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Neurons in the pontomedullary reticular formation receive converging inputs from the hindlimb and labyrinth

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Abstract

The integration of inputs from vestibular and proprioceptive sensors within the central nervous system is critical to postural regulation. We recently demonstrated in both decerebrate and conscious cats that labyrinthine and hindlimb inputs converge onto vestibular nucleus neurons. The pontomedullary reticular formation (pmRF) also plays a key role in postural control, and additionally participates in regulating locomotion. Thus, we hypothesized that like vestibular nucleus neurons, pmRF neurons integrate inputs from the limb and labyrinth. To test this hypothesis, we recorded the responses of pmRF neurons to passive ramp-and-hold movements of the hindlimb and to whole-body tilts, in both decerebrate and conscious felines. We found that pmRF neuronal activity was modulated by hindlimb movement in the rostral-caudal plane. Most neurons in both decerebrate (83% of units) and conscious (61% of units) animals encoded both flexion and extension movements of the hindlimb. Additionally, hindlimb somatosensory inputs converged with vestibular inputs onto pmRF neurons in both preparations. Pontomedullary reticular formation neurons receiving convergent vestibular and limb inputs likely participate in balance control by governing reticulospinal outflow.

Keywords

Multisensory integration; Limb; Balance; Posture; Locomotion; Reticulospinal tracts

2 Introduction

Multiple sensory inputs must be integrated to achieve postural stability, as no one sensory system provides all the inputs needed to reflect body position in space (Peterka 2002; Horak 2006). For example, the vestibular system exquisitely signals the direction and velocity of head movements in space, but vestibular signals alone do not indicate the relative position of the head with respect to the trunk and limbs (Wilson and Melvill Jones 1979). Previous studies showed that neck proprioceptive inputs converge with labyrinthine signals within the vestibular nuclei (VN) and reticular formation (Boyle and Pompeiano 1981; Pompeiano et al. 1984; Kasper et al. 1988b; Kasper et al. 1988a; Wilson 1991), to allow the nervous

system to distinguish whole-body from head-on-body motion and execute appropriate corrective motor responses (Tokita et al. 1989; Tokita et al. 1991; Kennedy and Inglis 2002; Kennedy et al. 2004). However, much less is known about the influences of proprioceptive signals from other body regions on the processing of vestibular inputs. Although recent work in human subjects showed that vestibulospinal responses are modified by feedback from the limbs (Welgampola and Colebatch 2001; Marsden et al. 2002; Brooks and Cullen 2009; Grasso et al. 2011), few studies have explored the convergence and integration of limb and vestibular signals within the nervous system.

A series of experiments from our laboratory showed that the activity of a population of VN neurons is modulated by hindlimb movement (Arshian et al. 2014; McCall et al. 2016), building upon earlier work demonstrating that VN neurons respond to electrical stimulation of hindlimb nerves (Wilson et al. 1966; Jian et al. 2002; McCall et al. 2013). This evidence leads to speculation that some VN neurons integrate limb somatosensory and labyrinthine inputs to adjust vestibulospinal outflow in accordance with limb state when a postural perturbation occurs (Arshian et al. 2014). Reticulospinal neurons also influence the motoneuron pool controlling the hindlimb musculature in a fashion complimentary to vestibulospinal neurons (Wilson and Yoshida 1969; Grillner et al. 1971), but convergence of vestibular and somatosensory inputs from the hindlimb has not previously been shown in the reticular formation.

Neurons in the pontomedullary reticular formation (pmRF) receive inputs from widely-distributed sensory receptors, including those in the vestibular periphery and limbs (Siegel and Tomaszewski 1983). Experiments using electrical stimulation demonstrated that pmRF neurons receive excitatory and inhibitory cutaneous input through both direct and indirect pathways (Segundo et al. 1967; Peterson et al. 1974; Eccles et al. 1975; Peterson et al. 1976; Maunz et al. 1978; Siegel and Tomaszewski 1983; Drew et al. 1996). In addition, VN neurons project to the reticular formation (Ladpli and Brodal 1968; Abzug and Peterson 1973; Peterson and Abzug 1975; Carleton and Carpenter 1983) and robustly modulate the activity of pmRF neurons (Manzoni et al. 1983; Bolton et al. 1992). Despite the presence of these inputs and the pmRF's well-established role in postural (Schepens and Drew 2003; Schepens and Drew 2004; Schepens et al. 2008; Stapley and Drew 2009) and locomotor control (Drew et al. 1986; Matsuyama and Drew 2000; Prentice and Drew 2001), convergence of vestibular and limb somatosensory signals onto single pmRF neurons has not previously been demonstrated. The primary objective of the present study was to determine if somatosensory signals from the hindlimb converge with vestibular signals onto pmRF neurons, and to characterize the responses of the neurons to limb movements and whole-body rotations that activate vestibular receptors.

3. Methods

The University of Pittsburgh's Institutional Animal Care and Use Committee approved the experimental procedures on animals. These procedures conformed to the National Research Council *Guide for the Care and Use of Laboratory Animals* (National Academies Press, Washington, DC, 2011). Experiments were performed on 11 purpose-bred felines of either sex obtained from Liberty Research (Waverly, NY, USA). Neuronal recordings were

obtained from 7 animals after decerebration and the removal of anesthesia. The other four felines were instrumented for chronic single-unit recordings using procedures described previously (Miller et al. 2008a; Miller et al. 2008b; McCall et al. 2015).

3.1. Surgical procedures

3.1.1. Decerebrate preparation—Animals were anesthetized using isoflurane (5% induction, 1–3% maintenance) vaporized in oxygen. The level of anesthetic was adjusted to maintain areflexia and a stable rate of respiration. Blood pressure was monitored using a transducer (Millar Instruments, Houston, TX, USA) inserted via the left femoral artery, and was maintained >100 mm Hg by saline infusion.

An endotracheal tube was inserted through a tracheostomy. The left femoral vein was cannulated to allow drug and fluid delivery. Rectal temperature was monitored and maintained at 36–38°C using a DC powered heating pad and an infrared lamp. The head was secured in a modified stereotaxic frame with the head pitched-down 30° in order to vertically align the vertical semicircular canals (Wilson and Melvill Jones 1979), and the body was secured using hip pins and a clamp placed on the T1 dorsal spinous process. This stereotaxic frame was then mounted on a servo-controlled hydraulic tilt table (Neurokinetics, Pittsburgh, PA, USA) capable of delivering simultaneous rotations in the roll and pitch planes.

The carotid arteries were ligated and a midcollicular decerebration was performed. Approximately 1 cm of tissue rostral to the transection site was aspirated to assure that the decerebration was complete. A second smaller craniotomy was made over the brainstem to permit neural recordings from the reticular formation. An L2 laminectomy was performed, and two stainless steel floating electrodes were placed on each side in the ventral spinal cord white matter, where the reticulospinal tracts are located (Steeves and Jordan 1980). Once decerebration was complete and at least 60 minutes before the start of the recording session, administration of isoflurane was discontinued. Then animals were paralyzed using an intravenous infusion of vecuronium bromide (0.1 mg/kg IV, every 20 minutes). During paralysis, the animals were artificially ventilated and end-tidal CO₂ was maintained near 4% by adjusting ventilation rate and tidal volume. At 6-hour intervals, intramuscular atropine sulfate (0.15 mg/kg) and intravenous dexamethasone (1mg/kg) were provided to reduce airway secretions and brain swelling, respectively.

At the conclusion of neural recording, electrolytic lesions were placed near recording sites in the reticular formation by passing a 0.5-mA (1-mA in one case) negative current through the recording electrode for 60 seconds. Subsequently, the animal was euthanized by an intravenous injection of Euthasol solution (120 mg/kg). The brainstem was removed, fixed in 10% Formalin, embedded in 2% agar, and cut transversely at a thickness of 50µm using a freezing microtome. Sections were stained with 1% thionine.

3.1.2. Conscious preparation—Animals underwent a recovery surgery using aseptic techniques in a dedicated operating suite. Anesthetic, surgical and post-surgical procedures are described in previous studies (Miller et al. 2008a; Miller et al. 2008b). In brief, animals were initially anesthetized using intramuscular ketamine (20 mg/kg) and acepromazine (0.2

mg/kg). The animals were intubated, and an intravenous catheter was inserted in the forelimb. During surgery, anesthesia was maintained using isoflurane (1–2%) vaporized in oxygen, and intravenous saline was used to replace fluid loss. A heating pad and an infrared heat lamp were used to maintain the rectal temperature between 36–38 °C.

A midline craniotomy (diameter, 1 cm) was made in the posterior aspect of the skull, and a recording chamber (David Kopf Instruments, Tujunga, CA, USA) was secured to the skull using Palacos bone cement (Zimmer, Warsaw, IN, USA) and stainless steel screws. A fixation plate was mounted on the skull rostral to the recording chamber to permit immobilization of the head during recording sessions. Post-surgical analgesia was provided continuously for 72 hours using a transdermal fentanyl delivery system (25 µg/h, Janssen Pharmaceutical Products, Titusville, NJ, USA). Amoxicillin (50 mg, twice daily) was administered orally for ten days after the surgery.

Animals were acclimated to head and body restraint while positioned on the tilt table during vertical plane rotations and hindlimb ramp-and-hold movements. The head was immobilized by attaching the fixation plate to a stereotaxic frame mounted on the tilt table. Additionally, the animal was placed in a modified restraint bag (Four Flags over Aspen, Aspen, CO, USA) with a hole cut in the rear of the bag that allowed access to the ipsilateral hindlimb. The torso was enclosed in a cylindrical tube that was secured to the stereotaxic frame, ensuring that body position did not change during table movement (Fig. 1).

After recordings were completed in each animal, two electrolytic lesions were made near recording sites in the brain stem by passing a 0.5–1 mA current through a 0.5 MΩ electrode for up to sixty seconds. One week later, the animals were anesthetized using an intramuscular injection of ketamine (20 mg/kg) and acepromazine (0.2 mg/kg), followed by an intraperitoneal injection of pentobarbital sodium (40 mg/kg) and transcardially perfused with 10% formalin. The brain stem was removed and then histologically processed in a manner identical to that in the decerebrate experiments.

3.3. Recording procedures

Extracellular recordings were made from pmRF neurons using 4–6 MΩ epoxy-insulated tungsten microelectrodes (Frederick Haer, Bowdoin, ME, USA). In conscious animals, an XY positioner (David Kopf Model 608Bb) was secured to the recording chamber, and the electrode was introduced via a 25-gauge stainless steel guide tube inserted through the dura into the cerebellum. In decerebrate animals, the electrode was maneuvered using a manipulator (David Kopf model 960) attached to the stereotaxic frame. In both preparations, the electrode was lowered into the medulla using a hydraulic microdrive (David Kopf Model 650). Neural activity was amplified by a factor of 10K, bandpass filtered at 0.3–10 kHz and sampled at 25 kHz using a Cambridge Electronic Design Micro 1401 mk2 data collection system and Spike 2 version 8 software (Cambridge, UK). Signals from potentiometers on the tilt table and servo-controlled leg rotator that respectively provided body and hindlimb position were sampled at 100 Hz. The template matching spike-sort feature of Spike 2 software was used to isolate the activity of particular units in the recording field. Trials in which it was difficult to segregate each unit's activity were discarded.

3.4. Vestibular stimulation and limb movement protocols

3.4.1. Vestibular stimulation—To ascertain if a neuron received vestibular inputs, we recorded neural responses during whole-body vertical plane rotations provided by a servo-controlled hydraulic tilt table (NeuroKinetics, Pittsburgh, PA, USA). The presence of responses to vestibular inputs was first ascertained by use of the “wobble” stimulus, a fixed-amplitude tilt whose direction moves around the animal at a constant speed (Schor et al. 1984). Wobble stimuli were delivered in the clockwise (CW) or counterclockwise (CCW) directions. For example, during the CW wobble stimulus, the animal was tilted sequentially through the following body positions: nose down, right ear down, nose up, and left ear down. The direction of rotation was reversed (nose down, left ear down, nose up, right ear down) during the CCW wobble stimulus. The response vector orientation (plane of maximum neural response to vestibular stimulation) lies midway between the stimulus direction producing maximal responses during CW and CCW stimuli (Schor et al. 1984).

Tilts were then delivered at or near the plane of the response vector orientation at a range of frequencies (0.02–2 Hz) and amplitudes (2.5–15°) to determine dynamics of the vestibular response (i.e., response gain and phase across stimulus frequencies). For each unit, neural activity recorded during whole-body tilts was binned (500 bins/cycle) and averaged over the sinusoidal stimulus period. Sine waves were fit to the neural responses using a least-squares minimization technique (Schor et al. 1984), and the signal-to-noise ratio was calculated as in Schor et al. 1984. The signal-to-noise ratio was defined as the amplitude of the sine wave fit to the response relative to the root mean square amplitude of frequency components above the second harmonic. Responses were considered significant if the signal-to-noise ratio was >0.5 and only the first harmonic was prominent.

3.4.2. Hindlimb movement—Ramp-and-hold hindlimb movements were delivered in the rostral-caudal plane by securing the hindlimb just proximal to the ankle joint to a servo-controlled motor (Fig. 1). As described in previous publications, this stimulus paradigm provides for flexion and extension movements of the limb only about the hip and knee joints (Arshian et al. 2014; McCall et al. 2015; McCall et al. 2016). The hindlimb is secured to the servo-controlled motor in a position comfortable for the animal with roughly orthogonal angles about the hip and knee joint; this position is referred to as midline (neutral) (Fig. 1b, middle panel). The servo-controlled motor was then ramped into 60° extension with resulting extension movements of the hip and knee (Fig. 1b, right panel). The hindlimb was then brought back to the midline position, ramped into 60° flexion (Fig. 1b, left panel), and again brought back to the midline position. Although joint range of motion was not measured with the current experiments, our group has previously measured these parameters using the same experimental protocol: average range of motion about the hip and knee was 27.5° and 40.8°, respectively (Arshian et al. 2014). In the decerebrate preparation, the limb was ramped into these positions at three different velocities (60°, 30°, 15° per second, 5 repetitions each) and held in a position for seven seconds before being moved to the next. In the conscious preparation, one velocity (60°/sec, ten repetitions) was used (because conscious animals acclimated better when the velocity was not altered). Ramp movements were performed over a period of one second and the limb was held in each position for six seconds prior to the next movement.

To determine if hindlimb movement resulted in an appreciable change in neuronal firing, spike counts were binned in 0.1 sec intervals and composite response histograms were generated, as in previous studies (Arshian et al. 2014; McCall et al. 2015; McCall et al. 2016). Each ramp segment was then analyzed for a change in activity relative to the baseline. Within each segment, peak maximums (excitatory responses) or peak minimums (inhibitory responses) were identified and bin counts from the 1-second time interval surrounding the peak or trough were calculated, and were compared with baseline bin counts (taken from the last 1 second interval of the limb held in midline). Responses to hindlimb movement were considered significant if: (1) there was greater than 20% change in firing from baseline; (2) the difference was statistically significant (Mann-Whitney U test, $p < 0.05$); and (3) responses were consistent trial to trial. In decerebrate animals, the effect of hindlimb velocity on neuronal responses was determined by comparing the average bin counts during movement across the three velocities tested (Friedman test with Dunn's multiple comparisons test). We also tested for sustained increases or decreases in activity during the hold portion of the stimulus (i.e. when the hindlimb was held in extension or flexion positions). Neurons were considered to have a sustained change in firing if the initial increase (or decrease) in firing during the movement phase continued during the hold phase, the bin counts from the last one second of the hold portion of the stimulus was $>20\%$ different from baseline counts, and those differences were statistically significant (Mann-Whitney U test, $p < 0.05$). Statistical analyses were performed using Prism 7 software (GraphPad Software, San Diego, CA, USA).

3.5. Electrical stimulation

Spinal electrical stimulation was performed only in the decerebrate preparation. Monopolar current pulses (pulse width up to 200 ms; pulse intensity up to 0.5 mA) were sequentially delivered to each spinal electrode. The anode was attached to muscle tissue adjacent to the stimulated spinal cord. Although our intent was to antidromically activate spinal projections, the stimuli typically produced strong orthodromic activation of reticular formation neurons, which confounded the use of the collision test to identify reticulospinal neurons.

4. Results

Hindlimb movement was used as the search stimulus during tracking in the decerebrate preparation. In conscious animals, we used whole-body vertical plane rotations (vestibular stimulation) as the search stimulus. This difference in experimental strategy was used because conscious animals often did not tolerate prolonged periods of passive limb movement, but could be acclimated to continuous body rotations.

In decerebrate animals, we identified 36 pmRF neurons whose activity was modulated by hindlimb movement, and then determined whether they also responded to vertical vestibular stimulation. In conscious animals, we identified 28 pmRF neurons that responded to whole-body rotations, and tested whether their firing rate was also altered by hindlimb movement. Examples of the responses of a pmRF neuron to hindlimb movement in a decerebrate animal are provided in Fig. 2. Responses to hindlimb movement were categorized as in previous studies (Arshian et al. 2014; McCall et al. 2015; McCall et al. 2016). The firing rate of

omnidirectional neurons changed similarly during both flexion and extension movements of the hindlimb, but returned to baseline levels when the limb was held in the new position (Fig. 3a). *Bidirectional* neurons responded with a change in firing rate as the limb moved in either direction away from the midline (Fig. 3b). However, unlike omnidirectional neurons, their firing rate was unaffected by limb movement towards midline. Thus, the firing rate of omnidirectional and bidirectional neurons did not reflect the direction of the limb movement. However, some of these neurons did have sustained alterations in activity while the limb was held in extension or flexion. *Complex* neurons combined omnidirectional response characteristics with positional sensitivity (Fig. 3c). Some bidirectional neurons also exhibited such static positional responses. Neurons exhibiting *unidirectional* or *reciprocal* responses were directionally tuned. Unidirectional neurons had activity that either increased or decreased during one direction of hindlimb movement away from midline, but no responses to the opposite direction of movement (Fig. 3d). The firing rate of *reciprocal* neurons increased during hindlimb movement in one direction, but decreased during movement in the opposite direction.

4.1. Responses of pmRF neurons to hindlimb movement and whole-body rotations in vertical planes

4.1.1. Decerebrate preparation—Most pmRF neurons in the decerebrate preparation (26/36, 72%) were classified as omnidirectional neurons (Fig. 3a). All of these cells exhibited increases in firing rate with respect to baseline during both flexion and extension limb movement. Eleven percent of neurons (4/36) exhibited bidirectional responses (Fig. 3b). Three of these neurons had increases in firing rate as the limb moved into extension or into flexion, and one neuron showed a decrease in firing. However, one omnidirectional and two bidirectional neurons also exhibited weak, sustained changes in firing when the limb was held in flexion or extension, as shown in Fig. 3c. The firing rate of most neurons (the 26 omnidirectional neurons and two of the bidirectional neurons) did not encode limb position during any phase of the stimulus cycle.

In contrast, six neurons (6/36, 17%) exhibited responses that explicitly encoded the direction of limb movement. Five of these neurons (5/36, 14%) had unidirectional responses (Fig. 3d). All of these unidirectional neurons responded only to hindlimb extension, four with a decreased firing rate and one with an increased firing rate.

We also examined the effect of varying hindlimb movement velocity on the peak firing rates of neurons (Fig. 4). The analysis was restricted to excitatory responses because inhibitory responses typically saturated at zero spikes per second (Arshian et al. 2014). Overall, responses were modestly velocity dependent. A four-fold increase in limb velocity, from 15°/s to 60°/s, resulted in a 29% increase in peak response magnitude (Dunn's multiple comparisons test, $p=0.0004$).

Of the 36 neurons whose activity was modulated by hindlimb movement, 31 were tested for responses to whole -body rotations in vertical planes. The activity of 11 of the neurons (11/31, 35%) was modulated by vestibular stimulation. Two of the neurons responded robustly to wobble rotations in one direction (CW or CCW), but not the other, and were classified as having spatial -temporal convergence (STC) behavior. Such responses are the

result of converging vestibular inputs with differing spatial and temporal characteristics (Baker et al. 1984; Schor et al. 1984; Schor and Angelaki 1992) Limited information is available about the responses to vestibular stimulation of the remaining neurons, as most (6/11) were lost before the vestibular testing battery was complete.

Another goal of these experiments was to ascertain whether pmRF units that responded to limb movement were reticulospinal neurons with projections to the lumbar spinal cord. The stimuli that we delivered to the L2 spinal cord elicited powerful orthodromic responses at short latency (mean of 10.7 ± 4.5 msec) in the eight tested units, as illustrated in Fig. 5. Since the strong, short-latency orthodromic responses to spinal stimulation may have masked antidromic activation, it is unclear what fraction of the sampled units were reticulospinal neurons. Nevertheless, the presence of these strong orthodromic responses confirms that pmRF neurons receive relatively direct inputs from the lumbar spinal cord.

4.1.2. Conscious preparation—In conscious animals, we identified neurons that responded to vestibular stimulation and then tested these neurons for responses to hindlimb movement. The firing rate of most pmRF neurons (28/35, 80%) that responded to wobble stimuli was also modulated by hindlimb movement. Most of these neurons had omnidirectional responses (17/28, 61%), and typically (14/17) exhibited an increase in firing rate during flexion and extension limb movements. Seven additional neurons (7/28, 25%) had complex responses to limb movement. Three of the complex neurons had sustained changes in firing rate when the limb was held in flexion, and the firing rate of three others increased when the limb was held in extension. The remaining neuron was excited by tonic limb extension and inhibited by tonic limb extension.

Three pmRF neurons (3/28, 11%) had unidirectional responses, two of which responded with an increase in activity as the limb was moved into extension and one that responded with a decrease in firing when the leg was moved into flexion. The activity of an additional neuron had reciprocal responses to hindlimb movement. Thus, 11 of 28 pmRF neurons (39%; 7 complex units, 3 unidirectional units, and 1 reciprocal unit) that responded to hindlimb movement in conscious animals encoded limb position in some fashion.

Sufficient data were collected from 12 neurons that responded to whole-body rotations and hindlimb movement to construct Bode plots illustrating responses to tilts at different frequencies. Fig. 6 illustrates the responses of one of the neurons to vestibular stimulation, and Fig. 7 depicts Bode plots for all the neurons. The gain and phase of the response are plotted relative to stimulus position, such that responses leading stimulus position by 90° are aligned with stimulus velocity. Average response gains increased 9.2 ± 5.7 spikes*s⁻¹*deg⁻¹ per stimulus decade (Fig. 7a). Response phases were typically aligned with stimulus velocity (Fig. 7b); they led stimulus position by $58^\circ \pm 47^\circ$ at 0.1 Hz (mean $\pm$ SD) and $67^\circ \pm 47^\circ$ at 1 Hz. The dynamics of responses of many pmRF neurons to vestibular stimulation in the conscious cat were similar to the response dynamics of primary semicircular canal afferents (steep gain increase with advancing stimulus frequency, response aligned with stimulus velocity) (Fernandez and Goldberg 1976; Anderson et al. 1978). However, the dynamics of responses of some pmRF neurons to vestibular stimulation resembled those previously reported in the decerebrate cat, which approximated the response dynamics of primary

otolith afferents, or an addition of responses to otolith and semicircular canal signals (Bolton et al. 1992).

4.2. Neuron location

It was possible to accurately reconstruct the location of 48 neurons (26 in decerebrate animals and 22 in conscious animals). Neuronal locations are shown on four standard coronal sections (Fig. 8) and are plotted as though they were recorded on the right side. All neurons except one were located on the same side as the limb that was moved. The locations of recorded neurons in the conscious preparation were plotted with respect to an electrolytic lesion in the same tract, or in an adjacent tract. In the decerebrate preparation, neuronal locations were plotted relative to electrolytic lesions and the obex, whose location was determined during the experiment. The majority of neurons (44/48, 92%) were located within 3 mm of the midline, and were scattered over a rostrocaudal extent of 2.8 to 7 mm rostral to the obex. We were unable to accurately plot the locations of 16 neurons (10 in decerebrate animals and 6 in conscious animals) due to either swelling of the brainstem or to difficulties in finding the lesion site, but these neurons were clearly in the PMRF given their positions relative to the neurons whose locations were reconstructed.

5. Discussion

We found that the majority of pmRF neurons (80%) in the conscious cat with vestibular inputs also receive convergent inputs from the hindlimb. Similarly, a substantial fraction (35%) of pmRF neurons in the decerebrate preparation with hindlimb inputs also receive convergent vestibular inputs. Note that the proportion of pmRF neurons with convergent vestibular and hindlimb inputs may be underestimated in these experiments because limb movement was restricted to one plane of testing (along the rostral-caudal axis); it is possible that additional vestibular responsive pmRF neurons may have been activated by other planes of limb movement. Although we were unable to verify that the units whose activity was recorded were reticulospinal neurons, the extensive convergence of vestibular and limb signals that we observed suggests that some reticulospinal neurons must integrate these two inputs. The positions of neurons in this study overlaps the locations of reticulospinal neurons reported in previous studies in cats (Bolton et al. 1992).

Siegel and Tomaszewski (Siegel and Tomaszewski 1983) examined reticular formation neuronal responses during forelimb movement in the awake behaving cat (i.e., during volitional limb movements) and found that they were directionally specific and resulted from movement about proximal joints. In contrast, we found that the majority of pmRF neurons responsive to hindlimb movement exhibited excitatory (or inhibitory) responses during all tested directions of passive limb movement (omnidirectional responses) in both decerebrate and conscious animals. Thus, there are two differences between the present study and that of Siegel and Tomaszewski: the limb movements were active in their study and passive in ours, and their study entailed forelimb movements whereas ours utilized hindlimb movements. Stapley and Drew (Stapley and Drew 2009) reported that a larger proportion of reticulospinal neurons became activated when the support surface was suddenly removed from underneath the forepaw than from the hindpaw, supporting the notion that pmRF

neurons have different responses to forelimb and hindlimb movements. However, at present it is unknown whether active and passive movement of a limbs have distinct effects on the activity of pmRF neurons, as occurs in other central nervous system vestibular processing areas (McCrea et al. 1999).

Interestingly, many neurons in CNS sites that participate in postural control exhibit omnidirectional responses to passive hindlimb movement, including 77% of neurons in the rostral fastigial nucleus of decerebrate cats (McCall et al. 2015), 51% of neurons in the caudal vestibular nuclei (51%) in conscious cats (McCall et al. 2016), and 61% of pmRF neurons in conscious cats. Thus, it is common for the activity of brainstem and cerebellar neurons to be modulated during limb movements in any direction. Moreover, these studies considered the effects of movement of a single limb, while most actions of quadrupeds entail movements of or afferent input from multiple limbs. Thus, it is possible that the activity of VN and pmRF neurons is altered anytime a limb is in motion, potentially to alter the sensitivity of the neurons to other signals related to movement, such as vestibular inputs and descending motor commands.

The pmRF is a key relay for signals from the mesencephalic locomotor center to spinal motoneurons (Orlovsky 1970; Shefchyk and Jordan 1985), such that the activities of pmRF neurons with spinal projections are phasically activated during fictive locomotion (Perreault et al. 1993). Neurons in the pmRF also transmit cortical signals to the spinal cord (Peterson et al. 1976; Keizer and Kuypers 1984; Kably and Drew 1998). We hypothesize that during locomotion and other voluntary movements, inputs from the limb and labyrinth are integrated by pmRF neurons that also receive motor commands, to shape and adjust the responses to those commands when unexpected changes in head and limb position occur.

Removal of supratentorial inputs to the brainstem through decerebration did not appreciably alter the responses of pmRF neurons to passive hindlimb movement. In both decerebrate and conscious animals, omnidirectional responses predominated. In contrast, previous studies showed that the responses of VN neurons to limb movement are considerably different in conscious and decerebrate animals (Arshian et al. 2014; McCall et al. 2016). In the decerebrate preparation, VN neurons responding to hindlimb input tended to encode movement direction much more frequently than in the conscious cat (McCall et al. 2016). At face value, these findings suggest that the processing of limb and vestibular signals by pmRF neurons is influenced less by supratentorial signals than is integration of these inputs in the VN. However, descending signals to the brainstem could play a much larger role in regulating the processing of limb and vestibular signals during active movements. Experiments comparing neural responses during active and passive limb movements will be necessary to address this question.

In summary, our data show that passive hindlimb movement alters pmRF neuronal activity, and that in conscious animals most pmRF neurons with labyrinthine inputs also respond to limb movements. Typically, pmRF neurons responded to both flexion and extension of the hindlimb, such that the responses reflected a change in limb position regardless of the direction of movement. These data suggest that reticulospinal outflow to the spinal cord is altered when the limbs are in motion. However, it is currently unclear how limb inputs affect

pmRF activity during active movements. Given the results of the current study, and the known roles that pmRF neurons play in locomotion and in compensatory postural reflexes, one possibility is that limb inputs shape the motor commands relayed to the spinal cord through reticulospinal pathways, particularly when a limb unexpectedly changes position, but this notion is yet to be examined experimentally.

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Figure 1. Experimental setup

A: Cats were placed into a restraining device that was mounted on a servo-controlled tilt table. The tilt table was used to provide vestibular stimulation, and could rotate the animal in the roll and pitch planes. Hindlimb movement was provided by a servo-controlled motor positioned behind the animal. The hindlimb was placed through a hole in the restraint bag and secured to the servo-controlled motor via a Velcro strap attached immediately proximal to the ankle. **B:** Hindlimb positions, with approximate angles of the knee and hip joints, during midline, extension, and flexion.

Figure 2.

The activity of a subpopulation of pmRF neurons is modulated by passive hindlimb movement in the rostral-caudal plane. These tracings show that the firing rate of this decerebrate pmRF neuron modulated with hindlimb movement regardless of the direction of movement. **(a)** Raw neural data sampled at 25 kHz. **(b)** Tracing showing the isolated neuron of interest. **(c)** Mean firing frequency, binned in 0.1-second intervals. **(d)** Potentiometer recording from the servomotor used to move the hindlimb. Hindlimb position started at midline (M), was then moved to extension (E), returned to neutral, moved to flexion (F), and returned to midline. Movements in this example were at 15°/s.

Figure 3.

pmRF neurons respond to hindlimb movement with a variety of response patterns. These examples are from recordings in decerebrate (**b**) and conscious (**a**, **c–d**) animals. **a**) Omnidirectional and **b**) bidirectional neurons encode hindlimb movement without overtly encoding the direction the hindlimb is moved away from midline (i.e., responses to flexion and extension movements are similar). Furthermore, omnidirectional neurons (**a**) exhibit a similar change in firing rate with all limb movements, including movements from extension back to midline and flexion back to midline. The bidirectional neuron depicted in (**b**) exhibited an increase in firing only as the hindlimb was moved away from midline regardless of whether moved into extension or flexion. Complex (**c**), unidirectional (**d**) and reciprocal neurons exhibited responses that encode the direction of limb movement and/or position. For example, the complex neuron shown in (**c**) has its activity modulated with all directions of hindlimb movement (resembling an omnidirectional neuron) but additionally has a sustained increase in firing over baseline when held in the extension position. The unidirectional neuron depicted in (**d**) exhibited an increase in firing rate only when the limb was moved toward extension.

Figure 4.

Decerebrate pmRF neurons are modestly responsive to the velocity of the hindlimb movement. Doubling limb movement velocity from 15°/sec to 30°/sec increased the magnitude of the peak response by 9% while quadrupling velocity to 60°/sec increased the magnitude of responses by 29% ($p < 0.05$, Friedman's test with Dunn's multiple comparisons test). Mean and standard deviation are plotted.

Figure 5.

Activity of a pmRF neuron elicited by electrical stimulation of the L2 spinal cord. A gray shaded area shows the 0.2 msec period over which the stimulus (stimulus intensity, 400 μ A; interstimulus interval, 0.8 sec) was provided. Responses to multiple stimulus presentations are plotted.

Figure 6.

Responses recorded from a pmRF neuron in the conscious preparation whose activity was modulated by rotations in vertical planes. **(a)** Responses to clockwise (CW) and counterclockwise (CCW) wobble stimuli delivered at 0.5 Hz with an amplitude of 5°. Signal-to-noise ratios were 1.2 for responses to CW rotations and 0.9 for responses to CCW rotations. The CW trace reflects the average of 6 sweeps, whereas the CCW trace reflects the average of 10 sweeps. **(b)** Responses to rotations in the roll plane delivered at various frequencies (0.1–1 Hz). Tilt frequency and stimulus amplitude are listed for each tracing. A dashed line indicated table position. The solid line represents the sine wave fit to the response. Sweep counts for each frequency were as follows: 0.1 Hz, 2 sweeps; 0.2 Hz, 4 sweeps; 0.5 Hz, 6 sweeps; 1 Hz, 17 sweeps. For this neuron, there was an increasing gain with increasing stimulus frequency (gain at 1 Hz was 5.96 times greater than at 0.1 Hz) and phase leads across all frequencies approached stimulus velocity. **Abbreviations:** CED contralateral ear down, CCW counterclockwise, CW clockwise, IED ipsilateral ear down, ND nose down, NU nose up, MID midline

Figure 7.

Bode plots illustrating the dynamic properties of pmRF neuronal responses to rotations across multiple frequencies. Bode plots were constructed using data from 12 neurons in the conscious preparation that responded to both whole-body stimulation and hindlimb movement. Response gain (a) and phase (b) are plotted relative to stimulus position. Thin gray lines designate responses of individual neurons. The thick black lines show averaged data and SEM across all conscious units.

Figure 8.

Location of neurons in the PMRF whose activity was recorded in this study. Symbol shapes indicate the response classification to limb movement. Filled symbols indicate neurons from the conscious preparation and unfilled symbols indicate neurons from the decerebrate preparation. Only the locations of neurons that could be positively identified are depicted. Numbers above each section designate distance in millimeters rostral to the obex. The distance between the red ticking along the midline of each section is equal to 1 mm.

Abbreviations: 7G, genu of the facial nerve; CB, cerebellar cortex; CX, external cuneate nucleus; DMV, dorsal motor nucleus of the vagus; FTG, gigantocellular tegmental field; FTL, lateral tegmental field; FTM, magnocellular tegmental field; INT, nucleus intercalatus;

IO, inferior olive; P, pyramidal tract; PH, nucleus praepositus hypoglossi; PR, paramedian reticular nucleus; RB, restiform body; SM, medial nucleus of the solitary tract; TB, trapezoid body; V4, fourth ventricle; VIN, inferior vestibular nucleus; VLD, lateral vestibular nucleus, dorsal division; VMN, medial vestibular nucleus

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