Central Sensitization of Nociceptive Neurons in Trigeminal Subnucleus Oralis Depends on Integrity of Subnucleus Caudalis

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INTRODUCTION

The subnucleus caudalis (Vc) of the trigeminal (V) spinal tract nucleus is generally considered to play an integral role in the brain stem processing of orofacial nociceptive information and to represent the V brain stem homologue of the spinal dorsal horn and has also been termed the medullary dorsal horn. However, recent studies have also documented the involvement in orofacial pain mechanisms of the rostral components of the V spinal tract nucleus, including subnucleus oralis (Vo). Lesioning of Vc or V tractotomy at the obex level does not completely abolish nociceptive responses evoked by noxious intraoral or perioral stimuli, including tooth pulp stimulation, and disruption of rostral components including Vo has been reported to interfere with nociceptive orofacial sensation or behavior (for review, see Bereiter et al. 2000; Sessle 2000). Electrophysiological studies also have demonstrated that nociceptive neurons responding to high-intensity electrical stimuli or formalin applied to the intraoral or perioral region occur in Vo, and some of these neurons are sensitive to diffuse noxious inhibitory controls (Azerad et al. 1982; Dallel et al. 1990; Hu and Sessle 1984; Hu et al. 1992; Parada et al. 1997; Raboissin et al. 1995). We have also recently shown that application of mustard oil (MO), a small-fiber excitant and inflammatory irritant, to the tooth pulp can produce “central sensitization” reflected in neuroplastic changes in nociceptive neurons of Vo as well as in Vc (the medullary dorsal horn) (Chiang et al. 1998; Park et al. 2001).

Earlier studies have documented that Vc neurons project to Vo and other rostral components of the V brain stem sensory nuclear complex via ascending intersubnuclear pathways (Gobel and Purvis 1972; Hu and Sessle 1979; Ikeda et al. 1982, 1984; Jacquin et al. 1990; Nasution and Shigenaga 1987; Panneton and Burton 1982) and that Vc exerts a net facilitatory influence on Vo neurons (Dallel et al. 1998; Greenwood and Sessle 1976; Khayyat et al. 1975; Woda et al. 2001; Young and King 1972). These considerations raise the possibility that the central sensitization recently documented in Vo nociceptive neurons (Park et al. 2001) is mediated by Vc. To address this issue, the present study used microinjection into Vc of CoCl2, an effective synaptic transmission blocker in the CNS (Allen and Prongch 1997; Hochstenbach and Cirillo 1997; Lee and Malpeli 1985; Malpeli 1983; Mooney et al. 1992; Nuseir et al. 1999), to determine whether the Vo neuroplastic changes induced by MO application to the tooth pulp depend on an ascending influence from Vc. The data have been briefly presented in abstract form (Chiang et al. 2000).

METHODS

Animal preparation

Thirty-two Sprague-Dawley male rats (285–410 g) were used. The methods used for animal preparation, stimulation, and neuronal recording and classification were similar to those described previously in detail (Chiang et al. 1998; Park et al. 2001) and so will only be briefly outlined. The animal was anesthetized by a mixture of alphachloralose (50 mg/kg ip) and urethan (1 g/kg ip). The trachea and the left external jugular vein were cannulated. To expose the pulp of the right maxillary first molar, a cavity was prepared with a low-speed dental drill and temporarily filled with a cotton pellet soaked with saline. The animal was then placed in a stereotaxic apparatus, a craniotomy was performed on the right side to expose the cerebral
cortex for insertion of the recording electrode into Vo, and the caudal medulla oblongata was also exposed. After 1 h, a supplemental dose of urethan (200–300 mg/kg iv) was administered, the animal was immobilized with gallamine triethiodide (initial dose, 35 mg/kg; maintenance dose, 14 mg/h; iv) and ventilated, and then microelectrode recordings were begun (see following text). An adequate level of anesthesia was confirmed periodically by the lack of spontaneous movements and responses to pinching the paw when the gallamine-induced muscle paralysis was allowed to wear off. In addition, pupil size and heart rate were routinely monitored to ensure their stability when nocuous pinch stimuli were applied. The expired percentage CO₂ and rectal temperature were also continuously monitored and maintained at physiological levels of 3.5–4.5% and 37–38°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).

Recording and stimulation procedures

Single neuronal activity was recorded extracellularly in the right Vo (2.5–2.7 mm lateral, P 1.3–2.6 mm) (Paxinos and Watson 1986) by an epoxy-resin-coated tungsten microelectrode with a rostral inclination of 26°. As the microelectrode was advanced through the Vo region, natural stimuli (see following text) were applied to the orofacial tissues to search for responsive neurons. The Vo was explored 2.5–2.7 mm lateral to the midline and between frontal planes P 1.3 and P 2.6 mm referred to the interaural line. Neuronal activity was amplified, displayed on oscilloscopes and also led to a window discriminator connected to an A/D converter (1401plus, Cambridge Electronic Designs (CED)) and a personal computer. Data were collected with Spike2 for Windows (CED) and analyzed off-line.

A wide range of mechanical (brush, pressure, and pinch), electrical and noxious thermal (radiant heat, 51–53°C) stimuli were applied to the orofacial skin or intraoral mucosa to classify each neuron as a low-threshold mechanoreceptive neuron (LTM) or a nociceptive neuron [wide dynamic range (WDR) or nociceptive-specific (NS)] (Chiang et al. 1998; Hu 1990; Park et al. 2001). The presence of a deep nociceptive input was considered to occur if the application of a blunt probe to muscle, bone, tendon, or temporomandibular joint (TMJ) evoked a neuronal response at a mechanical threshold >5 g but no response could be evoked by the wide range of cutaneous stimuli used (Chiang et al. 1994; Iggo 1960; Park et al. 2001; Schaible and Schmidt 1983; Yu et al. 1993). Electrical stimuli of constant-current single pulses (0.2 ms and <1 mA for A-fiber inputs; 2 ms and <5 mA for C-fiber inputs) were applied within the delineated mechanoreceptive field (RF) to determine the existence of A- or C-fiber afferent inputs, and monopolar stimuli of cathodal current single pulses (0.2 ms, <2 mA) were applied to the exposed maxillary molar tooth pulp to determine the existence of a dental afferent input (Chiang et al. 1998; Park et al. 2001); we did not attempt to determine if the evoked Vo neuronal responses reflected monosynaptic or polysynaptic afferent inputs.

The spontaneous activity, RF size, and responses to mechanical stimuli were assessed at the time intervals specified in the following text. Spontaneous activity was measured as the average frequency (Hz) of spikes occurring for 2 min. The RF of each neuron was determined through the use of a brush, blunt probe, and a pair of nonserrated forceps. As previously described (Park et al. 2001), the orofacial region was empirically divided into 20 small areas (see Fig. 1), and the size of an orofacial RF was quantified by summing the number of these areas included in the RF. To evaluate the reliability of this area-summing method, the extent of the neuron’s cutaneous RF was also outlined on a life-size drawing of the rat’s head and measured by a computer-aided device (SigmaScan, Jandel, CA) as previously described (Chiang et al. 1997, 1998; Yu et al. 1993). Mechanical threshold of the neuronal RF was not studied because of difficulty to access the intraoral RF of most neurons. Consistent with our other recent study of Vo neurons (Park et al. 2001), we tested for responses to graded mechanical pinch or pressure stimuli (5, 10, and 20 g for WDR neurons; 50, 100, and 200 g for NS neurons) that were applied to the neuronal orofacial RF were tested with a force-monitoring forceps; each stimulus was applied for 3 s at an interval of >30 s. As in our previous study (Park et al. 2001), the lower stimulus intensity range for WDR neurons was chosen because these neurons have a lower mechanical activation threshold and intensity for mid-range response than NS neurons (Chiang et al. 1994, 1998; Hu et al. 1981), and we chose an intensity that would elicit a mid-range suprathreshold response for each WDR neuron and NS neuron and applied it three times at the same site within the neuron’s RF. The responses were quantified as the average of the number of spikes produced by this standardized stimulus.

Drug administration

MO (allyl isothiocyanate, 95%; Aldrich Chemical) was applied locally to the exposed molar pulp to induce central sensitization of Vo nociceptive neurons that typically lasts >40 min (Park et al. 2001). Therefore to test for the possible Vc synaptic mediation of the MO-induced central sensitization of Vo nociceptive neurons, CoCl₂ (Sigma-Aldrich Canada) was freshly dissolved in saline (5 mM) and a 0.3 μl bolus was microinjected into a manual microinjector (Model 5000, David Kopf) over 45–60 s into the right Vc (1.0 mm behind the obex; 1.2 mm lateral to the midline; 1.0 mm below the medullary surface) at ~20 min after MO application. Vehicle (isotonic saline, 0.3 μl) application served as a control. In some experiments (see following text), CoCl₂ was instead microinjected into subnucleus interpolaris (Vi), 1 mm rostral to the obex and 1.5 mm lateral to the midline. To mark the injection site so that the extent of drug diffusion could be assessed, a 0.3 μl bolus of pontamine sky blue solution (2% in saline) was first loaded into the syringe, followed by an air gap (0.1 μl), and then the syringe filled with the CoCl₂ solution.

Experimental paradigm

In each animal, only one neuron was tested with saline or CoCl₂ after the pulp application of MO. Experimental animals were divided into three groups according to the drug or saline application: MO application followed by saline injection into Vc (Saline/Vc); MO application to the pulp followed by CoCl₂ injection into Vc (CoCl₂/Vc); MO application followed by CoCl₂ injection into Vi (CoCl₂/Vi).
The latter experiment was designed to test the possibility that any 
CoCl₂ blocking effect on Vo neurons might be caused by drug 
diffusion from the injection site (Vc) directly to Vo. A similar experi-
mental paradigm was applied for all three groups: 10 min after a Vo 
nociceptive neuron was identified, the neuronal spontaneous activity, 
orofacial RF size and responses to mechanical stimuli were deter-
mined and served as baseline values; a complete assessment of these 
properties took ~6–7 min. Then the saline-soaked cotton pellet in the 
prepared molar cavity was carefully replaced by a segment of dental 
paper point soaked with MO (0.2 μl), and the cavity quickly sealed 
with CAVIT (ESPE, Germany). Another assessment of neuronal 
properties started at 3 min after MO application, and repeated at 8- 
to 10-min intervals until 60 min. At ~20 min after MO application, 
saline or CoCl₂ was locally injected into right Vc (or Vi in the 
CoCl₂/Vi group).

**Histological and statistical analysis**

An electrolytic lesion (anodal current, 8 μA, 10 s) was made at the 
recording site at the end of the experiment. Ten minutes before the 
beginning of transcardial perfusion, the pontamine sky blue solution 
(0.3 μl) that was left in the injection syringe was slowly injected at the 
same site. Verification of the recording and injection sites and the 
dye’s approximate diffusion extent was assessed with conventional 
histological procedures.

Statistical treatments were performed on the normalized data. For 
assessing the drug effects, differences between baseline (predrug) 
values and values at different postdrug time points in each group were 
treated by repeated-measures ANOVA (RM ANOVA) or RM 
ANOVA on ranks followed by Dunnett’s method. Differences be-
tween groups were treated by ANOVA on ranks (Denenberg 1984). 
Differences between groups at a given time point were treated by a 
priori Mann-Whitney rank sum test or t-test. All values were ex-
pressed as means ± SE except for those measures of orofacial RF size 
that involved summing the areas (see preceding text); these were 
expressed as the median and 25th and 75th percentiles. The level of 
significance was set at \( P < 0.05 \).

**RESULTS**

Twenty-five nociceptive (18 WDR, 7 NS) Vo neurons were 
recorded and studied with the full range of tests, but 3 LTM 
neurons recorded did not show central sensitization to pulp 
application of MO and so were excluded from further analysis. 
The recording sites of all 25 nociceptive neurons were in the 
middle and ventral part of Vo, except for one site close to the 
border of its dorsomedial (DM) part (Fig. 2).

All 7 NS neurons and 16 of the WDR neurons had at 
baseline (i.e., before MO application) an ipsilateral RF that 
involved the maxillary and/or mandibular divisions and that 
was located in the intraoral and/or perioral regions; only 2 
WDR neurons had a cutaneous RF involving the ophthalmic 
division. In most WDR neurons, the location of the cutaneous 
or intraoral tactile RF was within the pinch/pressure RF, but in 
five WDR neurons, the intraoral tactile RF was separate from the 
intraoral pinch/pressure RF in the same division or in a 
different division. Most (56%) of the 16 WDR neurons and 
50% of the six NS neurons tested received electrically evoked 
A-fiber inputs from their cutaneous or intraoral mucosal RFs; 
33% of these responsive WDR neurons also received C-fiber 
inputs. Moreover, 56% of the 16 WDR neurons tested, but 
one of the 6 NS neurons, received electrically evoked A- 
or C-fiber molar inputs. Spontaneous activity was noted in 28% 
of the WDR and NS neurons; the spontaneous firing rate was 
<0.25 Hz for most of these neurons.

**OROFACIAL RF SIZE**

MO application produced a significant, long-lasting increase in tactile and pinch/pressure RF size in all nine nociceptive neurons tested. As shown in Fig. 4 and Table 1, the pinch/pressure RF size increased significantly through-
out the 40-min period following MO application (\( P < 0.001 \), 
RM ANOVA on ranks), with its peak around 26 min (median: 
200%; 25th–75th percentile: 161–338% of baseline; \( P < 0.05 \), 
Dunnett’s method), despite saline injection into Vc at 20 min. 
MO application produced a novel intraoral or periangular pinch 
or pressure RF in three WDR and two NS neurons having, re-
spectively, a baseline periangular or intraoral RF. In addition, 
the cutaneous RF size of six of the nine neurons was quantitatively 
assessed (see METHODS) before and after MO application. As 
shown in Fig. 5, the cutaneous pinch RF size showed a significant 
and prolonged increase following MO application, which 
peaked at 26 min (mean ± SE: 348 ± 80%; range: 120–592% 
of baseline; \( P < 0.001 \), RM ANOVA). The time course of the 
cutaneous pinch RF changes of these neurons was similar to 
the orofacial pinch/pressure RF changes measured by summing 
the number of areas in the orofacial RF (see preceding text and 
Fig. 4).

The orofacial tactile RF of all 6 WDR neurons also showed
induced significant neuroplastic changes in all Vo nociceptive (7 WDR, 3 NS) neurons tested. These changes involved increases in RF size and responses to pinch or pressure stimuli; no consistent changes in spontaneous activity occurred after MO application or CoCl₂ injection (Table 1). An example is shown in Fig. 3B. At baseline, 3 of the 10 neurons tested had both intraoral and perioral (including facial) RFs, 3 neurons had only a facial and/or perioral RF, and the remaining 4 neurons had only an intraoral RF. An example of the CoCl₂ injection site in Vc is illustrated in Fig. 7A in which the diffusion extent, as marked by the subsequent injection of dye, was confined within a region with a diameter of 1.2 mm around the end of the needle track.

**OROFACIAL RF SIZE.** After MO application, the neuronal pinch/pressure RF size increased from 8 min and peaked ~18 min (median:171%, 25th–75th percentile: 126–200% of baseline; P < 0.001, RM ANOVA on ranks). As in the Saline/Vc group, MO application produced a novel intraoral or perioral RF in four WDR and two NS neurons having, respectively, a baseline perioral or intraoral RF. CoCl₂ solution injected into Vc ~20 min after MO application produced a significant blockade of the increased RF size within 6 min in eight neurons and at 12–15 min in the remaining two neurons; the median value at 12 min after CoCl₂ injection was 105% (25th–75th percentile: 100–150% of baseline, P > 0.05, Dunnett’s method; Fig. 4). As shown in the example in Fig. 3B, the blockade was typically limited to the expanded portion of the RF, not the baseline RF, and lasted for 15–20 min. Thereafter, the MO-induced neuroplastic changes returned (median:129%, 25th–75th percentile: 114–167%; P < 0.05, Dunnett’s method) and then gradually diminished (at 60 min: median: 124%, 25th–75th percentile: 114–160% of baseline; P > 0.05, Dunnett’s method; Fig. 4). The CoCl₂-induced blockade and its time course were also reflected in the changes in orofacial RF size determined quantitatively in five of the neurons (Fig. 5).

The tactile orofacial RF of the seven WDR neurons also showed a significant increase in size that peaked at 18 min after MO application (median: 200%, 25th–75th percentile: 163–300% of baseline; P < 0.001, RM ANOVA on ranks). Six minutes after CoCl₂ injection into Vc, the tactile RF was abruptly reduced (median: 100%, 25th–75th percentile: 100–133% of baseline; P > 0.05, Dunnett’s method) and was maintained around this level for at least another 35 min (Table 1).

There were significant differences in both the orofacial tactile and pinch/pressure RF sizes between the CoCl₂/Vc and Saline/Vc groups (P < 0.001, ANOVA on ranks, Table 1); these differences included values at the 26-min time point after MO application, i.e., 6 min after CoCl₂ injection (P < 0.05, Mann-Whitney test; Figs. 4 and 5).

**RESPONSES TO SUPRATHRESHOLD MECHANICAL STIMULI.** After MO application, neuronal responses to standardized, mid-range suprathreshold pinch or pressure stimuli were increased in all eight nociceptive neurons tested. The response to noxious mechanical stimuli gradually increased to a peak at 18 min that was significantly different from baseline (333 ± 62% of baseline; range: 76–671%; P < 0.02, RM ANOVA; Fig. 6, Table 1), then declined slowly toward baseline level by 60 min. Saline injection into Vc at 20 min did not affect these changes.

**CoCl₂ injected into Vc reversibly blocks MO-induced neuroplastic changes in Vo nociceptive neurons.**

In the CoCl₂/Vc group, as in the Saline/Vc group and the CoCl₂/Vi group (see following text), pulp application of MO...
TABLE 1. Effects of microinjection of CoCl2 or saline into Vc or Vi on neuroplastic changes in spontaneous activity and RF and response properties of Vo nociceptive neurons in adult rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spontaneous Activity, Hz</th>
<th>Tactile RF, area</th>
<th>Pinch/Pressure RF, area</th>
<th>Pinch- or Pressure-Evoked Response, Spikes/3 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline/Vc experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron sample</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline value</td>
<td>6 WDR, 3 NS</td>
<td>6 WDR</td>
<td>6 WDR, 3 NS</td>
<td>5 WDR, 3 NS</td>
</tr>
<tr>
<td>Value 18’ after MO</td>
<td>0.09 ± 0.08</td>
<td>1.5 (1.0, 4.0)</td>
<td>4.5 (0.5, 6.3)</td>
<td>20.1 ± 7.5</td>
</tr>
<tr>
<td>Value 6’ after saline</td>
<td>0.22 ± 0.21</td>
<td>4.0 (3.0, 6.0)*</td>
<td>7.0 (3.8, 9.3)*</td>
<td>47.4 ± 8.6*</td>
</tr>
<tr>
<td>Value 20’ after saline</td>
<td>0.13 ± 0.12</td>
<td>5.0 (3.0, 7.0)*</td>
<td>9.0 (3.5, 10.0)*</td>
<td>54.0 ± 19.3*</td>
</tr>
<tr>
<td>CoCl2/Vc experiments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuron sample</td>
<td>7 WDR, 3 NS</td>
<td>7 WDR</td>
<td>7 WDR, 3 NS</td>
<td>5 WDR, 3 NS</td>
</tr>
<tr>
<td>Baseline value</td>
<td>0.40 ± 0.37</td>
<td>2.0 (1.0, 3.0)</td>
<td>3.7 (3.0, 7.0)</td>
<td>30.0 ± 7.4</td>
</tr>
<tr>
<td>Value 18’ after MO</td>
<td>0.26 ± 0.18</td>
<td>4.0 (3.0, 6.0)*</td>
<td>7.5 (4.0, 12.0)*</td>
<td>68.8 ± 14.4*</td>
</tr>
<tr>
<td>Value 6’ after CoCl2</td>
<td>1.16 ± 0.66</td>
<td>3.0 (1.3, 4.0)</td>
<td>3.5 (2.5, 7.0)*</td>
<td>29.9 ± 10.5</td>
</tr>
<tr>
<td>Value 20’ after CoCl2</td>
<td>2.57 ± 2.31</td>
<td>2.0 (1.3, 3.8)</td>
<td>4.5 (3.5, 9.0)*</td>
<td>65.7 ± 16.5*</td>
</tr>
<tr>
<td>CoCl2/Vi experiments</td>
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<td></td>
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<tr>
<td>Neuron sample</td>
<td>5 WDR, 1 NS</td>
<td>5 WDR</td>
<td>5 WDR, 1 NS</td>
<td>5 WDR, 1 NS</td>
</tr>
<tr>
<td>Baseline value</td>
<td>0.01 ± 0.01</td>
<td>5.0 (2.0, 6.0)</td>
<td>7.0 (4.0, 8.0)</td>
<td>16.6 ± 5.4</td>
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<tr>
<td>Value 18’ after MO</td>
<td>0.21 ± 0.17</td>
<td>6.0 (4.8, 7.8)*</td>
<td>9.5 (9.0, 12.0)*</td>
<td>40.3 ± 10.8*</td>
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<tr>
<td>Value 6’ after CoCl2</td>
<td>0.55 ± 0.53</td>
<td>7.0 (4.8, 8.0)*</td>
<td>11.5 (9.0, 12.0)*</td>
<td>42.9 ± 10.2*</td>
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<tr>
<td>Value 20’ after CoCl2</td>
<td>0.21 ± 0.20</td>
<td>6.0 (4.8, 7.8)*</td>
<td>11.0 (9.0, 11.0)*</td>
<td>37.8 ± 9.0*</td>
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<tr>
<td>ANOVA on ranks‡</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>H</td>
<td>6.98</td>
<td>28.82</td>
<td>45.59</td>
<td>31.66</td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ANOVA on ranks‡</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>4.19</td>
<td>28.68</td>
<td>35.14</td>
<td>39.83</td>
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<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

All values are shown as means ± SE or median (25th percentile, 75th percentile); Vc and Vi, subnucleus caudalis and interpolaris, respectively; RF, mechanical receptive field; Vo, subnucleus oralis; WDR, wide dynamic range; NS, nociceptive specific. * P < 0.05 for comparison between the baseline value and values at the different time after MO, CoCl2, or saline in each group (repeated-measures ANOVA on ranks). ANOVA on ranks was used for comparison of values between Saline/Vc and CoCl2/Vc groups (‡), and between CoCl2/Vc and CoCl2/Vi groups (‡). C represents the value 12’ after CoCl2.

Responses returned at ~20 min after the CoCl2 injection (243 ± 54%, range: 143–573% of baseline; P < 0.05, Dunnett’s method; Fig. 6) and slowly returned to baseline levels. The time course of response changes was similar to that of RF size changes (Figs. 4 and 6). An example is shown in Fig. 3B.

There were significant differences in responses to the standardized suprathreshold stimuli between the CoCl2/Vc and saline/Vc groups (P = 0.003, ANOVA on ranks; Table 1), including those at the 26-min time point after MO application (P = 0.03, t-test, Fig. 6).

CoCl2 injected into Vi does not affect MO-induced neuroplastic changes in Vo nociceptive neurons

In the CoCl2/Vi group, pulpal application of MO induced significant increases in orofacial RF size and responses to pinch or pressure stimuli in all six (5 WDR, 1 NS) neurons tested (Figs. 3C, 4, and 6, Table 1), but no consistent changes in spontaneous activity occurred after MO application or CoCl2 injection (Table 1). At baseline, all these neurons had an intraoral RF, and one WDR neuron also had a perioral RF and one NS neuron also had a RF in the TMJ region. An example of the CoCl2 injection site in Vi is illustrated in Fig. 7C.

Orofacial RF size. After MO application, the intraoral RF of the neurons expanded into the perioral region which was sensitive only to pinch stimuli (e.g., Fig. 3C) and was maintained throughout the 60-min observation period. Significant increases in the pinch/pressure RF size occurred from 18 to 60 min (P < 0.001, RM ANOVA on ranks) and peaked at 32 min after MO application (median: 171%, 25th–75th percentile: 147–225% of baseline; P < 0.05, Dunnett’s method) despite CoCl2 solution injected into Vi at 20 min after the MO application (Figs. 4, Table 1). As in the Saline/Vc and CoCl2/Vc groups, MO application produced a novel perioral RF in four WDR and one NS neurons having a baseline intraoral RF only; two of these WDR neurons also acquired a deep RF involving jaw muscle and TMJ.

The orofacial tactile RF of all five WDR neurons also showed a significant increase throughout the observation period (P < 0.001, RM ANOVA) with a plateau from 18 to 40 min after MO application (median: 167%, 25th–75th percentile: 119–225% of baseline; P < 0.05, Dunnett’s method; Table 1). CoCl2 injection into Vi at 20 min did not affect these changes.

There were significant differences in both the orofacial tactile and pinch/pressure RF sizes between the CoCl2/Vi and CoCl2/Vc groups (P < 0.001, ANOVA on ranks, Table 1); the pinch/pressure RF sizes of the two groups were also significantly different at the 26-min time point after MO application (P < 0.05, Mann-Whitney test).

Responses to suprathreshold mechanical stimuli. Significant increases in responses to suprathreshold mechanical stimuli of the six neurons occurred after MO application and were maintained despite the injection of CoCl2 into Vi at 20 min after MO application (P < 0.004, RM ANOVA). The increases peaked at 26 min after MO application and 6 min after CoCl2 injection into Vi (387 ± 97%, range: 167–667% of baseline; P < 0.05, Dunnett’s method) and returned toward baseline level by 60 min (Fig. 6; Table 1). The differences in responses between the CoCl2/Vi and CoCl2/Vc groups were significant (P < 0.001, ANOVA on ranks, Table 1), including those at the
DISCUSSION

Consistent with our previous findings (Park et al. 2001), we have documented that application of MO to the rat molar pulp produces central sensitization in Vo that is reflected in signific-
CoCl₂ is injected, (1997). However, if too great a volume or concentration of 40 min (Allen and Pronych 1997; Hochstenbach and Ciriello 1992). In addition, 100 – 250 nl 10 mM CoCl₂ microinjected into the medulla of orofacial primary afferents (Kobayashi and Matsumura 1996; Takemura et al. 1991). Given that the needle tip was placed at 1.0 mm below the medullary surface and we estimated that the extent of CoCl₂ diffusion was 1.2 mm in diameter, it is likely that the CoCl₂ injection blocked most of the intersubnuclear bundles projecting from Vc to Vo (Gobel and Purvis 1972; Ikeda et al. 1982, 1984; Jacquin et al. 1990; Nasution and Shigenaga 1987; Panneton and Burton 1982; Voisin et al. 2002). Furthermore, our findings that CoCl₂ injected into Vi did not affect the neuroplastic changes induced in Vo indicate that the effects on Vo neurons of the CoCl₂ injection into Vc cannot be accounted for by CoCl₂ diffusion directly from Vc to Vo. Although Vi as well as Vc contains intersubnuclear bundles projecting to Vo, the ineffectiveness of CoCl₂ blockade of Vi might be explained by findings that, compared with Vc, Vi lacks laminae I–II (which in Vc contributes to the deep bundles) and has a very limited density of putative neurotransmitters or receptors associated with nociceptive transmission (Mansour et al. 1994; Petralia et al. 1994; Sessle 2000; Tallaksen-Greene et al. 1992). If this explanation is correct, it points to the critical role played by the superficial laminae I–II of Vc in generating central sensitization that might then be mediated to Vo via V intersubnuclear bundles (see following text).

Central sensitization is thought to be reflected in neuroplastic changes that can be triggered by nociceptive afferent inputs and that are manifested in spinal and medullary dorsal horn nociceptive neurons as an increase in spontaneous activity, an expansion of the RF, and an increase in the responsiveness to stimuli (Chiang et al. 1998; Coderre and Katz 1997; Dubner 1991; Hu et al. 1992; Li and Woolf 2001; Ma and Woolf 1996; Morisset and Nagy 2000; Sotgiu and Biella 2000; Suzuki et al. 2000; Willis 1993; Woolf 1992; Yu et al. 1993). These neuroplastic changes have also been previously documented in Vo nociceptive neurons following MO application to the tooth pulp (Park et al. 2001). Of particular note are our present

autonomic pathways (Allen and Pronych 1997; Hochstenbach and Ciriello 1997; Lee and Malpeli 1985; Malpeli 1983; Mooney et al. 1992; Nuseir et al. 1999). It has several advantages over intracerebral injection of local anesthetics, such as lidocaine, and neurotransmitter modulatory agents, such as the GABA agonist muscimol, which have been used in some other studies. These include a selective block of synaptic transmission rather than fibers of passage (relative to lidocaine), an aqueous solution that is less diffusible to neighboring tissues, a faster onset and shorter duration of blocking action, less toxicity on primary afferent neurons and block of all synaptic transmission (whereas muscimol may not block firing in all neurons—in e.g., if they do not have GABA receptors) (Gold et al. 1998; Lee and Malpeli 1985; Malpeli 1983; Mooney et al. 1992). Furthermore, a 120 nl 4 mM CoCl₂ solution can safely be used repeatedly without any tissue damage (Lee and Malpeli 1985), and 25 nl 10 mM CoCl₂ can inactivate a volume of tissue with a diameter under 250 μm for 200 s (Mooney et al. 1992). In addition, 100–300 nl 5–10 mM CoCl₂ microinjected into the medulla can block pressor or depressor responses for 40 min (Allen and Pronych 1997; Hochstenbach and Ciriello 1997). However, if too great a volume or concentration of CoCl₂ is injected, fibers of passage or local tissues may be damaged (Lee and Malpeli 1985; Malpeli 1983).

With these findings in mind, we made only one 300 nl injection of a 5 mM CoCl₂ solution into Vc or Vi. We also avoided several smaller injections at different sites or depths because this would take several minutes to complete and be impractical in our experimental paradigm. The injection site selected was that region that contains Vc nociceptive neurons with an orofacial RF (Chiang et al. 1998) and corresponds to the central termination area in the rostral part of the caudal medulla of orofacial primary afferents (Kobayashi and Matsumura 1996; Takemura et al. 1991).
findings that the neuroplastic changes in RF size and response properties could be transiently and reversibly reduced by CoCl$_2$ microinjection into Vc. The findings are consistent with the earlier observations, although we did not observe a consistent long-lasting increase in spontaneous activity as previously reported (Park et al. 2001). The latter results could be explained by our present sample of nociceptive neurons tested with MO application; it comprised almost 50% of neurons having an RF involving only the intraoral region, and the Park et al. (2001) study showed no change in MO-induced spontaneous activity in such neurons.

Our findings that Vc blockade abolishes pulp-induced neuroplastic changes in Vo nociceptive neurons indicate that the blockade is likely interfering with the relay of MO-evoked pulp afferent inputs via Vc to Vo because Vc is the termination site of many pulp afferents (Clements et al. 1991; Nishikawa et al. 1997; Takemura et al. 1991) that are very effective in inducing central sensitization in Vc neurons (Chiang et al. 1998). The effect of Vc blockade could also be explained by its disruption of a tonic influence that Vc is exerting on Vc central sensitization induced by pulp afferent inputs directly to Vc, although it has been argued that Vc central sensitization most likely depends on the neuronal substrates in Vc (see Chiang et al. 1998; Dallel et al. 1998; Parada et al. 1997; Park et al. 2001; Voisin et al. 2002; Woda et al. 2001; for review, see Sessle 2000). As noted in the preceding text, our data are nonetheless consistent with the considerable anatomical interconnections that exist between Vc and the rostral components of V spinal tract nucleus, including Vo. It has been well documented that the main intranuclear projection of neurons in Vc is via ascending deep intersegmental pathways (Gobel and Purvis 1972: Ikeda et al. 1982, 1984; Jacquin et al. 1990; Nasution and Shigenaga 1987; Panneton and Burton 1982). Furthermore, our present data are also consistent with earlier documentation that Vc exerts a net facilitatory influence on Vo (Greenwood and Sessle 1976; Hu and Sessle 1979; Khayyat et al. 1975; Young and King 1972); this is also supported by recent findings that C-fiber-evoked responses of Vo nociceptive neurons can be depressed by morphine microinjection into the superficial laminae of Vc, but not into Vc, and that N-methyl-D-aspartate antagonist MK-801 microinjection into the lateral part of Vc can markedly depress “wind-up” in some Vc nociceptive neurons (Dallel et al. 1998; Woda et al. 2001). Nonetheless, local modulatory mechanisms in Vo itself cannot be neglected as possible factors influencing nociceptive transmission in the rat Vo because morphine microinjected into Vc can reduce the formalin-induced nociceptive behavior (Lucarini et al. 1995) and MK-801 microinjection into Vc can significantly reduce MO-evoked neuroplastic changes in Vc (Park et al. 2001).

Although CoCl$_2$ microinjected into Vc reversibly blocked the MO-induced neuroplastic changes in Vc nociceptive neurons, both orofacial RF size and responses to noxious stimuli of Vc nociceptive neurons were retained close to their baseline levels. This suggests that Vo orofacial nociceptive processing per se is not influenced by CoCl$_2$-induced block of Vc, whereas the baseline RF properties of LTM neurons in Vo can be reversibly influenced by cold block of Vc (Greenwood and Sessle 1976). Therefore it seems likely that the mechanisms underlying orofacial nociceptive processing may involve neural substrates in both Vo and Vc; this is consistent with earlier findings (Broton and Rosenfeld 1986; Graham et al. 1988; Lucarini et al. 1998; Pickoff-Matuk et al. 1986), while those underlying MO-induced central sensitization may mainly involve Vc. Thus the functional integrity of Vc appears to be a prerequisite condition for maintenance of the central sensitization in Vo and perhaps in other components of the V brain stem complex or higher brain centers that still have to be explored.

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REFERENCES


