Temporal and Spatial Profiles of Pontine-Evoked Monoamine Release in the Rat’s Spinal Cord

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INTRODUCTION

Numerous physiological and behavioral experiments have suggested that norepinephrine release in the spinal cord can enhance locomotor rhythms and suppress nociceptive transmission (Barbeau and Rossignol 1991; Grillner and Shik 1973; Ono and Fukuda 1995; Willis and Westlund 1997). Neurons in the dorsal pons provide the majority of noradrenergic spinal terminals and are particularly abundant in the cytoarchitecturally defined compact cell cluster of the locus ceruleus and the more diffuse adjacent subceruleus nucleus. Their terminals are found throughout the spinal gray matter, although they are more concentrated in the superficial dorsal horn, the intermediolateral column, and near motor neurons (Clark and Proudfoot 1991a,b; Fuxe et al. 1990; Grzanna and Fritschy 1991; Martin et al. 1999; Westlund et al. 1982). The time course and spatial pattern of pontine-evoked changes in norepinephrine concentration in the spinal cord are not well known. Such knowledge is not only fundamental to understanding spinal noradrenergic control, it may be useful when transplanting catecholamine-releasing cells to reverse spinal locomotor damage (Yakovleff et al. 1989, 1995) and to alleviate chronic pain (Hentall and Sagen 2000). Extrajunctional as well as subsynaptic concentrations are likely to be important because paracrine transmission could play a significant role in spinal noradrenergic transmission (Bach-y-Rita 1993; Ridet et al. 1993; Umeda et al. 1997).

The present work examined pontine-evoked changes in extrajunctional monoamine level in the rat’s lumbar gray matter across most of the major dorsal-ventral laminae. The experiments focused on locus ceruleus stimulation, although some effects from adjacent pontine regions were also studied. Measurements were made with fast cyclic voltammetry through carbon fiber microelectrodes, whereas previous studies of spinal norepinephrine release have employed extractive methods. Extractive methods give high qualitative accuracy, but their low temporal resolution means that prolonged conditioning stimulation is needed. Even when extraction is optimized with the use of microdialysis probes, the time resolution is more than several minutes, which implies spatial resolutions above a millimeter in the diffusional environment of the CNS. Microdialysis probes, moreover, are at least a quarter of a millimeter in diameter, which exacerbates the low resolution and may cause tissue damage. To see extrasynaptic changes in neurotransmitters due to diffusion from multiple sources, resolutions on the order of seconds and tenths of a millimeter are needed. These are presently provided only by microelectrode-based voltammetry (Nicholson and Philips 1981), which detects sub-
stances by the redox currents that they generate in electrode reactions at particular voltages. Subsynaptic changes in vivo, being faster and more confined, are inaccessible by any current method. Fast cyclic voltammetry has the best spatiotemporal resolution of the various voltammetric paradigms that scan a range of oxidation potentials (Stamford et al. 1992). Its main drawback, compared with extraction studies or slower voltammetric techniques, is imperfect qualitative discrimination among different monoamines. However, conclusions on the molecular species detected can be verified by comparing results from studies using other, diverse methods. Preliminary results have been presented elsewhere (Hentall et al. 2002).

METHODS

Animal preparation

Adult male rats (n = 9, Fisher-CDF strain, obtained from Charles River Labs) were initially anesthetized with 3% halothane in a mixture of 60% nitrous oxide and 40% oxygen. The anesthetic was first delivered in an induction chamber and then by face mask. Next the trachea was intubated for direct administration of the anesthetic. The halothane dose was reduced at this time to 1–1.3%. The left common carotid artery was cannulated and connected to a pressure transducer for blood pressure monitoring. The right external jugular vein was cannulated for the administration of fluids, including a bicarbonate solution (100 mM NaHCO₃ with 5% glucose) that was infused continuously at 0.5 ml/h. A laminectomy was performed over the lumbar spine, formed by back muscle and skin, was sealed with a spinal cord. The animals were next positioned in a stereotaxic head clamp amplifier (Millar Voltammetric, P.D. Systems). The leads were connected to the carbon-fiber working electrode, to a carbon-based reference electrode internally filled with KCl (Dri-Ref: WPI, Sarasota, FL), and to an Ag-AgCl auxiliary electrode, which applied the clamping current. The reference and auxiliary electrodes were located in the pool of saline covering the spinal cord. Measurement of monoamines was done by applying a triangular voltage waveform (or "scan") to the carbon-fiber microelectrode, which passed at a rate of 450 mV/s from 0 to –1 V, then to +1.4 V, then to –1 V and then back to 0 V, thus lasting ~30 ms. The amplifier’s output waveform during the first scan in a test sequence was stored and subtracted from subsequent output waveforms, giving a "subtracted voltammogram."

Microelectrodes were formed from commercially available carbon fibers of 33 μm in diameter (Textron Systems), which were inserted into micropipettes made from 2-mm-diam borosilicate glass whose tips were ~40–50 μm in inside diameter. The small gap between the glass tip and the carbon fiber was sealed with epoxy resin (Epon 815), which was hardened for 48 h. A bare silver wire (0.10 mm, A-M Systems, Carlsborg, WA) was inserted into the shaft of the capillary tube to make direct contact with the carbon fiber. This was secured within the shaft by a few drops of electrically conductive silver epoxy, which was allowed to cure overnight at room temperature followed by oven-drying at 60°C for 2 h. The microelectrodes were beveled at a 30° angle so that the carbon fiber surface was flush with the tip. They were then cleaned in ethyl alcohol, rinsed with deionized water. Just prior to use, they were electrically pretreated to increase their sensitivity (Stamford et al. 1992) by applying an offset 71 Hz triangular waveform for three consecutive 10-s periods: at –0.6 to +3.0 V, –0.6 to +2.0 V, and –0.6 to +1.0 V.

Ex vivo calibration

Microelectrodes were always calibrated, both before and after experiments, with norepinephrine and serotonin dissolved in phosphate-buffered saline at pH 7.4, which was made up in de-ionized water purged with nitrogen gas. Each substance was rapidly stirred into the control solutions at three or more different concentrations between 500 and 2,500 nM. Concentration changes in vivo were estimated from the linear regression of the peak oxidation current against norepinephrine concentration (Fig. 1B), obtained from calibrations performed after the experiments. The mean sensitivity to norepinephrine of the microelectrodes used was 60.9 ± 9.7 (SE) nM/nA (n = 9).

The addition of norepinephrine caused an oxidation current during the first ascending phase of triangular input waveform, which was maximum when the applied input waveform was near 940 mV. This peak current was subtracted from the current at one or two points in the subtracted voltammogram just before and after deflection, where the second time differential was near zero. One point, typically near 620 mV, was subtracted if the deflection was superimposed on an otherwise flat subtracted voltammogram (e.g., Fig. 1A). If the subtracted voltammogram was not flat outside the deflection, a line was interpolated between the two points, and the peak was subtracted from the point directly below it on that line (Fig. 2, A, curves 1 and 2, and B, curves 1 and 2). This subtracted peak was taken to reflect the

FIG. 1. Subtracted voltammograms. A: results of ex vivo calibrations in different concentrations of norepinephrine (NE) and serotonin (5-HT), and in the control medium (phosphate-buffered saline, pH 7.4). The subtracted voltammograms are averages (20–30 scans). Also shown is the triangular input waveform (top). B: calibration curves (peak oxidation current) of the carbon fiber microelectrodes obtained after each experiment. Linear regression lines are shown for each microelectrode. C: effect of acid shifts in the physiological range in the presence of 2.0 μM NE. D: effect a large increase in ascorbate concentration (100 μM) in the presence of 1 μM NE.
taken at peak amplitudes during each trial. The time course of the effects of stimulation are also shown (A3 and B, 3 and 4), where the thick arrows point to the start and finish of stimulation. In A3, results during the 1st (w1) and 2nd (w2) waves from 1 trial of stimulation of the locus ceruleus (LC) are displayed. In B, 3 and 4, are depicted results from 2 trials along the same pontine track, 1 stimulation of the nucleus cuneiformis (Cun) and the other of the locus ceruleus. Dotted lines indicate reference levels used to calculate peak current (see METHODS). The closed triangles show points of peak reduction current, where a downward deflection means an increase in reduction with respect to the preceding reference voltammogram. Oxidation peaks are indicated by the open triangles in the subtracted voltammograms. The subtracted voltammograms in A, 1 and 2, are averages (20–30 sweeps) taken at peak amplitudes during each trial.

norepinephrine concentration. All other maxima in oxidation or reduction currents were processed in the same way.

Serotonin gave an oxidation peak near 920 mV and therefore could not be distinguished from norepinephrine by this deflection. However, a postoxidation reduction current of slightly smaller amplitude was always observed during the second descending slope of the applied waveform, probably due to the electrode oxidation reaction reversing itself. These had different features in serotonin and norepinephrine. In norepinephrine, the postoxidation reduction currents peaked at approximately −400 mV, whereas in serotonin two peaks emerged, one near −100 mV and the other near −800 mV (Fig. 1A).

Because physiological alterations in pH or ascorbate concentration could potentially complicate the interpretation of results, these influences were studied ex vivo. Decreasing the pH by the physiologically large amount of −0.1 units in the presence of norepinephrine enhanced the amplitude of the subtracted voltammogram across most of the voltage scan (Fig. 1C) as reported by other investigators (Michael et al. 1998). The changes were readily reversed by alkalization. Adding 100 μM of buffered ascorbate slightly enhanced the norepinephrine oxidation current but did not alter the postoxidation reduction current (Fig. 1D).

Analysis of electrochemical findings in vivo

Pontine stimulation caused marked increases in the oxidation signal at most spinal sites tested. However, the peaks in the subtracted voltammograms were delayed compared with those for norepinephrine and serotonin ex vivo. They occurred between 1,000 and 1,100 mV during the first positive-going ramp of the applied triangular waveform. These peaks were nevertheless taken to represent a monoamine signal. Where the peak oxidation or reduction signal occurs depends theoretically on many factors, such as diffusion rates, pH, temperature, and redox environment, which can be expected to differ in vivo and ex vivo (O’Neill et al. 1998; Palij and Stamford 1994). The postoxidation reduction current measured in vivo on the subsequent descending slope showed a single peak near −500 mV (Fig. 2B, trace 1). The ratio of this current to the prior oxidation current was also lower and more variable in vivo, and the reduction current could even appear diminished by a pontine stimulus that raised the oxidation current (Fig. 2A, traces 1 and 2), which is also a possible result of the different physiochemical environment of tissue.

Electrical stimulation

Pontine sites were stimulated with a monopolar insulated tungsten wire electrode (A-M Systems). The electrode tapered gradually from 0.12 mm in diameter to a sharp tip. Bare metal was exposed for the last 50 μm. All stereotaxic coordinates will be expressed here in millimeters with respect to the interaural-line when the dorsal skull surface is held in the horizontal plane (Paxinos and Watson 1986). The stimulating electrode was aimed stereotaxically along a caudally directed track in the sagittal plane at 25°. This track passed through the stereotaxic target at 1.1 mm posterior, 3 mm dorsal, and 1.2–1.7 mm medial. Stimuli for all tests consisted of trains of rectangular cathodal pulses of 10– to 100-Hz frequency, 50- to 200-μA amplitude, and 0.5-ms width. Most trials consisted of 20 s of such stimulation given at 50 Hz. Monoamine samples were taken for 200 s at a rate of 2 Hz, starting 10 s before stimulation. A further 240 s was allowed to pass before repeating the pontine stimulation.

Lumbar spinal segments L1-L6 were mapped for pontine-evoked changes in monoamine level along vertical microelectrode trajectories. The microelectrodes entered the dorsal surface of the cord 400–800 μm from the midline, ipsilateral to the pontine stimulating electrode. Measurements were made at 100 or 200 μm spacing to a depth of 2,200 μm as measured by the digital readout of the stepping motor that controlled the micromanipulator. A vertical spacing of 100 μm was initially thought to be optimal and was used in earlier experiments. The 200 μm was used later to allow more tracks to be mapped and more parametric tests to be performed.

Histology

At the end of each experiment, the location of the stimulating electrode was marked with an electrolytic lesion (1 mA, 8–10 s). Spinal and brain stem tissue was fixed by immersion in 10% buffered formalin. Frozen sections in the transverse plane, 100 μm thick, were counterstained with Toluidine blue or cresyl violet. The distance between histologically observed electrode tracks was used to calculate shrinkage. Reconstructed stimulation sites were plotted on standard maps of brain sections. The locations of recording sites obtained from morphologically correlated micromanipulator readings were confirmed by the localized tissue damage along electrode tracks seen in stained sections.
**RESULTS**

**Optimal location and frequency of pontine stimulation**

The goal of the first tests on each preparation was to map the dorsal medial pons along a standard 3-mm trajectory (given in METHODS). Changes in monoamine level were monitored at a fixed depth in the dorsal horn (Fig. 3). At most sites along this track, a wave of increased monoamine signal could usually be evoked with latencies of a few seconds. If increases in monoamine signal were weak or absent along the evoked track, a wave of increased monoamine signal could usually be found 1 mm lower in the locus ceruleus itself. This site was used for subsequent mapping of the spinal cord and investigation of stimulation parameters.

With the carbon-fiber microelectrode still positioned in the deep dorsal horn (lamina V) and the pontine electrode at its optimal position in the locus ceruleus, a range of stimulus frequencies from 10 to 100 Hz was tested with rectangular cathodal pulses of constant width and amplitude (0.5 ms, 150 μA) applied for 20 s. The higher frequencies of 50 and 100 Hz elicited a strong wave of monoamine oxidation (Fig. 4). In contrast, the response to 10- or 20-Hz stimulation was disproportionately weak, and sometimes was undetectable.

Several tests were carried out to confirm an inhibitory effect of the locus ceruleus stimulus on synaptic transmission in the dorsal horn. In one experiment, the cord dorsum potential produced by sural or tibial nerve stimulation at 20 times threshold was monitored. Locus ceruleus stimulation (20 Hz, 150 μA, 0.5 ms) reduced the sural-evoked cord dorsum potential by 12.5% and the tibial-evoked cord dorsum potential by 10%. In two additional experiments, spontaneous single-cell firing was recorded through the carbon fiber microelectrode from neurons in laminae IV (n = 2) and V (n = 2), in the intervals between the 2-Hz triangular waveforms. These cells showed nonnociceptive cutaneous sensory responses characteristic of lamina IV, and the spontaneous firing was inhibited during locus ceruleus stimulation (20 Hz, 150 μA, 0.5 ms).

**Temporal pattern of changes in monoamine level evoked by locus ceruleus stimulation**

A total of 14 electrode tracks, incorporating four spinal segments (L3, L4, L5, and L6) and lateral positions between 400 and 800 μm from the midline, were tested with a standard electrical stimulus train (50- to 200-μA, 0.5-ms pulses at 50 Hz for 20 s) applied to the locus ceruleus. No recordings were made in lamina IX or X. In all tracks, at most depths between 100 and 2,200 μm below the dorsal surface of the spinal cord, a sharp rise in monoamine signal was observed (Figs. 5 and 6). The mean peak level estimated from postexperimental calibration in norepinephrine was 544 ± 82 nM. The average rise showed a latency of 4.5 ± 0.3 (SE) s after stimulus onset, and a steep initial climb to maximum lasting 12.0 ± 0.6 s. This response will be referred to here as the “first wave.” The only deviation from this highly consistent finding was found in laminae II and III at ~400 μm depth. In 50% of tracks, an increase similar to those in other laminae occurred, but in the remainder, there was a wave of lower monoamine oxidation (Fig. 5).

The peak positive concentration of the first wave, in the combined data from all depths, showed a significant inverse correlation with latency (ANOVA: P = 0.0004), greater release being associated with shorter latencies. However, the rise time was not significantly correlated with the peak concentration. The declining phase of the first wave was quite variable, ranging from quite rapid (<20 s) to prolonged (200 s) and sometimes combining a rapid initial drop followed by a slower downturn.

**Statistical analysis**

Measurement sites were assigned to cytoarchitectural laminae (Moland et al. 1984), and various measured parameters were averaged across tracks and subjects. Statistical significance was assessed by ANOVA followed by contrast analysis. The contrasts compared each site in turn with all other sites combined and with nearest neighbors. Bonferroni corrections were made to probabilities. A level of P < 0.05 (2-tailed) was considered significant.
decline (Figs. 5 and 6). After the first wave, a period of unchanging monoamine level was most common in the remainder of the 200-s trial. This level was sometimes considerably lower than before the stimulus, a phenomenon that did not occur with any obvious spatial or temporal pattern. A second wave of raised monoamine level starting between 35 and 145 s after stimulation, was noted in 18% of trials (seen at several points in Figs. 5 and 6). It too showed no spatial preference. Its rise time, but not its latency, was correlated directly with the final concentration attained (ANOVA, \( P = 0.022 \)), indicating a tendency for a constant rate of rise. In some trials, the later monoamine level oscillated for many cycles with a period of several seconds, more often in association with negative-going responses (e.g., lamina II in Fig. 5, and dorsal-lateral lamina VII in Fig. 6).

Laminar differences in the effects of locus ceruleus stimulation

The preceding data were further analyzed with respect to spinal laminae (Molander et al. 1984) as described in METHODS. Laminae II and III were combined for this analysis, and data assigned to lamina I included sites that were slightly dorsal to it (\( \pm 50 \mu m \)) within white matter. Various measured parameters from the first waves were averaged across tracks and

![Figure 4](image1.png)

**FIG. 4.** Effects of varying the frequency of locus ceruleus stimulation on monoamine oxidation currents in lamina V. Results of consecutive trials are shown on the left. Data from 3 different rats are graphed, in terms of norepinephrine concentration from postexperimental calibration, on the right.

![Figure 5](image2.png)

**FIG. 5.** Representative time courses of locus-ceruleus-evoked changes in monoamine level, mapped along a single spinal cord trajectory in the L3 segment during locus ceruleus stimulation. The curves show the amplitude of the sampled currents due to monoamine oxidation. Arrow, in this and Fig. 6, indicates higher frequency oscillation of monoamine level observed in some laminae after ceruleus stimulation. Measurements were made in 200-μm steps as indicated on the map.

![Figure 6](image3.png)

**FIG. 6.** Results of locus ceruleus stimulation along 2 spinal cord trajectories at the same rostrocaudal level on the L5/L6 boundary. The curves show the amplitudes of the sampled currents due to monoamine oxidation. Measurements were made in 100-μm steps.

![Figure 7](image4.png)

**FIG. 7.** Averages of parameters of locus-ceruleus-evoked 1st waves of monoamine concentration at different depths in spinal cord taken from all sampled trajectories (n = 14) in segments L3, L4, L5, and L6, at lateral positions 400–800 μm from the midline. Measurement sites were assigned to spinal lamina from histological reconstructions. Statistical significance was assessed by ANOVA followed by contrast analysis, in which the contrast was between each site and all other sites. Bonferroni corrections were made to probabilities. A level of \( P < 0.05 \) (2-tailed) was considered significant and is indicated by nearby *.
DISCUSSION

Interpretation of in vivo voltammetric findings

The experiments reported here showed that the locus ceruleus can evoke increases in monoamine level over a wide vertical extent within laminae I–VIII of the rat’s lumbar spinal cord. Exceptions were encountered in laminae II and III, where half of all sites showed decreases in the monoamine signal. Laminae IX and X were not examined. Although the molecular species that gave rise to the oxidation signal are not known, the noradrenergic nature of the spinal cord projection from the locus ceruleus and the absence of the distinct serotonin signature from the subtracted voltammograms favors the likelihood of a major contribution from norepinephrine. Detection of its metabolites is less likely because these take several minutes to influence the extracellular monoamine concentrations (Michael and Wightman 1999; Michael et al. 1985). The correspondence between the anatomically delineated noradrenergic innervation density and the mapped monoamine signal, as discussed later, further supports this conclusion.

Several microdialysis studies have also been performed to study physiological norepinephrine release in the spinal cord, although the direct effects of locus ceruleus stimulation on the ventral spinal cord have not been examined. Electrical stimulation of the midbrain’s periaqueductal gray matter, an antinociceptive region that activates cereulospinal and raphespinal efferents (Cedarbaum and Aghajanian 1978), was found to elevate the norepinephrine level in the rat’s dorsal horn to several times above the basal level (Cui et al. 1999). High basal levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylethylglycol were found in the rat’s ventral funiculus and were observed to rise by 150% during 60 min of treadmill exercise (Gerin and Privat 1998). A 20% reduction in norepinephrine in the lumbar ventral horn, conversely, was observed in association with inhibition of postural tone during prolonged activation of the cat’s mesolimbic reticular formation (Lai et al. 2001).

Several considerations tend to rule out pH changes in the presence of a constant monoamine level as the cause of the locus-ceruleus-evoked changes in the oxidation currents. Most brain regions studied show a triphasic sequence of pH changes (alkaline then acid then alkaline), each lasting at least several seconds, when an input tract is stimulated (Chesler and Kaila 1992; Sykova and Svoboda 1990). In the spinal cord, this sequence has been seen on peripheral nerve stimulation (Sykova and Svoboda 1990), although the influence of brain stem stimulation on spinal pH appears not to have been reported. In the present experiments, sural or tibial nerve stimulation had no effect on the monoamine signal in any lamina.

Furthermore, an alkaline-acid-alkaline sequence of pH shifts should produce a decrease-increase-decrease shift sequence in apparent oxidation signals, according to the present ex vivo studies. This is opposite to what was observed, except in laminae II and III, where the prolonged decreases may therefore conceivably be due to increased alkalinity.

Timing of locus ceruleus-evoked changes in monoamine level

The first waves of pontine-evoked monoamine release had latencies and rise times on the order of seconds. The dynamics need to be accounted in modeling release, diffusion and synaptic effects. Small molecules travel on average about 0.1 mm in 1 s by free diffusion from a point source in bulk aqueous solution, a distance that varies as the square of the time. This rule of thumb can also be applied to neural tissue. The three-dimensional distribution of transmitter sources, membrane reuptake mechanisms, bulk fluid flow systems (mainly blood), and metabolic enzymes will shorten the time to peak after pulsed release as will the constrained volume of extracellular space (volume fraction), but the tortuosity of extracellular space has a counterbalancing effect (Nicholson and Sykova 1998; Rice and Nicholson 1995). Considerations of microelectrode size and diffusion times suggest the following model of the observed events. Norepinephrine released by the first few descending volleys is restricted to synaptic clefts by nearby reuptake mechanisms and is not detected by fast cyclic voltammetry, even with a juxtaposed microelectrode. After a few seconds, especially at higher stimulus frequencies, continued release overwhelms reuptake, extrajunctional concentrations rise, and the microelectrode detects the overflow from thousands of release sites. Similar models have been proposed for other CNS regions (Bunin and Wightman 1998; Wightman et al. 1988).

Between the start of locus ceruleus stimulation and the initial detection of monoamines there was a mean latency of 4.5 s. This value could include conduction delays in the neuronal circuit from the pons, the time needed to saturate the reuptake mechanisms, and the mean diffusion time needed for detectable release. Delays caused by neuronal conduction are likely to be much less than 4.5 s even though cereulospinal fibers are mainly unmyelinated (Guyenet 1980; Nakazato 1987). The correlation between peak concentration and latencies suggest that diffusion times are a major factor because closer sources should deliver larger amounts. However, the largest peak seen had a 1.5-s latency, which suggests that overflow delay was equally important.

An explanation is also needed for the finding that monoamine levels peaked and then declined before the end of the 20-s stimulation train. Continuous build-up during the stimulus is the simplest expectation, given the widespread terminations of pontine monoaminergic fibers and hence the likely ubiquitous release of norepinephrine in spinal gray matter. It should be noted that in many tests the rise in monoamines did outlast the stimulus. Two possible mechanisms for the early peak are the onset of concentration-dependent reuptake (Nicholson 1995) and the onset of conduction block in the neural pathway due to activity-dependent elevation of thresholds for direct activation (Raymond and Lettvin 1978). Either hypothesis predicts that higher stimulation frequencies should tend to give earlier peaks, as in fact can be seen to have occurred in Fig. 4.
Because the time to peak was not significantly correlated with the concentration attained, concentration-dependent reuptake seems less likely. It should be noted, however, that rise times were significantly longer near white matter in lamina I, where the relevant membrane transporters are absent (Gerin et al. 1995). In either case, it appears that the neuronal pathways activated were typically capable of transmitting activity at 50 Hz for >10 s without serious conduction failure.

Functional significance of release patterns

The spatiotemporal patterns of monoamine release uncovered by this study are probably mirrored by local noradrenergic effects on firing. Effects are immediate when noradrenergic receptors of spinal interneurons are activated iontophoretically (Bras et al. 1990; Jankowska et al. 1997, 2000) or by electrical stimulation of descending monoaminergic pathways (Noga et al. 1992, 1995b). The presently reported dynamics of extrajunctional concentration after locus ceruleus stimulation suggest that paracrine noradrenergic modulation of locomotion can operate on a time scale of ~1 s. Preliminary evidence shows that extrajunctional monoamine concentrations rapidly increase during mesencephalic locomotor region-evoked fictive locomotion in the cat (Noga et al. 1999), beginning a few seconds prior to the onset of locomotion. Thus spinal monoamines seem to be partly modulatory, and it is likely that they preset the gain and quality of locomotion that is subsequently activated by fast reticulospinal command fibers (Jordan 1991; Noga et al. 1995a). The extrajunctional levels observed in the present study are well within the physiological range for receptor-mediated effects (Allgaier et al. 1992; Zoli et al. 1998), and a high proportion of spinal noradrenergic synapses are remote from synaptic contacts (Ridet et al. 1993). However, modulation probably also results from the more rapid and precise junctional synaptic transmission. Many monoaminergic neurons are rhythmically active during locomotion (Jacobs and Jordan 1984; Zemlan and Behbehani 1988), while providing receptor-mediated effects (Allgaier et al. 1992; Zoli et al. 1998), present study are well within the physiological range for re-

Spatial differences in effects within the lumbar spinal cord and from the dorsal pons

Axons from the locus ceruleus appear to terminate throughout the spinal gray matter in the rat. The ubiquity of this spinal innervation explains the robust locus ceruleus-evoked monoamine release found in most of the laminae presently examined. Furthermore spinal noradrenergic terminals have been reported to show greater density in lamina I, the intermedio-lateral column at the lateral edge of the border between laminae VI and VII, and the motor neuron pools of the ventral horn (lamina IX) (Clarke and Proud dit 1991a,b; Fuxe et al. 1990; Grzanna and Fritschy 1991; Martin et al. 1999; Westlund et al. 1982). The laminar differences observed here in monoamine release patterns to some extent reflect these anatomical differences. Thus the first waves of release were highest in lamina I on average and had shortest latencies in lamina VI. One apparently discrepancy was the finding of significantly lower first waves in lamina VII than elsewhere. But this was an apparent average across all segments, whereas the intermedialateral column is not present in lower lumbar segments. In the cat, noradrenergic-innervated locomotor-activated cells in lower lumbar segments are mostly concentrated in medial lamina VII and lamina X (Johnson et al. 2002), areas that were not sampled in this study. The first waves measured in upper lumbar segments near the intermedialateral column were always relatively high (Fig. 5).

The effects of different pontine regions also generally matched the previously reported anatomical projections. These correlations strengthen the conclusion that direct stimulation of monoaminergic pontine neurons or their afferent terminals, not fibers of passage, was responsible for the effects observed. The locus ceruleus has been reported to provide more of monoaminergic input to the dorsal horn than the A5 and A7 (including subcerebral) regions, while the latter provide more of the input to the intermedialateral column and ventral horn (Lyons et al. 1989). Correspondingly, release in the dorsal horn was larger on average when elicited from the locus ceruleus than from the subcerebral nucleus. The nucleus cuneiformis of the rat has a strong projection to the nucleus raphe magnus, which in turn sends many serotoninergic fibers to the spinal cord (Steeves and Jordan 1984; Zemlan and Behbehani 1988), while providing little direct innervation to the locus ceruleus (Luppi et al. 1995). The raphe magnus relay therefore can account for the high levels of evoked monoamine release with serotonin-like reduction currents observed in the dorsal horn when the nucleus cuneiformis was stimulated. That this response was more variable than locus-ceruleus-evoked increases in the monoamine signal can be ascribed to the electrode track being aimed near the caudal edge of the nucleus cuneiformis and hence sometimes missing it entirely.

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