	62.3±24.3	2.0±0.1	48.7±10.2	0.7±0.7
Fos	814±252	149±7.3	1415±116	194.7±23.0
FG	412±45.4	165±22.0	103±23.7	173.3±21.6
Female-ipsi				
Fos/FG	84.0±34.7	0.0±0.0	46.0±7.4	0.0±0.0
Fos	1082±323	255±27.2	1572±23.4	205±23.8
FG	433±23.4	298±29.5[b]	130±20.8	165±11.2
Male-contra				
Fos/FG	18.0±10.9*	0.0±0.0	2.2±0.9**	0.0±0.0
Fos	335±46.4*	64.0±9.8	138±24.5**	48.7±5.4
FG	210±18.8**	64.1±9.6**	18.7±9.6**	63.3±3.2**
Female-contra				
Fos/FG	7.4±5.7**	0.0±0.0	6.1±3.5**	0.0±0.0
Fos	208±52.6**	84.0±6.6	168±27.7**	60.0±2.6
FG	227±89.1*	93.0±10.1**	40.0±14.9**	66.0±5.5**

Total cell counts per brain region, mean±SEM. See methods for number of sections/CNS region. n=4–6 rats per treatment group. Ipsi=ipsilateral to TMJ stimulus (left side); contra=contralateral to TMJ stimulus (right side). FG injected into PAG contralateral to TMJ stimulus.
* $P<0.05$, ** $P<0.01$ ipsilateral vs. contralateral; [a]=$P<0.05$, [b]=$P<0.01$ male vs. female.