Steady State Levels of Monoamines in the Rat Lumbar Spinal Cord
– Spatial Mapping and the Effect of Acute Spinal Cord Injury

Brian R. Noga1*, Alberto Pinzon1,2, Riza P. Mesigil2, and Ian D. Hentall1,3

The Miami Project to Cure Paralysis, University of Miami School of Medicine
P.O. Box 016960, R-48, Miami, Florida 33101

1The Miami Project to Cure Paralysis, University of Miami School of Medicine, P.O. Box 016960, R-48, Miami, Florida 33101

2Department of Biomedical Engineering, University of Miami, Miami, Florida 33124

3Department of Biomedical Sciences, University of Illinois College of Medicine, Rockford, Illinois 61107-1897.

267 words in abstract, 6 figures

Running title: Spinal release of monoamines at rest

Key words: fast cyclic voltammetry, raphespinal, ceruleospinal, tonic descending inhibition

*Corresponding author: Telephone: (305) 243-6155, Facsimile: (305) 243-3921, E-mail address: bnoga@miami.edu

Copyright © 2004 by the American Physiological Society.
Abstract - Monoamines in the spinal cord are important in the regulation of locomotor rhythms, nociception and motor reflexes. To gain further insight into the control of these functions, the steady-state extracellular distribution of monoamines was mapped in the anesthetized rat’s lumbar spinal cord. The effect of acute spinal cord lesions at sites selected for high resting levels was determined over approximately one hour, to estimate contributions to resting levels from tonic descending activity and to delineate chemical changes that may influence the degree of pathology and recovery after spinal injury. Measurements employed fast cyclic voltammetry with carbon fiber microelectrodes to give high spatial resolution. Monoamine oxidation currents, sampled at equal vertical spacings within each segment, were displayed as contours over the boundaries delineated by histologically reconstructed electrode tracks. Monoamine oxidation currents were found in well defined foci, often confined within a single lamina. Larger currents were typically found in the dorsal or ventral horns and in the lateral aspect of the intermediate zone. Cooling of the low-thoracic spinal cord led to a decrease in the oxidation current (to 71-85% of control) in dorsal and ventral horns. Subsequent low-thoracic transection produced a transient increase in signal in some animals followed by a longer lasting decrease to levels similar to or below that with cooling (to 17-86% of control values). We conclude that descending fibers tonically release high amounts of monoamines in localized regions of the dorsal and ventral horn of the lumbar spinal cord at rest. Lower amounts of monoamines were detected in medial intermediate zone areas, where strong release may be needed for descending activation of locomotor rhythms.
INTRODUCTION

Several spinal neuronal systems are influenced by descending monoaminergic pathways. Monoamines such as norepinephrine, dopamine and serotonin, are capable of activating the spinal neurons involved in the production of walking (Antri et al. 2002; Barbeau and Rossignol 1991; Kiehn et al. 1992; Marcoux and Rossignol 2000) and of modulating nociceptive transmission (Millan 1999, 2002; Willis and Westlund 1997). Because virtually all spinal monoaminergic innervation originates supraspinally (Basbaum et al. 1978; Carlsson et al. 1964; Clarke and Proudfoot 1991a,b; Grzanna and Fritschy 1991; Martin et al. 1978; Westlund et al. 1982), spinal cord injury inevitably affects the normal function of these spinal systems. However, spinal neurons retain functional postsynaptic receptors on their cell membranes following denervation (Giroux et al. 1999; see also preliminary observations: Noga et al. 2002), so that monoamine transmitter replacement therapies may potentially improve locomotor function (Feraboli-Lohnherr et al. 1997; Yakovleff 1995) and alleviate chronic pain (Hentall and Sagen 2000; Horiuchi et al. 2003) following spinal cord injury.

We have recently measured monoamine release in the spinal cord during evoked locomotion (Noga et al. 1999) and stimulation of monoaminergic centers in the brainstem (Hentall et al. 2003a,b). These preliminary findings gave the first indications of the temporal and spatial patterns of changes in monoamine concentration in the spinal cord following activation of neurons of the noradrenergic dorsal pons and the serotonergic medullary raphe. Such data are essential both for delineating basic mechanisms of spinal monoaminergic transmission and for determining potential targets of monoamine transmitter replacement therapies. Our findings confirmed that the actions of monoamines in the spinal cord likely occur through non-synaptic diffusion as well as classical
Here we examine the distribution of monoamines released from terminals of descending monoaminergic fibers in the rat lumbar spinal cord in the absence of behavioral activation (i.e., at “rest”). In this study, a spatial mapping technique (Noga et al. 1995) was combined with fast cyclic voltammetry (FCV) (Armstrong-James and Millar 1984). The in vivo voltammetry technique is based upon the ability of some substances such as monoamine neurotransmitters and their metabolites to oxidize at the surface of carbon fiber microelectrodes (CFME) when an appropriate potential is applied to the electrode. This results in a release of electrons at the electrode surface that may be then measured as a current, the amount being proportional to the number of molecules oxidized. In contrast to extractive techniques of measurement such as microdialysis combined with high performance liquid chromatography, fast voltammetry with CFMEs allows one to determine extracellular neurotransmitter content with very high spatial and temporal resolution. However, since voltammetric measurements must be referred to some initial starting value, it is easier to map changes, such as those due to brainstem stimulation, than to map basal quantities. Here we solve this difficulty by using dorsal white matter as a spatial reference point, where relative concentrations are likely to be much lower due to the absence of nearby release sites. The work achieved three main goals. First, it indicated the basal monoamine levels upon which dynamic descending effects are superimposed. Second, it showed the extent to which tonic descending activity determines steady-state monoamine levels. Third, it revealed how spinal monoamine levels change during the first hour after spinalization, which has important implications for neuroprotection (Salzman et al. 1994), neuronal regeneration (von Euler et al. 2002), pain (Horiuchi et al. 2003) and hyperreflexia (Bennett et al. 2001) following spinal cord injury. A key experimental procedure for the last two
aims was voltammetric measurement from selected areas within the lumbar spinal gray matter during and after cooling and transection of the thoracic spinal cord. Preliminary results have been presented previously (Noga et al. 2001).

**MATERIALS AND METHODS**

*Animal preparation*

Experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23; revised 1996). The number of animals used, and their pain and distress, were minimized.

Adult male Fisher rats (n=9, Fisher-CDF strain, Charles River Labs, Inc.) 250-300 g were anaesthetized with 1-3% halothane in a mixture of 60% nitrous oxide and 40% oxygen. Animals were first placed in a closed chamber for anesthetic induction and then subsequently, the halothane mixture was applied through a facemask. The trachea was intubated for direct anesthetic administration. 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 (normal saline solution for fluid loss and bicarbonate solution with 5% glucose to maintain a normal pH balance). Body temperature was maintained at approximately 37°C using feedback-controlled heating lamps. CO₂, O₂ and tissue oxygenation were monitored throughout the experiment using a Datex Oscarxy airway gas monitor. The levels of anesthetic were adjusted to ensure that there was no cardiac or respiratory depression. Withdrawal reflexes were also monitored to ensure that the animal was appropriately anesthetized. A multi-level laminectomy was performed to expose the lumbar (L) spinal cord. In five animals, the 6th and 7th thoracic (T) segments were also exposed for acute transection at a later time during the experiment. The animals
were then transferred to a stereotaxic headframe and spinal cord fixation device. All four limbs were pendant. A back pool was formed with the skin flaps and the muscle surrounding the spinal cord area covered with Reprosil (Dentsply Caulk, Milford, DE). The spinal cord was then covered with warmed saline and the temperature of the pool maintained at 37°C using a feedback-controlled heating lamp. Voltammetric measurements were made in the L1-L5 segments (see below). In the five spinalized animals, the T6/7 cord segments were exposed by cutting and retracting the dura and in 3 cases, cooled with crushed ice prior to transection. The cord was transected in the medial-to-lateral direction using spring-mounted scissors under microscopic guidance. The completeness of the transection was confirmed visually by the slight separation of the proximal and distal stumps of the cord. Gelfoam was applied between the transected stumps when necessary to stop bleeding. No displacement of the cord was observed distally at the level of the recording site when the electrode was withdrawn at the end of the experiment.

**Fast cyclic voltammetry and calibration of carbon fiber microelectrodes (CFMEs)**

CFMEs based on a 33 µm diameter glassy carbon monofilament were constructed to established procedures (Armstrong-James and Millar 1984; O’Connor and Kruk 1991) and electrically pretreated as described previously (Hentall et al. 2003a). A Millar Voltammeter (P.D. Systems International, Ltd., UK) was used to generate the FCV scans. The Millar Voltammeter uses a 3-electrode potentiostat design including a CFME, a 5 cm long Ag-AgCl auxiliary electrode, and a carbon-based reference electrode, internally filled with KCl (Dri-Ref: WPI, Sarasota, FL). The oxidation of monoamines was caused by applying voltage ramp waveforms lasting approximately 20 ms (from 0 V to -1 V, then to +1.4 V, then to -1 V and back to 0 V) at a scan rate of ~ 450 V/s, repeated at a frequency of 1 Hz. The output voltage (or “voltammogram”) signaled the clamping
current in the CFME. This was in the form of a “subtracted voltammogram”, obtained by digitally (at 10-20 kHz) subtracting a reference voltammogram recorded at the cord surface and stored as a background signal.

The electrodes were calibrated \textit{ex vivo} (Fig. 1), in 0.25 to 2.5 µM of serotonin, dopamine and norepinephrine. The following monoamine metabolites and other potential contaminants were also tested in 25 to 250 µM concentrations: 5-hydroxyindoleacetic acid (\textit{5-HIAA}), homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxy-phenyl(ethylene)glycol (MHPG) and the purine derivative, uric acid (UA). All substances were dissolved in phosphate-buffered saline at pH 7.4. Calibrations were always done after each experiment since insertion into brain tissue significantly decreases electrode sensitivity (Stamford et al. 1992). In the presence of monoamines, oxidation currents were generated during the first ascending phase of the triangular input waveform. Oxidation peaks of all tested monoamines, which were maximal when the applied input waveform was around 950 mV, could not be distinguished from each other (Fig. 1). The sensitivity of the electrodes (n=11) in nM/nA was 12.7 ± 2.5 (mean ± standard error, SE) for serotonin, 47.9 ± 9.0 for dopamine and 118.7 ± 17.4 for norepinephrine. The CFMEs were much less sensitive to anionic metabolites of the transmitters (Commissiong 1985a,b; Kopin 1985; O’Neill et al. 1998). Specific values were 5-HIAA 830 ± 105 (n=7), HVA 919 ± 216 (n=4), DOPAC 5073 ± 888 (n=7), MHPG 692 ± 19 (n=3) nM/nA. The sensitivity to UA was also low: 2225 ± 407 (n=2).

Post-oxidation reduction currents of catecholamines and indoleamines showed quite different patterns, which was used as an aid to their identification. Catecholamines had single peaks near –400 mV, whereas indoleamines showed two peaks, one near –100 mV and the other near – 800 mV (Fig. 1). Reduction peaks were always of smaller amplitude, probably because diffusion of the products prevented complete reversal of the electrode oxidation reaction.
The pH dependence of the voltammetric measurements was evaluated as described previously (Hentall et al. 2003a). A physiologically large decrease of ~0.1 units in the presence of NE enhanced the current output across most of the voltage scan. Ascorbate slightly enhanced the oxidation current but did not alter the post-oxidation reduction current.

**In vivo voltammetry and isocurrent mapping**

FCV measurements were made at various recording sites within the L1-5 spinal segments of halothane anesthetized rats (Fig. 2) following localized removal of the pia matter. The reference electrode was positioned near the recording site in the spinal cord, and the auxiliary electrode was covered with saline-soaked gauze and placed into a nearby muscle. Changes in the concentration of the various monoamines at the different recording locations within the spinal cord were measured relative to the initial surface scan of each track (Fig. 2B, top trace). The depth of the electrode in all tracks in a given mapping session was referred to the surface depth of the first track. This was done so that measurements at each depth were always related to ones obtained at similar depths in the adjacent tracks. In penetrations where the surface of the spinal cord was different than the initial track, the first subtracted voltammogram was obtained slightly above the spinal cord (Fig. 5). The electrodes were advanced in steps of 2 µm at 1 Hz along vertical penetrations through the spinal cord (Fig. 3A). Signal amplitudes of oxidation peaks were stable at each depth when electrodes were advanced at this slow rate. In some cases, single voltammetric scans were recorded from each point, then averaged in 100 µm segments for the average depth. In other experiments, the microelectrode was stopped every 100 µm (Fig. 3B) and twenty triggered sweeps were then averaged. Tracks were equally spaced in the lateral direction (every 100 µm) and were made successively from the most
medial point to the most lateral point possible on the dorsal surface of the spinal cord. The final depth of penetration was selected to prevent microelectrode damage.

A new higher-resolution matrix was generated by fitting a cubic interpolating function (Matlab, Mathworks Inc.) to all data points in a given cross-section. Isocurrent contours were derived from this matrix (Fig. 3; see Noga et al. 1995) and color coded maps of the oxidation current generated. The histologically reconstructed maps of recording sites were re-scaled for tissue shrinkage and the angle of microelectrode penetration, then superimposed on the cross-sectional isocurrent contour maps. In cases where subtracted voltammograms obtained every 2 µm were averaged together for every 100 µm of the matrix (see track 4 of Fig. 3A), the midpoint depth was used when overlaying the histological outline onto the coordinates of the matrix and the maps appear to start slightly below the surface of the cord (Fig. 5E,F,H,K).

In the experiments with cooling and transection of the thoracic spinal cord, CFMEs were positioned at locations within the dorsal or ventral horns where oxidation peaks were large. Voltammograms applied every second were captured before, during and after cooling and/or spinalization. A xanthine oxidase inhibitor allopurinol (10 mg/kg dissolved in 1.0 ml of 0.9% NaCl) was slowly administered (over 100 s) i.a. to one animal to diminish the concentration of UA, a potential contaminant of the oxidation signal (Rivot et al. 1987). Allopurinol was only weakly electroactive (4065 nM/nA).

**Histology**

At the end of the experimental procedure, the cords were removed and immersed in 4% paraformaldehyde in phosphate buffered saline (PBS) solution (pH 7.4). Frozen serial sections (50 µm) of the relevant spinal cord segments were cut on a sliding microtome, collected in PBS and
counterstained with Toluidine Blue or Cresyl Violet (Hentall et al. 2003a). Camera lucida drawings were made to reconstruct the position of the electrode tracks. The distance between histologically observed electrode tracks was used to calculate shrinkage.

RESULTS

Characteristics of individual voltammograms

Peaks occurring in voltammograms during the first positive going ramp of the applied triangular waveform were delayed in vivo from those acquired ex vivo (Fig. 2; Hentall et al. 2003a), occurring at 1035 ± 7.1 mV (mean ± SE). The peaks were also somewhat broader than those seen ex vivo. They were nevertheless taken to represent a monoamine signal because similar signals were produced by microinjection or superfusion of monoamines while recording within the dorsal horn (see Fig. 2A for response to serotonin). Reduction currents were not always clearly observed and were delayed with respect to ex vivo calibrations. Reduction peaks of topically applied or intraspinally injected monoamines, when present, were also delayed. It was usually not possible to distinguish between indoleamines or catecholamines from reduction currents in vivo. The post-oxidation reduction current recorded in vivo typically showed a single peak (Fig. 2). This could be due to a mixture of indoleamines and catecholamines since the single reduction peak of catecholamines is found midway between the double reduction peaks of indoleamines (Fig. 1A).

Basal mapping

Peaks in the amplitude of the oxidation current were observed at various depths within the gray matter during electrode tracking. The localized nature of the peaks observed as electrodes were advanced downward were confirmed with measurements made at equal increments while the
electrodes were withdrawn upward at the same speed from the spinal cord (n=10). As can be seen from a representative example in Fig. 4, local maxima in monoamine oxidation current within the dorsal horn (at depths of ~400, 850 and 1130 µm) observed when the CFME was inserted, were also observed when the electrode was withdrawn. The amplitude of the oxidation current, however, was decreased during withdrawal.

Isocurrent maps, generated from the oxidation peak amplitude measurements of subtracted voltammograms and overlaid on histological reconstructions of each spinal segment, are illustrated in Fig. 5. The relative sensitivity of different electrodes to monoamines was quite variable, as gauged by their calibration curves, so the color code of each map is scaled according to its minimum and maximum current values. The oxidation current amplitude varied according to the depth of the recording site within the spinal cord (Fig. 2B). Relative to each initial surface scan, oxidation currents ranged from –40 to 260 nA, with the maximum range in any particular map not exceeding 240 nA (Fig. 5E). The entire range of measured current could be encountered across distances as short as 100-200 µm, thus the foci of maxima were well defined and confined to particular lamina(e) or portions of them. The location of oxidation current foci was quite variable in the different maps, although some general patterns emerged. In the dorsal horns of all segments (Fig. 5), large oxidation currents and foci were observed in various parts of laminae I-IV and in the central to lateral portion of laminae V/VI. Occasionally electrode penetrations through foci within the dorsal horn appeared to result in the adherence of electroactive substrate to the electrode tip and in the subsequent contamination of the electrode as it was advanced through the gray matter (Fig. 5H,J). However, foci could sometimes be observed along these tracks. In intermediate zone and ventral horn, the largest oxidation currents were observed in areas within lamina VII (central to lateral part), lamina VIII and
lamina IX (medial or lateral). Overall, the largest currents were found in dorsal or ventral horns and lateral aspect of the intermediate zone.

With regard to segmental differences, the largest oxidation peaks in L1 were observed predominantly in lateral areas of laminae II, III and V. Smaller foci were found in the lateral or central portion of laminae IV, V, VII-VIII (Fig. 5A,B). In L2, the largest oxidation currents were observed in medial or central portions of laminae I-III, VIII and IX. Lesser currents were found in central-to-lateral parts of laminae IV, V, VII and lateral lamina IX (Fig. 5C,D). In L3, a zone of high oxidation current was observed along the medial aspect of the entire dorsal horn (Fig. 5E) and in central-to-lateral portions of laminae IV-IX (Fig. 5F,G). Zones of lesser current were found separating these areas (Fig. 5E-G). In L4 the largest foci were located primarily along medial areas of the spinal gray matter in laminae I-IV and in the medial nucleus of lamina IX, with lower currents in the central and lateral parts of laminae IV-VII (Fig. 5H-J). In L5, areas of highest oxidation currents were found along lateral aspects of the dorsal horn, intermediate zone and ventral horn and in laminae IV/V (medial), VIII, and the medial nucleus of lamina IX (Fig. 5K,L).

**Interruption of descending activity**

The contribution of neurotransmitters released from tonically active descending monoaminergic pathways to oxidation currents measured either within the dorsal or ventral horns was determined by cooling and/or transection of the thoracic spinal cord. The results are illustrated in Fig. 6. Cooling of the spinal cord with crushed ice (n=3) decreased the oxidation signal recorded within the dorsal horn to 71-85% of control values (Fig. 6A,C,D). Transection of the cord usually resulted in a brief (20-45 s) injury-evoked increase in the oxidation signal (Fig. 6A,B,D,E) followed by a drop in the oxidation current amplitude to levels lower than pre-transection values, either
immediately (Fig. 6C-E) or after a short delay (Fig. 6A,B). Oxidation current amplitudes attained a low of 17-86% of control values [mean 57.4% +/- 11.9 (SE), n=5]. In 1 case, oxidation current values returned to normal control (pre-cooling) values, 72 minutes after transection (Fig. 6A). The maximal current drop was 64, 125, 47, 29, and 10 nA for A-E, respectively.

Assuming an equal contribution from serotonin and norepinephrine and half of that from dopamine (see Discussion), the peak concentrations for the serotonin and norepinephrine at each recording site (Fig. 6A-E) calculated from the decrease in current following transection would be approximately 1200, 1200, 500, 200 and 200 nM, as determined from ex vivo calibrations. The highest concentrations of monoamines released from tonically active descending fibers were thus in laminae I-III, with progressively lower amounts released in more ventral areas of the dorsal horn (medial lamina IV) and in the intermediate zone (laminae VII). Following transection, residual currents of 138, 25, 40, 69 and 59 nA were observed for A-E, respectively. An upper limit on the concentration of metabolite at each of these sites can be obtained if one assumes that residual currents originate from the monoamine metabolites (see Discussion below). Assuming that monoaminergic transmitter metabolites are present in the same ratio as their parent transmitters, that the CFMEs had similar sensitivities to 5-HIAA, MHPG and HVA in these five experiments (~700 nM/nA), and that contributions from DOPAC can be excluded by virtue of its very low oxidation current ex vivo, the concentrations for 5-HIAA and MHPG at these sites was estimated to be approximately 39, 7, 11, 19 and 17 µM (with half that amount for HVA), respectively. Allopurinol administration (Fig. 6D) resulted in a brief rise in measured current, likely due to the weak oxidation current that this substance generates by itself. Subsequently, an additional ~ 5nA (5%) drop in oxidation current (11.1 µM) was noted.
DISCUSSION

The present study combined a spatial mapping technique (Noga et al. 1995) with fast cyclic voltammetry to measure extracellular concentrations of monoamines within the lumbar spinal cord of the anesthetized rat. Salient findings included large gradients in oxidation current, which can go from highest to lowest in ~100-200 µm. Such high gradients were not detected in previous studies, probably because measurements were made either with microdialysis probes (e.g., Bowker and Abhold 1990; Lisi et al. 2003; Matos et al. 1992; Men et al. 1996; Sorkin et al. 1991) or larger carbon fiber electrodes (Rivot et al. 1983, 1995) that span several spinal laminae. Also, a significant amount of the monoamine content was shown to be released from tonically active descending pathways. However, transection of the spinal cord led initially to a transient increase in monoamine levels, which may be a significant factor in the induction of acute and chronic neurological response to spinal cord injury.

What substances contribute to the steady-state oxidation currents within the lumbar cord?

The present voltammetric data show high spatial resolution, but the chemical species contributing to the signal are best deduced in conjunction with previous analyses using chemical extraction (principally microdialysis) and with the known chemical neuroanatomy. Measurements of endogenous monoamine levels in the rat, using tissue extracts (Basbaum et al. 1987; Commissiong 1985a; Commissiong et al. 1979, 1984; Ko et al. 1997) or in vivo microdialysis (Abhold and Bowker 1990; Bowker and Abhold 1990; Lisi et al. 2003; Men et al. 1996) indicate that the spinal serotonin concentration is several times higher than dopamine and is relatively similar to norepinephrine. Our electrodes were more sensitive to serotonin than to norepinephrine or dopamine (respectively 9 and 4 times more). Thus serotonin is likely to have produced most of the current in the electrochemical
signal. In support of this view, the largest oxidation currents were also observed where descending serotonergic terminals are most dense (Jones and Light 1992; Maxwell et al. 1983, 1996).

Norepinephrine probably also provided a significant proportion of the oxidation current at many sites, even if less than that provided by serotonin. The spinal cord is densely innervated by descending noradrenergic fibers, in the dorsal horn (laminae I-IV), intermediate zone and ventral horn (lamina IX) (Clarke and Proudfit 1991a, b; Grzanna and Fritschy 1991), which are all regions where large oxidation currents were observed. Dopamine, on the other hand, probably contributed less to the electrochemical signal given the relatively low levels reported previously (see above), although we cannot rule out that a few individual oxidation foci were due to dopamine release. Dopaminergic pathways descend within the dorsolateral funiculus (Comissiong et al. 1979; Skagerberg et al. 1982) and terminate in the dorsal horn (primarily laminae III-V), the intermediolateral cell column, close to the central canal and sparingly within the ventral horn (Ridet et al. 1992; Skagerberg et al. 1982).

A significant portion of the total current could also have come from the serotonin metabolite 5-HIAA, even though the electrodes were 65 times less sensitive to 5-HIAA than to serotonin. 5-HIAA is found in much higher concentrations than serotonin in dialysates from the dorsal horn (Matos et al. 1992; Men and Matsui 1994; O’Neill et al. 1998; Sorkin et al. 1991). If the amounts of serotonergic and noradrenergic metabolites were in equal concentrations (Comissiong et al. 1984), and only metabolites were present following transaction, then post-transection measurements suggest that levels of 5-HIAA and MHPG (the major metabolite of norepinephrine) were between 7 and 39 µM. For 5-HIAA, this is in general agreement with differential pulse voltammetry measurements of indoleamines in the rat dorsal horn (Rivot et al. 1987, 1995). The assumption that only metabolites remain after transaction is based on the improbability of continued transmitter
release from the terminals of the severed monoaminergic projections due to injury-evoked depolarization, since severed axons seal within 30 to 60 minutes (Shi et al. 2000). The steady drop in oxidation current over the one hour period following transection could reflect this sealing. It is possible, however, that monoamine uptake mechanisms are reduced after transection due to energy deprivation. No evidence is available on this point, and non-myelinated axons are less vulnerable than larger axons to oxygen and energy deprivation following injury (Shi and Pryor 2002). Another untested possibility is an extraneuronal (vascular) source of monoamines (Salzman et al. 1987) that would maintain transmitter levels within the distal cord immediately after injury.

Metabolites of dopamine are found in much higher concentrations in the rat dorsal horn compared to dopamine (Men and Matsui 1994), but our CFMEs were 19 and 106 times less sensitive to HVA and DOPAC than to dopamine and extracellular dopaminergic metabolites are at much lower levels than 5-HIAA (Matos et al. 1992; Men and Matsui 1994). Norepinephrine and dopamine, being cations, diffuse slower than their anion metabolites (Rice et al. 1985), supporting the notion that the present highly differentiated maps mostly reflect the primary neurotransmitter.

Other neurotransmitters, principally amino acids or peptides, do not contribute to the current registered by fast cyclic voltammetry (Stamford et al. 1992). Uric acid (UA), a product of purine metabolism found within the spinal cord (Basbaum et al. 1987; Rivot et al. 1987,1995), and ascorbate are more likely to be contaminants. Our CFMEs were weakly sensitive to UA (185 times less than to serotonin). Also, allopurinol administration resulted in only a 5% drop in the oxidation current within the dorsal horn (Fig. 6B). Hence the contribution of UA to the recorded signals within the spinal cord is likely to be low. High concentrations of ascorbate enhance the oxidation current generated by monoamines (Hentall et al. 2003a). But powerful homeostatic mechanisms maintain
ascorbate at relatively fixed levels in the extracellular space (Schenk et al. 1982), so this substance is unlikely to have given rise to the present spatially differentiated map.

**Functional considerations**

Oxidation current foci were found in all laminae, with little intersegmental differences in the regions examined. Thus several descending functions appear to be continuously operative. One is the tonic control of vasomotor tone and arterial pressure (Millan 2002) within the intermediolateral horn of the thoracic and upper lumbar spinal cord, where terminals of monoaminergic pathways innervating preganglionic sympathetic neurons are found (Hosoya et al. 1991; Fuxe et al. 1990). In laminae I, II (outer), IV, V, and VI, the high concentrations of monoamines may be related to their importance in the control of nociceptor transmission from C and Aδ fibers which project to these areas (Craig and Dostrovsky 1999, Millan 1999) and in lamina IX to the control of motoneuron activity (Schmidt and Jordan 2000). The medial part of lamina VII, on the other hand, showed low oxidation currents, despite the concentration of monoaminergic terminals here. This can be explained by the involvement of neurons in this area of the rat (Kjaerulff and Kiehn 1996), like the cat (Huang et al. 2000; Noga et al. 1995; see also preliminary observations: Johnson et al. 2002), in the control of walking and other voluntary movements, functions that are not active continuously but rather as the need for the specific movement activation occurs.

The highest monoamine concentrations observed (in the superficial dorsal horn) were well above those needed by endogenous ligands to activate serotonergic and catecholaminergic receptors (Bunin and Wightman 1998; Calado and Stamford 2000; Cragg et al. 2001). Given that the CFME cannot resolve concentrations confined to the synaptic cleft, this implies that non-synaptic “volume” or diffusion neurotransmission is likely (Bach-y-Rita 1999; Vizi 2000). This concept is supported by
observations that serotonergic and noradrenergic terminals within the dorsal horn of the rat exhibit little synaptic specialization (Marlier et al. 1991; Maxwell et al. 1983; Ridet et al. 1993, 1994). In the ventral horn, in contrast, synaptic contacts are predominant for serotonergic terminals (Privat et al. 1988), so that serotonergic volume transmission may here be less important. The well-defined oxidation current maxima, however, and the steep concentration gradients, indicate that transporter uptake likely restricts neurotransmitter diffusion a few hundred microns at most.

**Effects of acute spinal transection**

Spinal cooling or transection resulted in a complex pattern of rise and fall in the oxidation currents within the spinal cord. The drops strongly suggest ongoing release from terminals of descending monoamine pathways, as shown by microdialysis studies (Abhold and Bowker 1990; Bowker and Abhold 1990; Lisi et al. 2003; Men et al. 1996). The reduction amounted on average to approximately 43% of the oxidation current (range of 14-83% of control values). Tonic activity through descending monoaminergic pathways is known to modulate the activation of spinal neurons by a variety of segmental afferents (Millan 1999, 2002; Rivot et al. 1987). Cells within the nuclei of origin of these pathways frequently display a steady discharge rate (Faiers and Mogenson 1976; Fields et al. 1983; Valentino and Aston-Jones 1995) that could account for the release of these transmitters at the spinal level. Surgical exposure of the spinal cord should result in increased noxious stimulation of multireceptive dorsal horn neurons (Li et al. 1998), and increased activation of descending monoaminergic pathways (Dickenson and Goldsmith 1986; Valentino and Aston-Jones 1995), with consequent increased monoamine release. This idea is supported by the observation that peripheral nerve stimulation can increase the release of serotonin, norepinephrine and dopamine within the spinal cord (Men and Matsui 1994; Men et al. 1996). The present, well-
defined foci of monoamine oxidation current may therefore reflect “localized alterations in nociceptive processing” due to recruitment of descending monoaminergic pathways (Millan 2002). Such foci may be further enhanced by the anesthetic (Keita et al. 1999; Pashkov and Hemmings 2002).

The transient changes in the extracellular monoamine signal in the distal stump of the cord over the first few hours following spinal cord injury have not previously been reported. However, the increases observed are not entirely new, since a small increase of serotonin or related compounds (but not neuropeptide or dopamine) has been observed near cord impact injuries (Salzman et al. 1987; Liu et al. 1990). The rapid and massive efflux of monoamines seen here immediately after injury in distal cord segments is likely to affect both local vasomotor control (Saruhashi et al. 1990) and, via its action on autonomic preganglionic neurons, arterial pressure (Hosoya et al. 1991; Fuxe et al. 1990; Millan 2002). Serotonin may also support the initiation of trophic effects that can influence fiber regeneration (Salzman et al. 1994; Bregman et al. 2002). On a longer time-scale, tissue levels of serotonin and dopamine have been reported to rise 1 day following transection, followed by decreases to near zero levels by 5 and 9 days, respectively (Magnusson 1973). Norepinephrine has been reported to show no initial increase but rather to decrease gradually to near zero levels by 15 days (Magnusson 1973; Commissiong and Toffano 1989).

In conclusion, high-resolution spatial mapping of monoaminergic substances with fast-cyclic voltammetry provides insight into the dynamic nature of monoaminergic control of spinal function. The detection of spatial maxima and minima over distances as short as a few hundred microns indicate that monoaminergic control of sensory and motor function may be more precise than suggested by measurement techniques utilizing larger diameter microdialysis probes or CFMEs with
exposed lengths of several hundreds microns (which are essential for slow scanning across a range of oxidation potentials with pulse voltammetry). This implies that combining the spatial mapping of transmitter release with temporal measurements from multiple implanted CFMEs is a useful way to study the dynamic control of neural function by descending modulatory pathways in different states of behavioral activation.

Acknowledgements. We wish to thank M. Riesgo for assistance during the experiments. The study was supported by: The Miami Project to Cure Paralysis, by a grant from the State of Florida, and by the Campus Research Board of the University of Illinois Chicago.

REFERENCES


Clark FM and Proudfit HK. The projection of noradrenergic neurons in the A7 catecholamine cell group to the spinal cord in the rat demonstrated by anterograde tracing combined with immunocytochemistry. *Brain Res* 547: 279-288, 1991b.


FIGURE LEGENDS

**Fig. 1.** Subtracted voltammograms obtained *ex vivo*. **A:** Results of *ex vivo* calibrations in different concentrations of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in the control medium (phosphate-buffered saline, pH 7.4). The subtracted voltammograms are averages (50 scans). Also shown is the triangular input waveform (*top*). Note the concentration dependence of the oxidation peak current. *Inset:* Scanning electron micrograph of beveled tip of CFME. The carbon fiber can be seen surrounded by the shaft of the pulled micropipette. Calibration bar: 100 µm. **B:** Voltammograms of monoamine neurotransmitters, their metabolites (5-HIAA, MHPG, DOPAC, and HVA) and UA at the indicated concentrations.

**Fig. 2.** Comparison of *in vitro* and *in vivo* subtracted voltammograms relative to the applied input waveform. **A:** Voltammograms obtained from 5-HT *in vitro* (0.5 µM), 5-HT *in vivo* (CFME in the dorsal horn; 10 µM 5-HT added to the bath solution over the surface of cord) and from a recording site (foci) in the ventral horn of the L3 segment. The input waveform is shown in the top trace. 40 nA calibration bar for first and second voltammograms; 100 nA calibration bar for third voltammogram. *Dotted lines* indicate reference levels used to calculate the peak current (see Methods). The oxidation peaks are indicated by the *open triangles* in the subtracted voltammograms. The oxidation current was obtained by measuring the distance from the base to the peak (*arrow*). *Dashed line* through peaks obtained *in vivo* illustrates the delay in the oxidation peak when compared to that obtained *in vitro*. The *closed triangle* shows point of peak reduction current. **B:** Successive voltammograms (from surface to a depth of 2200 µm) obtained from a middle track in L3
(Fig. 5G). The subtracted voltammograms (averages of 20 sweeps) were taken every 100 µm. Calibration for current traces as in A (100 nA). Time calibration in B applies to all voltammograms.

**Fig. 3.** Alternate methods used to generate isocurrent maps from subtracted voltammograms taken at different depths of the spinal cord. **A:** The electrodes were advanced 2 µm/s along vertical tracks in the cord. Tracks were separated 100 µm from each other. 50 sweeps were averaged together (25 sweeps on either side of the midpoint depth) for each 100 µm position of the matrix (see track 5). The midpoint depth of the averages was used when overlaying the histological outline onto the coordinates of the matrix. **B:** The electrodes were advanced at evenly spaced coordinates (100 µm) along each penetration to form a matrix. Oxidation current amplitudes were measured from averages of 20 sweeps made at each depth. In both cases cubic interpolation techniques were used to generate a matrix of higher resolution by fitting a continuous surface through the given points over the entire area of the matrix. Regions of equal current were then connected to form isocurrent lines and the map fitted into the most dorsal or ventral points of the most medial and lateral electrode tracks (respectively) of camera lucida drawings of cross-sections of the spinal cord containing the reconstructed electrode tracks (see Methods). Regions of similar current values were then color-coded (Fig. 5).

**Fig. 4.** Reproducibility of recorded oxidation currents with penetration and withdrawal of the microelectrode. Intraspinal foci of relatively high current, were similar during penetration and withdrawal of the CFME, albeit smaller during the latter.
Fig. 5. A-L: Isocurrent maps of oxidation peak amplitude measured from subtracted voltammograms obtained from all lumbar segments (L1-5). The colored scale is optimized for each map due to the high difference in sensitivities of the electrodes. Areas of lowest current are depicted as blue, and the gradient increases to yellow in areas with higher current, and to a red gradient at foci (color codes indicated at bottom of each map). Post-experimental calibration curves for serotonin, dopamine and norepinephrine for each electrode, indicated in M, place an upper limit on the estimations of monoamine concentration. See Methods for detailed description of the generation and plotting of the isocurrent maps. Cytoarchitecture adapted from Molander and Grant (1995).

Fig. 6. Effect of cooling and/or transection of the thoracic spinal cord (T6/7) on amplitude of monoamine oxidation peaks recorded from laminae I-IV of the lumbar dorsal horn (A-D) or from lamina VII (E). Sites of recording for each trial are indicated on camera lucida drawings. Periods of cooling, packing, transection and application of gelfoam to the transection site are indicated for each trial. A,C,D: shows effects of prior cooling of the cord plus the transection procedure. Cooling resulted in a drop in oxidation current that was further compounded by transection of the cord. Manipulation of the spinal cord accounts for sudden changes in the oxidation peaks at several points during the recordings (ice packing or application of gelfoam), possibly due to compression or injury-evoked discharge from monoaminergic axons. Transection of the cord resulted in a brief injury-evoked increase in the oxidation signal in 4 cases (A,B,D,E). A drop in the oxidation current amplitude to levels lower than pre-transection values was observed in all cases. Allopurinol administration (2.5 mg i.a.) following transection (D) resulted in a brief increase in oxidation current followed by an additional drop of 5 nA relative to the pre-drug level. Calibrations for A-E are found in Fig. 5M (calibration B, L, D, A and C, respectively).
Figure 1
Figure 2

A

Volts

5-HT in vitro
0.5 µM
40 nA

5-HT in vivo

in vivo

in vivo

0

-1

-1

5 ms

B

5 ms

Figure 2
Figure 3
Figure 4
Figure 5 - Part 1
Figure 5 - Part 2
Figure 6

- A: Peak Oxidation Current (nA) for Caudal L1 with cooling and transection events.
- B: Peak Oxidation Current (nA) for Rostral L4 with transection event.
- C: Peak Oxidation Current (nA) for L2 with transection and cooling events.
- D: Peak Oxidation Current (nA) for L1 with transection, ice packing, and allopurinol events.
- E: Peak Oxidation Current (nA) for Rostral L4 with transection and gelfoam events.