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Anatomic patterning in the expression of vestibulosympathetic reflexes

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Kerman, I. A., B. J. Yates, and R. M. McAllen. Anatomic patterning in the expression of vestibulosympathetic reflexes. *Am J Physiol Regulatory Integrative Comp Physiol* 279: R109–R117, 2000.—To investigate the possibility that expression of vestibulosympathetic reflexes (VSR) is related to a nerve's anatomic location rather than its target organ, we compared VSR recorded from the same type of postganglionic fiber [muscle vasoconstrictor (MVC)] located at three different rostrocaudal levels: hindlimb, forelimb, and face. Experiments were performed on chloralose-anesthetized cats, and vestibular afferents were stimulated electrically. Single MVC unit activity was extracted by spike shape analysis of few-fiber recordings, and unit discrimination was confirmed by autocorrelation. Poststimulus time histogram analysis revealed that about half of the neurons were initially inhibited by vestibular stimulation (type 1 response), whereas the other MVC fibers were initially strongly excited (type 2 response). MVC units with types 1 and 2 responses were present in the same nerve fascicle. Barosensitivity was equivalent in the two groups, but fibers showing type 1 responses fired significantly faster than those giving type 2 responses (0.29 ± 0.04 vs. 0.20 ± 0.02 Hz). Nerve fibers with type 1 responses were most common in the hindlimb (21 of 29 units) and least common in the face (2 of 11 units), the difference in relative proportion being significant ($P < 0.05$, χ^2 test). These results support the hypothesis that VSR are anatomically patterned.

muscle vasoconstrictor fibers; vasomotor pathways; sympathetic nerves

ASSUMPTION OF AN UPRIGHT POSTURE in humans or a vertical posture in quadrupeds can severely challenge normal cardiovascular function. Unless compensation takes place, such postural changes can lead to orthostatic hypotension and decreased blood flow to the brain. Several lines of evidence point to the possible role of vestibulosympathetic reflexes (VSR) in counteracting the onset of orthostatic hypotension. Bilateral eighth nerve transection leads to blood pressure instability during nose-up, whole body tilts in anesthetized (7) and awake (13) cats, suggesting that vestibular inputs are necessary for blood pressure stability during assumption of a vertical posture. In addition, activity of sympathetic nerves is altered by electrical (15) or natural stimulation of vestibular afferents (31), with rotations in the sagittal plane (nose-up pitch) being most effective in eliciting cardiovascular responses (30, 31).

There is considerable evidence to suggest that only a subset of sympathetic efferents responds to vestibular stimulation. For example, in humans sympathetic outflow to muscle but not skin may be selectively affected by vestibular stimulation (22). In a previous study (15), we reported that expression of VSR in a variety of sympathetic nerves is attenuated by baroreceptor stimulation during blood pressure increases. Because stimulation of baroreceptor afferents inhibits the activity of vasoconstrictor sympathetic efferents but not that of most other sympathetic fibers (12), this finding suggests that VSR are mediated predominantly by vasoconstrictor sympathetic efferents. However, these responses were not homogeneous across sympathetic nerves. When compared in two classical "vasoconstrictor" nerves (those whose ongoing activity is completely abolished by baroreceptor stimulation), VSR were significantly greater in magnitude in the renal than in the external carotid nerve (15). This finding raised the possibility that expression of VSR might be patterned not only with respect to the type of tissue supplied by the sympathetic nerve, but also by its location in the body, such that caudally located sympathetic neurons respond more strongly to vestibular stimuli than rostrally located sympathetic neurons.

The goal of the present study was to examine whether expression of VSR depends specifically on the anatomic location of the efferent pathway. To achieve this aim, we compared responses to vestibular stimulation of sympathetic efferents of the same functional type, muscle vasoconstrictor (MVC), located at three rostrocaudal levels: hindlimb, forelimb, and face. Electrical stimulation was used to activate vestibular afferents in these experiments. This method of vestibular stimulation was chosen for several reasons. First, it powerfully excites vestibular afferents and thus could be used to reliably determine if simultaneous activation of fibers from all vestibular end organs elicits a

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differential pattern of responses in MVC fibers at several rostrocaudal levels. If maximal excitation of vestibular afferents elicits a patterned response in these sympathetic efferents, it is likely that activation of subsets of vestibular afferents by more natural vestibular stimuli would also elicit patterned responses. Second, use of electrical stimulation in this study is a logical extension of our former experiments in which we demonstrated that electrical vestibular stimulation elicits a specific pattern of responses in sympathetic nerves (14, 15). Third, many studies have shown that electrical stimulation applied within the labyrinth to vestibular nerves can be used to selectively activate vestibular afferents (15, 25). In contrast, extensive denervations are required to eliminate nonlabyrinthine inputs that might be elicited during natural vestibular stimulation (head movements or whole body rotations), thus potentially compromising the preparation. Furthermore, it would have been technically impossible to accomplish the aims of this study (which involved recording MVC activity from both the face and limbs) during natural vestibular stimulation, which involves movement of either the head or body.

METHODS

Experiments were conducted on nine cats anesthetized with α-chloralose (70 mg/kg) administered intravenously after premedication with a mixture of ketamine (11 mg/kg) and xylazine (0.1 mg/kg) given intramuscularly. Studies were conducted in accordance with the Australian National Health and Medical Research Council guidelines and approved by the Animal Experimentation Ethics Committee of the Howard Florey Institute.

A tracheostomy was performed, and the right femoral artery and vein were cannulated for blood pressure measurement and drug delivery, respectively. The common carotid arteries were dissected bilaterally, and a loop was tied around each one to allow for baroreceptor sensitivity testing (see below). All animals were artificially respired with oxygen-enriched air throughout the experiment at a level sufficient to suppress spontaneous ventilation in these unparalyzed animals; end-tidal CO_2 was maintained at ~3.5%. Rectal temperature was monitored and maintained between 36 and 38°C with the use of an electric blanket and a heating lamp. The bladder was catheterized and drained.

The vestibular nerves on one or both sides were prepared for bipolar electrical stimulation with the use of a previously described method (15, 25, 32). The tympanic bulla was dissected with the use of a ventrolateral approach and was opened to expose the promontory. The anterior wall of the promontory was opened to gain access to the scala vestibuli. One silver-silver chloride ball electrode, insulated except at the tip, was inserted through the round window into the scala vestibuli in the direction of the vestibule. The second electrode was placed 1–2 mm away, in the vicinity of the oval window. The effectiveness of vestibular nerve stimulation was assessed by monitoring eye movements and neck contractions, which occur as part of vestibular-ocular and vestibular-collic reflexes (29). These reflexes were elicited with the use of a train of 50 shocks with a pulse width of 0.2 ms and a 3-ms separation repeated every 0.5–2 s. The position of the electrodes was adjusted to produce a large differential between the stimulus intensity required to produce eye movements and that which resulted in facial twitching. The facial nerve runs just outside the labyrinth, and it is the first target to be affected by stimulus spread (29). Previous studies have shown that stimulation of the vestibular nerve using intensities that are subthreshold for activating facial efferents selectively activates vestibular inputs (15, 25). The electrodes were fixed in place with silicone gel (Wacker).

After implantation of labyrinthine electrodes, the left peroneal nerve (in all 9 cats) and either the left radial nerve (6 cats) or the left facial nerve (3 cats) were dissected. Skin edges were tied to a metal ring, and a mineral oil pool was constructed at each incision. Individual whole fascicles were dissected from each nerve and placed intact over platinum hook electrodes, and activity was recorded after amplification (20,000 times) and filtering (500–3,000 Hz band pass) to determine receptive fields of afferents within the fascicle. Fascicles were classified as supplying muscle if their afferent activity increased in response to stretching or pulling of surrounding muscles but not in response to gentle stroking of and blowing on the nearby skin and hair. This process was unnecessary for facial nerve fascicles, which supply only muscle and contain no afferents. Typically, one pure muscle fascicle was chosen from each nerve. It was crushed distally, desheathed, and laid across a laryngeal mirror for microdissection. Small filaments were teased away and were laid across one of the wires of a platinum wire electrode. A strand of connective tissue was laid across the other wire of the electrode as reference. Few-fiber efferent activity was recorded monophasically under oil, amplified 10,000 times and filtered (15- to 1,000-Hz band pass). Nerve recordings, blood pressure, and an event marker were stored on magnetic tape for off-line analysis; limited online data analysis was also performed during the experiment with the use of an IBM-compatible computer. Nerve recordings and blood pressure were digitized at 10,000 and 100 Hz, respectively (1401 Plus Interface and Spike2 Program; Cambridge Electronic Design, Cambridge, UK). Single-unit activity was extracted off-line with the use of the spike shape-sorting algorithm in the Spike2 Program and testing the success of single-unit isolation by autocorrelation analysis (see below).

Baroreceptor sensitivity of efferents was evaluated by observing responses to bilateral carotid artery occlusion, carotid stretch, and the fall in blood pressure caused by brief expiratory outflow occlusion. Additionally, cardiac periodicity in the spontaneous activity of efferents was evaluated by creating event correlation histograms (cross correlations with a bin width of 20 ms) triggered from the systolic peak in blood pressure. Recordings were presumed to be from postganglionic sympathetic efferents if activity was silenced by systemic administration of hexamethonium (10–20 mg/kg iv) at the end of the experiment. For facial nerve recordings, stimulation of the cervical sympathetic trunk, in continuity, through a pair of platinum wire hooks (2- to 5-V intensity, 1 Hz, 0.2-ms duration) was used as a search stimulus to aid in identification of sympathetic efferents.

After stable few-fiber MVC recordings were obtained from the hindlimb and either the forelimb or the face, vestibular afferents were electrically stimulated (train of 5 square-wave pulses, 0.2 ms in duration, 3-ms separation) at an intensity two to four times the threshold needed to elicit eye movements. In cases where forelimb (and hindlimb) MVC units were recorded, responses to contralateral or ipsilateral vestibular activation were studied. In experiments where facial (and hindlimb) MVC units were recorded, only contralateral (right) vestibular afferents were stimulated to minimize stimulus artifacts. Each stimulus train was repeated 200–400 times and delivered every 2–5 s. Poststimulus time histograms (PSTH; 50-ms bin width) were created online

from multiple-unit responses to these stimuli. Single-unit activity was extracted off-line by matching spike shapes with the use of the Spike2 Program, and the unitary nature of recorded activity was confirmed by autocorrelation analysis (2- or 10-ms bin width). Spikes were considered as single units if their action potentials maintained a constant shape and if there was a clear gap, corresponding to the refractory period, spanning zero time on the autocorrelogram.

Differences in the distribution of MVC fibers exhibiting distinct response patterns to vestibular stimulation were evaluated with the use of the χ^2 test. Firing rate differences were evaluated with the use of the Student's *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Characteristics of few-fiber recordings. In all animals, activity was simultaneously recorded from sympathetic fibers in fascicles supplying muscle in the hindlimb and either the face or forelimb. Each fascicle contained 2–18 active fibers (estimated from the number of single units separated as described below). Barosensitivity was the primary criterion used to test whether MVC fibers were being recorded (12). The baroreceptor reflex was activated either by bilateral traction (stretch) of the common carotid arteries (in 4 cats, Fig. 1*A*), bilateral carotid occlusion and release (4 cats, including 2 in which the carotid arteries were also stretched; Fig. 1*B*), or lowering blood pressure by temporary occlusion of the expiratory outflow (3 cats, Fig. 1*C*). In all cases, the activity of efferent fibers responded appropriately to the induced baroreceptor stimulus. Additionally, spontaneous activity was demonstrated to be modulated by the cardiac cycle in all of the nerve fascicles recorded in each experiment (Fig. 2).

In six of the animals, all efferent activity was silenced after systemic hexamethonium administration (10–20 mg/kg iv, Fig. 1*D*). In one of the animals, this test was not performed; in the other two cats, activity of one forelimb fiber (in each experiment) persisted after ganglionic blockade, although all other activity was abolished.

Few-fiber responses to vestibular stimulation. The minimal intensity of vestibular nerve stimulation required to produce eye movements was 0.4 ± 0.1 V (mean ± SE), whereas the stimulation intensity required to produce twitches of facial muscles was over five times greater, 2.1 ± 0.3 V. This separation in threshold intensities allowed us to stimulate vestibular afferents in each experiment at two to four times the minimum vestibuloocular reflex threshold (T) without current spread to the facial nerve. The average intensity of vestibular afferent stimulation employed in these experiments was 1.5 ± 0.3 V (~3.5 T).

In all animals, alterations of few-fiber MVC activity at each recording site were observed in response to electrical stimulation of the labyrinth. Few-fiber re-

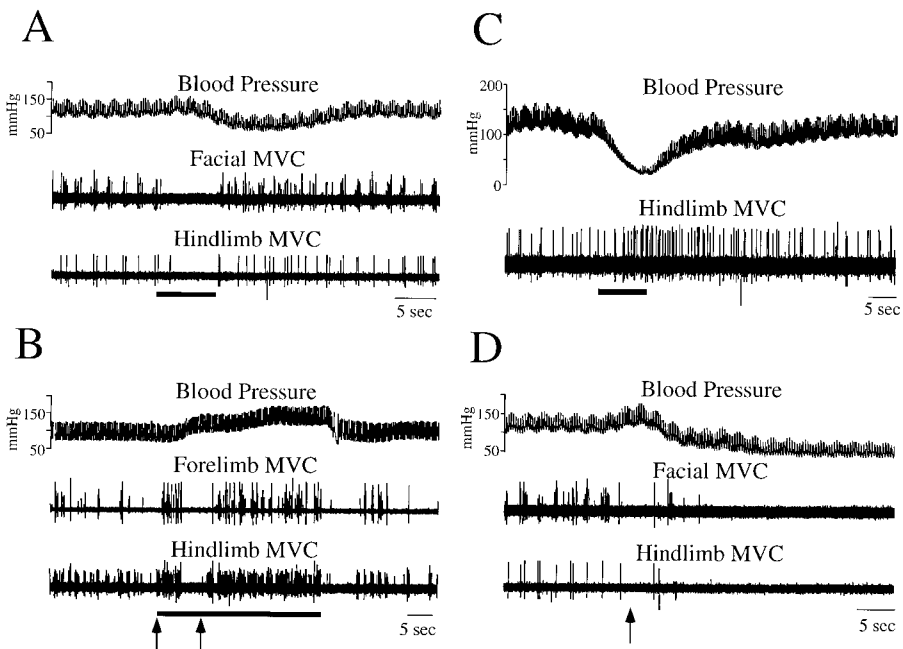

Fig. 1. Muscle vasoconstrictor (MVC) few-fiber responses to baroreceptor stimulation and hexamethonium administration. *A*: activity of both facial and hindlimb MVC fibers was silenced by bilateral and intermittent stretch of the carotid arteries; note the subsequent depressor response and accompanying increase in MVC activity. Stimulation period is indicated by the bar at *bottom*. *B*: bilateral carotid occlusion led to unloading of carotid baroreceptors, an increase in MVC activity, and a subsequent pressor response. The occlusion period is indicated by bar at *bottom*, and the times at which the two carotid arteries were clamped are indicated by arrows. Note the decreases in activity before occlusion of each artery and after release of occlusion; the former is presumably due to the carotid stretch before placement of the clamp, whereas the latter reflects reexposure of the carotid sinuses to the (now raised) arterial pressure. *C*: occlusion of expiratory outflow leads to a decrease in blood pressure, triggering an increase in MVC activity. *D*: systemic administration of hexamethonium (14 mg/kg iv; at the arrow) silenced facial and hindlimb MVC activity.

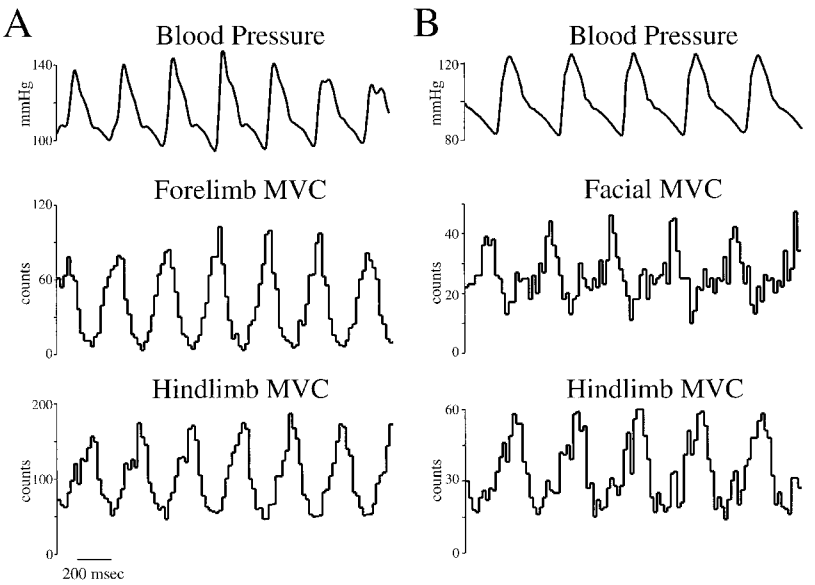

Fig. 2. Examples of cardiac rhythmicity of few-fiber MVC activity. Data were obtained from 2 separate experiments in which hindlimb and either forelimb (*A*) or facial (*B*) MVC activity was recorded. Event correlations were triggered by systolic peaks in the blood pressure wave; the bin width in traces illustrated is 50 ms. The number of cycles used for each histogram were 632 (*A*) and 1,340 (*B*).

sponses always included a long period of inhibition, typically followed by a smaller "rebound" excitation. In some cases, however, the inhibitory period was preceded by a short duration excitation (see Fig. 3 for examples). Those waveforms including early excitation were classified as type 2 responses, whereas type 1 responses did not include this component. Of the nine animals in which responses were recorded from a hindlimb nerve fascicle during vestibular stimulation, an early excitatory component (type 2 response) could only be discerned in two cases. Early excitation was more commonly recorded from forelimb nerve filaments (4 of 6 cases) and was always present in facial nerve filaments (3 of 3 cases).

Figure 3 also illustrates that no changes in blood pressure were elicited by vestibular nerve stimulation. This finding suggests that sympathetic nerve responses were due directly to activation of the vestibular system and were not secondary to changes in blood pressure produced by vestibular stimulation.

Single-unit responses to vestibular stimulation. The analysis of few-fiber MVC responses raised the possibility that a majority of rostrally located single MVC efferents exhibits a different response pattern to vestibular stimulation than those units located in the hindlimb. To further investigate this possibility, single-unit activity was discriminated from few-fiber recordings, and responses of single MVC efferents to vestibular stimulation were determined. Recorded activity was considered to originate from a single nerve fiber if the spike shape remained constant and a clear gap around *time zero*, corresponding to the unit's refractory period, was present in the autocorrelogram. Figure 4*A*, *right*, shows an autocorrelogram taken from a discriminated single fiber and, for comparison, one from a few-fiber recording (Fig. 4*A*, *left*). Only units for which barosensitivity had been demonstrated and which were silenced by hexamethonium administration were selected for this analysis. With the use of this approach, 50 such single units were identified, of which 37 had activity that was strongly entrained to the cardiac rhythm (e.g., Fig. 4*B*, *right*). The other 13 nerve fibers exhibited weaker cardiac rhythmicity, but showed robust and appropriate responses to carotid stretch (12 units) or bilateral carotid occlusion (1 unit), and were thus also classified as vasoconstrictors.

Individual MVC nerve fibers differed in their responses to vestibular stimulation. Of the 50 single units studied, 27 responded with the type 1 pattern (as discussed above), whereas the remaining 23 units exhibited type 2 responses (see Fig. 5 for examples). Although qualitative criteria were used to classify a unit as either type 1 or 2, each nerve fiber fell clearly into one of these two categories (early excitation was either prominent or absent). Units exhibiting both response types could be present at the same time in the same fascicle (Fig. 5).

Figure 6, *A* and *B*, illustrates pooled responses of nerve fibers exhibiting types 1 and 2 responses. For type 1 responses, the inhibition had a latency of 0.2–0.35 s and a duration of about 1 s (Table 1) and was followed by a period of "rebound" excitation of similar duration. Type 2 responses consisted of an initial excitatory period with a latency of ~0.2 s, which was followed after a duration of ~0.1–0.3 s by an inhibitory phase of ~1-s duration (Table 1) and subsequent rebound excitation. When types 1 and 2 responses recorded at all anatomic locations were pooled, the difference in the onset latency between the two types of responses was statistically significant ($P < 0.05$, Student's *t*-test). However, differences in the onset latencies of types 1 and 2 responses recorded at each anatomic location did not reach statistical significance ($P > 0.05$), perhaps due to small sample sizes. In particular, because type 1 responses were prevalent in the lower body and type 2 responses were prevalent in the upper body (see below), such statistical comparisons of parameters of evoked activity at an individual

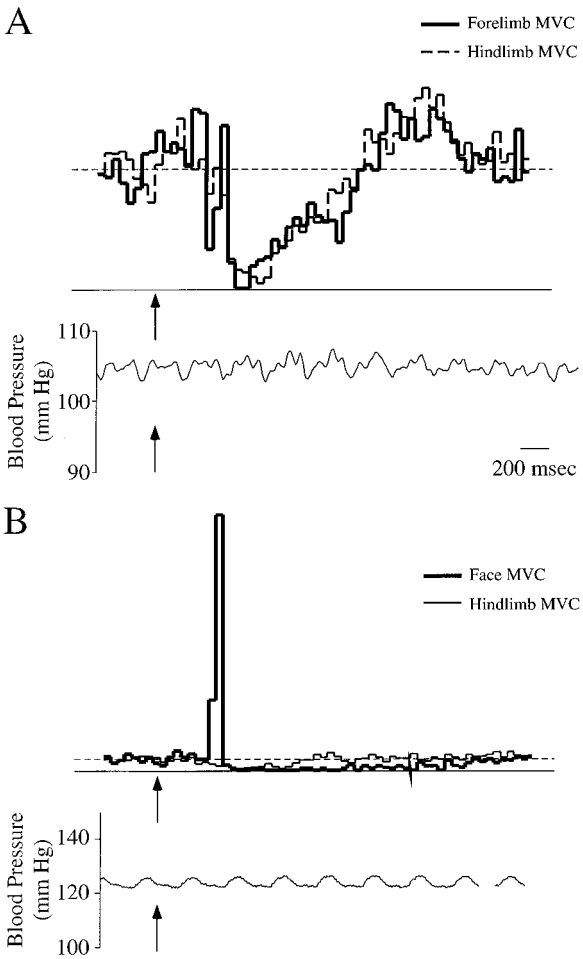

Fig. 3. Examples of few-fiber MVC responses to vestibular stimulation. *A* and *B*, *top*: traces show averaged recordings from sympathetic nerve filaments. *A* and *B*, *bottom*: traces show an averaged recording of blood pressure. Data were obtained in 2 separate experiments in which hindlimb and either forelimb (*A*) or facial (*B*) MVC activity were recorded. In both experiments, hindlimb MVC responses consisted of inhibition, typically followed by weaker "rebound" excitation; this response pattern was classified as type 1. Forelimb and facial responses, however, consisted of a brief excitatory phase that preceded the inhibition (classified as type 2); the early excitation is particularly prominent in the facial nerve recording (*B*). Counts within each bin were normalized with respect to the mean activity level of the unit during the prestimulus period (i.e., the bins represent the firing rate at a particular time as a percent of baseline firing rate). Stimulus onset is shown by arrows at *bottom* of each graph. Dashed horizontal lines indicate baseline activity level; solid horizontal lines refer to zero activity level. Stimulus intensity: 0.8 V [4 times threshold (T); *A*], 1.2 V (2.4 T; *B*). Responses were averaged using 50-ms bins over 339 (*A*) and 224 (*B*) stimulus trials.

site were constrained by limited numbers of units of one type.

For units exhibiting type 2 responses, activity increased by an average of 554 ± 226% during the initial excitatory period. As mentioned above, this excitatory phase was of short duration and did not occur at exactly the same time in each nerve fiber. Nonetheless, when all type 2 responses were pooled, a distinct response peak (~350% above baseline activity) was apparent at ~0.2 s after stimulus onset (Fig. 6*B*, *top*). With the exception of this initial excitation, types 1 and 2 responses were of similar shape and magnitude with complete silencing of both types of nerve fibers during the long inhibitory period (Fig. 6, *A* and *B*, *top*).

Although two types of responses to vestibular stimulation were recorded from MVC fibers, units showing types 1 and 2 responses appeared to be equivalent in their barosensitivity. Figure 6, *A* and *B*, *bottom*, illustrates averaged cardiac rhythmicity in the activity of all single MVC fibers showing types 1 and 2 responses; the extent of their modulation by the cardiac cycle was indistinguishable (Fig. 6, *A* and *B*, *bottom*). Firing rates of MVC nerve fibers exhibiting types 1 and 2 responses were also compared over the entire testing period. Units exhibiting type 1 responses were significantly ($P < 0.05$, 2-tailed Student's *t*-test) more active than those with type 2 responses, with firing rates of 0.29 ± 0.04 Hz for the former and 0.20 ± 0.02 Hz for the latter. This finding suggests a difference in spontaneous firing rates of MVC nerve fibers that exhibited the two types of responses to vestibular inputs.

We also investigated whether a particular unit could exhibit both types 1 and 2 responses, depending on the parameters of vestibular stimulation. Twenty-three units were tested to determine whether their responses could be converted from one type to another by varying either lateralization or intensity of vestibular stimulation. Of these nerve fibers, 19 responded to both ipsilateral and contralateral stimulation. For 16 of them, stimulation on either side elicited a similar response: type 1 responses in 12 cases and type 2 responses in 4 other units. However, three nerve fibers exhibited type 1 responses during ipsilateral labyrinthine stimulation and type 2 responses with contralateral stimulation. In addition, responses of 10 units to multiple intensities of vestibular nerve stimulation were also tested. All of these nerve fibers exhibited type 1 responses, and in no case did the response pattern change as stimulus intensity was lowered from 4 to 3 or 2 T. Overall, only 3 of 23 units could be converted from one response type to another by altering the parameters of vestibular nerve stimulation.

Differences in anatomic distribution of nerve fibers showing types 1 and 2 responses. Types 1- and 2-responding nerve fibers differed in their anatomic distribution. Units exhibiting type 1 responses were most prevalent in the hindlimb, whereas those giving type 2 responses were predominantly found at the rostral sites. Of the 27 type 1-responding nerve fibers recorded in this study, 21 were found in the hindlimb, 4 in the forelimb, and 2 in the face. In contrast, of the 23 type 2-responding nerve fibers, 8 were in the hindlimb, 6 in the forelimb, and 9 in the face. Differences in the distribution of the two types of units were statistically significant (χ^2 test, $P < 0.05$) for comparisons between hindlimb and face and between caudal (hindlimb) and rostral (forelimb plus face) locations (Fig. 7).

DISCUSSION

Previous studies on the organization of VSR revealed that vestibular inputs influence the activity of sympa-

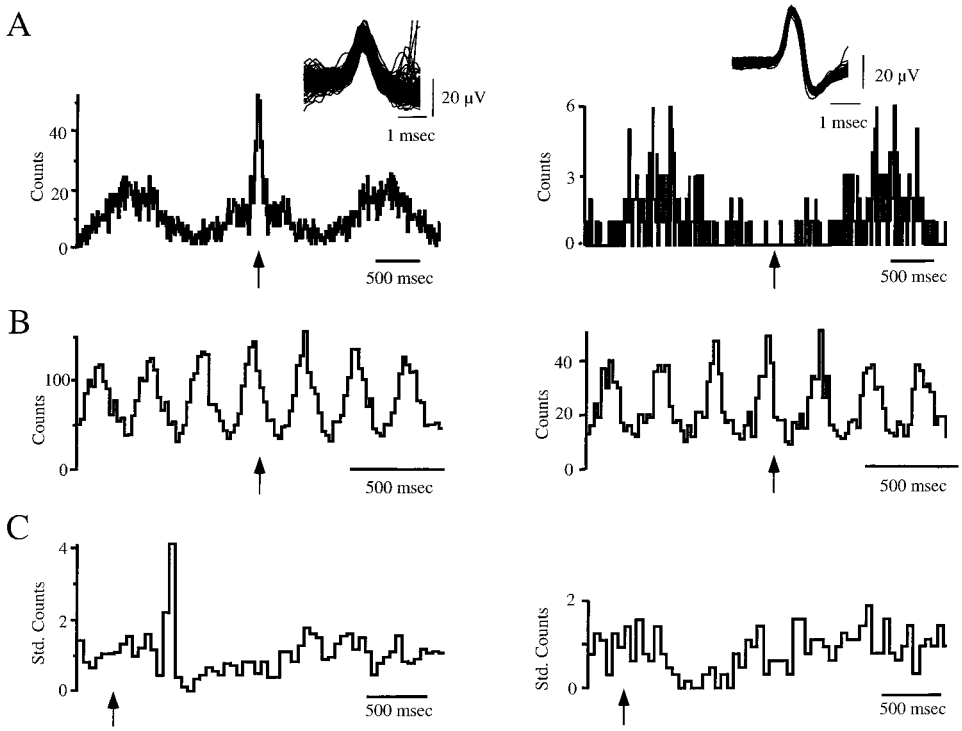

Fig. 4. Multiple- and single-unit MVC responses to vestibular stimulation. Data were extracted from the same few-fiber MVC recording in the left radial nerve. Spike shape was maintained in both cases (*A insets*). However, a large peak at the autocorrelation trigger (*A*, *left*) indicated that recordings were multiunitary, whereas a gap spanning zero time of the autocorrelogram (which corresponds to the unit's refractory period) indicated that recordings on the *right* (*A*) were from a single unit. Clear cardiac rhythmicity was present in both multiple- and single-unit recordings (*B*). The single-unit response consisted of inhibition followed by weaker "rebound" excitation (*C*, *right*), whereas the multiple-unit response included a brief excitatory phase that preceded the inhibition (*C*, *left*). Standard counts plotted in *C*, *left* and *right*, represent bin counts divided by the mean baseline firing rate before the stimulus (e.g., a standard count of 1 represents 100% of baseline firing, whereas a standard count of 2 represents two times the baseline firing rate). Bin widths: 10 ms (*A*), 20 ms (*B*), and 50 ms (*C*). The numbers of triggers used to construct each histogram were: *A*, *left*: 1,108; *A*, *right*: 319; *B*, *left* and *right*: 4,050; and *C*, *left* and *right*: 380.

thetic nerves located throughout the body, including those that innervate the head, heart, adrenal gland, gut, kidney, and bladder (15). Despite the widespread distribution of these responses, vestibular inputs appear to selectively influence activity of vasoconstrictor efferents. This conclusion is on the basis of the observation that the expression of vestibular-elicited responses in sympathetic nerves, including those that contain both vasoconstrictor and nonvasoconstrictor efferents (e.g., superior mesenteric nerve), is strongly attenuated by blood pressure increases (15). Previous studies in humans have shown that MVC efferents

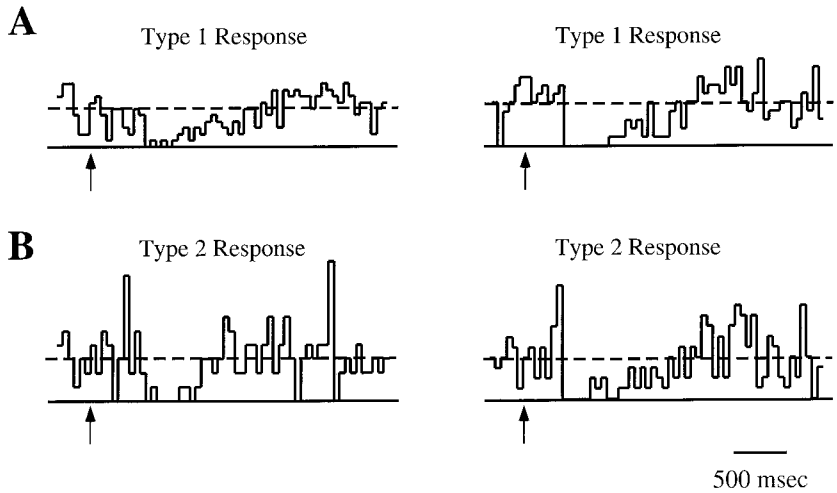

Fig. 5. Single-fiber MVC responses to vestibular stimulation. Single-unit activity was extracted from few-fiber MVC recordings of peroneal (*A*) and radial (*B*) nerve fascicles. All recordings were made in the same animal, and barosensitivity of all 4 units was confirmed. Units within the same fascicle exhibited 2 distinct patterns of responses to vestibular stimulation. Type 1 responses consisted of inhibition followed by excitation, whereas type 2-responding units exhibited an additional short latency excitatory phase. Counts within each bin of the histograms were normalized with respect to the mean activity level of the unit during the prestimulus period (i.e., represent the firing rate at a particular time as a fraction of baseline firing rate). Dashed horizontal lines indicate baseline activity level; solid horizontal lines refer to zero activity level. Stimulus intensity: 0.8 V (4 T); time of stimulus is indicated by arrows. Response histograms were summed over 379 stimulus trials with the use of 50-ms bins.

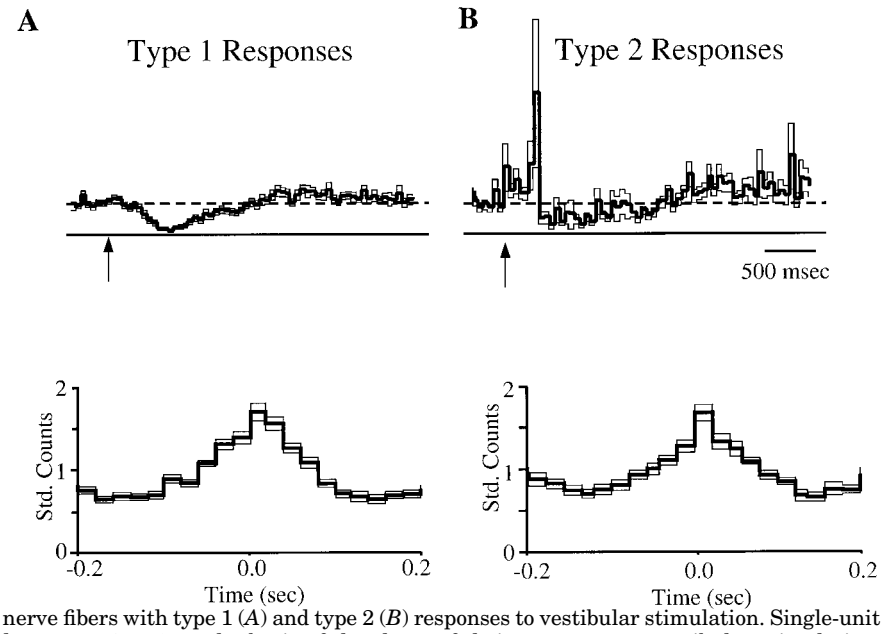

Fig. 6. Pooled data from nerve fibers with type 1 (*A*) and type 2 (*B*) responses to vestibular stimulation. Single-unit responses were classified as types 1 or 2 on the basis of the shape of their responses to vestibular stimulation. Responses were pooled by averaging individual poststimulus time histograms (50-ms bin width) (*A* and *B*, *top*); before averaging, counts within each bin were normalized with respect to the mean activity level of the unit during the prestimulus period (stimulus onset is shown by arrows at the bottom of each graph). Horizontal dotted lines indicate baseline activity levels, whereas solid horizontal lines correspond to zero activity levels. Baroreceptor sensitivity of MVC units with types 1 and 2 responses to vestibular stimulation was compared by averaging the first cycle of cardiac rhythmicity event correlations (*A* and *B*, *bottom*). Event correlations were triggered off the peaks of the blood pressure wave and constructed with 20-ms bins; before averaging, counts within each bin were standardized (std) by dividing by the mean activity level. The data are represented as means (thick lines) ± SE (thin lines). The two classes of MVC units differed in their responses to vestibular stimulation but were equivalent in their cardiac rhythmicity.

respond to caloric vestibular stimulation (4) and to head-down neck flexion (21, 22). Results of the present study extend the earlier findings by demonstrating that activity of MVC efferents is altered by selective electrical stimulation of vestibular afferents. These experiments also add further evidence that vestibular signals affect vasomotor outflows to a wide variety of organs throughout the body.

All of the fibers analyzed in detail in the present study were evidently sympathetic, supplied skeletal muscle, and were inhibited by baroreceptor stimuli. These criteria have been used elsewhere to identify MVC units (8, 12, 19). It is unlikely that the units we studied belonged to the other known class of sympathetic efferents that supply skeletal muscle, vasodilator fibers, which do not show spontaneous activity and are not inhibited by baroreceptor stimulation (2, 10–12). It was therefore a surprise to find that MVC nerve fibers fell into two distinct categories based on their type of response to vestibular stimulation. The fact that a particular fiber responded with one pattern rather than the other was not simply explainable by experimental factors (e.g., stimulating electrode position or the condition of the animal at the time) because fibers of both categories were frequently present at the same time in the same fascicle. Moreover, the proportions of fibers in each category systematically differed between rostral and caudal nerve fascicles recorded at the same time.

One possible explanation for these findings is that two distinct types of MVC neurons mediated the two types of responses to vestibular stimulation. It is feasible that these may represent hitherto unrecognized subclasses of MVC neurons, perhaps innervating different targets within the skeletal muscle's vascular tree, by analogy with the way that different types of sympathetic neurons innervate different segments of the rabbit's ear vasculature (20). The finding that most MVC fibers could not be converted from one category to the other by altering either the strength or the lateralization of vestibular stimulation is consistent with this notion. Alternatively, it is also possible that the two response patterns were mediated by MVC nerve fibers of the same functional class (and with the same vascular target), but in different physiological states. If so, this seems unlikely to have been a simple difference in neuronal excitability because the magnitudes and shapes of the inhibitory components of types 1 and 2 responses were similar (Fig. 6).

One possibility is that two distinct pathways relay vestibular signals to MVC neurons, one that influences all of the units (eliciting the long duration inhibition after electrical stimulation of the labyrinth) and another that influences only some of the cells (eliciting the short latency excitation that was recorded from type 2 nerve fibers in this study). The latter pathway appears to mainly provide inputs to MVC units that affect vasculature in the rostral parts of the body; this pathway may

Table 1. *Characteristics of types 1 and 2 responses to vestibular stimulation*

	Hindlimb	Forelimb	Face
	Type 1 responses		
Phase 1			
Latency	0.344 ± 0.028	0.275 ± 0.043	0.210
Duration	0.928 ± 0.051	1.218 ± 0.050	1.605
	n = 21	n = 4	n = 2
	Type 2 responses		
Phase 0			
Latency	0.223 ± 0.065	0.225 ± 0.036	0.191 ± 0.023
Duration	0.134 ± 0.039	0.073 ± 0.011	0.314 ± 0.171
Phase 1			
Latency	0.428 ± 0.052	0.485 ± 0.144	0.547 ± 0.189
Duration	1.151 ± 0.287	0.905 ± 0.147	1.464 ± 0.287
	n = 8	n = 6	n = 9

Data are means ± SE (s) from 50 single muscle vasoconstrictor units, with 27 of them exhibiting type 1 responses and 23 units showing type 2 responses; n = no. of units recorded at each location. Responses recorded from units in the hindlimb, forelimb, and face are represented in separate columns. Phase 0 is the initial excitation that was part of type 2 but not type 1 responses, whereas phase 1 consisted of inhibition that was present in all responses. SE values were not calculated for nerve fibers located in the face giving type 1 responses due to low n.

also be lateralized, which explains our observation that in a few cases MVC neurons were excited by only contralateral vestibular stimuli. Whether there are other intrinsic differences between MVC neurons that receive inputs from the two pathways remains to be established. But in either case, such a mechanism could account for the present finding of rostrocaudal differences in the expression of VSR.

Despite uncertainty regarding precise neural mechanisms, the current observation that there may be qualitative and quantitative differences between MVC outflows at different rostrocaudal levels bears light on an issue that has been debated in the literature during the past 20 years: whether the activity of MVC fibers innervating different body regions can be selectively controlled. The null hypothesis would be that MVC fibers in all body regions respond homogeneously to central and reflex drives [at least those mediated by the brain stem; an exception may be made for segmental spinal reflexes (24)]. This view is supported by the finding that when microinjections of sodium glutamate were used to activate neuronal cell bodies in the ventrolateral medulla of anesthetized cats, no separation could be found between sites that caused vasoconstriction in forelimb and hindlimb muscles (17). This was so despite the fact that the vasomotor drives to different types of tissue could be readily separated (3, 5, 6, 16–18). The hypothesis that all MVC efferents are activated simultaneously is also supported by the observation that human MVC discharges recorded simultaneously from the arm and leg show a remarkable similarity in the timing and amplitude of bursts in activity both at rest (26) and during activation by lower body negative pressure (23). Against this idea, however, are studies that have found differences between limbs in human MVC activity, particularly during muscle contraction and mental stress. For example, activity of MVC fibers located on the two sides of the body, although strongly correlated at rest (26, 27), is diminished in coherence by contraction of muscles on one side (27). Additionally, although activity of arm and leg MVC increases in parallel form during a static handgrip task (28), when blood supply to the exercising arm muscle is occluded, the resulting postcontraction ischemia induces greater increases in MVC activity in the radial nerve than in the peroneal nerve (28). These findings may be due to a superimposing of spinal reflex vasomotor drives [some of which are known to show "local signs" (24)] elicited by stimulation of metaboreceptors in active muscle (9), with generalized vasomotor drives descending from the brain. Such an explanation is much less likely, however, in the case of differences between arm and leg MVC responses to mental stress. Anderson and co-workers (1) reported that during a mental arithmetic task, leg MVC activity increases, whereas that of the arm remains unchanged. Against this background, the current findings provide further evidence for brain stem influences exerting at least partly independent control of muscle blood vessels in different body regions.

Although this study has shown that activity of rostrally and caudally located MVC fibers can be influenced independently by vestibular inputs, the interpretation of the functional significance of the present observations is limited by methodological constraints. Electrical vestibular stimulation was chosen in this study because it can reliably produce a powerful and effective activation of vestibular afferents, without stimulating other sensory systems. The drawback is that this type of stimulation simultaneously activates all vestibular afferents, thus encoding a nonphysiological afferent input that signals head movement in all directions at once. Such a complicated afferent activa-

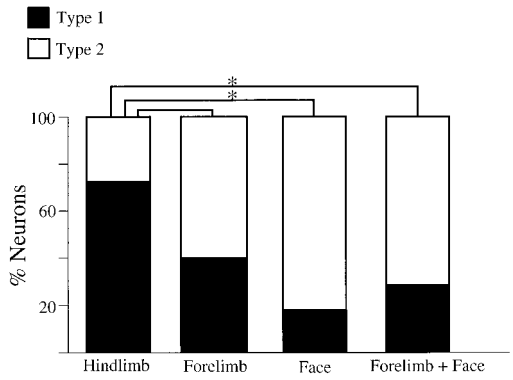

Fig. 7. Differences in the prevalence of MVC single units with types 1 and 2 responses to vestibular stimulation at different anatomic locations. Nerve fibers with type 1 responses made up over 70% of units in the hindlimb population, whereas their proportion was 40% in the forelimb and <20% in the face. When nerve fibers located at rostral levels (in the face and forelimb) were pooled together, the prevalence of those with type 1 responses was <30%. *Differences between the hindlimb and face as well as between the caudal (hindlimb) and rostral (forelimb and face) locations reached statistical significance (χ^2 test, $P < 0.05$).

tion is likely responsible for eliciting the complex patterns of MVC responses observed in this study, which consisted of a combination of excitation and inhibition. Thus it is not possible to definitely conclude how head movements in a particular direction may affect MVC activity in different body regions. However, because simultaneous activation of all vestibular afferents elicits patterned MVC responses, it seems likely that stimulation of subsets of vestibular afferents through natural head movements would also elicit patterned changes in MVC activity. Further experiments must be performed to examine this possibility. It also seems probable that either electrical or natural stimulation of vestibular afferents would lead to patterned changes in blood flow to muscles at different rostrocaudal levels. The companion manuscript (13a) examines hemodynamic changes elicited by electrical stimulation of the vestibular nerve.

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