ALPHA-1 ADRENOCEPTOR AGONISTS GENERATE A “FAST” NMDA-RECEPTOR INDEPENDENT MOTOR RHYTHM IN THE NEONATAL RAT SPINAL CORD.

Gabbay H and Lev-Tov A.

Dept. of Anatomy and Cell Biology
The Hebrew University Medical School
P.O.Box 12272, Jerusalem 91120
ISRAEL.

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Correspondence:
Dr. A. Lev-Tov
Dept. of Anatomy and Cell Biology
The Hebrew University Medical School,
P.O. Box 12272, Jerusalem 91120,

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ABSTRACT
Noradrenaline, a potent activator of rhythmogenic networks in adult mammals has not been reported to produce functional rhythmic patterns in isolated spinal cords of newborn rats. We now show that a “fast” (cycle time 1-4s) transient rhythm was induced in sacrococcygeal (SC) and rostral-lumbar spinal segments of the neonatal rat by bath-applied noradrenaline. The “fast” rhythm was blocked by 1µM of the α1-adrenoceptor antagonist prazosin but not by 1-20µM of the α2-adrenoceptor blocker yohimbine, it could be initiated and maintained by α1-adrenoceptor agonists, and was accompanied by a slow non-locomotor rhythm. Transection at the lumbosacral junction abolished the “fast”-thoracolumbar (TL) rhythm while the fast-SC and slow-TL rhythms were unaffected. The NMDA-receptor antagonist AP5 abolished the slow- and did not interrupt the “fast”-rhythm. Thus, α1-adrenoceptor agonists induce an NMDA receptor-independent rhythm in the SC cord, and modulate NMDA receptor-dependent rhythmicity in TL segments. Injection of current steps into S2 and flexor-dominated L2 motoneurons during the “fast” rhythm revealed a 20-30% decrease in input-resistance ($R_N$), coinciding with contralateral bursting. The $R_N$ of extensor-dominated L5 motoneurons did not vary with the “fast” rhythm. The rhythmic fluctuations of $R_N$ in L2 motoneurons were abolished, but the alternating left-right pattern of the “fast” rhythm was unchanged in midsagittally-split TL cords. We suggest that the locomotor generators were not activated during the “fast” rhythm, that crossed-inhibitory pathways activated by SC projections controlled the rhythmic decrease in $R_N$ in L2 motoneurons, and that the alternating pattern of the split TL cord was maintained by excitatory SC projections.
INTRODUCTION

Monoamines have long been known as potent modulators of rhythmogenic networks. Lundberg and colleagues have shown that L-DOPA, a precursor of dopamine and noradrenaline, initiates coordinated rhythmic activity in spinal cats (Jankowska et al. 1967, for review see Hultborn et al., 1998). This rhythmic activity exhibited complex locomotor patterns and could be induced in the absence of phasic sensory input (Grillner and Zangger, 1979). The monoamine noradrenaline (NA) has also been reported to initiate locomotor activity in cats and rabbits (Barbeau and Rossignol, 1991, Forssberg and Grillner 1973, Jankowska et al. 1967, Kiehn et al, 1992, Viala and Buser, 1969). It triggers locomotion in cats few days after spinalization by activation of mainly $\alpha_2$ and to lesser extent $\alpha_1$ adrenoceptors (Chau et al, 1998), and it can modulate locomotion, muscle tone and reflex excitability in spinal cats that established spontaneous locomotion (“late” spinal cats, for review see Rossignol et al, 1998). The noradrenergic innervation of the spinal cord is mediated by descending projections from the locus coeruleus and the subcoereleus nucleus and their terminations are distributed throughout most of the spinal gray matter, with the highest densities at the superficial dorsal horn, the thoracolumbar intermediolateral column, and the ventrolateral ventral horn (Clark and Proudfit 1991a,b; Fuxe et al. 1990; Grzanna and Fritschy 1991; Martin et al. 1999; Westlund et al. 1982). While it is clear that monoamines are important regulators of locomotion in the adult spinal cord, their role in the neonatal spinal cord is less clear. Although many noradrenergic terminals are present in the cervical and lumbar cords of rats during the first postnatal week (Rajaofetra et al, 1992, Tanaka et al, 1996), it has been difficult to establish a clear functional role for noradrenaline or adrenoceptor agonists in locomotion.
For example, bath application of noradrenaline failed to produce a locomotor rhythm in the neonatal rat spinal cord preparation (Kiehn et al, 1999, Sqalli-Houssaini and Cazalets 2000). Interestingly, dopamine, the precursor of noradrenaline has been shown to induce a slow and organized motor pattern in the neonatal rat spinal cord (Smith et al, 1988, Kiehn et al 1996), and its presence facilitates the expression of the locomotor rhythm in isolated spinal cords of young adult mice (Jiang, Carlin and Brownstone, 1999, for review see Bonnot et al, 2002b).

The apparent differences in the response of the adult and neonatal cord to adrenoceptor agonists may reflect the relative immaturity of the central pattern generators in the younger cords. However, we now show, in contrast to earlier studies, that adrenoceptor agonists can produce a transient rhythm with a 1-4s cycle time and an organized alternating left right pattern in rostral lumbar and sacrococcygeal segments of the neonatal rat spinal cord. In this paper we investigate the receptors associated with the generation of the rhythm, its segmental origin, the organization of the networks involved in its generation, and the induced motor behavior. Our findings provide further insights into the maturation of vertebrate locomotor pattern generating circuitry.

**METHODS**

**Preparations:** Spinal cord preparations (T6-Co3) were isolated from P2-P4 ether anesthetized rats with or without an intact tail (Lev-Tov et al., 2000, Lev-Tov and Delvolvé, 2000, Delvolvé et al 2001). The cord was transferred to a recording chamber and superfused continuously with an oxygenated Krebs saline (e.g. Kremer and Lev-Tov 1997; Lev-Tov et al. 2000, Delvolvé et al, 2001).
Stimulation and recordings: ventral root potentials (VRPs) were recorded by suction electrodes from pairs of lumbar and sacral ventral roots (DC or 0.1Hz-10 KHz) using a high gain DC/AC amplifier. Sharp electrode intracellular recordings were obtained from L2, L5 and S2 motoneurons impaled from the ventral or ventrolateral aspect of the cord, and identified by the presence of antidromic spikes. Microwire EMG recordings (100Hz-10KHz) were obtained from left and right tail flexors and extensors (see Lev-Tov et al, 2000, Delvolve et al., 2001, Gabbay et al. 2002). Rhythmic activity was induced by bath application of noradrenaline (NA) or the α1 adrenoceptor agonist methoxamine or phenylephrine.

Data acquisition and statistical analysis: Recorded data, were digitized (Digidata 1320A, Axon Instruments Inc.), and stored on the computer’s hard disk for subsequent analyses (see Gabbay et al, 2002, Strauss and Lev-Tov, 2003). VRP data were low pass filtered at 50-100Hz and analyzed using either Clampfit 9 (Axon Instruments Inc.) or the time series routines of STATISTICA 6 (StatSoft, Inc. 2001). A detailed description of the time series analysis can be found in Strauss and Lev-Tov, 2003. Briefly, rhythmic data were divided into equal consecutive segments, the frequency of the rhythm and the phase shift between any given pair of time series variables were extracted using Fourier bivariate (cross spectral) analysis. The cycle time data were analyzed using linear statistical methods. Data were pooled when required if one way ANOVA revealed no significant differences between the data samples. The phase data were analyzed using circular statistics to calculate the mean phase-lag and the r-vector describing the concentration of phase-lag values around the mean under each experimental condition (Lev-Tov et al, 2000, Delvolve et al, 2001, Gabbay et al, 2002). Data were pooled when
required if the Watson and Williams test revealed no significant differences between the tested samples. The Rayleigh's test (Zar 1984) was used to determine whether the phase values were uniformly distributed around the circle (see Delvolve et al, 2001, Gabbay et al, 2002). Multi-sample testing was performed to compare the mean phase values of any pair of tested factors (the Watson-Williams test, Zar 1984).

RESULTS

**Noradrenaline induces a “fast” alternating rhythm in lumbar and sacrococcygeal segments of the spinal cord**

Noradrenaline has been reported to produce irregular bursting or a very slow rhythm in the lumbar cord of neonatal rats (Kiehn et al, 1999, Sqalli-houssaini and Cazalets 2000). Figure 1 shows that NA also produced a “fast” alternating left-right rhythm in lumbar and sacral segments of the spinal cord (A). Analysis of this rhythm revealed a mean cycle time of 2.8±1.2s, a robust alternating pattern with a mean left-right phase shift ($\phi_{L-R}$) of 0.51±0.09 cycles, r-vector=0.84 (7 exp.), and a coupled activity of the rostral-lumbar and ipsilateral sacrococcygeal segments with a mean phase shift ($\phi_{LL2-LS2}$) of 0.92±0.07 cycles (r-vector=0.92, 6 exp.).

**FIGURE 1 NEAR HERE**

Intracellular recordings from an S2 motoneuron (Fig 1B) show that the “fast” rhythm produced by NA is expressed as rhythmic oscillations of transmembrane potential superimposed on a tonic depolarization (~20mV) and that the bursts of spikes elicited during the depolarizing phases of these oscillations, were in phase with the ipsilateral ventral root firing. The “fast” rhythm induced by NA was short-lasting, it slowed down
within 1-2 minutes, decomposed into irregular packets of short alternating bursts mixed with occasional longer bursts (Fig1C), and finally (~5min) blocked.

**The “fast” rhythm is mediated mainly by activation of \(\alpha_1\)-adrenoceptors**

The pharmacological basis of the “fast” NA-induced rhythm was examined in a series of 8 experiments. The “fast” rhythm (Fig.2A) could be induced when NA was added to the bath 20min after application of the \(\alpha_2\)-adrenoceptor blocker yohimbine (1\(\mu\)M, Fig2B). The rhythm persisted in the presence of 2, 5 and 10\(\mu\)M yohimbine and in some of the experiments, in the presence of 20\(\mu\)M of the drug, and exhibited various perturbations when the concentration of yohimbine exceeded 20\(\mu\)M. By contrast, application of the \(\alpha_1\)-adrenoceptor blocker prazosin (1\(\mu\)M) to the preparation prevented the ability of NA to initiate the rhythm (Fig.2C). Similar results were obtained in each of the experiments performed in this series. These results suggested that the “fast” NA induced rhythm is accounted for mainly by activation of \(\alpha_1\)-adrenoceptors, a suggestion that was further supported by the finding that the “fast” rhythm could be initiated by bath application of the \(\alpha_1\)-adrenoceptor agonists methoxamine (Fig. 2D) or phenylephrine (not shown). The methoxamine-induced rhythm was superimposed on a tonic depolarization (11.7mV this experiment; mean=13.8±5.4mV, \(n=18\), pooled methoxamine and NA data) and was accompanied by a significant increase in input resistance (48.2±21.3%, sampled during non-rhythmic epochs in the 5 tested cases, \(P<0.01\), two tailed t-test). The cycle time of the methoxamine induced rhythm was 2.7±0.85s and the left-right phase was 0.5±0.001cycles, \(r\)-vector=0.89 (10 exp.). Unlike the NA-induced rhythm, the rhythm produced by methoxamine was long lasting. It slowed down gradually and persisted for tens of minutes (30-60min). To test whether the rhythm
produced by $\alpha_1$-adrenoceptors required an activation of NMDA receptors, we tried to induce the rhythm by methoxamine in the presence of the NMDA-receptor blocker AP5 (200µM).

FIGURE 2 NEAR HERE

Figure 3 shows that a robust rhythmic activity could be produced in S2 (B) as well as L2 (C) by methoxamine in the presence of AP5. The cycle time of the rhythm was slower than the control rhythm (5 exp.; histograms, D, $P<0.001$, two tailed t-test) and its alternating left-right pattern was unchanged (Watson and Williams test for multiple comparisons), circular diagrams (D, Control: $\phi_{LL2-RL2}=0.49\pm0.02$ cycles, r-vector=0.83, $\phi_{LS2-RS2}=0.49\pm0.09$ cycles, r-vector=0.86; AP5: $\phi_{LL2-RL2}=0.51\pm0.1$ cycles, r-vector=0.81, $\phi_{LS2-RS2}=0.47\pm0.1$ cycles, r-vector=0.83, respectively, 5 exp.)

FIGURE 3 NEAR HERE

**The pattern and segmental source of the methoxamine induced rhythm**

In a previous study we have shown that NA induced rhythmic tail movements characterized by left-right alternations of the tail musculature and by coactivation of flexors, extensors, and abductors on a given side of the tail (Gabbay et al, 2002). Similar movements and patterns were induced by bath-applied methoxamine (not shown). To determine the pattern of flexor-extensor activation at the lumbar cord during the rhythm, we recorded the activity produced by methoxamine from flexor (L1-L4) and extensor (L5) dominated segments of the lumbar spinal cord. Recordings from the left L2, L3, L4, and L5 ventral roots and from the left and right S2 ventral roots are shown in Fig. 4. Immediately (2-3min) after addition of methoxamine to the bath, “fast” rhythmic bursts appeared in L2, L3, L4 and S2 (cycle time $3.14\pm0.4$s, 5 exp.) but not in L5 (A). Analyses
of the temporal relation between L2, L3 and S2 (5 exp.) revealed that $\phi_{LL2-LS2}$ was 0.96±0.003 cycles, r-vector=0.99, meaning that the L2 rhythm lagged by 125±8ms after that of the ipsilateral S2, thereby, suggesting a caudorostral spread of the rhythm. The phase shift between L2 and L3 ($\phi_{LL2-LL3}$) was 0.003±0.0001 cycles (r-vector=0.99), and the phase shift between the left and right S2 ($\phi_{LS2-RS2}$) was 0.49±0.001 cycles, r-vector=0.98. At a later stage, (5-10 min after addition of methoxamine, B), the “fast” rhythm in the flexor dominating lumbar segments was accompanied by a superimposed slow rhythm (B, arrows). The slow rhythm which had a cycle time of 64±34.4s (data pooled from 5 exp. in this series and 9 additional exp.) compares to 3.14±0.4s, 5 exp. of the “fast” rhythm (see histograms in C), appeared in all the recorded lumbar (L2-L5) and sacral segments, exhibiting ipsilateral synchronicity of flexor and extensor dominated segments of the lumbar cord ($\phi_{LL2-LL5}$=0.02±0.03 cycles, r-vector=0.98, 5 exp. in this series and 4 additional exp.)

FIGURE 4 NEAR HERE

Thus, the “fast” lumbar rhythm developed mainly in sacral and flexor-dominated segments of the lumbar cord, while the slow rhythm appeared in all lumbar segments, and it showed no flexor-extensor alternation. Recordings from the left and right L2 and S2 segments (Fig. 5) show that the “fast” rhythm exhibited an alternating left-right pattern and that the slow superimposed rhythm had a bilaterally synchronous pattern (Fig. 5B, 7/13 experiments, $\phi_{L-R}$=0.998±0.06 cycles, r-vector=0.93), an alternating left right pattern (Fig. 5D, 3/13 experiments, $\phi_{L-R}$=0.55±0.18 cycles, r-vector=0.76) or a mixed and irregular (uniformly distributed, Rayleigh's test) pattern (3/13 exp. Not shown).

Transection of the spinal cord at the lumbosacral junction or at mid L5 blocked
immediately the “fast” alternating lumbar rhythm without impairing the slow lumbar rhythm. The “fast” alternating left-right rhythm of the sacral cord persisted under these conditions with a robust alternating left right pattern. Analysis of 8 transection experiments revealed that the cycle time of the S2 rhythm was 2.7±0.8s and 2.6±1.1s, and the left right phase shift \( \phi_{LS2-RS2} \) was 0.5±0.07 cycles, r-vector=0.91, and 0.51±0.09 cycles, r-vector=0.86, before, and after the transection, respectively.

To test whether the “fast” rhythm of the detached sacrococcygeal spinal segments (SC) and the slow rhythm of the detached thoracolumbar cord depended on activation of NMDA receptors, we transected the spinal cord at the lumbosacral junction in 5 different experiments, and recorded the activity induced by methoxamine from L2 and S2 before, and 30min following addition of 200\( \mu \)M AP5 to the preparation. The results of one of these experiments are shown in Fig. 5D. The slow lumbar rhythm (alternating left-right pattern in this case) could not be produced in the presence of 200\( \mu \)M of AP5 (upper pair, right), while a robust sacrococcygeal rhythm with a “fast” cycle time and an alternating left-right pattern was initiated under these conditions (lower pair, right; cycle time=2.5±0.96s (methoxamine) and 2.49±0.88s (AP5 and methoxamine), left right phase shift \( \phi_{LS2-RS2} = 0.5±0.07 \) cycles, r-vector=0.92, and 0.47±0.1 cycles r-vector=0.82, in the absence and presence of AP5, respectively). The unchanged frequency of the “fast” SC rhythm in the presence of AP5, suggests that AP5 did not affect the SC CPGs and that the prolonged cycle time observed in preparations with attached SC segments in the presence of AP5 (Fig. 3), was probably due to changes in interactions between the TL and SC cords following the suppression of NMDA receptor depended connectivity.

FIGURE 5 NEAR HERE
As mentioned above, the “fast” rhythm generated in the SC spinal segments, appeared also in flexor- but not extensor dominated segments of the lumbar cord. In a previous study we have shown that changes in input resistance of motoneurons and their relation to efferent firing of the segmental ventral roots could be used to detect rhythmic changes in somatic inhibition produced by the sacrococcygeal rhythmogenic circuitry in neonatal rats (Lev-Tov et al, 2000). Therefore, to infer about the organization of the pattern generating circuitry in the SC spinal segments and the recruitment of flexor and extensor half centers in the lumbar cord, we used repetitive intracellular injection of hyperpolarizing current steps to examine changes in input resistance of motoneurons in S2, the flexor-dominated L2, and the extensor-dominated L5 segments during the methoxamine induced rhythm. Our results are described below:

**Rhythmic fluctuations in input resistance of S2 and lumbar flexor motoneurons**

Figure 6A shows simultaneous ventral root recordings from S2 and intracellular recordings from an S2 motoneuron in a surgically detached SC cord, in the presence of methoxamine (one of three experiments performed in this series). A “fast” alternating rhythm with similar characteristics to the rhythm described in S2 motoneurons of intact TL-SC preparations (e.g. Fig. 2) was produced under these conditions. The firing of the recorded motoneurons was blocked by injection of hyperpolarizing current (-0.75 nA), and the voltage transients produced by repetitive injection of hyperpolarizing current steps (-0.5nA, 100ms, 2Hz) to the motoneuron were monitored. Continuous recordings of these voltage transients during the rhythm are shown with DC recordings of the left and right S2 VRPs, in Fig. 6B. A substantial decrease in the amplitude of the voltage transients was evident during the troughs of the rhythmic oscillation in membrane
potential. To analyze the relation between the voltage transients and the rhythmic activity, we averaged the voltage transients and the left and right S2 VRPs produced during the last 20ms of each current step and plotted the resultant mean amplitudes as a function of time in Fig. 6C. The rhythmic variations in voltage transients and their relation to the contralateral ventral root potentials (VRPs) were examined using cross correlation analysis (Fig. 6 C, right). This analysis revealed significant rhythmic changes in the amplitude of the voltage transients (reflecting rhythmic changes in input resistance) and an inverse correlation between the amplitude of the transients and that of the contralateral VRPs, i.e., the drop in input resistance was in phase with contralateral activity.

FIGURE 7 NEAR HERE

Figure 7 shows recordings from L2 motoneurons and the left and right L2 ventral roots in a bilaterally intact (A left) and a midsagittally split TL cords (A, right) during injection of hyperpolarizing current steps in the presence of methoxamine. The cross correlation analyses of the relation between the amplitude of left and right L2 VRPs, and between the amplitude of the voltage transients and that of the contralateral VRPs under these conditions are shown in B (top and bottom correlograms, respectively). These data show that the rhythmic fluctuations in amplitude of the voltage transients observed in the bilaterally intact thoracolumbar cord were inversely correlated to the amplitude variations in the contralateral VRPs, and that these oscillations in input resistance were not evident when the current steps were injected to L2 motoneurons in the midsagittally split TL cord. Interestingly, the rhythmic activity in the midsagittally split TL cord exhibited a significant alternating left-right pattern under these conditions. Similar pattern was found in 3 experiments in this series and 4 additional extracellular experiments. The relation
between the amplitude of the voltage transients and contralateral ventral root activity was further examined using regression analyses of pooled standardized data (for details see Fig. 7 legend). Summary plots of the standardized amplitude of the voltage transients vs. that of the contralateral VRPs in the bilaterally intact (3 exp.) and midsagittally split (3 exp.) TL cord are shown with the linear regression lines in Fig. 7C (left and right, respectively). A significant negative slope ($p<0.0001$) was found only for the bilaterally intact preparations (the slope and coefficient of determination ($R^2$) were $-0.56\pm0.04$, and 0.32 in the bilaterally intact, and $+0.09\pm0.51$ and 0.008, respectively, in the midsagittally split TL cords).

**FIGURE 8 NEAR HERE**

**Lack of rhythmic fluctuation in input resistance of lumbar extensor motoneurons**

Recordings from motoneurons in the extensor dominated L5 segments were performed in 6 different experiments to examine whether these motoneurons exhibit rhythmic inhibition during the activation of lumbar flexors. Figure 8 shows the voltage transients produced in a left L5 motoneuron and the efferent activity recorded from the left and right L5 and L2 ventral roots in the presence of bath applied methoxamine (A). The displayed records show no indication for systematic variations in input resistance of the recorded L5 motoneuron during the rhythm. This notion is supported by the cross-correlation analysis of these data (B). A clear and significant alternating left-right pattern of the L2 rhythm is evident from the cross-correlogram of the amplitude of the left vs. right L2 VRPs (Fig. 8B, left). At the same time there was no significant correlation between the amplitude of the voltage transients produced in the recorded L5 motoneuron and the amplitude of the ipsilateral L2 VRPs (B, right). Similar findings were observed in each of the experiments performed in this series. A summary plot of a regression
analysis of the pooled standardized data obtained in these experiments is shown in Fig 8C. The slope of the regression line between the amplitude of the voltage transients produced in L5 motoneurons and the ipsilateral rhythmic activity -0.03±0.024, was not significantly different from 0 ($R^2=0.001$ $P<0.15$).

**DISCUSSION**

*Initiation and modulation of rhythmic patterns by activation of adrenoceptors*

The effects of noradrenaline and adrenoceptor agonists on rhythmogenic networks in the mammalian spinal cord have been described in detail in a number of preparations (*vide ante*). In contrast to the ability of adrenoceptor agonists to induce locomotion or locomotor-like activity in spinal cats (for review see Rossignol, 1998), bath application of noradrenaline to isolated spinal cords of neonatal rats has been shown to induce a slow rhythmic activity with irregular patterns in the lumbar (Kiehn et al, 1999, Sqalli-houssaini and Cazalets 2000) and cervical cord (Morin et al, 2000). These effects of NA have been reported to be mimicked by bath applied $\alpha_1$- (Sqalli-houssaini and Cazalets 2000, Morin et al 2000), but not $\alpha_2$ or $\beta$-adrenoceptor agonists (Sqalli-houssaini and Cazalets 2000).

In the present work we showed that in addition to the slow and irregular rhythmic activity, bath applied NA produced a “fast” alternating transient rhythm in the sacral (e.g Gabbay et al, 2002) and rostral lumbar segments of the neonatal rat spinal cord. This rhythm has not been reported in previous studies (Kiehn et al, 1999, Sqalli-houssaini and Cazalets 2000), probably due to its short-lasting nature, and the appearance of the slow accompanying rhythm. We also showed that the “fast” rhythm could be induced and maintained for tens of minutes when the $\alpha_1$-adrenoceptor agonist methoxamine was used.
instead of NA. Thus, the transient nature of the “fast” NA-induced rhythm may be ascribed to activation of $\alpha_2$ and/or $\beta$-adrenoceptors which have been shown to slowdown and inhibit the 5HT/NMDA induced rhythm in the neonatal rat spinal cord (Sqalli-houssaini and Cazalets 2000).

The slow and “fast” rhythms induced by $\alpha_1$-adrenoceptor agonists differ not only in their cycle-times, but also in their segmental origin and the receptors involved in their generation. Our findings that the “fast” rhythm persisted in the SC spinal segments and was abolished in the TL segments after transecting the cord at the lumbosacral junction, and that the slow rhythm continued in the detached TL and not the SC cord, suggested that the “fast” rhythm originates in the SC segments and the slow one is produced by the TL circuitry. The finding that the slow rhythm was blocked by the NMDA antagonist AP5, while the “fast” rhythm persisted in the presence of high concentrations of AP5, indicated that the slow rhythm reflects modulation of the NMDA-receptor dependent rhythmicity of the TL cord similar to the suggestion of Kiehn et al, 1999, Sqalli-houssaini and Cazalets, 2000 and Morin et al, 2000, whereas the “fast” rhythm does not depend on NMDA receptors, and it may be actually initiated by activation of $\alpha_1$-adrenoceptors in the SC cord.

The reasons for the differential responsiveness of the TL and the SC central pattern generators (CPGs) to $\alpha_1$-adrenoceptor agonists are not known mainly due to lack of data concerning noradrenergic innervation of the tail moving networks in neonates. The differential responsiveness may arise from regional differences in the density and spatial distribution of adrenoceptors, from differences in the proportion of the known subtypes of adrenoceptors over constituents of the TL and SC CPGs, and from differential properties
of the pattern generating circuitry of the TL and SC spinal segments. Further understanding of the ability of monoamines to induce NMDA-independent rhythmic patterns in the isolated SC and not in the isolated TL spinal segments should await additional physiological and histochemical studies of noradrenergic innervation of these regions.

The mechanisms involved in the generation of the “fast” rhythm by NA are not known. Noradrenaline has been shown to decrease the potassium conductance and the input resistance of neonatal rat motoneurons, to depolarize them and increase their excitability (White et al, 1991, Parkis et al, 1995, Morin et al, 2000). These effects of noradrenaline were mimicked by $\alpha_1$ adrenoceptor agonists (Parkis et al 1995, Morin et al 2000). In adult motoneurons NA has been shown to increase the voltage dependent persistent inward currents ($I_{\text{PIC}}$, Lee and Heckman, 1999) involved in generation of plateau potentials which are capable of producing a prolonged neuronal discharge in response to a transient activation and thereby shape the motor output (for review see Perrier et al 2002). Plateau potentials have been described in various spinal neurons, including putative pattern generating interneurons of the neonatal rat spinal cord (Kiehn et al, 1996), and their possible activation in the presence of NA may contribute to rhythmogenesis. Our attempts to detect plateau potentials in P2-P4 spinal motoneurons in the presence of NA, and to affect the SC rhythmicity by application of nifedipine, a blocker of the L-type calcium channel whose activation is a major source of $I_{\text{PIC}}$ (Hounsgaard and Kiehn, 1985, 1993, Russo and Hounsgaard, 1996, for review see: Perrier et al, 2002), have not been successful (Medini and Lev-Tov unpublished results), possibly due to the immature nature of the L-type channel at this postnatal age (Jiang,
Rempel et al 1999). Additional studies in this regard are currently performed in our laboratory.

**The source of the “fast” rhythmic drive potential**

As mentioned above, the “fast” lumbar rhythm is significantly delayed comparing to the SC rhythm, and it depends on the presence of SC spinal segments (e.g. Fig. 4). An important question is whether its generation involves activation of the TL locomotor pattern generators by SC projections. The pattern of the “fast” lumbar rhythm is different from that of the locomotor rhythm. The locomotor rhythm is characterized by alternating flexor-extensor and by alternating left-right patterns, while the “fast” lumbar rhythm is manifested as an alternating activation of the flexor dominating segments of the cord.

Generally speaking a hypothetical agonistic half-center can be activated separately from its antagonist half-center (Hochman and Schmidt 1998). If this were the case, the anticipated rhythmic drive potential of lumbar flexor motoneurons would be either composed of phasic-excitation alone (see Hochman and Schmidt 1998), or phasic excitation alternating with phasic inhibition (originating from the contralateral half-center, see Kjaerulff and Kiehn 1997). In either case one would expect evidence of phasic inhibition of the extensor motoneurons (e.g. in L5). However, intracellular recordings of extensor dominated L5 motoneurons and injection of current steps into these motoneurons revealed no rhythmic changes in their input resistance in the presence of methoxamine (Fig. 8). This finding suggests a lack of rhythmic inhibitory input in L5 motoneurons from components of an ipsilateral half-center as well as an absence of crossed inhibitory input.

In addition, a substantial decrease in input resistance of both sacral (Fig. 6) and the flexor dominated L2 motoneurons (Fig. 7) was detected during the troughs of the
rhythmic drive potential induced by methoxamine. This rhythmic inhibition of L2 motoneurons coincided with the contralateral ventral root firing and was abolished in the midsagittally split TL cord. This suggests it was due to activation of crossed inhibitory pathways, as has been proposed for the sacral rhythm produced by repetitive stimulation of sacrocaudal afferents (Lev-Tov et al, 2000). Interestingly, the alternating pattern of the “fast” lumbar rhythm could be maintained for many minutes in the absence of the phasic inhibitory drive in the midsagittally split thoracolumbar cord (Fig. 7). These observations suggest that the purely excitatory rhythmic drive potential observed in L2 motoneurons of midsagittally split thoracolumbar segments originates from ascending/propriospinal projections of the ipsilateral sacral cord, while the left-right phase is maintained under these conditions by crossed inhibitory pathways between the left and right sacral pattern generators. Given that axons of commissural neurons cross the midline at the segmental level of their somata (Eide et al, 1999, Stokke et al, 2002), this suggestion implies that the crossed inhibition found in rostral lumbar motoneurons of bilaterally intact TL cords during the rhythm, is not due to axons from the sacrocaudal inhibitory interneurons that project rostrally and cross at the lumbar cord, but rather due to lumbar commissural interneurons activated by ascending/propriospinal excitatory projections from the sacral cord. Schematic representation of this hypothesis is illustrated in Fig. 9.

**Functional implications**

What is the functional significance of the “fast” rhythm? As mentioned above, the “fast” rhythm was generated primarily in the SC spinal segments by adrenoceptors activation and it produces rhythmic tail movements and rhythmic activation of the
regional axial musculature. In this way, descending noradrenergic projections onto the sacrococcygeal cord may be able to control tail movements during different behavioral repertoires. Moreover, in the present study we showed that the “fast” rhythm appeared also in flexor dominating segments of the lumbar cord. Thus, the activated SC circuitry is capable of increasing the probability of recruiting lumbar flexors, a feature that may be used to facilitate the combined appearance of rhythmic tail movements and locomotor activity in response to stimulation of sacrocaudal dermatomes (Lev-Tov et al, 2000, Lev-Tov and Delvolve, 2000, Delvolve et al 2001, Smith et al, 1988, Bonnot et al, 2002a, Strauss and Lev-Tov 2003, Whalen et al, 2000), during a nocifensive/escape type of behavior.

Finally, the ability of the NMDA/5HT dependent rhythmicity of the TL pattern generators to drive the SC networks in the presence or absence of NA (Kremer and Lev-Tov, 1997, Cazalets and Bertrand, 2000, Gabbay et al. 2002) may be used to produce coordinated limb and tail movements with the required frequency during swimming (Gabbay et al, 2002, Cook et al. 2001), climbing and turning (Bennett et al., 1999; Wada and Shikaki, 1999; Walker et al., 1998).
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FIGURE-LEGENDS

Fig. 1- Noradrenaline (NA) induces a “fast” rhythm in the TL and SC-cord.
Alternating left-right rhythmic activity induced by bath application of 5µM NA was recorded from the left (L) and right (R) L2 and S2 ventral roots at 100Hz-10 KHz (A). Simultaneous intracellular recordings from a right S2 motoneuron (R-S2MN) and extracellular recordings from the left (L) and right (R) S2 ventral roots are shown immediately (A), and 3min after (B, compressed time scale), addition of 5µM NA to the bath. Note the rapid prolongation of the cycle time (B) and the breakdown of the rhythm (C) toward the end of the record.

Fig. 2- The “fast” rhythm is induced by activation of α1-adrenoceptors.
Recordings from the left and right S2 ventral roots (100Hz-10 KHz) are shown after addition of 5µM NA to the bath (A). NA was washed out for 60min and reapplied 20min after addition of the α2-adrenoceptor antagonist yohimbine (1µM) to the bath (B). After a 60min washout, the α1-receptor blocker prazosin (1µM) was added to the bath, and the application of NA 20min later, failed to induce the rhythm (C).
Intracellular recordings from a left S2 motoneuron (L-S2MN) and extracellular recordings from the left and right S2 ventral root are shown immediately after addition of 100µM of the α1-adrenoceptor agonist methoxamine to the bath (D). The regions marked by bars (1, 2) are shown in an expanded scale below. The dashed line denotes the resting membrane potential before application of methoxamine.

Fig. 3- The “fast” rhythm does not depend on activation of NMDA receptors.
An alternating left right rhythm produced by bath application of 100µM methoxamine was recorded from the left and right S2 ventral roots (DC recordings, A) and from the left
and right L2 and S2 ventral roots (100Hz-10 KHz recordings, C left). After a 60min of wash, the NMDA receptor blocker AP5 (200µM) was applied to the bath, followed 20min later, by addition of 100µM methoxamine (B, and C right). The regions denoted by bars in A and B are shown in an expanded scale below.

Histograms of the normalized cycle time of the methoxamine induced rhythm in the absence and presence of 200µM AP5 in 5 experiments are shown in D (left). The circular distributions of phase values measured between left and right S2 under these conditions, are shown superimposed with the r-vectors (arrows) describing the concentration of phase values around the mean. The inner and outer circles denote 5 and 10 events for the circular histogram display, and r-vector values of 0.5 and 1, respectively.

**Fig. 4-** The spatiotemporal pattern of the rhythm.

Simultaneous recordings from the left L2-L5 and the left and right S2 ventral roots are shown, immediately (A) and 10min after (B, different experiment) addition of 100µM methoxamine to the bath. Note the short synchronous rhythmic bursts in flexor dominating segments and their absence in the extensor dominated L5 in A, and the appearance of a synchronous slow bursting in the left flexor and extensor dominating segments in B (arrows). Frequency distribution of the cycle time of the methoxamine induced rhythm is shown in C. Data were pooled from a series of 5 exp. (slow and “fast” rhythm) and 9 additional exp. (slow rhythm).

**Fig.5-** The pattern and segmental source of the α1-adrenoceptor induced rhythms.

A-B: Recordings from the left and right L2 (DC) and S2 (100Hz-10 KHz) ventral roots are shown 5min (A) and 15 min after (B) bath application of 100µM methoxamine. C: After 60min of wash, the cord was transected at the lumbosacral junction and
methoxamine has been added to the bath. Note that the “fast” lumbar rhythm and the slow sacral rhythm were blocked following the transection. Vertical bars are superimposed to demonstrate the alternating pattern of the “fast” rhythm.

D: Recordings of the slow and “fast” methoxamine induced rhythms from the left and right L2 (DC) and S2 ventral roots (100Hz-10 KHz), are shown in the absence (left) and presence (right) of 200µM AP5. Note that the slow lumbar rhythm was blocked and the “fast” sacral rhythm persisted in the presence of AP5. AP5 was added to the bath following 60 min of wash with Krebs saline; methoxamine (100µM) was added 20 min after AP5.

**Fig. 6-** Crossed inhibition in sacral motoneurons during the “fast” methoxamine rhythm.

A- Intracellular recordings from a left S2 motoneuron (L-S2MN) and extracellular recordings from the left and right S2 ventral roots are shown in the presence of 100µM methoxamine. Dashed line denotes the pre-methoxamine resting membrane potential.

B- Recordings of the voltage transients produced in the same motoneurons by intracellular injection of -0.5nA 100ms current steps at 2Hz. Motoneuron firing was blocked by injection of a continuous hyperpolarizing current (-.75nA) to the cell. Computer averaged records of the voltage transients produced during ipsi- and contralateral ventral root bursts are shown as insets (arrows), the mean drop in R_N during contralateral activity was 25±16%, P<0.001, (3 exp.).

C- The amplitude of the voltage transients (multiplied by -1 for convenience, circles) and the left and right S2 VRPs (lines) recorded during the last 20ms of each current step is plotted as a function of time (left). Cross-correlogram of the amplitude of voltage transients (LS2IC) vs. that of the contralateral VRPs (RS2) is shown superimposed with
±2SE (dashed lines) on the right. The drop in the amplitude of the voltage transients was in phase with contralateral VRPs.

**Fig. 7-** Crossed inhibition in lumbar flexor motoneurons during the “fast” rhythm. A-The voltage transients produced by repetitive (2Hz) intracellular injection of current steps (-0.5nA, 100ms) to right L2 motoneurons and the left (L) and right (R) ventral root recordings of L2 are shown before, (left) and after (right, different experiment) midsagittal section of the entire thoracolumbar cord (T6-L6). Extracellular data were recorded at 0.1-5 KHz (left) and DC (right). The rhythm was induced by bath application of 100µM methoxamine. The insets (arrows) are computer averaged records sampled during ipsi- and contralateral activity, respectively. The mean decrease in RN of L2 motoneurons in the bilaterally intact preparations was 28±14%, P<0.001, (3 exp.)

B-Cross-correlograms of the LL2 vs.RL2 (upper plot in each pair) and the amplitude of the voltage transients (RL2IC) vs. the contralateral L2 VRPs (LL2, lower plot in each pair) are plotted ±2SE (dashed lines). Data were obtained using the measurements described in Fig. 6C.

C- Summary plots of the amplitude of voltage transients vs. that of the contralateral VRPs in the bilaterally intact (left) and midsagittally split (right) thoracolumbar cord. Data obtained in 6 (3+3) experiments were standardized by subtracting the mean and dividing by the standard deviation of each variable, and then pooled. The slope of the regression line of data obtained from bilaterally intact cords was -0.56±0.04, with R² of 0.32 (p<0.0001). The slope of the regression line of the data obtained from midsagittally split cords (+0.09±0.51, R²=0.008) was not significantly different from 0.
**Fig. 8**- The $R_N$ of lumbar extensor motoneurons does not vary with the “fast” rhythm.

The voltage transients produced by repetitive (2Hz) intracellular injection of current steps (-0.5nA, 100ms) to a left L5 motoneuron (L-L5MN) and left (L) and right (R) ventral root recordings of L2 (A) are shown with cross-correlograms of the amplitude of the left and right L2 VRPs (B, left) and the amplitude of the voltage transients (LL5IC) vs. the ipsilateral L2 VRPs (LL2, B, right). Upper and lower dashed line denote ±2SE. The L2 recordings (top) were rectified and low-pass filtered at 50Hz. The insets (arrows) are computer averaged records sampled during ipsi-and contralateral L2 bursting, respectively. Summary plot of the normalized amplitude of the voltage transients produced in L5 motoneurons vs. the amplitude of the ipsilateral L2 and S2 VRPs (the activity of the ipsilateral L2 and S2 was highly synchronized (in phase) during the “fast” rhythm, see Figures 1, 3, 4, 5) is shown in C. The plot is based on standardized data (see Fig. 7C) pooled from 6 experiments (L2 and S2 VRPs were recorded in 2 and 4 of the experiments, respectively). Regression analysis of these data revealed a slope which was not significantly different from 0, see text.

**Fig. 9**- Hypothetical organization of the circuitry accounting for the alternating left-right rhythm produced in sacral and flexor dominating segments of the lumbar cord by activation of $\alpha_1$-adrenoceptors. The sacrococcygeal pattern generating circuitry is organized as left and right half centers, driving the coactivated flexors-extensors and abductors at the respective side of the tail. The crossed inhibitory pathways between the half centers include direct projections to contralateral motoneurons. These projections
account for the rhythmic drop in $R_N$ of sacral motoneurons during the “fast” methoxamine rhythm. Excitatory projections (ascending/propriospinal) of the sacral half centers to lumbar-flexor motoneurons and to crossed inhibitory lumbar interneurons produce alternation of phasic excitation and inhibition in the rhythmic drive potential of bilaterally intact L2 motoneurons. After a thoracolumbar midline split, the phasic inhibition onto L2 motoneurons is abolished and the alternating pattern of the split lumbar cord is maintained by the sacral crossed-connectivity.