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Late-Developing Rostral Ventrolateral Medullary Surface Responses to Cardiovascular Challenges During Sleep

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Abstract

Pressor and depressor manipulations are usually followed by compensatory autonomic, respiratory, somatomotor or arousal responses that limit the extent of blood pressure change. Of neural sites participating in blood pressure control, the rostral ventrolateral medullary surface (RVLMS) contributes significantly, and exhibits rapid-onset overall activity declines and increases to pressor and depressor challenges, respectively. In addition, longer-latency physiological responses develop that further compensate for the homeostatic challenge; some of these later influences are associated with arousal. Late-developing RVLMS activity changes accompanying physiologic responses that normalize a cardiovascular manipulation may provide insights into compensatory neural mechanisms during sleep following sustained or extreme blood pressure changes. We used intrinsic optical imaging procedures in seven unanesthetized adult cats to examine RVLMS and control site responses during sleep to pressor and depressor challenges during sleep that resulted in somatomotor, respiratory, heart rate or electroencephalographic indications of late-developing (post-baroreflex) compensatory responses. Although initial RVLMS responses differed in direction between pressor and depressor challenges, neural activity increased later in both manipulations, coincident with overt physiological manifestations indicative of compensatory responses, including arousal. Arousal occurred in 44% of blood pressure challenges. Comparable late-developing neural activity increases were not apparent in control sites. Latencies of late RVLMS responses during rapid eye movement sleep were significantly longer than in quiet sleep for pressor challenges. The pattern of the late RVLMS responses was not

dependent on arousal, and suggests that the RVLMS participates in both the early baroreflex response and the late-developing compensatory actions.

Theme: Endocrine and Autonomic Regulation

Topic: Cardiovascular Regulation

Keywords: optical imaging, arousal, sympathetic, blood pressure, baroreflex

Introduction

Marked changes in blood pressure are typically associated with compensatory physiological responses that serve to limit the extent of blood pressure elevation or lowering to maintain homeostasis. Of neural sites involved in mediating compensatory action, the rostral ventrolateral medullary surface (RVLMS) plays a significant role. Cells close to the surface serve as sympathetic pre-motor (bulbo-spinal) neurons [3] and contribute to sympathetic adjustments to blood pressure changes [5,9,11] , as well as peripheral and central chemoreceptor activation [2,10,23,34,37]. RVLMS neural activity is also modulated by sleep/wake state [33]. Pressor and depressor blood pressure challenges evoke significant rapid-onset lowering and elevation in overall RVLMS activity, respectively, presumably an expression of the baroreflex, and these changes are also modulated by sleep/wake state [12,14,31]. Compensatory responses during sleep are important because failure to recover from momentary changes in blood pressure has been implicated in problematic events and a number of clinical disorders [32,39].

Moderate-to-large changes in blood pressure are typically followed by alterations in heart rate, breathing or somatomotor activity to maintain perfusion in hypotension or limit extreme hypertension; respiratory pattern changes include depression of diaphragmatic and upper airway patterning to pressor challenges [25,38], and enhancement of breathing rates to blood pressure declines [29]. Somatomotor activation can include marked contraction of the peripheral musculature as a mechanism to maintain blood pressure levels [14]. Blood pressure manipulations can also, after a few seconds, lead to activation of the electroencephalogram (EEG) and arousal, presumably recruiting more-rostral brain areas to assist in stabilization of blood delivery, or enhancing the

responsiveness of mechanisms that compensate for a perceived error signal between baroreceptor input and cardiovascular or respiratory output [7,16,18,20]. Furthermore, a later-developing compensatory response which may or may not accompany arousal can act to further modulate changes in blood pressure or other cardiopulmonary changes [6,18,43]. These late-developing responses are not found in the anesthetized preparation [13].

Expression of late-developing physiological responses to blood pressure manipulation requires several seconds, and the neural mechanisms involved in those compensatory efforts are unknown. Common features of these late responses are sudden increases in heart rate and blood pressure, regardless of the presence or absence of accompanying EEG arousal [6,18,43]. The RVLMS may contribute to those late-emerging responses, just as the early RVLMS neural responses participate in the baroreflex action to the challenge. Evaluation of participation and time course of RVLMS activity changes in restoration of homeostasis would be useful in determination of mechanisms responsible for that restoration, particularly those responses necessary for recovery from severe challenges during sleep which typically require longer-term compensatory responses. We therefore evaluated late-developing responses in RVLMS activity to pressor and depressor challenges using intrinsic optical imaging procedures during both quiet sleep (QS) and rapid-eye-movement sleep (REM).

Materials and Methods

Studies were conducted on seven unanesthetized, freely-moving adult cats (3-4 kg) using intrinsic optical imaging to assess RVLMS and control site neural activities.

These same animals were the subjects of other investigations which have been previously published [14,23,31,33,34]. Each cat was anesthetized with sodium pentobarbital [25 mg/kg, intravenously (IV)] for aseptic surgical implantation of the recording devices and maintained throughout the surgery with supplemental administration (20 mg/kg, IV), as needed. Respiratory activity was recorded with two sets of multi-stranded stainless-steel insulated diaphragmatic electromyographic (EMG) leads. In each cat, an abdominal incision was made and the leads were placed in that side of the costal diaphragm. Neck EMG leads were placed in the dorsal cervical musculature to monitor postural muscle activity and stainless steel screws were implanted in the dorsal cranium to record EEG and eye movement; these signals were used for sleep-state scoring [41]. The electrocardiogram (ECG) waveform was recorded between a diaphragmatic and a neck EMG lead. One jugular vein and one carotid artery were cannulated to administer drugs and to record arterial blood pressure, respectively. Post-operatively, antibiotics (Bicillin 1cc, intra-muscular [IM]); Chloramphenicol 50 mg/kg, IM; neomycin/polymyxin B, topically) and anti-inflammatory steroids (Dexamethosone 0.4 cc, IV) were administered daily for 4-6 days. An analgesic (buprenorphine HCL 0.01 mg/kg, IM) was administered for the first two days after surgery. At the end of each study the cats were euthanized with an overdose of anesthesia. All procedures were approved by the UCLA Institutional Animal Care and Use Committee.

The optical imaging device consisted of a coherent fiber-optic conduit attached to a Charge-Coupled Device (CCD) which was equipped with two narrow bandwidth light emitting diodes (LEDs) to illuminate the RVLMS. The conduit had a diameter of 3 mm (field of view = ~7.1 mm^2) and was constructed of approximately ten-thousand 12 μm

glass fibers encased in glass cladding. Illumination fibers from the two LEDs were attached around the periphery of the conduit. Two different wavelengths of light were used to illuminate the RVLMS, one at 660 $\pm$ 10 nm (red) and the second at 560 $\pm$ 10 nm (green). Red light was used to assess changes in neural activity, since this wavelength is optimal for detecting neural membrane conformational changes that occur with depolarization [24,30]. Green light was used to detect changes in hemoglobin/deoxyhemoglobin ratios, thereby producing an index of perfusion of the tissue. Green signals in conjunction with the red signals were used to identify potential movement or other artifact in the images. Illumination at the two wavelengths was alternated at 100 Hz, *i.e.*, 50 periods of red illumination/s interleaved with 50 periods of green illumination/s. For each illumination period, an 80 x 80 pixel grayscale intensity image was recorded.

The optical device was placed on the RVLMS using a ventral approach involving a midline incision and retraction of the trachea and esophagus. The ventral surface of the skull was exposed between the foramen magnum caudally, the anterior edge of the tympanic bulla rostrally, the midline, and the medial edge of the tympanic bulla laterally. A small opening that was sufficiently large to accommodate the camera conduit was drilled through the cranium (sparing the *dura mater*). The adjacent bulla was opened to allow filling with dental cement to anchor the camera. The camera was then cemented in place with the tip of the conduit resting on the dura (scattered light readily penetrates the dura from neural tissue). The camera cable, vascular cannulae and electrophysiological leads were routed subcutaneously to a headcap on the superior surface of the cranium. The animal was allowed to recover for one week before data acquisition to avoid

anesthetic effects. All data were derived from unanesthetized, un-restrained animals. At the end of each study, immediately after euthanasia, the camera was removed from the cranium. The dura mater was excised and the brain surface underlying the light conduit was marked with ink. The brain was then removed from the cranium and the camera field-of-view (indicated by ink) was evaluated for position by using surface landmarks of the exit of the 6^{th} and 12^{th} cranial nerves and midline.

Multiple recording sessions were conducted on each cat across several weeks, until the optic probe deteriorated or until the data signals degraded beyond usefulness. Initial analyses included sleep/wake stage scoring of each entire record [41], derivation of heart rate from R-wave peak detection on the ECG waveform, integration of the diaphragmatic EMG to achieve a respiratory waveform and determination of breathing rate from peak detection of the respiratory waveform.

Experimental sessions consisted of an initial recording without manipulation or challenge to gather baseline sleep/wake data and RVLMS activity characteristics. Two cardiovascular challenges were used, 20-30 μg phenylephrine (PE; pressor challenge), and 30-40 μg sodium nitroprusside (NP; depressor challenge). Challenges were administered in each of the sleep/wake states: quiet waking, QS and REM sleep; only challenges delivered in sleep are reported here. Phenylephrine and sodium nitroprusside injections were administered IV (< 1 cc) through tubing that extended into the recording chamber, and were followed with saline flush (~2 cc). The effectiveness of those challenges was assessed by evaluation of blood pressure, heart rate and respiratory patterns. Sham delivery of 3 cc saline was administered to control for effects of bolus IV

injections. Challenges that did not evoke blood pressure changes were not used for analysis. A recovery period of at least 5 minutes was allowed between challenges.

Several seconds after drug effect and baroreceptor reflex onset, additional compensatory changes in heart rate and blood pressure were noted. The onset of the late-developing response was identified by an abrupt increase in heart rate, subsequent to the initial baroreflex-mediated heart rate effects of a drug (> 5 s), as in other studies [6,18,43]. These late-developing events were examined for effects on RVLMS activity and blood pressure. The events were also sub-divided into those with and without accompanying EEG arousal for additional analysis. Cortical arousal was defined as a state change to waking using standard criteria [41]. The criteria included increased EEG desynchronization and heightened EMG activity from QS levels or increased EMG activity during REM sleep, with elevation sustained for $\geq$ 30 s.

The signals from red illumination were averaged for all pixels in each frame, producing a single value for each frame, thereby indexing overall neural activity on the region of the RVLMS under the probe. These RVLMS activity traces were then tagged with the onset times of the late-developing increases in heart rate resulting from pressor or depressor challenges. The RVLMS traces were grouped by initial sleep state and type of drug challenge and then aligned by the onset tags. The RVLMS traces were acquired as relative intensity (0 – 4095; 12-bit resolution) and were normalized as percent change from the baseline period.

In addition, reflected and refracted light, collected by the CCD, was analyzed with ANOVA on a pixel-by-pixel basis to view individual or averaged frames. For every challenge, data from a baseline period of 15-20 s were collected immediately prior to

pharmacologic administration and averaged. Post-baseline frames were averaged over contiguous 1 s windows (50 frames/s) during challenges and compared to the averaged baseline image. ANOVA significance levels were set at $p < 0.05$; pixel values reaching that level were color-coded by extent of change, with green pixels representing no significant change from baseline, yellow-orange-red-white colors indicating progressively larger increase in activity, and blue-violet-black colors indicating progressively larger declines in activity. Alterations in physiological variables including blood pressure, heart rate and event latencies were statistically assessed with non-parametric procedures because of non-normal distributions of the data; statistical significance was assigned when $p < 0.05$. Un-paired data were assessed with the Mann-Whitney test while paired data were analyzed with the Wilcoxon-Signed Ranks test (SPSS; Chicago, IL). Both blood pressure and heart rate were evaluated as percent changes from the baseline period. Differences in red signal intensities between specific time points were also evaluated with these non-parametric tests.

Results

Of the seven cats studied, camera placements in five were in the RVLMS. The other two placements were on the caudal lateral medulla and the caudal pons, and were used as control sites.

Fifty-eight phenylephrine (PE) and 49 sodium nitroprusside (NP) challenges were administered across the seven cats during QS and REM. Challenges were approximately evenly distributed across experimental cats. Challenges in the five experimental cats are categorized by sleep state in Table 1. For each category in the Table, data were not

included if: arousals occurred prior to drug administration or before the drug-induced changes in arterial pressure, unconsolidated sleep stages were present, drugs exerted no effects on arterial pressure, arousals were transient (< 30 s), or arousal-related movement artifacts occurred. Several seconds after baroreceptor reflex onset, heart rate increased abruptly, and RVLMS neural activity increased concurrently. This increase in neural activity occurred regardless of whether the initial RVLMS response to blood pressure manipulation was a fall (pressor) or an increase (depressor). These late-developing responses exerted substantial influences on blood pressure and occurred whether or not the challenges elicited an EEG arousal. These post-baroreflex changes followed all PE challenges during QS and REM; for NP challenges, these changes occurred in all trials in REM sleep and all but one (22 of 23) trial in QS.

Responses to pressor challenges

The initial neural response in the RVLMS to the pressor challenge in both QS and REM was a significant decrease in activity, as previously reported [31]. This decrease was typically found over the entire field of view in all camera locations over the RVLMS. A later-developing physiological response emerged from both QS and REM and was accompanied by significant RVLMS activation (see below). An example of RVLMS and physiological responses to a pressor challenge in QS which was not followed by an EEG arousal is shown in Figure 1. The traces in Figure 1B illustrate the baroreflex-mediated decline in heart rate and then an abrupt increase in heart rate in spite of the continuing pressor response, (*i.e.,* reversal of the baroreflex-mediated bradycardia), and also show two augmented breaths. A small *further* increase in blood pressure can be seen just after 0 s (the onset of the late-developing response). Panels in Figure 1A show RVLMS

images from selected time points in the response. Figure 2 shows averaged traces of RVLMS responses in QS (A and C) and REM (B and D), aligned by the onsets of the late-developing component (pressor challenges in A and B; depressor responses in C and D). Regardless of sleep state or the presence of arousal, RVLMS activation occurred at or near the onset of the late-developing response. No significant differences in magnitude of RVLMS activation occurred between the arousal categories or sleep states, although differences often emerged in subregions in the field-of-view.

Changes in RVLMS activity from baseline were assessed at sequential times around the onset of the late-developing response: -5, 0, +5, +10, +20, and +30 s. In both sleep states and regardless of accompanying arousal, higher neural activity developed as the late response progressed. However, only the RVLMS changes at the later time points significantly differed from values before the response onset (see Fig. 2). In QS, activity at +20 s and +30 s was significantly higher than activity at -5 s for both arousal and non-arousal trials. In REM, activity was higher at +30 s (*vs.* -5 s), both with and without accompanying arousal.

The topography of RVLMS activation/deactivation during the late-developing response following hypertension exhibited two phases. The early phase, beginning at the onset of the late-developing event, was characterized by a complex topography of neural activation, deactivation and no change. Subregions of activation and deactivation had relatively static loci, and the activity pattern expanded and contracted from those distinct subregions. The second phase of RVLMS responses emerged abruptly from the first phase and was characterized by stronger activation across the entire field-of-view. This

pattern appeared in both sleep stages and arousal categories, and is shown in the panels of Figure 1A.

Neural activity responses to a pressor challenge in the two control sites were often characterized by a small area of relatively modest activation that appeared at various times in the post-injection period. These activity changes did not show temporal relationships with the onset of late-developing responses, and activation in the control sites never covered the entire field-of-view.

Responses to depressor challenges

Late RVLMS responses to depressor challenges consisted of a further intensification of activation initiated by the baroreflex. These late patterns were qualitatively similar to those elicited by pressor challenges. An example of the responses to a depressor challenge in QS is shown in Figure 3. Late response-mediated heart rate was slightly higher than the early baroreceptor-mediated heart rate. Initial RVLMS responses to NP-induced depressor challenges were more varied than those for pressor challenges. In QS, the late-developing response induced a further neural activity increase in all animals, such that activation spread over the entire area late in the response (Fig. 3A). This increased activity occurred whether or not EEG activation occurred. At response onset, a transitional period emerged. In this period, both activated and deactivated regions co-existed (as with the pressor challenges), or an isolated short period of deactivation occurred or rapid (1-2 s) alternation between deactivation and activation was observed. During both sleep states, RVLMS neural activity significantly increased during the late-developing responses compared with just prior to those events, as shown in Figure 2 C and D. These differences reached significance at +10 s and +20 s during

QS trials and at 0 s, +20 s and +30 s in REM trials (*vs.* -5 s). No significant differences emerged related to sleep state or the presence of arousal.

Neural activity responses to NP in the control sites consisted of activation in a portion of the field-of-view. As with control pressor responses, activation was not temporally related to the late-developing response. Figure 4 illustrates the camera signal from the control site in the caudal lateral medulla, with physiological changes, to an NP trial in QS that elicited an EEG arousal. In this case, deactivation occurred well after the onset of the late-developing response (as opposed to activation in RVLMS sites), as is shown in the panels of Figure 4A.

Blood pressure modification by the late-developing response

Before the onset of the late-developing response, blood pressure increases to PE were greater in REM trials than in QS trials for those challenges that did not subsequently elicit an arousal (-15 s, $p < 0.05$; -10 s, $p < 0.05$). Drug-induced blood pressure changes were reversed concurrently with the late-developing RVLMS patterns of activity and increases in heart rate, as shown in Figure 5. Development of the late-developing responses following PE was characterized by a significant reduction in the magnitude of the elevated blood pressure (Fig. 5B). Blood pressure peaked at time 0 (or +5 s for REM trials not eliciting an arousal) and then fell significantly (beginning at +10 s) from peak values towards baseline, except for pressor challenges in QS that elicited arousals. For that subgroup, blood pressure did not significantly change after the onset of the late-developing response (Fig. 5A).

For the NP challenges, the main effect of the late-developing response was to reverse the fall in pressure (see Fig. 5). Regardless of sleep state or the presence of

arousal, the nadir in blood pressure occurred at time 0. Subsequently, blood pressure significantly increased after time 0 for QS and after +20 s for REM, returning toward baseline (Fig. 5C and D). No state-related or arousal-related significant differences were detected.

Latency to onset of late-developing response

Latencies to the late-developing response, computed from the onset of the drug-induced change in blood pressure to the onset of the late-developing compensatory response, were significantly longer in REM than QS for pressor trials only, and in those trials not associated with arousal, where REM > QS (25.4 s $\pm$ 6.2 *vs.* 6.5 s $\pm$ 0.9, respectively; Fig. 6).

Discussion

Late-developing responses to changes in blood pressure exerted a significant activating influence on the rostral ventrolateral medullary surface (RVLMS), a pattern which was opposite to the immediate RVLMS response to a pressor challenge and which accentuated the RVLMS response to a depressor challenge. This activity pattern was not apparent in control recordings from nearby sites. In contrast to the opposite effects of pressor and depressor trials on initial RVLMS activity, the late-developing responses increased RVLMS activity in a remarkably similar fashion, suggesting a different role for the RVLMS during these late responses. We speculate that the neural activation during the late response accounts for part of the associated sympathetic effects of those events, and suggests that the RVLMS plays a role in both the classic baroreflex and the longer-latency actions compensating for altered blood pressure.

We previously described early RVLMS activity changes to blood pressure challenges that were not accompanied by EEG arousal responses [31], and showed that the most prominent initial falls in activity occurred in more rostral camera placements to pressor trials in QS. When trials with arousal were included, the initial activity fall was prominent at all camera locations. Furthermore, inclusion of trails with arousal showed that: 1) the late-developing response exerted a powerful modulating effect on blood pressure, 2) the RVLMS responses were temporally correlated with the abrupt increase in heart rate, 3) the late-response was physiologically distinct from the initial baroreflex response, 4) the late-developing response was largely independent of any accompanying EEG arousal, sleep state, or type of blood pressure challenge, and 5) late RVLMS activation occurred regardless of the direction of initial changes in neural activity. The inclusion of trials with arousal in the current study involved more detailed analysis of cardiopulmonary variables and demonstrated that the late-developing response did not constitute the classic baroreflex, since heart rate increased during hypertension (in the PE trials). Finally, the late-developing response was more effective than the initial baroreflex in returning blood pressure towards baseline conditions.

Late-developing responses as a standard component of blood pressure homeostasis

Although some of the blood pressure responses were relatively modest (< 10 %), nearly all manipulations resulted in a late response that increased RVLMS activity, increased heart rate, and returned blood pressure towards baseline. The incidence of waking from sleep accompanying these late responses ranged from half of the trials (REM-PE) to nearly all (REM-NP), as shown in Table 1. A comparable incidence of EEG arousal to PE challenges has been described by Kesler *et al.* [18]. In addition,

Horne *et al.* [15] reported a similar rate of cortical arousal to both hypo- and hypertensive challenges during sleep in neonatal lambs.

Studies in sleeping humans and animals suggest that a secondary, late response is the normal result of cardiovascular challenge, and is only loosely associated with EEG arousal. Most apnea in infants (~ 60%) and children (> 90%) are terminated without an EEG arousal, but are accompanied by increased heart rate [27]. Adults with sleep apnea/hypopnea syndrome can also resolve respiratory events without an EEG arousal, and those events which are terminated without conventionally defined arousals are always accompanied by an increase in blood pressure and, for some, an increase in EMG activity [32]. Other stimuli have similar effects; in one study, nearly half of vibrational and auditory stimuli in sleeping adult humans were not followed by EEG arousal, but resulted in increased blood pressure and heart rate [6]. These results are consistent with the proposal that long-latency secondary responses are a normal component of blood pressure homeostasis. It is unclear how EEG arousal is related to these late-developing adjustments, with the single exception of pressor trials during QS that elicit arousal, where the increases in mean arterial pressure were higher than the same trials not associated with arousal.

Late-developing responses are unique to un-anesthetized preparations and the RVLMS

The present data provide insights into how late-developing responses may compensate for sustained changes in blood pressure and affect the baroreflex by actions on the RVLMS during sleep. The effectiveness of the late component is particularly apparent when these data from intact, sleeping cats are compared to the same challenges in anesthetized animals. Pressor trials in the anesthetized preparation lower RVLMS

activity for several minutes and depressor trials increase neural activity, also for long periods [12]. Therefore, unlike the initial baroreceptor RVLMS responses, later-developing responses are not found in the anesthetized preparation. The rise and fall in RVLMS activity during depressor and pressor trials with anesthesia, respectively, are similar to those initial changes reported here. These effects likely represent the influence of the baroreceptor reflex on RVLMS activity, since complete baro-denervation abolishes the RLVMS responses to NP and PE [*ibid*]. Late-developing responses modified the ongoing baroreceptor-related RVLMS responses by increasing activation. This RVLMS neural activation is presumably translated into sympathetic activation, accounting for at least part of the increase in heart rate and modulation of drug effects on blood pressure. The differences between anesthetized and un-anesthetized challenges also indicate that the changes reported here result from an active process (*e.g.,* an additional component to the mechanism of blood pressure control) and not to the normal extinction of drug effects.

Two control sites near, but outside, the RVLMS were also examined. In both cases, activation patterns were occasionally present, but were not similar to the patterns of RVLMS activation (Fig. 5). In addition, autonomic changes related to the late-developing responses were temporally linked to RVLMS activation, but not to control site activity changes. These differences in control site responses suggest that the RVLMS activity patterns that accompany the late responses are specific to this area, and not a generalized activation of all brain structures or a recording artifact.

Manipulation of blood pressure in the intact preparation affects many variables in addition to heart rate; respiration, arousal and hormonal outflow are all altered, and

impact RVLMS activity. However, blood pressure manipulation was followed by specific and repeatable RVLMS and heart rate changes, providing a reasonable basis to assume that the activity changes responded to the altered systemic arterial pressure. The non-specificity of arousal, respiratory, and tonic EMG results also suggest, by default, that blood pressure-induced changes were responsible for the RVLMS activation during the late-developing response. Other changes, not monitored in this study, may have been present and may have impacted RVLMS activity (*e.g.*, altered levels of Angiotensin II). The mechanisms associated with the late-developing responses and increases in RVLMS activity which returns blood pressure towards baseline levels are unclear.

Functional anatomy of the imaged RVLMS areas

Several types of baro-sensitive and/or cardiovascular-related neurons exist close to the ventral medullary surface (< 400 μm), including bulbospinal, presumably sympathetic pre-motor, neurons [3,10,36]. Many of the superficial bulbospinal neurons belong to the C1 cell group, although not all bulbo-spinal or baro-sensitive RVLM cells belong to C1, nor are all adrenergic [22,36]. The RVLM surface also contains glial cells and processes [40]; both glia and processes may also contribute to the optical images reported here [24].

Topography and time course of the late RVLMS response

RVLMS activation during the late-developing responses generally occurred in two phases, distinguished by the topography of neural activity under the probe. In the early phase, activated regions were intermingled with deactivated regions, while the later phase was characterized by more intense and widespread neural activation, *i.e.*, already activated neurons became more activated as did initially deactivated and unaffected

neurons. This pattern was similar between late responses associated with and without EEG arousals, and between sleep states. The early, sub-divided phase may represent a "ramping up" of the response in which the late-developing influence competes with the baroreceptor reflex for control of sympathetic premotor neurons. The later phase would then reflect the dominance of the second component on RVLMS neural activity. Since blood pressure remained affected by the drugs (albeit to a lesser extent during the late response), baroreceptors were still functioning and presumably sending information to the RVLMS. This competition was most distinct in the pressor response, where the hypertensive baroreflex and the late-developing response exerted inverse effects on RVLMS activity (and heart rate); late-developing effects to a depressor challenge followed the same sequence of competition and subsequent homogenous activation.

Longer latencies to the late-developing responses in REM, compared with QS, occurred only in pressor trials and only in the sub-group not associated with arousal. Longer latencies to EEG arousal from REM, compared to non-REM sleep, were found by others following manipulation of blood pressure [7,15]. The mechanisms underlying this difference in late-developing response latency are unknown, but may relate to the reduced interplay between forebrain structures and brainstem systems during REM sleep [8]. These data may suggest, however, that longer latencies to EEG arousal from REM sleep are not entirely dependent on a relative "disconnection" between cortical and brainstem areas, since the autonomic events not associated with EEG arousal are presumably less dependent on cortical involvement. Instead, the longer latencies to late-developing responses during REM may involve different state-related reflex sensitivities in brainstem structures or different properties of integration of input [28,45]. In light of

the modulatory effect of the secondary responses on baroreceptor reflexes, this difference in latency may represent a state-related vulnerability in adequate homeostatic control.

The late-developing response – arousal implications

There are many similarities between the late-developing events associated with cortical arousals and those without arousal, such as their effects on RVLMS activity, heart rate and blood pressure. Several investigators have noted the suspension or reversal of baroreflex actions by arousal. In cats, arousals by electrical stimulation of the amygdala and by naturally-arousing stimuli (social confrontation) alter baroreceptor reflex sensitivity and increase heart rate [35]. Other non-baroreflex influences can also affect baroreflexes [21], and in a sleep state-dependent manner [45]. The concept of "sub-cortical," "autonomic," or "brainstem" arousal has been introduced by others to provide a mechanism for explanation of homeostatic recovery [6,15,18]. Our findings suggest recruitment of circuits to normalize blood pressure that do not necessarily involve processes that activate the EEG, although, on occasion, EEG arousal processes are triggered. The extent of this recruitment outside the RVLMS is unknown, but until the structures involved are outlined, it may be premature to invoke the term "arousal" as an explanatory concept.

The means by which EEG arousal factors into the late-developing compensatory responses to sustained blood pressure changes remain unclear. A temporal relationship emerged between EEG arousal and the onset of the late response when such activation did occur. Since we found no differences in blood pressure between trials with or without EEG arousal, it appears that arousal is not a necessary component for homeostatic control of blood pressure in this experimental design. The latter issue is of

some clinical importance, since several studies report that different cardiopulmonary challenges (*e.g.,* pressor response or obstructive apnea) resolve as effectively without, as with, EEG arousal [27,32,43].

It is possible that some other brainstem reflex accounts for the changes in heart rate used in the present study to indicate the onset of the late-developing response. For example, Daly and colleagues [4] found that respiratory activity (central respiratory drive and slowly-adapting pulmonary stretch receptors) can significantly modulate chemoreceptor and baroreceptor reflexes that are cardio-inhibitory. However, it is unlikely that these respiratory factors account for the changes in heart rate in the present study, since most of the late-developing events were not accompanied by changes in respiration. In addition, heart rate increases at the late response onset were similar between pressor (cardio-inhibitory) and depressor (cardio-excitatory) challenges.

The agents employed here, phenylephrine and nitroprusside, are often used to elicit vasoconstriction and vasodilatation, respectively. However, each drug can exert other effects which could impact cardiovascular regulation. PE causes tracheal smooth muscle contraction [42], increased mucous secretion in the trachea [17], and at high doses, pulmonary edema [1]. Some of these additional actions, particularly those of mucous secretion, likely take a different time course from that of the blood pressure response. Nitroprusside is a nitric oxide (NO) donor that significantly affects many different cardiopulmonary control areas, including the RVLMS [19,26]. The role(s) of NO in cardiovascular control is extraordinarily complex and is expressed as both excitatory and inhibitory neural influences [44] which make any evaluation of NO effects here problematic.

In summary, the initial RVLMS responses to pressor and depressor challenges included neural activity decreases and increases, respectively. These effects were then modified by subsequent later-developing responses, such that the RVLMS deactivation and bradycardia to pressor challenge were reversed, and the activation and tachycardia to depressor challenge were augmented. These changes in RVLMS neural activity are apparently compensatory, since blood pressure began to return towards baseline at the onset of the late-developing response. The RVLMS activation during the late-developing responses typically occurred in two phases, with the first phase characterized by regionalized deactivation and activation (pressor) or mildly increased (depressor) activity patterns, and the second phase by more intense and widespread activation. RVLMS responses to the late-developing events were similar across challenges with and without awakening. The findings suggest that the RVLMS, in addition to a role in the baroreceptor reflex, contributes to enhanced sympathetic activation during secondary (long-latency) compensatory responses.

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TABLE 1
Summary of Challenges

	Pressor		Depressor	
	QS	REM	QS	REM
Total	44	14	34	15
Excluded	22	4	11	4
Sample Size (n)	22	10	23	11
Secondary Response – Full Arousal	10 (45.5)	5 (50)	7 (30.4)	7 (63.6)
Transient Arousal	8 (36.4)	0 (0)	9 (39.1)	3 (27.3)
Secondary Response – No Arousal	4 (18.2)	5 (50)	6 (26.1)	1 (9.1)
No Secondary Response	0 (0)	0 (0)	1 (4.3)	0 (0)

Number of trials in quiet sleep (QS) and rapid-eye-movement sleep (REM) for both types of blood pressure challenge. Number in parentheses () are percent of total sample size. Data on transient arousals are not included in this report.

Figure 1

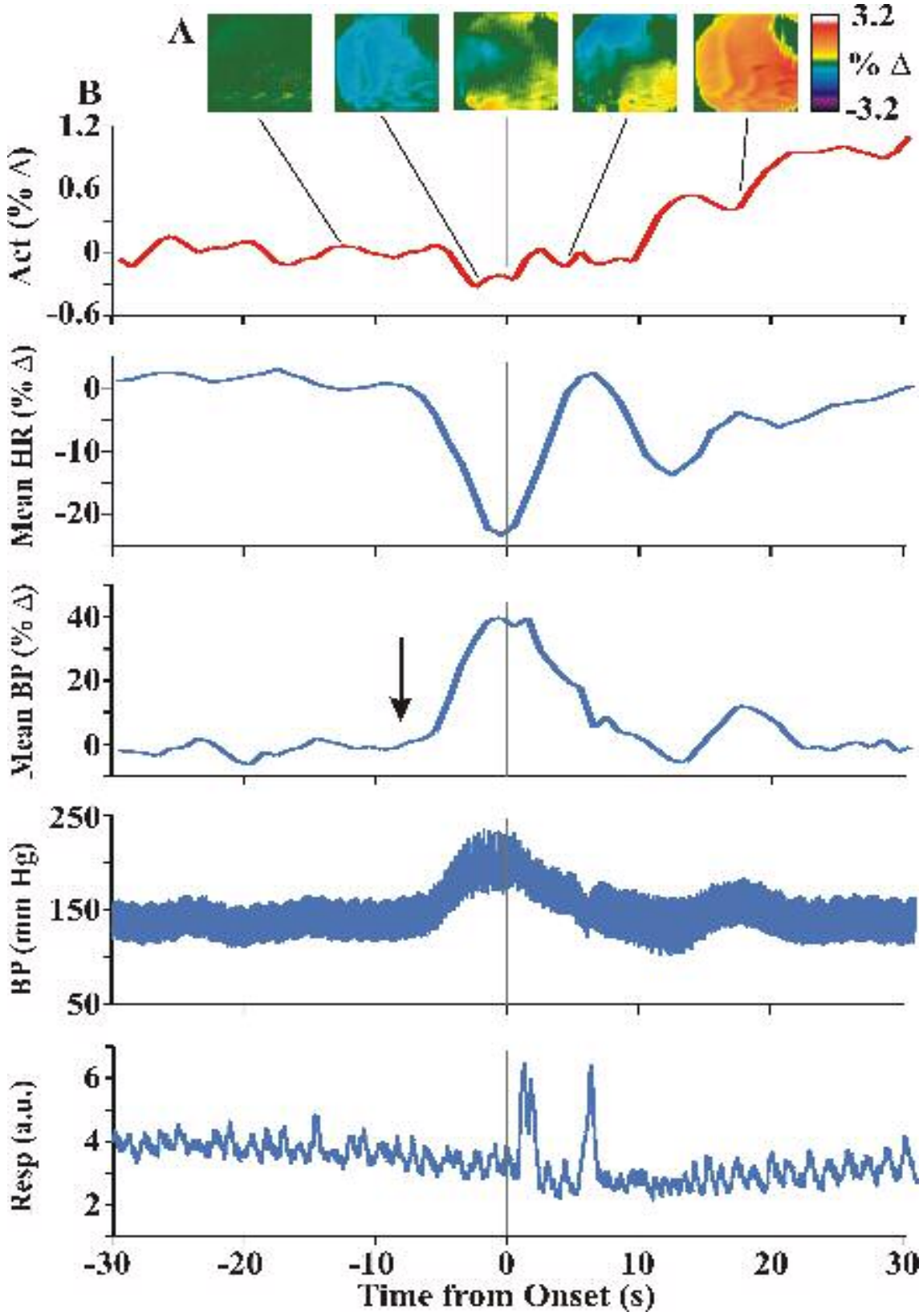
A
3.2
% Δ
-3.2
B
Act (% Δ)
1.2
0.6
0
-0.6
Mean HR (% Δ)
0
-10
-20
Mean BP (% Δ)
40
20
0
BP (mm Hg)
250
150
50
Resp (a.u.)
6
4
2
-30
-20
-10
0
10
20
30
Time from Onset (s)

Figure 2

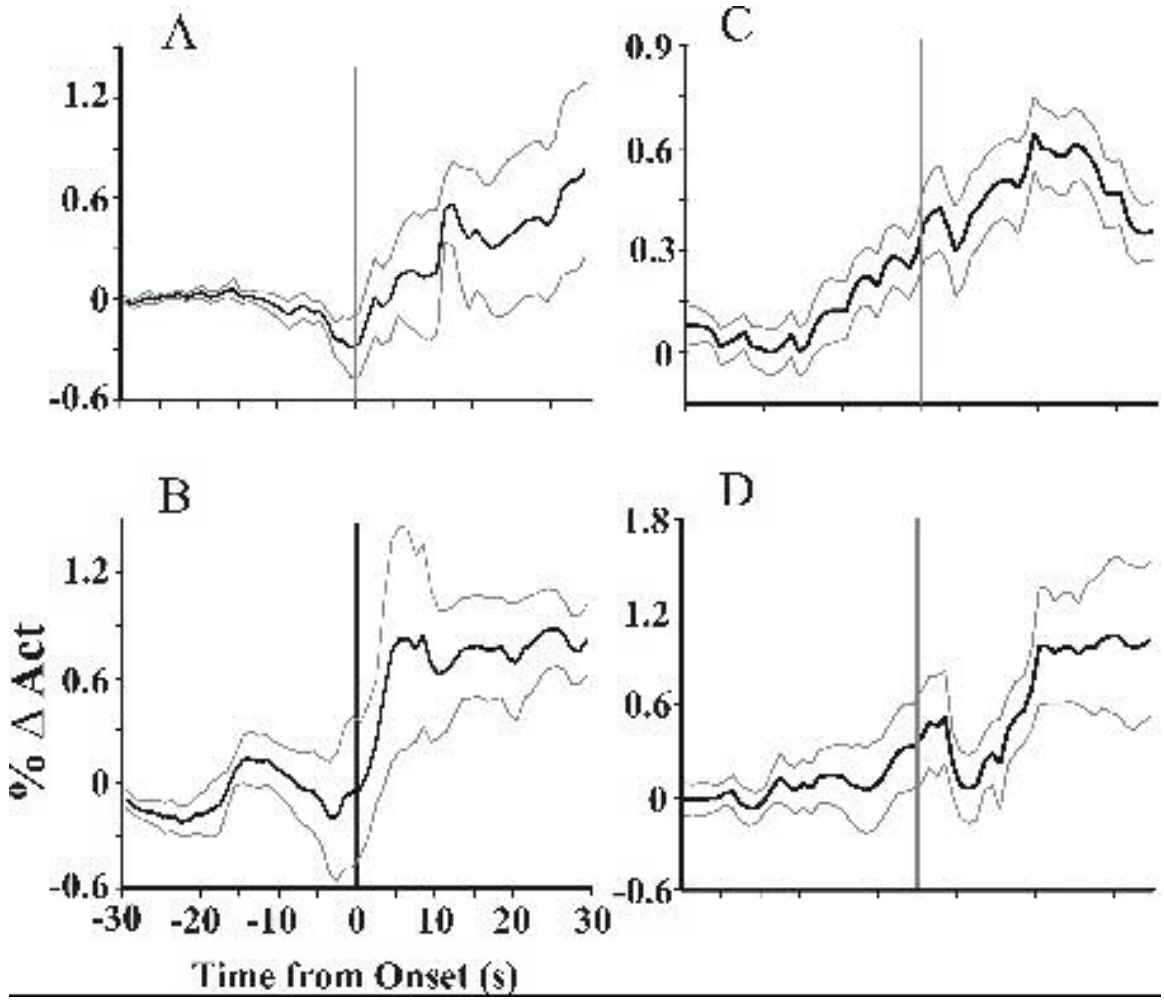

Figure 3

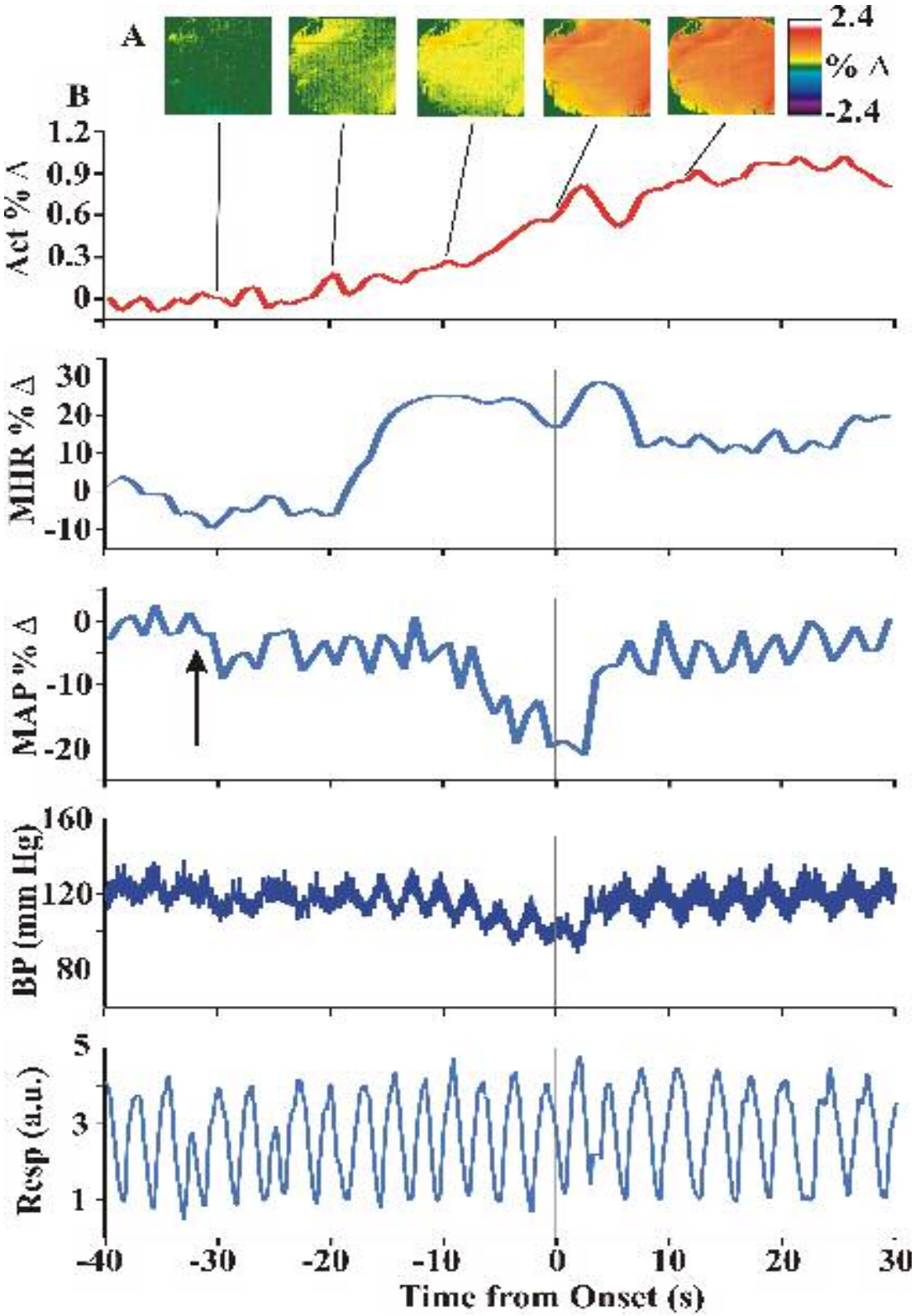
A
B
2.4
% Δ
-2.4
Act % Δ
1.2
0.9
0.6
0.3
0
MHR % Δ
30
20
10
0
-10
MAP % Δ
0
-10
-20
BP (mm Hg)
160
120
80
Resp (a.u.)
5
3
1
-40
-30
-20
-10
0
10
20
30
Time from Onset (s)

Figure 4

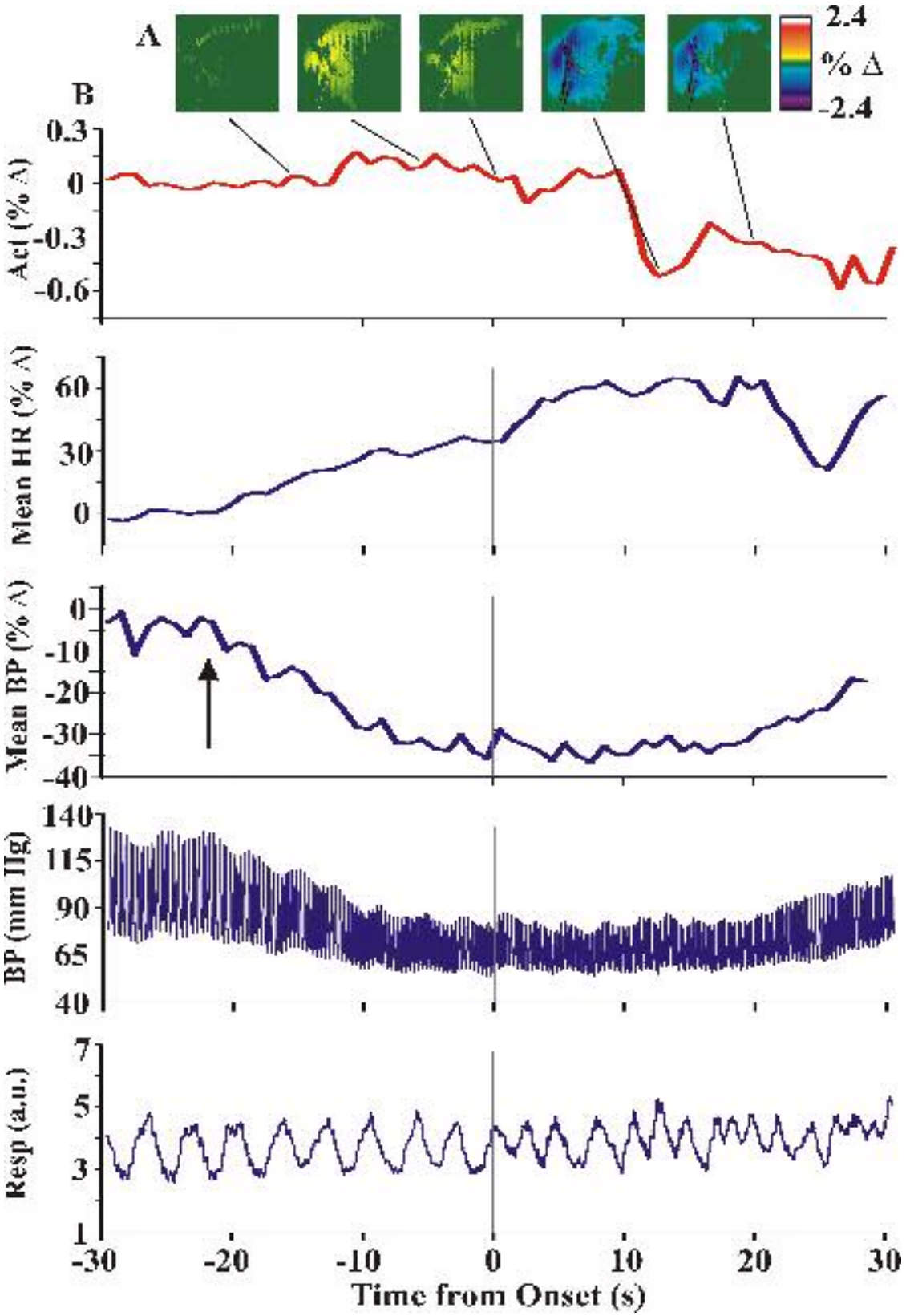
A
2.4
% Δ
-2.4
B
Acl (% Δ)
0.3
0
-0.3
-0.6
Mean HR (% Δ)
60
30
0
Mean BP (% Δ)
0
-10
-20
-30
-40
BP (mm Hg)
140
115
90
65
40
Resp (a.u.)
7
5
3
1
-30
-20
-10
0
10
20
30
Time from Onset (s)

Figure 5

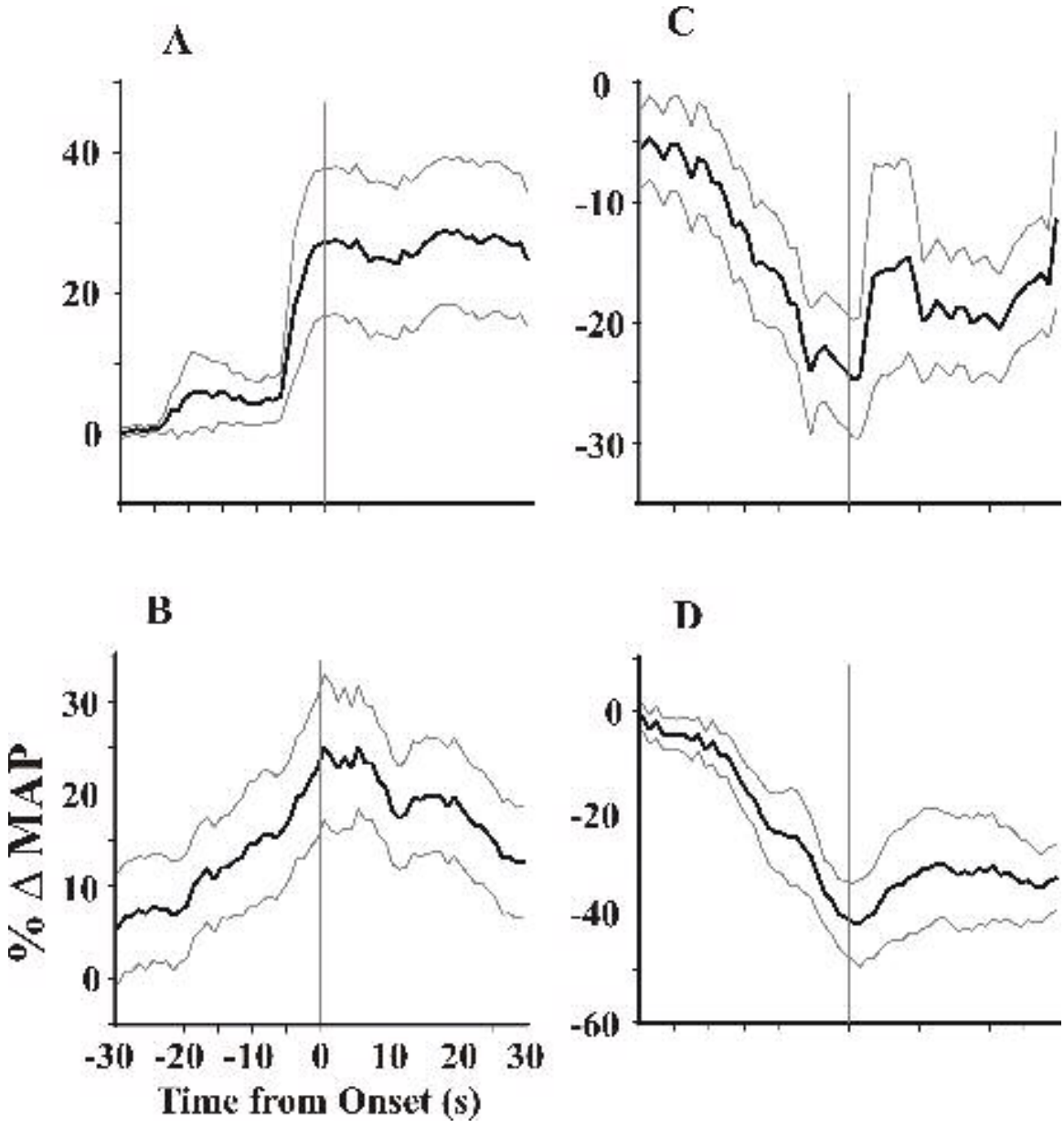
A
C
B
D
40
20
0
0
-10
-20
-30
30
20
10
0
-20
-40
-60
% Δ MAP
-30 -20 -10 0 10 20 30
Time from Onset (s)

Figure 6

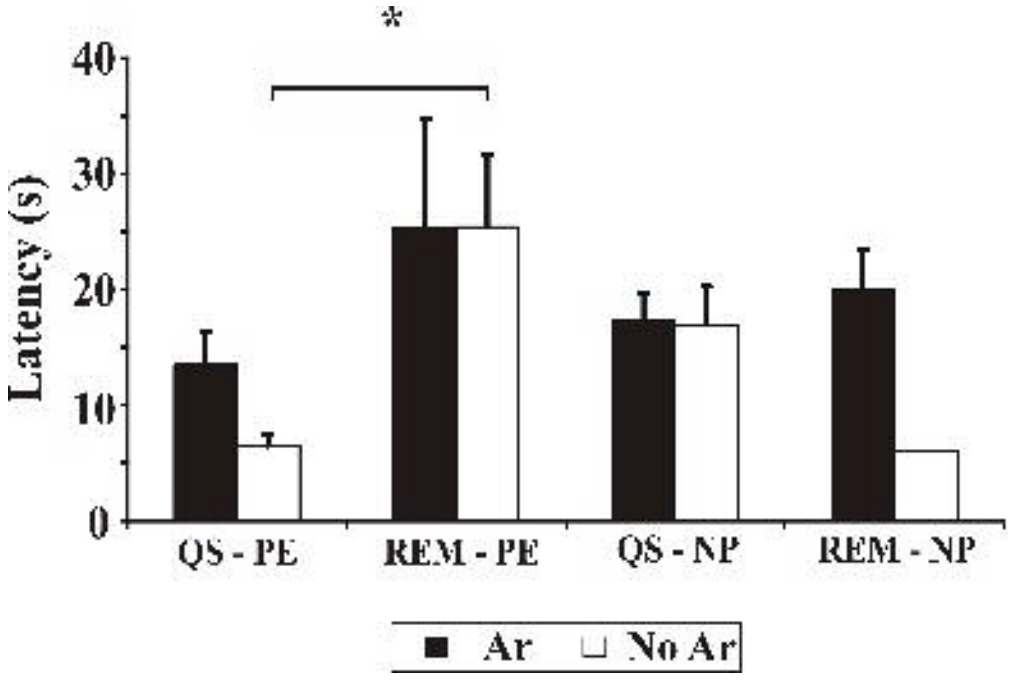

FIGURE LEGENDS

FIGURE 1 – Pressor challenge in quiet sleep without arousal. Example of neural (A) and physiological (B) changes to a pressor challenge during quiet sleep that did not result in an EEG arousal; the onset of hypertension (arrow) occurred 9 s before the onset of the late-developing response (vertical gray lines at time 0). Note the reversal of the decline in RVLMS activity (Act) and later, a strong increase in neural activity. Panels (A) above the activity trace show color-coded regional and extent of change data in neural activity at selected time points as indicated by lines. Each frame is averaged over one second and compared to baseline. Green pixels indicate no significant change; see color bar for color-coding of significant changes. % Δ= percent change; HR=Heart Rate; BP=arterial blood pressure; Resp=respiration; a.u.=arbitrary units.

FIGURE 2 – Averaged RVLMS activity traces during late-developing responses. Averaged traces of RVLMS activity for all trials, separated by drug challenge and sleep state (A – PE/QS; B – PE/REM; C – NP/QS; D – NP/REM). Mean activity changes (percent of baseline) are shown as black lines; $\pm$ SEM are shown as light gray lines. Traces are aligned by the onset of the late-developing response (time 0, vertical gray lines).

FIGURE 3 – Depressor challenge in quiet sleep without arousal. Example of the RVLMS (A) and physiological (B) responses to hypotension (NP) which was not accompanied by EEG arousal. This trial was initiated in quiet sleep with the onset of

hypotension (arrow) 31 s before the onset of the late-developing response. Image panels show significant increases in RVLMS activity. Early in the late-developing response both heart rate (HR) and blood pressure (BP) increase but with no respiratory effect. Abbreviations and axes as in Fig. 1.

FIGURE 4 – Control site response to depressor challenge in quiet sleep. Example of RVLMS neural activity (A) at a control site with physiological responses (B) to a depressor challenge during quiet sleep that evoked a cortical arousal. The control site was in the caudal medulla and lateral to the experimental placements. Hypotension began at -19 s (arrow). Late changes in neural activity at this control site (right two panels) were opposite to those in the RVLMS (see Figs. 1 and 3).

FIGURE 5 – Averaged blood pressure changes to late-developing response. Time course of percent changes (% Δ) in mean arterial pressure (MAP), averaged over challenge and sleep state (A – PE/QS; B – PE/REM; C – NP/QS; D – NP/REM). See Fig. 2 legend for other details. Except for QS-AW (A), maximal drug effects on MAP occurred at time 0 (onset of the late-developing responses) and MAP values were significantly altered (towards baseline) during the secondary response.

FIGURE 6 – Latency from drug effect to late-developing response. Mean (+SEM) latency (s) from the onset of drug-induced blood pressure change to the onset of the late-developing response for all trials. Data are grouped with and without arousal (Ar, No Ar, respectively), by types of sleep (QS, REM), and by challenge types (PE, NP). Significant

differences (*) were detected for a sleep-state effect in the PE challenges eliciting a secondary response without any accompanying arousal, where latency in REM was greater than in QS.

Summary of Challenges

	Pressor		Depressor	
	QS	REM	QS	REM
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Table 1 – Number of trials in quiet sleep (QS) and rapid-eye-movement sleep (REM) for both types of blood pressure challenge. Number in parentheses () are percent of total sample size. Data on transient arousals are not included in this report.