Effects of Chronic Dorsal Column Lesions on Pelvic Viscerosomatic Convergent Medullary Reticular Formation Neurons

Charles H. Hubscher and Richard D. Johnson

INTRODUCTION

The release of semen, urine, and feces is a complex process involving spinal reflexes that are under both conscious and unconscious control. Many brain regions along the entire neural axis are likely involved in the circuitry mediating ejaculation, micturition, and defecation. The focus of our studies is on the medullary reticular formation (MRF; includes the nucleus reticularis gigantocellularis and surrounding nuclei), which is part of a spinobulbo-spinal loop mediating ejaculation (Marson and McKenna 1990; Yells et al. 1992). MRF neurons receive convergent inputs from multiple cutaneous, mucocutaneous, and visceral territories, which include regions innervated by spinal and cranial nerves (Hubscher and Johnson 1996; Hubscher et al. 2004). Using both acute (1 h) and chronic (30 day) lesions, the location of spinobulbo-spinal pathways to/from the male urogenital tract and the type(s) of information being conveyed was previously shown, using electrophysiological techniques, to be located in the dorsal half of the cord at the T8 spinal level (Hubscher and Johnson 1999b, 2000). Acute lesions restricted to the dorsal columns revealed that low threshold penile input to MRF is conveyed in the dorsal columns. To address a number of issues surrounding the use of acute lesions, including spinal shock and the potential for reorganization of the neural circuitry, the location of the ascending projections within the mid-thoracic white matter was examined, in this study, with chronic lesions restricted to just the dorsal columns (DCx; recording of MRF neurons 30 days after injury).

METHODS

Surgeries were performed on nine male Wistar rats (90 days of age) under aseptic conditions as previously described (Hubscher and Johnson 1999b). Each animal was anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg, ip). A long-acting antibiotic (Ami-Pen: 0.5 ml sc; Butler, Columbus, OH) was administered before surgery. The spinal cord was exposed at the T8 level via removal of the overlying T7 vertebral lamina. DCx’s were made through a longitudinal dural incision using a 21-gauge needle (7 animals). All surgical procedures (except the lesion) were done on two sham surgical controls. All animals recovered as previously described (Hubscher and Johnson 1999b). An analgesic (Ketoprofen; 2.5 mg/kg sc; Fort Dodge) was administered twice daily for the first 48 h and then as needed to alleviate postoperative discomfort.

Electrophysiological recordings were made 30 days after injury using previously described protocols (Hubscher and Johnson 1996, 1999b). Each animal was anesthetized with urethane (1.2 g/kg, ip) and intravenous supplements of 5% urethane were given as needed. The common carotid artery, jugular vein, and trachea were intubated for the purposes of blood pressure monitoring, intravenous infusion route, and end expired pCO2 monitoring. Body temperature was maintained at 37°C and mean blood pressure 75 mmHg or above throughout the experiment. The head was clamped in a stereotaxic holder, and the brain stem exposed as previously described (Hubscher and Johnson 1996, 1999b). The pelvic nerve (PN) and dorsal nerve of the penis (DNP) were exposed bilaterally and specifically fabricated bipolar silicon-cuff microelectrodes were implanted around each of the exposed nerves (Hubscher and Johnson 1996). The stimulus consisted of trains of 14 pulses at 70 pps (100-ms train duration, 1 train/s), with a pulse strength set at approximately five times pudendal reflex threshold (i.e.,
30–50 μA, 0.1-ms duration). This stimulus intensity level is supramaximal for the myelinated PN/DNP nerve fibers in the Aβ and Aδ range (Johnson and Murray 1992). For the abdominal branch of the vagus nerve, a bipolar ring electrode was threaded down the esophagus and positioned in the abdominal cavity just caudal to the esophageal hiatus (Hubscher et al. 2004; Khasar et al. 1998). Stainless steel microelectrodes (FHC; impedance, 6–8 MOhms) attached to a hydraulic probe were used as previously described (Hubscher and Berkley 1994; Hubscher and Johnson 1996). The MRF region containing the highest percentage of DNP/PN responsive neurons (Hubscher and Johnson 1999a) was searched for neurons responsive bilateral stimulation of the PN. Single identified neurons (somato-dendritic) were recorded extracellularly and the spikes stored on videotape and replayed off-line using a computer based software package.

Latency of the response, bilaterality of the response, and the degree of excitation or inhibition was determined. As previously described (Hubscher and Johnson 1996, 2002), a neuronal response was counted if the number of spikes firing was at least two times (excitation) or one-half (inhibition) of background firing levels that occur immediately prior to stimulus onset. The doubling/halving criteria was chosen because the increase/decrease was clearly audible through the audiometer and is clearly distinguishable as a response in comparison to the random and slight increases/decreases in firing frequencies that were not associated with a stimulus. In units that did not have a spontaneous discharge, a minimum of three spikes was required for it to count as an excitatory response. Convergent cutaneous receptive fields were tested over the entire body, using hand-held probes. Colonic distension was made using a 10-mm-long latex balloon (Berkley et al. 1993; Hubscher et al. 2004). At the end of the experiment, the animal was killed with an anesthetic overdose and perfused transcardially with 0.9% saline followed by 10% formalin. The block of brain stem tissue containing the recording sites was removed and stored overnight in a 10% formalin/30% sucrose solution. Recording sites were visualized in 50-μm vibratome sections stained with cresyl violet and reconstructed under light and dark field illumination (Paxinos and Watson 1998). All animal procedures were reviewed and approved by the Institutional Animal Use and Care Committee at the University of Louisville and the University of Florida.

### TABLE 1. Summary of MRF recordings

<table>
<thead>
<tr>
<th>Properties of PN/DNP-responsive MRF neurons</th>
<th>Intact*Sham-DCx (N = 150)</th>
<th>DCx (N = 80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline/Activity, impulses/s</td>
<td>18.9 ± 1.8 (for 41% of N)</td>
<td>14.3 ± 1.6 (for 53% of N)</td>
</tr>
<tr>
<td>Excitatory (+)</td>
<td>68%</td>
<td>59%</td>
</tr>
<tr>
<td>Inhibitory (−)</td>
<td>21%</td>
<td>28%</td>
</tr>
<tr>
<td>Complex (+/−)</td>
<td>11%</td>
<td>13%</td>
</tr>
<tr>
<td>RF responses of PN/DNP-responsive MRF neurons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pinch</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>stroke</td>
<td>32%</td>
<td>0%†</td>
</tr>
<tr>
<td>Colon-distend</td>
<td>26%</td>
<td>30%</td>
</tr>
<tr>
<td>Vagus stimulation</td>
<td>48%</td>
<td>39%</td>
</tr>
<tr>
<td>Hindpaw</td>
<td>99%</td>
<td>99%</td>
</tr>
<tr>
<td>Whole body</td>
<td>38%</td>
<td>31%</td>
</tr>
</tbody>
</table>

*Hubscher et al. 2004. †Significantly different than controls (χ²; P < 0.01). MRF, medullary reticular formation; DCx, dorsal column lesion; PN, pelvic nerve; DNP, dorsal nerve of the penis; N, total of neurons; RF, receptive field.

In the MRF, a total of 109 neurons responsive to bPN were found in nine animals along 30 electrode tracks. All of these neurons also responded to bDNP stimulation. A summary of the response properties of these neurons is presented in Table 1. Since there were no significant differences (t-test; P > 0.05) found between neurons in the sham-DCx and intact control (Hubscher et al. 2004) groups, the data were combined (Table 1). Note that 47% of the PN/DNP-responsive MRF neurons in the DCx group and 59% of the neurons in the controls had no background activity. No significant differences (t-test, P > 0.05) in response latencies were found between DCx and sham-DCx or with intact controls (Hubscher et al. 2004). In addition, no significant differences were found in response latency between neurons with excitatory and inhibitory responses (so data were combined). The mean response latency to bPN and bDNP stimulation was 149.4 ± 11.0 and 132.1 ± 11.0 (SE) ms, respectively.

A summary showing the location of the PN-responsive neurons for three animals with DCx lesions at one anterior-posterior level within the restricted search area is shown in Fig. 1A. The results from intact control animals (Hubscher et al. 2004) are also shown (for comparison). As shown in Fig. 1, these neurons were located throughout the MRF search area (in Gi, GiA, DPGi, LGPi; Paxinos and Watson 1998).

For the DCx group of animals, 100% of the neurons responded to noxious levels of penile stimulation (pinch) compared with 0% responding to non-noxious levels (i.e., touch/stroke). Examples showing typical excitatory responses of single MRF neurons to PN/DNP stimulation following a chronic DCx lesion is provided in Fig. 2. As shown in Table 1, only MRF neurons in the control groups responded to touch/stroke of the penis.

A small subpopulation of the neurons, not previously described in the intact control group (Hubscher et al. 2004), had “complex” response patterns; i.e., MRF neurons with excitatory or inhibitory responses to PN/DNP stimulation occasionally had opposite responses (inhibitory or excitatory, respectively) from stimulation of one of the convergent territories, particularly the face. These neuronal response patterns, found on occasion in both controls and DCx lesioned animals, were located in all MRF subdivisions within the search territory (see outlined areas in Fig. 1). Two examples obtained from record-
ings in the MRF of an intact control animal are provided in Fig. 3 (see also data in Table 1).

A summary showing the location of neurons responding to colon distention in three DCx animals is also presented in Fig. 1B. No significant differences \((P > 0.05)\) were found between groups for convergent input from the distal colon. Although no significant differences \((P > 0.05; \text{see Table 1})\) were found across groups for responses to vagal stimulation \((P > 0.05)\), the mean vagal response latency for the DCx group \((305.1 \pm 33.4 \text{ ms})\) was significantly different \((t\text{-test}; P < 0.01)\) from the sham-DCx group \((166.9 \pm 31.4 \text{ ms})\) and intact controls \((166.8 \pm 21.3 \text{ ms})\).

**DISCUSSION**

One result of this study is the finding that the dorsal columns convey low-threshold input from the glans penis to the MRF. The MRF has been shown in the rat to be interconnected with the dorsal column nuclei \((Odutola 1977; Tomasulo and Em-\text{mers} 1972)\). Previous studies using female rats have shown that the nucleus gracilis receives pelvic-visceral inputs from the reproductive organs \((Berkley and Hubscher 1995; Hubscher 1994)\). Given that responses to bDNP and noxious mechanical stimulation of the penis are lost following both acute and chronic dorsal hemisection of the cord at the T8 spinal level
A second finding is that pathways other than the dorsal columns convey noxious input from the distal colon to MRF. Possibilities include the spinoreticular pathway (Chao and Johnson 1999b) but not following a DCx lesion, the dorsal portion of the lateral funiculus is most likely the location of these ascending projections to MRF. These findings are consistent with Li et al. (1998), who showed the loss of responses of GiA neurons to noxious heat applied to cutaneous regions following transection of the contralateral dorsolateral funiculus.

A third finding in this study is the change in response latency of MRF neurons to electrical stimulation of the abdominal branches of the vagus following DCx. The effect of DCx on the vagal input to MRF may reflect the possibility of reorganization of the inputs from the colon. Vagal afferents do not normally respond in the noxious range (Ozaki et al. 1999). Perhaps the C-fibers in the vagus became mechanosensitive to colon distention could therefore be due to the intact projection via the abdominal branch of the vagus.

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GRANTS

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REFERENCES


