Cerebellar fastigial nuclei activity during blood pressure challenges

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Rector, D. M., C. A. Richard, and R. M. Harper. Cerebellar fastigial nuclei activity during blood pressure challenges. J Appl Physiol 101: 549–555, 2006. First published May 11, 2006; doi:10.1152/japplphysiol.00044.2006.—The cerebellar fastigial nuclei (FN) assist in regulating compensatory responses to large blood pressure changes and show structural injury and functional impairment to cardiovascular challenges in syndromes with sleep-disordered breathing. The patterned time course of FN responses to elevation or lowering of blood pressure and location of responsive regions within the nuclei are unclear. We evaluated FN neural activity in six anesthetized rats using optical imaging procedures during elevation and lowering of arterial pressure by phenylephrine and nitroprusside, respectively. Hypertension diminished optical correlates of FN neural activity, while measures of activity increased to hypotension, with peak neural responses occurring 5–10 s later than peak blood pressure changes. Blood pressure responses were followed by heart rate changes, and peak respiratory rates developed even later, in close temporal proximity to FN activity patterns. Although overall topographical response trends were similar, regional patterns of altered neural activity appeared to both hypertension and hypotension. The extent of neural change was greater during recovery from hypertension than for hypotension at high-dose levels. Blood pressure levels saturated with increasing phenylephrine doses, while FN activity continued to decline. No saturation appeared in heart or respiratory rate trends. The findings suggest that the FN compensate for large blood pressure changes by sympathoexcitatory and inhibitory processes, which accompany late-developing somatic or respiratory adjustments.

hypertension; hypotension; intermittent hypoxia; obstructive sleep apnea

THE CEREBELLAR FASTIGIAL NUCLEI (FN) serve significant roles in autonomic nervous system regulation, receiving input from vestibular structures and from cerebellar Purkinje cells, which mediate vestibular and other afferent signals necessary for blood pressure compensation (9, 41). The nuclei project to medullary structures, which ultimately influence spinal intermediolateral column autonomic neurons (3, 8). In the monkey, the FN project directly to the inferior and lateral vestibular nuclei, the gigantocellular reticular nucleus, the paramedian reticular nucleus, the lateral reticular nucleus, and the anterior gray horn in the spinal cord (1). Functional roles for the FN in cardiovascular and respiratory regulation have been demonstrated by electrical and chemical stimulation and by lesion studies (20, 23, 37), and by functional magnetic resonance imaging (fMRI) studies in developing and adult animals (14, 15). FN lesions exert no significant effects on resting blood pressure or heart rate, but reduce compensatory responses to hemorrhage or endotoxic shock to the point that a fatal outcome can ensue (20). Electrical stimulation of the rostral FN in anesthetized animals elicits a pressor response with tachycardia, while chemical activation of the FN in anesthetized animals results in a depressor response with bradycardia (6, 7, 25). These findings have been interpreted to suggest that pressor responses are mediated by fibers passing through, or very close to, the FN, while depressor responses are mediated by intrinsic FN neurons.

The manner in which the FN mediate depressor and pressor responses is of clinical interest, since cerebellar injury with blood pressure and respiratory consequences is a common developmental occurrence (5), and cerebellar cortex and deep nuclei damage appears routinely with syndromes of sleep-disordered breathing, including obstructive sleep apnea [OSA (22)], congenital central hypoventilation syndrome [CCHS (19)], and heart failure [the latter condition frequently associated with Cheyne-Stokes breathing and OSA (33)]. The sleep-related syndromes associated with cerebellar injury show characteristics of impaired chronic blood pressure regulation (17, 29), as well as an inability to respond appropriately with breathing, heart rate, and blood pressure responses to dynamic blood pressure challenges (12, 18, 34). It remains unclear, however, whether the FN injury found in these syndromes plays a role in the disturbed blood pressure outcomes found among affected patients, since other brain sites also show significant damage.

The processes by which the FN normally mediate changes in blood pressure are unclear. The apparent unresponsivity to moment-to-moment changes in pressure suggests little or no contribution from the FN to modest changes in blood pressure, but point to a significant role in compensation for extreme depressor alterations (20). Severe hypotension is accompanied by compensatory somatic responses (13); transient hypertension is associated with suppression of somatic activity (31). The FN may play an essential role in mediating such somatic compensation. The nuclei are sensitive to respiratory control (38); yet, if lesioned, compensation for blood pressure fails, with fatal consequences (20). Since responses to major changes in blood pressure would follow a different time course than momentary corrections (i.e., would likely be slower to emerge since large blood pressure changes take some time to develop), outlining the FN temporal relationships in optical signal changes to blood pressure, heart rate, and respiratory patterns would help determine the role of these nuclei in blood pressure and breathing interactions.

Although single-neuron recording studies allow high temporal resolution evaluation of regional FN responses to chal-
lenges, it is difficult to obtain more than a small sample of cells for a sustained period of time. fMRI studies allow whole animal evaluation of FN responses to cardiovascular responses, but the low spatial and temporal resolution of magnetic resonance imaging precludes adequate evaluation of rapid signals occurring in a dynamic test.

The objective of this study was to examine, at high temporal and spatial resolution, the extent and time course of FN responses to graded elevation or lowering of blood pressure and to relate the drug-induced cardiac, vasopressor, and respiratory responses to those neural changes in the FN. Optical imaging procedures are an ideal method to record patterns of neural activity over a wide area, because several optical parameters correlate with neural activity (28). We used optical imaging procedures, which allowed rapid measurement of neural activation over the FN. We hypothesized that FN neural responses to hypertension and hypotension would be temporally linked to changes in respiratory pattern associated with compensatory responses to blood pressure manipulation.

**METHODS**

Six adult male rats were anesthetized with isoflurane in 100% O2 and intubated for delivery of anesthesia throughout the experiment; animals were allowed to breathe spontaneously. A femoral artery and vein were cannulated for monitoring of arterial pressure and for drug delivery, respectively, and ECG leads were placed on either side of the thorax. Expired CO2 was monitored continuously through a sampling port in the endotracheal tube. After instrumentation, each rat was placed in a stereotaxic head-holder, with the head flexed at a 30° angle, thus providing access to space between the cerebellum and medulla with a minor incision in the posterior cervical tissue and dura mater. A 1.5-mm-diameter coherent fiber-optic imaging probe (26, 27) was inserted through the caudal opening of the fourth ventricle. The tip of the fiber-optic probe was positioned to rest on the roof of the ventricle to image the left FN, while the charge-coupled device recording chip and other imaging hardware remained outside the brain (Fig. 1).

The intrinsic optical imaging technique uses narrow-bandwidth light at 660 nm (±10 nm) to illuminate brain tissue to a depth of ~500 μm and collect reflected and refracted light. Use of the 660-nm (red) wavelength optimizes the use of differences in collected photons to index neuronal activity under the fiber-optic probe (27). Image frames were collected at 100 Hz, together with electrophysiological recordings of ECG, expired CO2, and arterial blood pressure during hyper- and hypotensive episodes, as well as baseline periods. Cardiac R-waves were identified by amplitude discrimination of the ECG signal and used to derive heart rate on a beat-by-beat basis. Increased blood pressure was elicited with 20, 40, and 80 μg/kg iv phenylephrine (PE), and decreased blood pressure was produced with 10, 20, and 40 μg/kg iv sodium nitroprusside (NP); both agents were delivered in 0.1-ml saline, followed by 0.1-ml saline flush. Control injections used saline (0.2 ml); at least 15 min of recovery were allowed between drug challenges.

FN image frames were analyzed by averaging the value of all pixels within a frame and comparing the values before and after drug administration. Trends of defined regions within frames that showed significant signal change over other areas were also plotted. Peak mean frame values, peak mean arterial pressure, and heart rate were represented as means ± SE and compared using t-tests and Kruskal-Wallis tests; the nonparametric test was used when normal distributions could not be assumed. Regression techniques were used to evaluate co-varying measures. A P < 0.05 was required for statistical significance. All procedures were approved by the Institutional Animal Care and Use Committee of the University of California at Los Angeles.

**RESULTS**

PE administration increased blood pressure by 30, 45, and 40% with low, medium, and high doses, respectively, and decreased FN neural activity in a dose-dependent fashion by 16, 27, and 62% (Kruskal-Wallis, P = 2 × 10−6). PE-induced hypertension also reduced heart rate by 8, 12, and 23% with increasing doses and slowed respiratory rate (increased respiratory interval) by 18, 30, and 51% (P < 0.05, Kruskal-Wallis; Fig. 2). The dose-dependent peak changes in amplitude of the heart and respiratory rates were highly correlated with the peak neural responses (r² = 0.98 and 0.94, respectively), but not correlated with the peak change in blood pressure (r² = 0.02). Trends in dose-dependent group data of blood pressure, cardiac and respiratory interval, and optical responses are shown in Fig. 3. The peak neural responses for PE challenges significantly lagged peak blood pressure and heart rate responses (P < 0.04, paired t-test; Figs. 2 and 3). The lags were dose dependent, with longer times, especially to optical and respiratory peaks, with higher doses (P < 0.002, Kruskal-Wallis). The dose-dependent time to peak between the neural and respiratory responses was highly correlated (r² = 0.99), while the time to peak of the blood pressure response was not correlated with the neural reactions (r² = 0.33).

NP administration significantly decreased blood pressure in a dose-dependent fashion (r² = 0.92; Figs. 4 and 5) and increased FN neural activity at all dose levels (t-test, P < 0.001). Both heart and breathing rates also increased significantly for the highest dose (t-test, P < 0.001) (decreased R-R and respiratory interval); these values were variable with lower doses (Fig. 5). The peak neural response lagged the nadir of the decline in blood pressure at the highest dose, 40 vs. 30 s (t-test, P < 0.001; Fig. 4); timing of maximal changes in the remaining physiological variables was highly variable. FN neural responses showed a greater range of variability across pixels during 40 μg/kg PE challenges compared with those for 40 μg/kg NP (paired t-test, P < 0.01). The extent of neural change...
was greater during recovery from hypertension than for hypotension at high dose levels ($P < 0.01$).

Tracings of FN activity from individual trials during PE and NP challenges are shown in Fig. 6. The tracings represent trends of averages of three defined regions of interest (regions A, B, and C; first frames) and the overall average of optical signal during the course of the PE and NP challenges from the same animal. Seven individual averaged frames ($n = 100$ averages) following a baseline frame are shown at equispaced times (arrows) along the time course. The trend curves and the individual frames demonstrate that responses did not necessarily occur uniformly across the entire field, but that regional differences emerged. Although NP administration resulted in increased activity over virtually the entire field of view, areas of enhanced change appeared (region A), while changes in the two other areas (regions B and C), although larger than the surround, are less pronounced. The regional traces from PE challenges showed similar patterns of enhanced signal change (signal decline to PE) over the field of view. The response change was doubled in subregion A over the C site.

**DISCUSSION**

We found that the FN significantly deactivated to hypertension and activated to hypotension in isoflurane-anesthetized rats. Certain regions responded more vigorously to bothpressor and depressor challenges over others. Neural responses developed later than peak blood pressure and heart rate changes and were more temporally related, with respiratory patterns accompanying the blood pressure challenges. Although blood pressure changes saturated at high pharmacological doses, neural responses continued to change in magnitude.
The findings suggest that the FN compensate for large blood pressure changes, that the action appears late in a challenge, is likely related to respiratory or other somatic components of blood pressure compensation, and serves a sympathoexcitatory role for hypertensive challenges and sympathoinhibitory capacity for pressor challenges. The long-latency response precludes a significant role for the FN in initiating the immediate heart rate changes associated with blood pressure elevation or lowering and focuses attention on the somatic respiratory patterns or long-term autonomic compensatory responses to already elevated or lowered blood pressure.

**Respiratory relationships.** The high correlation between peak changes in the neural signal and respiratory and heart rate without significant correlation to blood pressure argues for a significant role in late-developing physiological processes that would provide somatic behaviors capable of assisting in blood pressure restoration. Recruitment of somatic muscular activity, increased respiratory rate, or tidal volume, and enhanced heart rate are used to overcome the hypotension induced by hypovolemia (13). Such an interpretation would be congruent with the evidence associating FN action with control of breathing. Neurons within the nuclei are chemosensitive (39), lesions of the rostral FN result in impaired responses to hypercapnia (36), and the respiratory-related responses appear independent of cardiovascular-related aspects. Activation of the FN, usually by electrical means, increases respiratory rate and frequently tidal volume, while lesions do not affect ordinary breathing, except by diminishing ventilatory responses to lowered O₂ or increased CO₂. A number of medullary areas contribute to both afferent and efferent components of FN responses to blood pressure challenges. The FN uses vestibular, especially medial vestibular input (16) and olivary afferent activity, to assist output to medullary structures; gigantocellularis neurons of the medulla are the principal recipient. Inhibitory input from Purkinje neurons, especially from the medial vermis, likely plays a critical role in modifying respiratory patterning, since stimulation of the vermis inhibits ventilation (2). The Shaker mutant rat model with hereditary Purkinje cell degeneration shows enhanced eupneic breathing and augmentation of ventilation to hypercapnia, presumably reflecting a loss of inhibition to the FN (40). The collective evidence suggests a major role for the FN, especially the rostral FN, in respiratory control. The FN neural response may reflect a principal modulating respiratory response to the blood pressure challenge.

It is unclear whether carotid baroreceptors directly project to the FN, or whether chemoreceptor information reaches the deep nuclei via other means. At least an indirect pathway from carotid chemoreceptors reaches the FN by way of the ventral medulla, and functional data (35) support that relationship. The animal fMRI evidence (15) suggests that baroreceptor information reaches the deep nuclei rather quickly, although the temporal resolution of magnetic resonance signals precludes adequate determination of transmission delays and pathways. The late development of FN signal changes suggests an integrative process rather than an immediate stimulus response (i.e., multiple-pathway circuitry).

**Potential artifacts.** The long lags between peak blood pressure and peak neural effects for both hypertension and hypotension also show that the optical signal representing neural activity changes are not contaminated by an optical “perfusion signal” related to drug-induced changes in mean arterial pressure. Small physiological and optical responses developed from saline injections. These responses likely reflect reflex responses to the injected volume and lower (room) temperature of the control fluid.

**Asymmetric responses.** The extent of neural change was larger during hypertensive challenge recovery than from hypotensive recovery. This finding, however, should be considered in the context that the increase in mean arterial pressure to PE was greater than the decrease in pressure to NP. However, other indications of asymmetry in FN responses to hypotension vs. hypertension appear in single-neuron responses to cardiovascular challenges. Some neurons respond only during recovery to hypotension (21), although most cells responded consistently to challenges. Others have reported asymmetric responses to hypertension and hypotension. FN lesions can reduce heart rate responses to hypotension (lowered baroreflex sensitivity), but augment the heart rate response to hypertension, while prolonging recovery from a hypotensive, but not hypertensive, challenge (4). The processes underlying the asymmetry are unclear; protection from hypotension serves an obvious teleological advantage for survival, with the FN involved in vasopressin release from the pituitary/hypothalamus in response to hypotension and hemorrhage (30). The FN protection against hypotension serves an obvious immediate survival role, presumably by instigating mechanisms to activate somatic musculature to assist restoration of blood pressure. FN processes that protect against the other extreme of
blood pressure, transient hypertension, likely mediate the profound suppression of breathing and other somatic activity to compensate against such elevation and may play a critical role against stroke. These processes may be at risk during the transient hypertension associated with cessation of airflow in OSA. OSA patients show injury in FN as well as regions with Purkinje cells in cerebellar cortex.

Relationship to sleep-disordered breathing. An impetus for these studies was the consistent finding of cerebellar cortex and deep nuclei damage in syndromes with sleep-disordered breathing. The areas of cerebellar cortex injury include regions of the vermis from which Purkinje neurons send inhibitory influences to FN neurons. All of the sleep-related syndromes are characterized by sustained periods of intermittent hypoxia, with obstructed upper airways in OSA, Cheyne-Stokes breathing with regular periods of sustained central apnea and obstructive events in heart failure, and episodic periods of hypoxia from failure to ventilate in CCHS. Chronic, intermittent hypoxia in rodents results in severe injury to Purkinje neurons and FN cells, even after relatively short (5 h) exposure (24); this

Fig. 4. Histograms of changes in optical signal, BP, cardiac RRI, and Ttot, and time from onset of challenge to maximum change in measure (time to peak) from 3 doses of sodium nitroprusside (NP) of 10, 20, and 40 μg/kg. Labels are as described in Fig. 2 legend. Optical changes were inverted in sign to reflect correlates of neural activity.
Damage may develop earlier than the intermittent hypoxia-related injury in hippocampal and frontal cortex sites (10). Damage to cerebellar Purkinje cells releases substantial inhibitory influences on the deep nuclei, including the FN. The processes by which cerebellar cortex neurons are injured in intermittent hypoxia are unclear, but may stem from excitotoxic mechanisms from climbing fibers of the inferior olive to the Purkinje neurons (32). Apoptotic mechanisms may also be involved (11). The inhibitory effects of Purkinje neurons on fastigial cells would be lost with such injury, with a resultant loss of response or exaggerated FN response to baroreceptor or vestibular stimulation. Exaggerated and dampened autonomic responses occur to a range of challenges in OSA, CCHS, and heart failure patients (12, 18, 34), possibly stemming from the FN injury accompanying the syndromes.

In conclusion, this study demonstrates the close integration of autonomic and somatic control processes normally found in intact animals reacting to a cardiovascular challenge. A substantial body of evidence illustrates a significant life-protecting role for the FN in blood pressure regulation during hypotension from hypovolemia or shock. In addition, evidence from multiple sources supports an important FN role in breathing control. The common clinical advice provided to orthostatically hypotensive patients, i.e., to tense postural muscles and breathe forcefully to maintain blood pressure on standing, and the observational findings of extreme muscular extension and

![Fig. 5](image-url) Averaged traces of optical and physiological signals during the course of sodium NP administration, with low (10 μg/kg, green), medium (20 μg/kg, blue), and high (40 μg/kg, red) dose traces, color-coded for clarity. The vertical dotted lines indicate 5-s spacing. The onset of the challenge is indicated with an arrow. Optical, BP, ECG, and RESP labels are as described in Fig. 2 legend. Optical traces were inverted in sign to reflect correlates of neural activity.

![Fig. 6](image-url) Individual tracings of FN activity [optical (OPT)] and physiological responses (RESP ΔTot and ECG ΔRRI, BP) during PE and NP trials (40 μg/kg dose) in 1 animal representing trends within 3 defined regions of interest (ROI) (A, B, C; outlined in first frames). Seven individual averaged frames of neural activity changes (n = 100 averages) following a baseline frame are shown at equispaced times (arrows) along the traces. Correlates of neural activity changes in region B during the NP challenge decline below zero earlier than most other areas. Images were pseudocolored such that cool colors (blue or purple) represent regions of decreased neural activity, and warm colors (orange to red) represent regions of increased neural activity. Green-colored pixels represent regions of no change from baseline. Optical traces were inverted in sign to reflect correlates of neural activity.
tachypnea found in awake animals during hypovolemia emphasize the role of somatic recruitment in blood pressure regulation. The temporal patterning of the FN neural response, in close timing relationship to the somatic respiratory pattern, suggests that a somatic compensatory role in support of extreme blood pressure change is the principal reaction found here.

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GRANTS

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