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Short Communication
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Responsive Property of Nociceptive Neurons in Ventrolateral Orbital Cortex to Noxious Stimulation and Nucleus Submedius Microinjection of Glutamate

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Abstract

The present study investigated the response property of neurons in ventralateral orbital cortex (VLO) to peripheral mechanical noxious stimuli and nucleus submedius (Sm) injection of glutamate. Fifty-two neurons in VLO with spontaneous and evoked response to noxious stimuli were studied. In these responsive neurons, 38% (20/52) were excited and 56% (29/52) inhibited by noxious stimuli. Three neurons showed mixed responses to noxious stimuli. The excitatory or inhibitory receptive fields of most neurons (71%, 37/52) were very large and bilateral, and in some cases included the whole body. The remaining neurons (29%, 15/52) only responded to orofacial stimulation. In most of these neurons, the responses only occurred during the stimulation, but in some outlasted the stimulation as a long after-discharge or after-inhibition. In 12 responsive VLO neurons, the responses to ipsilateral Sm glutamate injection were further studied. The response to glutamate injection was usually similar to that of mechanical noxious stimuli in most neurons. Four neurons excited by noxious stimuli were also excited by Sm injection of glutamate and six neurons inhibited by noxious stimuli were also inhibited by Sm injection of glutamate. The other two responsive neurons showed no apparent response to Sm injection of glutamate. These results suggest that VLO is involved in nociception and this involvement is related with the glutamatergic activation in Sm.

Keywords: Ventrolateral orbital cortex; Submedius nucleus; Nociception

Short Communication

Neuro-anatomical studies have established that, in rat and cat, the prefrontal ventrolateral orbital cortex (VLO) primarily receives projections from the spinal and medullary dorsal horn via the thalamic nucleus submedius (Sm) [1-4] and that the VLO neurons specially respond to peripheral noxious stimulation and may participate in the non-discriminatory aspect of pain [5-9], which suggest that the VLO may be involved in nociception. However, the VLO may also be involved in nociceptive modulation since many studies have indicated that the VLO contains neurons that project to the periaqueductal gray (PAG) [3,10], a region that is intensively involved in descending modulation of nociception [11], and that electrically or chemically induced activation of the VLO depresses the tail flick reflex and the jaw opening reflex and these effects are eliminated by lesions or functional depression of the PAG [12,13]. There are evidences showing that the glutamatergic neurons located in the spinal cord dorsal horn lamina I project to the Sm [14,15] and microinjection of glutamate into the Sm induces antinociception which is eliminated by γ-aminobutyric acid (GABA) application to VLO or PAG [16]. These studies suggest that Sm, VLO and PAG consist of a nociceptive modulation pathway and the excitatory neurotransmitter glutamate may play an important role in the activation of this pathway. However, it is unclear that the functional connection between the Sm and the VLO in transmission of the nociceptive information induced by glutamate activation of Sm neurons. Therefore, the present study examined the responses of neurons in the VLO to mechanical noxious stimulation and determined the effect of microinjection of glutamate into Sm on the VLO neurons in anesthetized rat.

The experiments were carried out on 24 male adult Sprague-Dawley rats (260-300g, provided by Shaanxi experimental animal center). The experimental protocol was approved by the Institutional Animal Care Committee of the University and was accordant to the guidelines of the International Association for the Study of Pain [17]. The animal was anaesthetized with urethane (90 mg/kg) and chloralose (50 mg/kg) i.p. for the initial surgical procedure. After a tracheotomy was performed to allow artificial ventilation, the animal`s head was positioned in a stereotaxic apparatus. A

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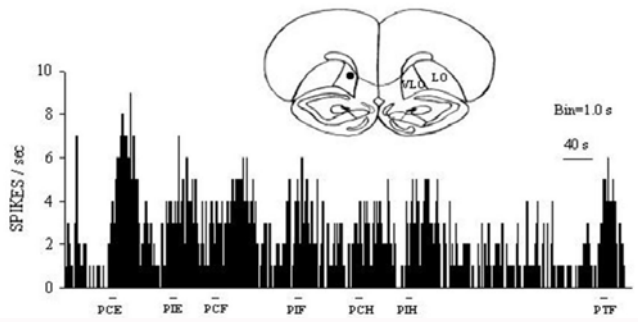

Figure 1A: An inhibitory VLO neuron to mechanical noxious stimuli with after-inhibition.

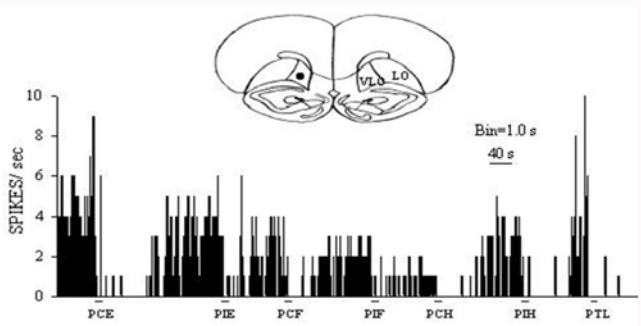

Figure 1B: An excitatory VLO neuron to noxious stimuli and Sm glutamate injection.

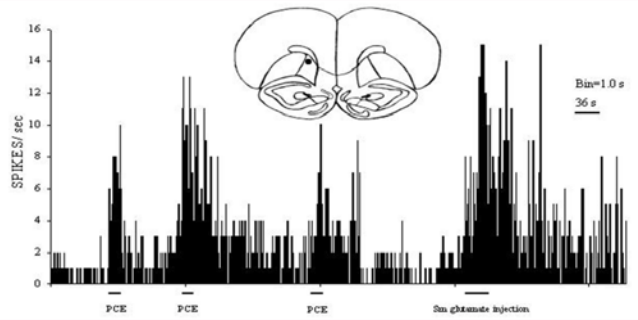

Figure 1C: An VLO neuron with inhibitory responses to mechanical noxious stimuli and Sm glutamate injection.

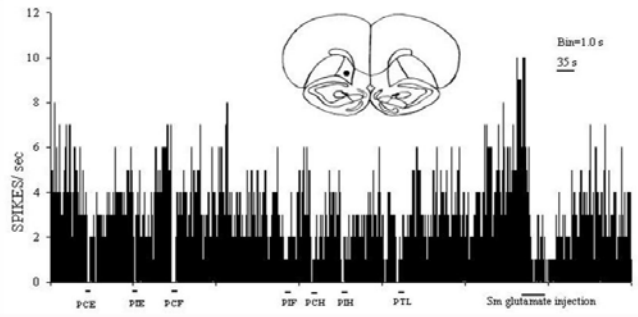

Figure 1D: Right-upper coronal section showing histologically confirmed neuronal recording site.

Abbreviations: Bin=1.0s. PN: Pinching Nose; PIE: Pinching Ipsilateral Ear; PCE: Pinching Controlateral Ear; PIF: Pinching Ipsilateral Fore paw; PCF: Pinching Controlateral Fore paw; PIH: Pinching Ipsilateral Hind paw; PCH: Pinching Controlateral Hind paw; PTL: Pinching Tail.

Figure1: Histogram showing an excitatory VLO neuron to mechanical noxious stimuli with a long after-discharge.

small craniotomy was made over the frontal cortex and the dura and arachnoid were removed carefully to expose the surface of the prefrontal cortex and then covered with warm mineral oil. Another craniotomy was performed over the thalamus. The VLO and the Sm coordinates were AP +3.2~3.7, ML 1.5~2.5, DV 4.0~5.0 and AP -2.5, ML 0.6~0.9, DV 6.5, respectively, according to the atlas of Paxions and Watson [18]. A guide cannula with the tip 2 mm above the Sm was fixed to the skull. The rectal temperature was monitored and maintained at 37°~38° with a thermostatically controlled blanket and the electrocardiogram was monitored continuously on an oscilloscope throughout the experiment. Supplemental i.p. injections of urethane were given as required to maintain the animal in a state without voluntary movements but still mildly responsive to noxious stimuli.

Single-unit extracellular recordings were made with glass microelectrodes (tip diameter <1.0 μm) filled with 2% Potamine Sky Blue in 0.5 M sodium acetate and having resistance of 8-12 MΩ. The neuronal activity was amplified, filtered (1-3 kHz), displayed on an oscilloscope and synchronously introduced into a window discriminator and a computer system that allowed continuous monitoring of neuronal firing rate and construction of a peri-stimulus time histogram. When a stable unit was obtained, the mechanical noxious stimulus was delivered by grasping a fold of skin and pinching with a toothed forceps for 10~20 sec [5]. The pinched skin regions included nose, bilateral ears, bilateral forepaws, bilateral hind paws and tail to determine the receptive characteristics. A noxious stimulus was considered to be that which elicits intense discomfort when applied for 10~20 sec to the digits of the experimenters [9]. Neurons were considered responsive (activated or inhibited) only if there was a at least 10% change from the pre-stimulus baseline firing rate during stimulus application [7]. In some neurons, after determining the receptive fields, glutamate (0.5 μl, 200 mM) was slowly injected into Sm over 20 s using a syringe inserted into Sm via the guide cannula. The neuronal response to Sm injection of glutamate was recorded continuously.

At the end of experiment, the recording site in VLO was marked by electrophoresis of Pontamine Sky Blue from the microelectrode with a cathodic current of 10 uA for 20 min and the Sm injection site was marked by injection of 0.5 μl Pontamine Sky Blue with syringe. Under deep anesthiesia, the animal was perfused transcardially with saline followed by 10% buffered formalin. The brain was then removed and fixed in fresh formalin for 3~7 days. The brain section was cut with a freezing microtome, mounted, and stained with Cresyl Violet for histological verification of the recording and injection sites being within VLO and Sm [18], respectively.

Data were expressed as the mean±SEM and analyzed for statistical significance by using paired *t*-test to compare the differences between pre- and post-stimuli or glutamate injection. A *P*-value less than 0.05 was considered statistically significant.

Sixty-three neurons in VLO were successfully isolated on the basis of spontaneous activity and fifty-two (82%) neurons with response to mechanical noxious stimuli were further studied. These responsive neurons had a mean firing rate of 2.41±0.17 Hz. Twenty (38%) of these responsive neurons were excited by noxious stimuli. The firing rate significantly increased from 1.83±0.27 before stimulation to 5.63±0.68 Hz during stimulation ($P< 0.01$). Figure 1 shows an example of VLO neuron excited by mechanical noxious stimuli applied throughout the body. Twenty-nine (56%) of these

responsive neurons were inhibited by mechanical noxious stimuli. The firing rate decreased significantly from 2.20±0.51 Hz before stimulation to 0.70±0.31 Hz during stimulation ($P< 0.01$). Figure 1B shows the inhibitory responses of a VLO neuron to mechanical noxious stimuli. Three (4%) of these responsive neurons had a mixed response to stimuli, i.e., excited by noxious stimulation applied to some sites while inhibited by other sites. Furthermore, eleven of excited and twenty-three of inhibited neurons had wide receptive fields, even through whole body; while the other remaining neurons had an excitated (9) or inhibited (6) receptive field limited to orofacial region. In the 3 neurons with mixed responses, two had opposite responses when stimuli were applied to the orofacial versus limbic regions and one was excited by ipsilateral limb stimuli and inhibited by stimuli applied to other body regions. Some neurons increased or decreased their firing rate during the application of the mechanical noxious stimuli and recovered following the termination of the stimuli, but some neurons had a prolonged after-discharge or after-inhibition (Figure 1A and 1B).

In the responsive neurons of VLO, twelve were further tested for the responses to glutamate (0.5 μl, 200 mm) injection into Sm. Four neurons excited by noxious stimuli were also excited by glutamate injection and the mean firing rate increased from 1.04±0.67 Hz before glutamate injection to 4.93±0.42 Hz during glutamate injection ($P< 0.05$). (Figure 1C) shows one excitatory VLO neuron excited by Sm injection of glutamate. Six of the neurons inhibited by noxious stimuli were also inhibited by glutamate injection. The mean firing rate decreased from 2.09±0.95 Hz before glutamate injection to 0.88±0.37 Hz during glutamate injection ($P< 0.05$). (Figure 1D) shows an example of VLO neurons that was inhibited by glutamate injection. The neuronal excitatory and inhibitory responses to Sm injection of glutamate continued for and, in some, outlasted the injection duration. The remaining two responsive neurons had no significant alteration in response to the Sm injection of glutamate.

The results of the present study showed that most VLO neurons are excited or inhibited by mechanical noxious stimuli and have large receptive fields. These results are consistent with these of previous studies [5-7,9]. The minor differences occurring in different laboratories relate primarily to the ratio of excitatory and inhibitory neurons, which may be due to the differences in animal species and anaesthetics. In the present study, most neurons have one large receptive field and some neurons outlasted the stimuli. There are reports [8,9] that the VLO neurons mainly respond to the prolonged application of intense noxious stimuli and require modality and spatial convergences. These results suggest that VLO is involved in modulation of nociception and in mediating critical to the affective-motivational aspect of the pain experience. This hypothesis is supported by the fact that the VLO receives projection from spinal and medullary dorsal horn and contains neurons projecting to the PAG [3,10] which is one important pain modulation center [11]. Our previous studies have also indicated that electrical or chemical activation of VLO inhibits the tail-flick reflex and jaw-opening reflex and these effects are abolished by lesion or inhibition of the PAG [12,13] while lesion or inhibition of VLO abolishes the antinociceptive effects evoked by activation of Sm or intense electro-acupuncture stimulation applied to Aδ and C fibers [19,20]. In addition, we also found that microinjection of opioid receptor agonist into VLO can inhibit the formalin-induced nociceptive behaviors [21]. So these studies provide support for above-mentioned hypothesis that the VLO, as one higher center, is involved in the nociception modulation mediated by the VLO-PAG-spinal cord pathway. Thus, it can be hypothesized that the nociceptive information transferred to the VLO produces the nociception and simultaneously activates the descending inhibiting system and depresses the nociceptive inputs at the spinal cord level.

Although the anatomical studies have indicated that the VLO receives directly inputs from Sm [2-4], the detailed transmission mechanism of noxious information from the lamina I of spinal and medullary dorsal horn via Sm to VLO remained unclear. The present results indicated that Sm microinjection of glutamate evoked the same excitatory or inhibitory response in VLO neurons as that evoked by peripheral mechanical noxious stimuli and complement the findings briefly reported by El-Yassir and Dostrovsky [6] who found that electrical stimulation of Sm excites VLO neurons excited by noxious peripheral stimulation and inhibits neurons inhibited by noxious peripheral stimulation. The results also provide physiological evidence for the projection from Sm to VLO. Immunohistological studies have indicated that there are axonal terminal distributions in Sm originating from spinal cord glutamatergic neurons, but no glutamatergic neurons in Sm [14,15]. So it is proposed that the noxious stimuli may activate the spinal glutamatergic neurons which release glutamate into Sm from the axonal terminals acting on the output neurons and subsequently excite some neurons while inhibits other neurons in VLO simultaneously. The mechanism underlying the inhibitory responses of some VLO neurons is less clear but one possibility is that it is due to the activation of inhibitory interneuron which is excited by glutamate released from Sm. And the integrated effects of these two kinds of neurons in VLO further activate the descending inhibiting system which prevents the noxious input at spinal cord level. The present result is also consistent with other reports about the information transmission between thalamus and cortex. Pirot et al. [22] provided anatomical and electrophysiological evidence for an excitatory amino acid pathway from the thalamic mediaodorsal nucleus (MD) to the prefrontal cortex (PFC) in the rat, mainly mediated via AMPA receptor but not NMDA receptors. Salt et al. [23] also reported that in the precruciate cortex the excitatory postsynaptic potentials (EPSPs) elicited by thalamic (VL) stimulation appear to be mainly mediated by AMPA-type receptors. These results were similar to our previous report that VLO was involved in the antinociception induced by Sm glutamate injection [12] and the antinociception is mainly mediated by AMPA/KA and metabotropic glutamate receptors but not by NMDA receptor [24].

Taken together with previous studies, the present study suggests that VLO is involved in nociception closely related to affective-motivational aspect of pain and provides a physiological support for anatomical finding that the VLO receives the projection from the Sm which relays nociceptive information from spinal and medullary dorsal horn to the VLO via glutamatergic activation.

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