Real-time imaging of the medullary circuitry involved in the generation of spontaneous muscle sympathetic nerve activity in awake human subjects

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ABSTRACT

In order to understand the central neural processes involved in blood pressure regulation we recorded muscle sympathetic nerve activity (MSNA) via a tungsten microelectrode in the common peroneal nerve while performing functional Magnetic Resonance Imaging (fMRI) of the brainstem at 3T. Blood Oxygen Level Dependent (BOLD) changes in signal intensity were measured over 4 s every 8 s (200) volumes. Using the total MSNA recorded during the previous 4 s epoch as the input model, we found that increases in sympathetic outflow were associated with robust increases in signal intensity in the region of the rostal ventrolateral medulla (RVLM). Reciprocal decreases in signal intensity occurred in the regions of the nucleus tractus solitarius (NTS) and caudal ventrolateral medulla (CVLM). We show for the first time that this combined approach of recording sympathetic neural activity and fMRI can provide “real-time” imaging of the neural processes responsible for the generation of sympathetic nerve activity in awake human subjects.

The automatic control of diverse organ systems provided by the sympathetic and parasympathetic divisions of the autonomic nervous system is vital for the homeostatic adjustments essential in life, and there are many pathophysiological conditions in which disorders in autonomic control have been identified. While much has been learnt about the brainstem and hypothalamic circuitry largely responsible for this control from studies in anaesthetized experimental animals, studies in awake human subjects have mostly been limited to non-invasive studies on the effector organs of the autonomic nervous system (by measuring changes in heart rate, blood pressure, skin blood flow, sweat release, etc) or invasive studies in which the activity of sympathetic postganglionic neurons is recorded via percutaneous intraneural microelectrodes (microneurography). However, little is known about the central nervous control of the autonomic nervous system in awake human subjects.

A primary determinant of resting blood pressure is the level of sympathetically-mediated vasoconstriction within skeletal muscle. Microneurographic studies have shown that muscle sympathetic nerve activity (MSNA) at rest, which occurs as pulse-synchronous bursts and is measured as burst frequency (bursts per minute) or burst incidence (bursts per 100 heart beats) is consistent in a given individual from day to day, year to year[10]. Some subjects have high levels of resting MSNA and some have low levels; identical twins have similar levels, whereas the burst incidence in fraternal twins differs widely[26]. Given that there is a poor relationship between the level of resting MSNA and resting blood pressure, one cannot predict the level of MSNA in a given healthy individual by recording blood pressure (or heart rate) alone[17]. However, it is clear that bursts of MSNA occur when diastolic pressure is low – and hence baroreceptor input is low - during the spontaneous fluctuations in arterial pressure that occur at rest[24]. The purpose of the present study was to determine whether we could identify sites within the brainstem responsible for these spontaneous fluctuations in resting MSNA.

It is known from experimental animal investigations that the *nucleus tractus solitarius* (NTS) within

the medulla oblongata is the primary receiving area of inputs from the arterial baroreceptors. The NTS sends excitatory projections to the *caudal ventrolateral medulla* (CVLM), which exerts tonic inhibitory control of the *rostral ventrolateral medulla* (RVLM) - the primary output nucleus responsible for the sympathetic vasoconstrictor control of arterial pressure[1,4,5,8,11,16,23]. By recording spontaneous MSNA concurrently with blood oxygen level dependent (BOLD) signal intensity changes within the human brainstem, we aim to identify the medullary nuclei involved in the baroreflex, and hence the generation of spontaneous bursts of MSNA, in human subjects. Based on animal work we predict that a decrease in activity of the NTS will occur during spontaneous falls in arterial pressure, resulting in decreases in activity in CVLM and hence increases in RVLM activity. This combination of direct recording of sympathetic nerve activity and functional magnetic resonance imaging (fMRI) is unique, and has not been attempted previously, yet promises to provide us with important information on the central processes responsible for autonomic control in awake human subjects.

RESULTS

Concurrent recordings of muscle sympathetic nerve activity (MSNA) and fMRI of the brainstem were achieved in seven experiments (six subjects); in one of the remaining experiments the recording site was lost before the sequence was completed, in another the subject had too little MSNA at rest to provide sufficient data for analysis. A sample of the neural data obtained from one subject is shown in Fig. 1. Despite the large scanning artifacts stable neural recordings were obtained, although the background electrical noise levels were higher in the scanner. It can be seen in Fig. 1 that spontaneous bursts of MSNA were observed in some, but not all, of the 4 s intervals between scans. Given that the apparent burst incidence during scanning was no different to that observed during the resting period, recorded prior to or following the scanning sequence, we can reasonably conclude that the scanning *per se* did not change a subject's resting level of MSNA.

Fig. 1 near here

Significant correlations between brainstem signal intensity and MSNA, obtained from seven experiments, are illustrated in Fig. 2. Using the mean levels of electrical activity recorded from the peroneal nerve – which reflects the spontaneous fluctutations in MSNA - as the input model we could identify brainstem regions responsible for moment-to-moment regulation of MSNA and hence blood pressure and heart rate. The most robust correlations occurred in the dorsolateral and dorsomedial medulla, in the regions of the human equivalent of *rostral ventrolateral medulla* (RVLM), *caudal ventrolateral medulla* (CVLM) and *nucleus tractus solitarius* (NTS).

Fig. 2 near here

Given the inverse relationship between spontaneous MSNA and diastolic pressure at rest, we reasoned that when MSNA is high - and the input from the baroreceptors low – NTS AND CVLM activity would be low and RVLM activity would be high. Figure 3 shows changes in BOLD signal intensity within the RVLM and CVLM during periods in which MSNA was high (low baroreceptor input) and periods in which it was low (high baroreceptor input). It can be seen that signal intensity within RVLM was higher when MSNA was high than when it was low; this difference was statistically significant for all six subjects. Conversely, there was an inverse relationship between MSNA and signal intensity in CVLM; activity in CVLM was higher for those periods in which MSNA was low. This relationship was expressed in four subjects, though the difference between the two conditions failed to reach statistical significance for one subject.

Fig. 3 near here

Time-series data, illustrating covariation of MSNA and signal intensity in RVLM and CVLM, are shown for two subjects in Fig. 4. In both subjects fluctutations in signal intensity within RVLM largely occurred in parallel with the fluctuations in MSNA. Conversely, fluctuations in signal inensity in CVLM essentially mirrored those occurring in the nerve signal.

Fig. 4 near here

DISCUSSION

By recording spontaneous MSNA concurrently with BOLD signal in the brainstem we aimed to identify the medullary baroreflex circuitry in human subjects and hence identify regions responsible for the generation of spontaneous muscle vasoconstrictor nerve activity. Based on animal work we predicted that a decrease in activity of the NTS would occur during spontaneous falls in arterial pressure, resulting in decreases in activity in CVLM and hence increases in RVLM. Our data demonstrate the feasibility of undertaking intraneural recordings from awake human subjects in a high-field MRI environment, and confirm the predicted changes in signal intensity in NTS, CVLM and RVLM.

Identification of the medullary nuclei responsible for the baroreflex in experimental animals

The fundamental circuitry responsible for the baroreflex has been examined over many decades in anaesthetized experimental animals. Early work had shown that excitation within circumscribed regions of the RVLM could induce increases in blood pressure and activation of vasoconstrictor neurons supplying skeletal muscle, while activation within other areas could induce increases in activity in cutaneous sympathetic neurons yet no increase in blood pressure[3,6,22]. Moreover, electrolytic destruction of neurons within RVLM was shown to cause a precipitous fall in blood pressure, arguing that ongoing activity within RVLM is essential for resting vasomotor tone[7]. It is known from intracellular recordings from RVLM neurons that they exhibit an irregular tonic firing that decreases with increases in blood pressure and increases with decreases in blood pressure; it is also known that this tonic activity is modified by excitatory and inhibitory inputs, but that in the absence of such inputs this tonic activity continues[8]. The current understanding of the baroreflex circuitry defined primarily in anaesthetised animal preparations, is that primary afferent axons from the baroreceptors project to the caudal region of the NTS where they synapse onto second-order neurons, which in turn send excitatory (glutamatergic) projections onto GABAregic neurons within

the region of the CVLM. These CVLM GABAergic neurons synapse directly onto excitatory neurons within the RVLM and serve to inhibit the spontaneous activity of RVLM premotor sympathetic neurons[1]. In addition to this basic baroreflex arc, it is known that other brain regions, such as the caudal pressor area, located in the most caudal part of the ventrolateral medulla, can also influence baroreflex activity by altering the activity in these baroreflex medullary nuclei[8]. Studies in conscious animals, using c-fos expression as a marker of neuronal activiation, have confirmed the operation of the NTS-CVLM-RVLM serial pathway during manouevres that increase or decrease arterial pressure[4,23].

Identification of medullary cardiovascular nuclei in human subjects

While fMRI in the anaesthetized cat has revealed changes in signal intensity within these same regions of the medulla[14,15], relatively few studies have applied fMRI to investigate the role of the brainstem in human cardiovascular control. One of the first studies to address this used the Valsalva manoeuvre, a forced expiratory effort that causes a sustained increase in muscle sympathetic nerve activity and blood pressure. Significant changes in BOLD signal intensity occurred in many areas of the brain, including the dorsal pons and medulla[12,13]. We recently showed that a maximal inspiratory breath-hold caused significant changes in BOLD signal intensity in three discrete regions of the medulla: robust increases in signal intensity occurred in the region of the RVLM and decreases in signal intensity occurred within the regions of the CVLM and NTS[21]. These signal intensity changes were expected, given that the increase in MSNA during this maneuver is believed to be due to unloading of the low-pressure baroreceptors[20,21]. In humans, the RVLM and CVLM are displaced dorsally by the large inferior olivary nuclei[2].

In the present study, we examined *spontaneous* fluctuations in muscle sympathetic outflow and documented the same changes within the medulla as we had previously identified during the

sustained increases in MSNA produced by a maximal inspiratory breath hold. Although investigations into brainstem sites involved in producing sustained changes in sympathetic drive are important, the changes in brainstem activity evoked by these challenges can be influenced by higher brain centres regulating functions such as arousal and attention. By measuring spontaneous fluctuations in MSNA we are circumventing these issues and can therefore measure a more accurate representation of moment-to-moment baroreflex activity. This is important, as it may be the case that in some autonomic diseases the performance of a particular maneuver may result in similar brain activity changes and similar changes in blood pressure and MSNA even though the underlying baroreceptor reflex arc may be significantly altered. The use of concurrent recordings of spontaneous MSNA and brainstem fMRI signal changes creates an opportunity to investigate the activity of human brainstem nuclei in baseline autonomic states in subjects with and without a range of autonomic conditions.

Methodological considerations:

We used an ON-OFF scanning protocol so that we could record MSNA during the 4 s period between each 4 s scan. This interval was chosen to take advantage of the temporal delays inherent in BOLD imaging. It is known that the microvascular responses to an increase in neuronal activity lag by some 5 s[19], and that we need to allow ~1 s for a muscle vasoconstrictor volley to travel from the brainstem to the peripheral recording site at the knee[9]. Accordingly, we reasoned that changes in BOLD signal intensity would reflect changes in neural activity associated with emission of sympathetic volleys recorded in the previous 4 s epoch.

There is convincing evidence that increases in BOLD signal intensity reflect the underlying electrical events within the brain[19], though whether this reflects neuronal firing or synaptic events is a matter of contention[25]. Moreover, there is controversy as to what a decrease in signal intensity

reflects, with different authors referring to these decreases as “inhibitions” or, more correctly, “deactivations,” though recent data indicates that both activation and deactivation patterns of BOLD signal intensity correlate well with neuronal glucose oxidation in glutamatergic neurons[18]. Based on our results, we would argue that a decrease in signal intensity, such as observed in NTS and CVLM, reflects a decrease in neuronal firing rather than synaptic events *per se*, given that the synaptic events within RVLM are related to the *active* release of the inhibitory neurotransmitter GABA. Finally, given that we could document spontaneous fluctuations in brainstem BOLD signal intensity that were time-locked to changes in muscle sympathetic nerve activity (and by inference, changes in blood pressure), we believe that much of the “baseline noise” in brainstem BOLD signal is simply a reflection of these underlying physiological processes.

CONCLUSIONS

We have demonstrated that it is possible to record muscle sympathetic nerve activity while undertaking fMRI of the brain. Moreover, we have demonstrated the operation of the fundamental medullary circuitry involved in the baroreflex and the generation of spontaneous muscle vasconstrictor drive.

METHODS

Nine experiments were performed on 7 subjects (6 male, 1 female), under approval of the Human Research Ethics Committee of the University of New South Wales. All experiments were conducted at the Clinical Research Imaging Centre at Prince of Wales Medical Research Institute (Achieva 3T, Philips Medical Systems, Netherlands). Subjects lay supine on an MRI bed in a laboratory. The left knee was supported on a foam block and the common peroneal nerve located at the fibular head by electrical stimulation through a surface probe (3-10 mA, 0.2 ms, 1 Hz; Stimulus Isolator, ADInstruments, Sydney, Australia). An insulated tungsten microelectrode (FHC, Maine,

USA) was inserted percutaneously into the nerve and manually guided into a muscle fascicle of the nerve while delivering weak electrical stimuli to evoke muscle twitches (0.01-1 mA, 0.2 ms, 1 Hz)). A nearby subdermal microelectrode, with 1 mm insulation removed, served as the reference electrode and a surface AgAgCl electrode on the leg as the ground electrode. Once a muscle fascicle had been entered neural activity was amplified (gain 10^4, bandpass 0.3-5.0 kHz) using a low-noise, electrically isolated, headstage (NeuroAmpEX, ADInstruments, Australia). The innervation territory of the muscle fascicle was identified by tapping over the muscle belly or relevant tendon, and the position of the microelectrode tip manually adjusted until spontaneous bursts of muscle sympathetic nerve activity (MSNA) were identified. Because the amplifier headstage is encased in stainless steel, and contains no iron components, it was intrinsically safe to operate within the high-field MRI environment. Pilot experiments confirmed that the amplifier does not block during the application of the high-frequency radio frequency (RF) pulses during the scanning sequences, and that the frequencies of interest within a nerve recording lie within the RF band and therefore cannot be retained by RF filters. Neural activity was acquired, RMS-processed (200 ms) and analysed on computer (Chart 5, PowerLab 25T; ADInstruments, Australia). A high-pass digital filter at 300 Hz was applied to the recorded signal to remove artifacts picked up by the cable from the headstage to the amplifier.

The subject's head was enclosed in an 8 channel SENSE head coil and stabilised with foam pads to minimise head movement. Headphones were provided to minimise noise and to allow communication with the subject. A continuous series of 200 gradient echo echo-planar imagies, sensitive to Blood Oxygen Level Dependent (BOLD) contrast and encompassing the entire brainstem were collected over a 27 minute period (46 axial slices, TR=8 s, TE=40 ms, flip angle=90 deg, raw voxel size =1.5 mm^3). All 46 axial slices were collected sequentially from caudal to rostral during the first 4 seconds of the 8 second TR . Sections of the MSNA recordings that contained the scanning artifacts were removed and the remaining data, which contained unaffected MSNA

signals, were spliced together. Mean voltage levels of the RMS nerve signal were measured every second during each 4 s inter-scan period.

Functional images were motion corrected and a brainstem template created from the functional image set of one subject. All subjects' images were then normalized to this template, intensity normalized to remove slow signal intensity drifts and smoothed using a 3mm FWHM Gaussian filter. Changes in fMRI signal intensity were assessed during the subsequent 4 s period to take into account the ~5 s neurovascular coupling delay and the ~1 s required for conduction of the sympathetic bursts from the brainstem to the peripheral recording site (see Discussion). As we were interested in defining medullary regions responsible for baroreflex regulation, correlations between MSNA during the first second of the subsequent 4 second recording period and BOLD signal changes in medullary slices, which were collected during the first second of the brainstem image collection period, were made. Medullary regions correlated to MSNA were then determined for all 7 trials and overlaid onto an individual subject's functional image set (fixed effects, $p<0.05$, corrected for multiple comparisons, medulla only mask). In addition, signal changes correlated to MSNA were assessed in individual subjects (uncorrected $p>0.005$). For significantly correlated clusters in individual subjects, the mean signal intensity during the 25 volumes corresponding to the highest MSNA was compared to the mean signal intensity during the 25 volumes corresponding to the lowest MSNA (2 sample t-test, $p<0.05$). Finally, the signal intensity changes of these clusters were plotted over time.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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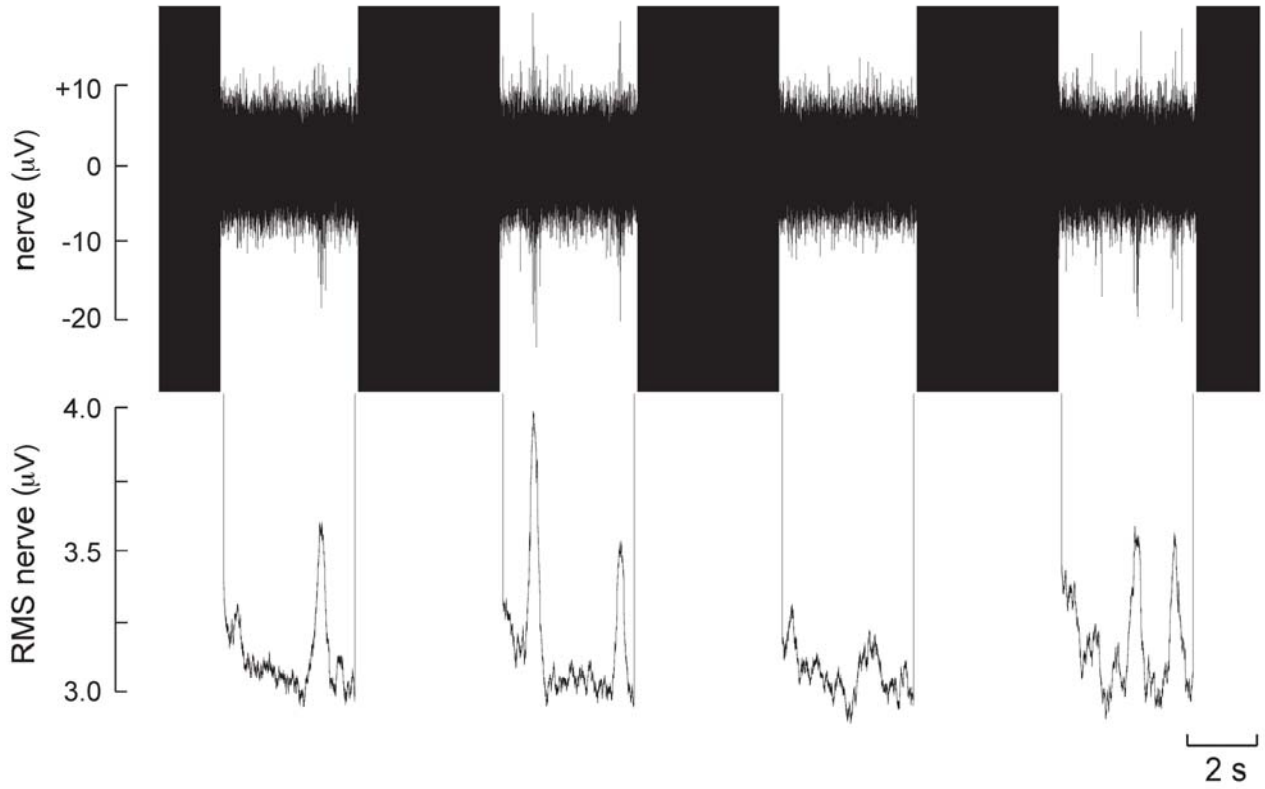


Figure 1: Spontaneous MSNA recorded during scanning. Filtered data are shown in the top trace, RMS-processed data in the lower trace. The dense areas are scanning artifacts.

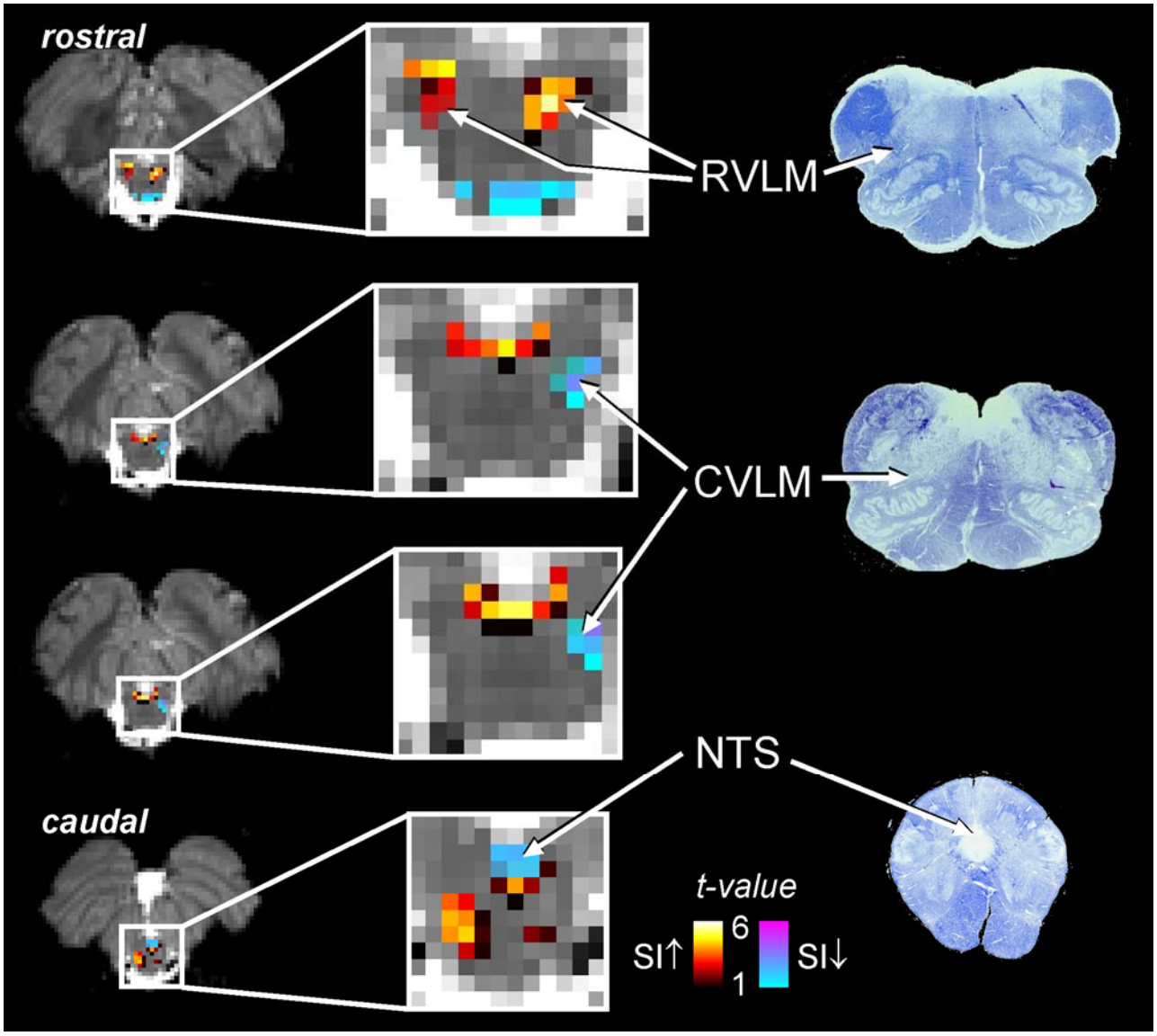


Figure 2: Signal intensity changes positively (warm colour scale) and negatively (cool colour scale) correlated with MSNA total burst activity in the resting state overlaid onto an individual subjects fMRI image set. Group data from 7 experiments. Each fMRI voxel is 1.5 x 1.5 x 1.5 mm. Equivalent myelin-stained histological sections are shown on the right. RVLM, rostral ventrolateral medulla; CVLM, caudal ventrolateral medulla; NTS, nucleus tractus solitarius (solitary tract nucleus); SI, signal intensity.

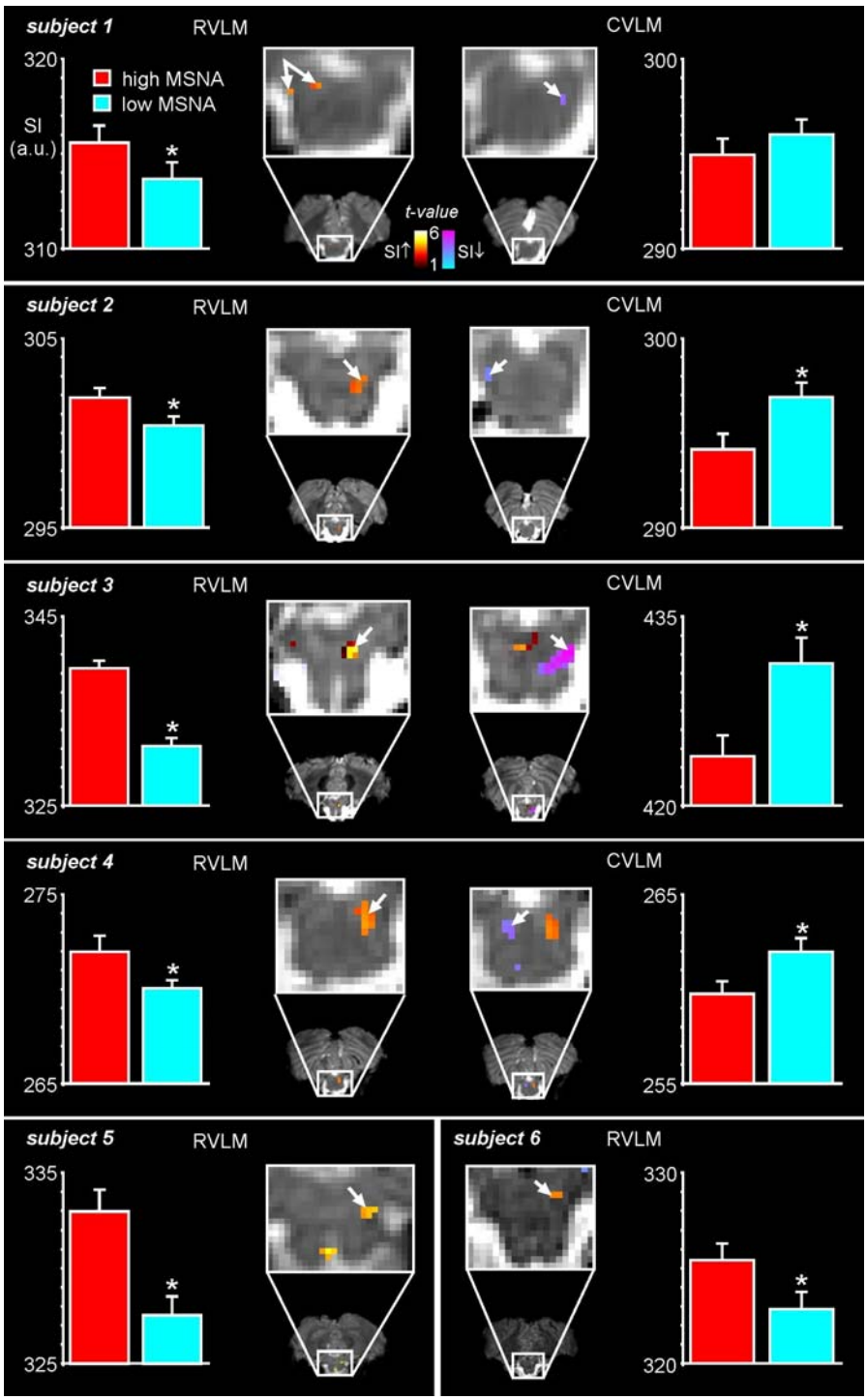


Figure 3: Changes in signal intensity within rostral ventrolateral medulla (RVLM) and caudal ventrolateral medulla (CVLM) after dividing the data into periods associated with high muscle sympathetic nerve activity (MSNA) (red) and low MSNA (blue). Regions in which signal intensity is positively (hot color scale) and negatively (cool color scale) correlated to MSNA, overlaid onto functional MR images are shown in the left of each panel. Data from all subjects show that the increases in signal intensity within RVLM were significantly higher when MSNA

was high. Data from subjects 1-4 show that the *decrease* in signal intensity in CVLM was smaller when MSNA was high, though this failed to reach statistical significance for subject 1. SI, signal intensity. * represent a significant difference in signal intensity during high and low MSNA conditions.

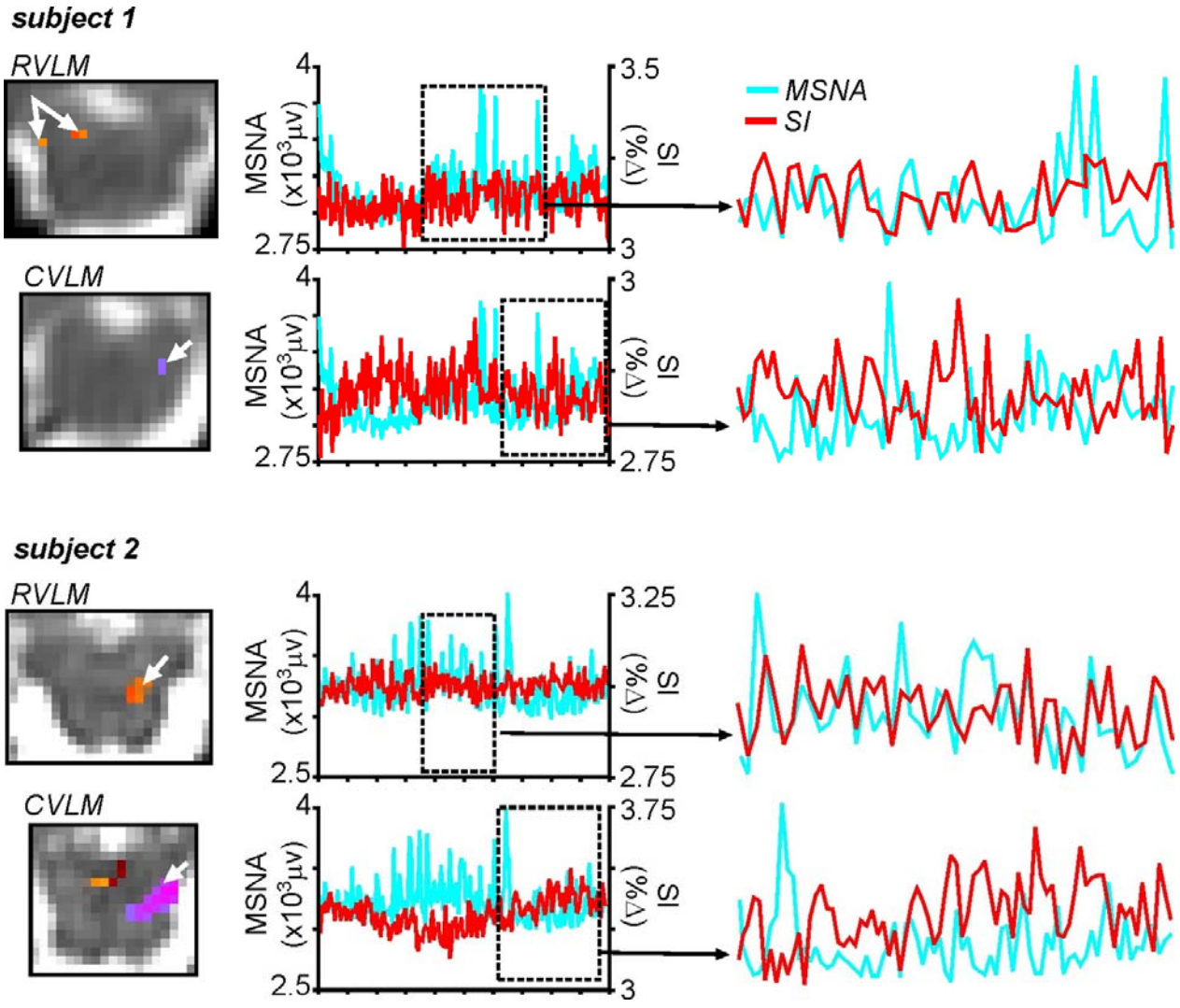
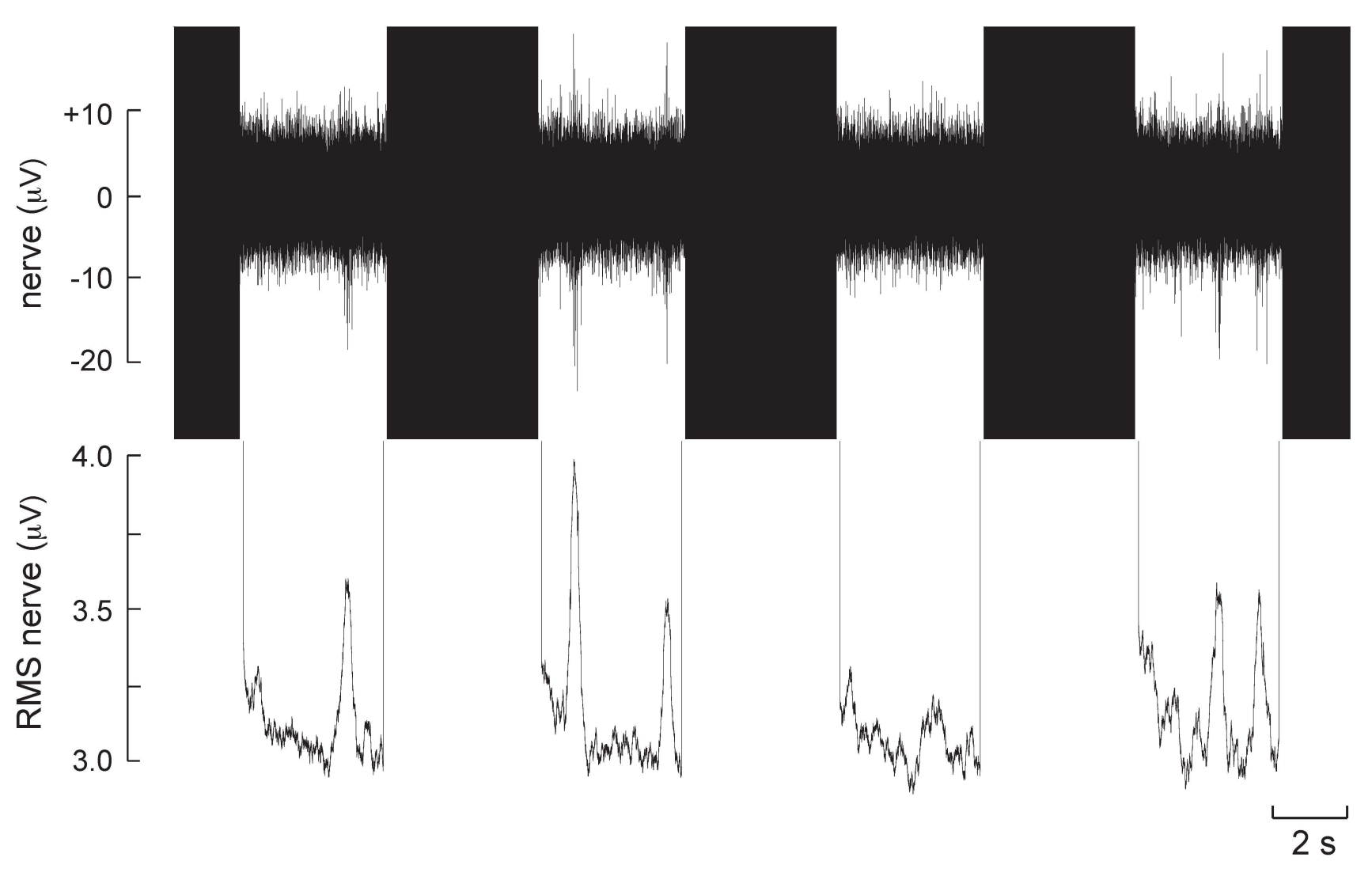


Figure 4: Covariation of signal intensity within rostral ventrolateral medulla (RVLM) and muscle sympathetic nerve activity (MSNA) and caudal ventrolateral medulla (CVLM) and MSNA in two subjects. Regions in which signal intensity is positively (hot color scale) and negatively (cool color scale) correlated to MSNA, overlaid onto functional MR images are shown to the left and the changes in signal intensity within these regions to the right. In both subjects, the fluctuations

in MSNA and signal intensity in RVLM occurred essentially in parallel, while fluctuations in MSNA and signal intensity in CVLM essentially mirrored each other. Expanded sections are shown in the far right.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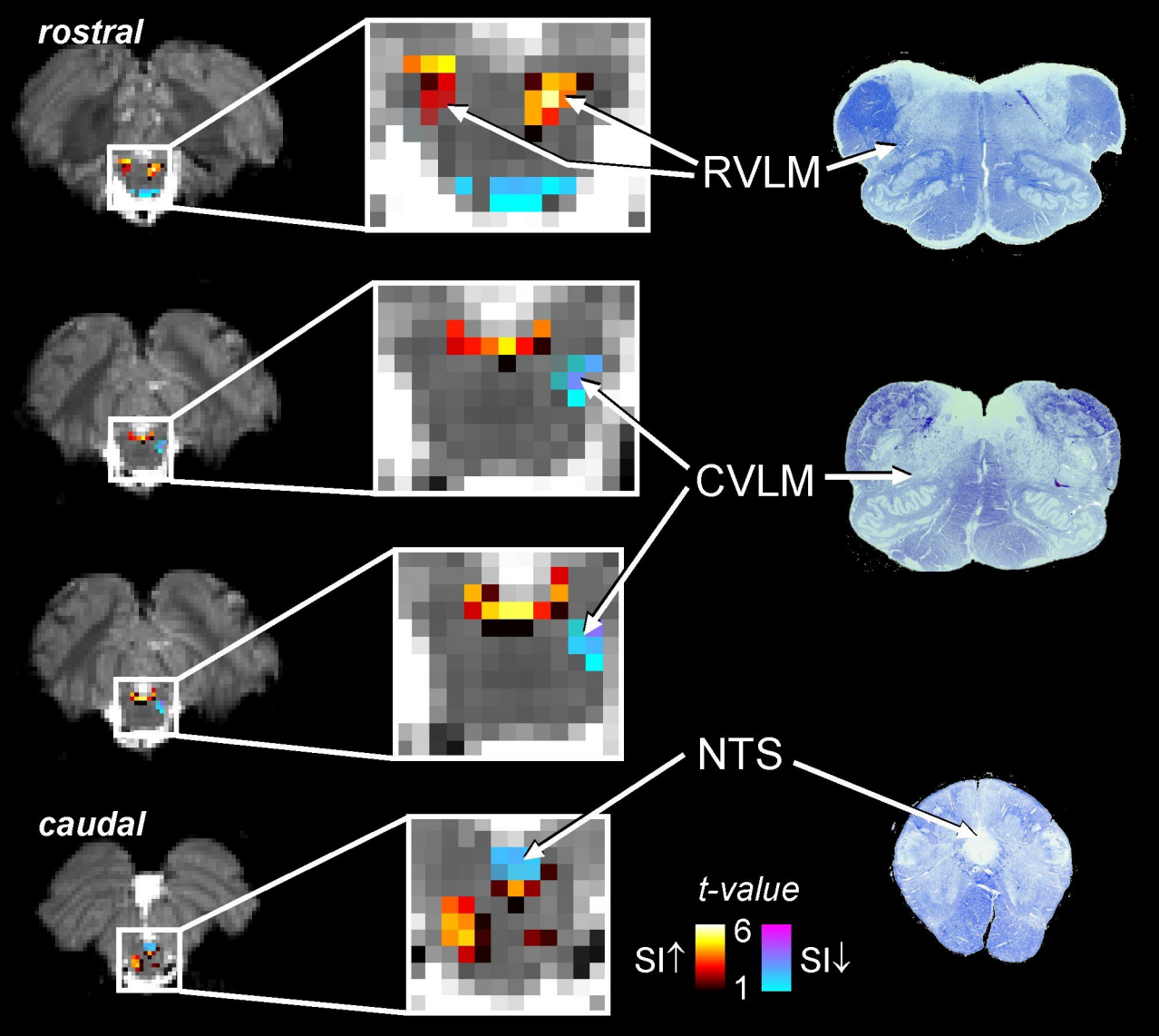
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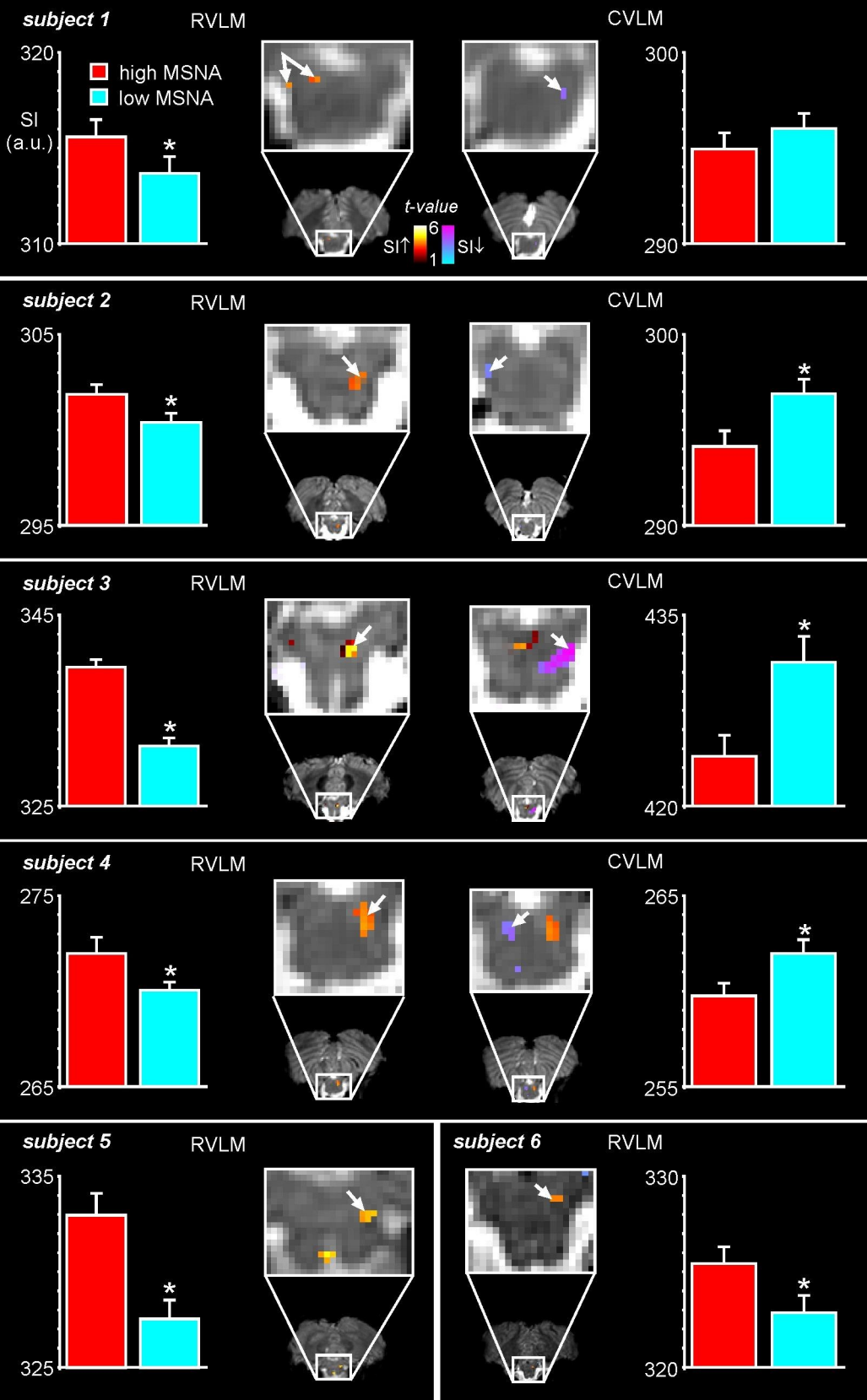
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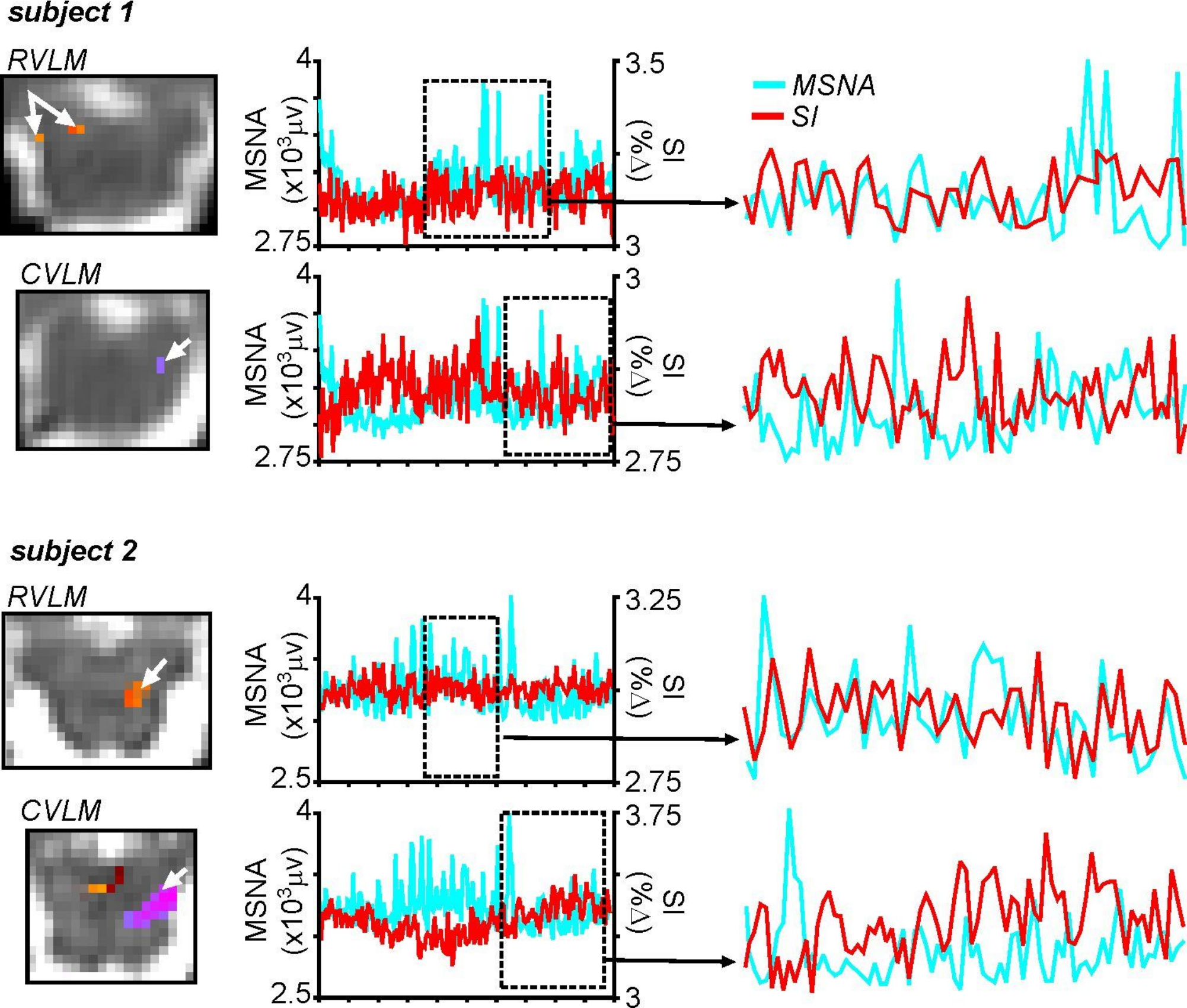
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