Rhythmic activity of feline dorsal and ventral spinocerebellar tract neurons during fictive motor actions

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Running Head: Spinocerebellar activity during fictive motor behaviors

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Abstract

Neurons of the dorsal spinocerebellar tracts (DSCT) have been described to be rhythmically active during walking on a treadmill in decerebrate cats, but this activity ceased following de-afferentation of the hindlimb. This observation supported the hypothesis that DSCT neurons primarily relay the activity of hindlimb afferents during locomotion, but lack input from the spinal central pattern generator (CPG). The VSCT neurons, on the other hand, were found to be active during actual locomotion (on a treadmill) even after de-afferentation, as well as during fictive locomotion (without phasic afferent feedback).

In this study we compared the activity of DSCT and VSCT neurons during fictive rhythmic motor behaviors. We used decerebrate cat preparations in which fictive motor tasks can be evoked while the animal is paralyzed and there is no rhythmic sensory input from hindlimb nerves. Spinocerebellar tract cells with cell bodies located in the lumbar segments were identified by electrophysiological techniques and examined by extra- and intracellular microelectrode recordings. During fictive locomotion 57/81 DSCT and 30/30 VSCT neurons showed phasic, cycle-related activity. During fictive scratch 19/29 DSCT neurons showed activity related to the scratch cycle. We provide evidence for the first time that locomotor and scratch drive potentials are present not only in VSCT but also in the majority of DSCT neurons. These results demonstrate that both spinocerebellar tracts receive input from the CPG circuitry often sufficient to elicit firing in the absence of sensory input.
Introduction

The feline dorsal and ventral spinocerebellar tracts (DSCT and VSCT) are two main ascending pathways which have been perceived to be serving different functional roles. They are different entities because of their anatomical organization and they have also been thought to convey different input to the cerebellum (Bosco and Poppele 2001; Lundberg 1971; Oscarsson 1965). The commonly accepted view is that the DSCT neurons primarily relay sensory input from afferents and they are less influenced by the activity of other spinal neurons than the VSCT cells (Arshavsky et al. 1986; Bosco and Poppele 2001). There is strong evidence that VSCT cells monitor premotoneuronal activity in relation to motor commands (Lundberg 1971; Mann 1973; Oscarsson 1965). Cortical input via disynaptic pathways to DSCT cells (Hongo and Okada 1967; Hongo et al. 1967) and some unidentified spinal input to DSCT cells has been recognized that may serve to maintain their tonic activity in the absence of sensory input following section of the dorsal roots (Holmqvist et al. 1956). Recently, cortical control of DSCT cells in neonatal mice has been recognized (Hantman and Jessell 2010) and it was suggested that DSCT neurons may also be important components of spinal circuits used for planning and evaluation of motor actions.

Early reports on the activity of spinocerebellar tract cells during locomotion described DSCT cells to be rhythmically active during actual walking on a treadmill in decerebrate cats, but de-afferentation of the hindlimb by dorsal root transection abolished the step-related modulation (Arshavsky et al. 1972b). This observation supported the hypothesis that the function of the DSCT neurons is to relay the activity of hindlimb sensory afferents during locomotion while not receiving excitation from the spinal neurons comprising the central pattern generator (CPG) for locomotion. VSCT neurons, on the other hand, were found to be active not only during actual locomotion on a treadmill, (Arshavskii et al. 1972a) but also
following deafferentation (Arshavskii et al. 1972b), as well as during fictive locomotion
without rhythmic sensory feedback (Orsal et al. 1988). In addition, VSCT neurons have been
found to be phasically active during fictive scratch (Arshavskii et al., 1975; Arshavsky et al
1978), while DSCT neurons were reported to have no rhythmic modulation of firing during
fictive scratch (Arshavskii et al. 1975). Some years later, indirect evidence lead to the
hypothesis that spinal timing generators may also convey information to the cerebellum via
DSCT neurons (Perciavalle et al. 1995) but it is still unclear whether signals from the CPG
circuitry reach DSCT cells. Therefore the role of DSCT cells during rhythmic motor actions,
namely during fictive locomotion and fictive scratch was examined in this study.

Fictive locomotion and scratch refers to motor output monitored by
electroneurograms from hindlimb nerves in decerebrate animals that are paralyzed by
pharmacological blockade of the neuromuscular junctions. The fictive motor output closely
resembles that during real locomotion and scratch activity but there is no movement and
therefore no rhythmic sensory feedback. In this state the activity of the spinocerebellar tract
cells can be ascribed to inputs from the neuronal networks involved in the generation of the
motor activity. In this study, we examined the activity pattern of DSCT and VSCT neurons
during two different types of fictive motor activity by extra- and/or intracellular recordings of
identified tract cells. Fictive locomotion was induced by the electrical stimulation of the
mesencephalic locomotor region (MLR) and fictive scratch was induced by mechanical
stimulation of the skin covering the ears or the face in decerebrate cats following application
of curare and/or bicucculine at the dorsal root entry zone of the first and second cervical
segments. Preliminary results have been presented as abstracts (Fedirchuck et al. 1995;
Stecina et al. 2008).
Materials and Methods
Preparation

Experiments were performed during two series of studies. The first series consisted of 15 animals in which fictive locomotion was evoked, and the second series consisted of 7 animals in which fictive scratch was evoked as rhythmic motor activity. Thus a total of 22 adult cats of either sex weighing 3.0 – 4.6 kg were used. All surgical and experimental procedures were conducted in accordance with EU regulations (Council Directive 86/609/EEC) and with National Institutes of Health guidelines for the care and use of laboratory animals (National Institutes of Health publication no. 86-23, revised 1985). All procedures were approved by the Danish Animal Experimentation Inspectorate. There were 4/7 animals in the second series from which data were also used for other studies (Stecina et al. 2007).

In the first series of studies (experiments on fictive locomotion) anesthesia was first induced by an intravenous injection of Saffan (1 ml/kg; alphaxalone 9 mg/ml + alphadolone 3 mg/ml; for n=15, 3.0 - 4.4 kg animals). In the second series (experiments on fictive scratch) induction was attained by halothane (2-3% halothane, 70% N₂O and 30% O₂; for n=7, 3.2 – 4.6 kg animals). After a tracheotomy and intubation, anesthesia was maintained throughout the surgery with Halothane (0.8-1.5%) delivered in an oxygenated mixture of nitrous oxide (60% N₂O, 40% O₂). The blood pressure was monitored continuously via a carotid artery catheter and cannulae were also placed in both forelimb brachial veins for administration of drugs. Atropine (0.1 mg/kg, subcutaneous), dexamethasone (1.0 mg/kg, intravenous) or solumedrol (2.5 mg/kg i.v.) and a glucose/bicarbonate buffer solution (10% dextrose and
1.7% NaHCO₃) at a rate of 2.5 to 4.0 ml/hr, intravenously were routinely administered early in the experiment.

In both series of experiments, the nerves innervating the following hindlimb muscles were dissected on the left side: the multifunctional hamstring muscles posterior biceps and semitendinosus (PBSt) that are often active during both flexion and extension of fictive locomotion and during extension of fictive scratch, the semimembranosus and anterior biceps (SmAB) active during extension, the medial and lateral gastrocnemious and soleus (GS), plantaris (Pl) that are both active during extension, the tibialis anterior (TA) and extensor digitorum longus (EDL), often TA and EDL together as deep peroneal (DP) both active during flexion of fictive locomotion and during both flexion and extension of fictive scratch, the and digit mover flexor digitorum and hallucis longus (FDHL) that is often active during both phases of fictive locomotion and of fictive scratch. In addition, the posterior tibial (Tib) nerve innervating ankle extensor muscles and carrying cutaneous input; the cutaneous sural (Sur) nerve, and the cutaneous superficial peroneal (SP) nerve were also dissected. In the second series of experiments, the nerve innervating the peroneus longus (PerL) muscle that is an ankle flexor during fictive locomotion but it becomes synergist of ankle extensors during fictive scratch was also dissected in some animals. In both series of experiments, the sartorius (Sart), and quadriceps (Q) branches of the femoral nerve were dissected and they were placed in implanted plastic cuff electrodes. The Sart (depending on the nerve branch dissected) is active mostly during flexion of fictive locomotion and scratch but it can be also active during both phases. On the right side the PBSt, SmAB and Sart nerves were dissected in some of the experiments. All peripheral nerves were cut distally and the proximal stump was freed from connective tissue.
In the first series of experiments both sciatic nerves and all the dissected branches were sufficiently dissected to allow the hindlimbs to hang pendant thus avoiding extension of the hip when constructing the hindlimb paraffin pool, which could prevent locomotor activities while the dissected nerves were laid in a plastic tray, filled with mineral oil, where they were placed on bipolar silver hook electrodes to be either stimulated or recorded.

In the second series of experiments the hindlimbs were fixed in an extended position to allow for the construction of a paraffin pool using the skin covering the hindlimbs and the dissected nerves were placed on bipolar silver hook electrodes. In this case all branches from the femoral, obturator and sciatic nerves were sectioned and the tendons of muscles crossing the hip joint (which were not denervated) were cut in order to prevent sensory feedback signaling the hip extension. All the hindlimb nerves as well as the exposed spinal cord were covered with mineral oil. The temperature of the animal’s core and the mineral oil pools were maintained at physiological levels using a feedback heating system.

In both series of experiments, laminectomy of the L3-L6 vertebrae exposed the lumbo-sacral segments, and in 2 animals the lower thoracic segments were also exposed by a laminectomy of the 12th to 13th thoracic vertebrae. The first cervical vertebra was also removed and in the second series the second cervical vertebrae was removed as well.

In both series of experiments, a craniotomy was performed and the animal was mechanically decerebrated at a precollicular postmamillary level and all brain tissue rostral to the transection was removed. At this time the anesthetic was discontinued and decreases in blood pressure associated with the decerebration were countered by intravenous administration of Oxypherol (an oxygen carrying volume expander, <10ml) and/or Gentran (3000 mM dextran solution, <10ml). The animal was paralyzed with intravenous Pavulon
(pancuronium bromide; 0.2mg/kg, supplemented every 40-60 min) and ventilated to maintain end tidal CO$_2$ at 4-6%. The tentorium was removed to expose the brainstem and the cerebellum for later electrical stimulation. When the blood pressures became less than 80 mmHg the drop was counteracted with intravenous administration of a volume expander (see above) or noradrenaline as needed.

**Evoking fictive motor behaviors**

Fictive locomotion was elicited in the paralyzed preparation by electrical stimulation of the mesencephalic locomotor region. Insulated monopolar steel electrodes were placed bilaterally in the midbrain (Horsley-Clarke coordinates: P1 - 2; L3.5 - 4; H0 -1.5 and electrical stimulation (30-200 μA, rectangular current pulses delivered at 15-20 Hz) elicited fictive locomotor activity, which was recorded from the peripheral nerves. The location of the electrodes was adjusted to obtain the lowest electrical threshold for locomotion and the most stable locomotor pattern possible. Normally stimulation was unilateral, but occasionally bilateral stimulation was required to improve locomotor activity.

Fictive scratch was evoked by topical application of D-tubocurarine solution 0.1 – 1%, and/or bicuculline solution 0.1 – 1% onto the first and second cervical dorsal root entry zone on the left side followed by mechanical stimulation of the skin of the ear on the left side.

**Recording Techniques**

Both extracellular and intracellular recordings were obtained using pulled-pipette glass microelectrodes filled with 2 M potassium acetate (1.4 to 2.0 μ tips; 3 to 10 MΩ) and amplified with an Axoclamp 2A microelectrode amplifier. Electroneurograms (ENGs) of hindlimb nerves were also recorded, or alternatively, each nerve could be stimulated. A ball electrode was placed on the dorsal surface of the spinal cord typically at the L6-L7 segment.
to record the incoming afferent volley associated with peripheral nerve stimulation (0.1 ms pulse) or descending volley evoked by supraspinal stimulation. The strength of peripheral nerve stimulation was given in multiples of the threshold that is the stimulus strength necessary to recruit the most excitable fibers (i.e. that produced an incoming volley recorded on the surface of the spinal cord at lumbar levels).

Data capture and analyses

In the first series of experiments, the microelectrode recordings, incoming volley recordings and rectified ENG recordings were digitized usually at a rate of 20 KHz, 5 KHz, and 650 Hz, respectively, on a Concurrent/Masscomp 5400 series computer and a custom made software from the Spinal Cord Research Center at the University of Manitoba (Winnipeg, Canada). In the second series ENG signals were digitized at a rate of 10 kHz and filtered (5 Hz to 1 kHz) while the microelectrode and spinal cord potential recordings were digitized at a rate of 20 kHz by using CED 1401 and Spike 2 version 5.21 Software (Cambridge Electronic Design, Cambridge, UK) and a personal computer with Pentium processor.

Post-hoc analysis of the data (i.e. offline analysis) consisted of calculating the averaged instantaneous firing frequencies of extracellular units or averaged membrane potential change of intracellularly recorded cells based on normalized and averaged locomotor cycles. Built-in spike sorting algorithms within the software identified specific cells which were determined to fulfill the criteria of being spinocerebellar tract cells (see paragraph below). The analysis of the locomotor cycles was based on flexor and extensor bursts of identified muscle nerves (TA, EDL or Sart as markers for onset of the flexor phase and GS, SmAB and sometimes PerL during scratch as markers for the extensor phase). Cycle
duration was determined as the time between consecutive onsets of flexor activity;

flexor/extensor phase duration was determined as the time period when the flexor/extensor ENG activity exceeded a set threshold. This threshold was visually determined by inspecting typically 1 – 3 min long activity with consecutive alternating flexor and extensor bursts.

Locomotor and scratch cycles were divided into 30 bins and normalized. The instantaneous firing frequency (IFF) of each identified neuron was calculated in each of the bins.

Overlaying the averaged ENGs with the graph of the IFF was used to visually determine the phase of activity of each neuron (i.e. when the IFF was maximal).

Criteria for identification of units

DSCT neurons are known to ascend in the ipsilateral spinal white matter and VSCT neurons ascend in contralateral spinal white matter (Eccles et al. 1967; Lundberg and Oscarsson 1961; Mann 1973). Therefore, spinocerebellar tract cells were identified by differential electrical stimulation applied at either ipsilateral or contralateral sites with respect to the intraspinal microelectrode as illustrated schematically in Fig. 1A-C. Stimulation on the surface of the cord at the first cervical (C1 stim see in Fig. 1C) segment was applied to identify ascending projections of a unit. Units were classified to be spinocerebellar tract neurons if they could be antidromically activated either by electrical stimulation at the surface of the cerebellum (surface stim in Fig. 1A) or by intra-cerebellar stimulation (intra-CB stim in Fig. 1B). Spinocerebellar tract neurons projecting on the ipsilateral (ipsi) and contralateral (contra) sides were compatible with DSCT and VSCT origin, respectively (see review by Mann 1973).

Surface stimulation was applied using insulated monopolar steel electrode placed on...
the anterior cerebellar cortex with the anode placed in the neck muscles near the base of the
head, thus presumably activating deeper structures such as the peduncle. Surface stimulation
was used in all experiments of the first series (i.e. those with fictive locomotion). Furthermore, we often verified that in addition to antidromic activation from the cerebellar
surface, VSCT neurons could be antidromically activated by a high strength stimulus pulse
(up to 300 μA, 1 ms pulse) from the contralateral MLR electrode since most VSCT fibers
enter the cerebellum through the superior cerebellar peduncle (Oscarsson 1965) and this
structure is close to the cuneiform nucleus and the area stimulated for evoking fictive
locomotion albeit some VSCT neurons are known to enter via the inferior cerebellar peduncle
as in e.g. (Kitamura and Yamada 1989).

Intra-CB stimulation was applied by using parylene-coated tungsten electrodes (0.1 –
0.3 MΩ, World Precision Instruments, Sarasota, FL, USA) initially inserted into the
cerebellum 1-2 mm dorsal and caudal to its junction with the inferior colliculi and about 2-3
mm lateral from the midline as shown in Fig. 1B. In Fig. 1D we illustrate cord dorsum
potentials recorded at the 6th-7th lumbar segment following intra-cerebellar stimulation
applied in one preparation. The most prominent descending volleys were seen at intra-CB
depth of 5-7 mm. Extracellular recordings in Fig. 1E illustrate the spike evoked by the intra-
CB stimulation at 6 mm depth. The collision of the antidromic spike with a spontaneous
spike is shown by the arrow. The area where the approximate location of our identification
points were in the cerebellum corresponds well with previous reports on the optimal
antidromic activation sites used for DSCT neurons (Edgley and Gallimore 1988).

Results
Not only the VSCT neurons, but the majority of the DSCT neurons was found to be active during fictive locomotor activity in a cyclic, phase-related manner even though there was no actual hindlimb movement or phasic afferent input. In addition, we also found that about two-thirds of the DCST neurons show phase-related activity during fictive scratch.

**Activity of DSCT neurons during fictive locomotion**

Extracellular or intracellular recordings were obtained from 81 DSCT neurons. 69 of these neurons were recorded in the L3 - L5 spinal segments and 12 neurons were recorded in the L1 or L2 spinal segments. Figure 2 shows the extracellular recording of a DSCT neuron during MLR-evoked fictive locomotion. The period shown in A starts just as the MLR stimulus (100 μA, 20 Hz) was turned off and shows that the rhythmic activity is related to the fictive motor pattern and not directly linked to the MLR stimulus. After normalizing and averaging the fictive step cycles (n=12) using the onset of Sart ENG activity as the cycle onset, the occurrence of action potentials during different times of the step cycle could be plotted. The upper trace of Fig. 2B shows the averaged instantaneous firing frequency (IFF) for the normalized and averaged step cycle in relation to the averaged ENG activity from the flexor (Sart) and the extensor (SmAB) muscle nerves. Then the step cycle-related change in instantaneous firing frequency was measured as shown in Fig. 2B for this cell. The maximal firing frequency was 78 Hz. Similar analysis was done for each cell recorded extracellularly during fictive locomotion.

Intracellular recordings were obtained from 12 DSCT neurons during fictive locomotion. Figure 3 illustrates oscillations of the membrane potential associated with the fictive step cycle on the intracellular microelectrode recording shown in the top panel (A).
When such oscillations of postsynaptic potentials were recorded from motoneurons they were called locomotor drive potentials or LDPs (Shefchyk and Jordan 1985). Spikes were absent in this trial because the sodium channels had been inactivated by a prolonged depolarizing current injection just before this recording period. The lower panels (B) show the averaged membrane potential and flexor and extensor ENG activity during the normalized and averaged step cycles (n=8). The activity of 9/12 cells was deemed to be modulated in phase with the step cycle based on analysis of intracellular recordings; but not all of these cells were recorded from an extracellular position and their firing patterns could not be analyzed. However, all 9 cells exhibited some degree of LDPs ranging from 0.7 to 6.0 mV.

A total of 57/81 (70%) of DSCT cells in this study showed activity that was modulated in relation to the fictive step cycle. There was no difference in the propensity for DSCT neurons from different spinal segments to exhibit phasic modulation during fictive locomotion, or to be active during a particular phase of the step cycle (see Table 1). Of the extracellularly recorded cells (n=48), 8 fired exclusively during flexion as in Fig. 2; 20 fired only in extension, and 20 fired throughout the fictive step cycle but at a higher frequency in one phase compared to the other. There was no significant difference in the cycle related changes of the instantaneous firing frequency between extension and flexion related DSCT neurons (Mann-Whitney rank sum test p>0.05). Of the intracellularly recorded DSCT (n=9) cells, 3 were depolarized (and/or had action potentials) during extension, 4 were excited during flexion and 2 were excited during flexion as well as during part of extension. The remaining 24/81 of DSCT cells tested had tonic activity without phasic modulation in relation to fictive locomotion. Prior to the onset of fictive locomotion (i.e. MLR stimulation) 33
DSCT cells showed tonic background activity with a mean of 22 Hz IFF while the mean IFF of the same cells during fictive locomotion was 20 Hz. The background firing frequency of 7/33 cells was comparable to the peak firing rates seen during fictive locomotion. In 2/33 cells the firing frequency during fictive locomotion was actually lower than the background rate. In the remaining 24 cells the firing frequencies during fictive locomotion were higher than the background rates.

Activity of VSCT neurons during fictive locomotion

Extracellular and intracellular recordings were obtained from 30 VSCT neurons within the L2 to L5 spinal segments. Fig. 4 illustrates an extracellular recording of the activity of a VSCT neuron during fictive locomotion. This unit started firing at the peak of ipsilateral extension, i.e. before the onset of Sart ENG activity (see Fig. 4B). In the example in Fig. 4, the cell had a 300 Hz change in instantaneous firing frequency during the averaged (n=12) fictive step cycle (see Fig. 4B). Overall, 100% of the VSCT cells recorded showed phasic activity with fictive locomotion. A total of 19 extracellularly recorded VSCT units fired exclusively in flexion, 8 fired only during extension and 3 units fired throughout the fictive step cycle but at a higher frequency in one phase (2 in flexion and 1 in extension).

There was no significant difference in the degree of the cycle related changes in instantaneous firing frequency between extension and flexion-coupled VSCT neurons (Mann-Whitney rank sum test p>0.05).

Prior to the onset of fictive locomotion there were 14/30 VSCT cells with tonic background activity. For 13 of these VSCT cells, the firing rates during fictive locomotion
were much greater than the background frequencies prior to locomotor activity. The remaining one VSCT cell had comparable firing rates prior to and during fictive locomotion. Locomotor drive potentials in VSCT cells are illustrated in Fig. 5A and during the normalized and averaged steps (n=24) the peak-to-peak amplitude was 6.8 mV (Fig. 5B). All of the intracellularly recorded VSCT cells (n=7) were depolarized during the flexion phase of fictive locomotion. In 4/7 cells the action potential generation ceased spontaneously (i.e. without hyperpolarization of the membrane potential by current injection) so the LDPs could be averaged, and their amplitude was measured. For the other 3 cells, hyperpolarizing current injection (1.0, 6.4 and 7.8 nA) was used to transiently suppress action potential production in order to measure LDP amplitude. Each of the 7 VSCT neurons recorded intracellularly displayed LDPs ranging from 1.4 to 9 mV peak-to-peak amplitude.

**Activity of DSCT neurons during fictive scratch**

Figure 6A illustrates the extracellular recording of the activity of a DSCT cell with action potentials during the extension phase of a 4 s long bout of fictive scratch activity. This is the same cell as illustrated in Fig. 1C and D while identifying it with intra-cerebellar stimulation. The ENG recordings were normalized and averaged (n=25) based on the onset of an extensor (PerL) ENG activity as the start of the cycle and the averaged and normalized instantaneous firing frequency is shown in Fig. 6B.

Intracellular records from one DSCT cell during fictive scratch are illustrated in Fig. 7. Note that the depolarization occurs in phase with the activity of the ankle extensor Pl.
nerve. This membrane potential modulation during fictive scratch is similar to those described previously in hindlimb motoneurons (Perreault 2002). The averaged modulation of the membrane potential during the scratch cycles (n=7) was based on the Pl ENG activity is shown in Fig. 7B with the averaged ENGs of the Pl and TA nerves. The extracellularly recorded IFF of this DSCT cell averaged and normalized during another bout of scratch (n=25) is overlaid on the membrane potential and it shows that the modulation of the IFF was 44 Hz.

Overall, extracellular recordings were made from 29 DSCT cells (16 from within the L3-L4 spinal segments and 13 in L2), and 19/29 DSCT were phasically active during fictive scratch. The phasic activity of 3 DSCT cells coincided with flexion, 6 cells were active during extension and 10 cells fired throughout both phases of the scratch cycle but at a higher frequency in one phase compared to the other. Prior to fictive scratch 12 DSCT cells had tonic activity and their mean IFF was 79 Hz while during fictive scratch the mean IFF of the same cells was 85 Hz. There were 4/12 DSCT cells which had lower IFF during fictive scratch than prior to it. Intracellular records during fictive scratch without action potential generation in DSCT cells were collected from 2 other cells in addition to the one illustrated in Fig. 7B and the “scratch-drive” potential amplitudes were 0.7 and 3.8 mV.

Differences in firing frequency between VSCT and DSCT cells
Our data of the DSCT and the VSCT cell activity during fictive locomotion were collected as interspersed recordings with similar robustness of locomotor network activity while recording from one or the other cell types. We have not made a systematic comparison
of cellular activity from one locomotor or scratch bout to the next, but in 13 DSCT cells we have recordings during two or more bouts of fictive scratch and none of these cells showed changes in their firing pattern from one bout to another. There were no attempts made to quantify the ENG recordings in relation to the firing frequency for either cell type.

It became apparent that although DSCT and VSCT neurons could both be phasically active during fictive locomotion, there were differences in the degree of modulation of activity exhibited by the two cell types. Fig. 8A is a summary graph showing the change in instantaneous firing frequency during the fictive step cycles of all DSCT and VSCT cells. Those DSCT cells that were not phasically modulated with fictive locomotion are shown with a step cycle-related frequency change of "0" Hz. VSCT neurons tended to have larger changes in instantaneous firing frequency than DSCT neurons (see also Figs. 2 and 4) and this difference was statistically significant (Mann-Whitney rank sum test p<0.001; non-modulated DSCT neurons were excluded from the test sample). The amplitudes of the LDPs of the few intracellularly recorded VSCT neurons also tended to be larger than those recorded in DSCT neurons. However, the small sample size in this study precludes the verification of this difference by statistical means. The firing frequency modulation of the DSCT neurons during fictive scratch is illustrated in Fig. 8B and it was found to be in the same range as that during fictive locomotion.

Relation of excitatory peripheral input and phase of rhythmic activity

We have categorized the DSCT cells examined in the present study based on the synaptic input from peripheral afferents as it has been described in the reviews by Mann
(1973) and Oscarsson (1965). Table 1 shows the source of excitatory synaptic input to the DSCT neurons tested in this study. There was no relation between the source of afferent excitation to a DSCT neuron and whether or not the activity of the neuron was modulated with the fictive step cycle. For those DSCT neurons with activity that was modulated with the fictive locomotion, there was no relation between the source of synaptic excitation and the cycle phase that the unit was active—even in the case when DSCT cells had monosynaptic input from group I afferents. There was no difference in the degree of modulation between the DSCT neurons with different patterns of inputs (one way ANOVA, p=2.97). As shown in Table 1, DSCT cells with excitation from extensor group I afferents were not only active during extension, but 7/18 were active during flexion.

Table 1 here

The DSCT neurons with phasic activity during fictive locomotion could belong to any of the categories that we have defined (see Table 1). The DSCT cells examined during fictive scratch were not included in the Table due to the small sample size. During fictive scratch 2/3 flexor-related DSCT cells had no discernible sensory input and the third cell had group II muscle afferent input from Sart and Quad. There were 2/6 of the extensor-related cells that had no discernible sensory input and 2 with input from Tib and FDHL (and 2 that were not tested for sensory inputs). In, 6/10 excitation from multiple sources (Sart, Q, PBS or Tib) was evoked, 2/10 had no sensory input and 1/10 had only cutaneous input from Sural (1/10 cell was not tested for inputs).

The VSCT neurons were also divided into groups based on the pattern of synaptic input that they receive. In our sample, 6 of the VSCT neurons received excitation from extensor group I muscle afferents, 2 from flexor group I muscle afferents, 6 received
excitation from higher threshold group II muscle and cutaneous afferents, while 9 received polysynaptic inhibition from various sources. Seven VSCT neurons had no discernible sensory inputs. As with DSCT neurons, we detected no trend for a difference between the degrees of modulation within the fictive step cycle for the different categories of the VSCT neurons, but note our small sample size.

Discussion

The results presented in this paper show that not only VSCT but also DSCT cells discharge phasically during fictive locomotion evoked by electrical stimulation of the MLR in pre-collicular/post-mamillary decerebrated cat preparations. We also demonstrate that many DSCT cells are phasically active during fictive scratch. Given the absence of phasic sensory activity during these fictive motor outputs, the results imply that inputs from the CPG are often sufficient to induce firing in these ascending tract neurons.

Comparison of DSCT and VSCT activity during fictive motor actions

The vigorous rhythmic activity of VSCT neurons during fictive locomotion reported in this study supports previous findings (Arshavskii et al. 1972a; b) and the concept that VSCT cells convey information about the activity of spinal interneurons as well as about input from sensory afferents (Arshavsky et al. 1972a; Lundberg 1971). Previously DSCT neurons in the upper lumbar segments have been described to have phasic activity during over-ground locomotion, but also that the phasic activity was abolished by sectioning the ipsilateral dorsal roots, although a tonic activity of around 9 Hz remained (Arshavskii et al. 1972c). During the fictive locomotion used here, no phasic sensory input is generated, thus
our observations on the phasic activity in the 70% of the recorded DSCT neurons are contrary
to previous conclusions. In addition, our results demonstrate that 66% (19/29) of the DSCT
cells are rhythmically active during fictive scratch. We did not record from VSCT cells
during fictive scratch since an extensive study by Arshavsky and colleagues (Arshavskii et al.
1975) has demonstrated that virtually all VSCT cells in thalamic (74 cells) and decapitate cats
(44 cells) were discharging rhythmically in relation to the scratch cycle. There are no
previous published results on DCST cells during fictive scratch, but in the discussion on the
activity of VSCT cells during actual and fictive scratching (Arshavsky et al. 1978) it is
mentioned that DSCT cells “were found to have no rhythmical modulation during fictious
scratching”. These observations by Arshavsky and colleagues (Arshavskii et al. 1972c;
Arshavsky et al. 1978) imply that the phasic activation of DSCT neurons during locomotion
and scratch was attributable solely to their activation by hindlimb proprioceptive systems and
are therefore seemingly contradictory to our present observations of rhythmically active
DSCT neurons during fictive locomotion and scratch.

In the single report on the lack of phasic DSCT activity post-deafferentation (during
locomotion) there were only 11 cells investigated so there may have been a sampling bias of
the DSCT neurons recorded. The differences between the preparations used i.e. thalamic or
decapitate (Arshavskii et al. 1972c; Arshavsky et al. 1978) vs. pre-collicular/post-mamillary
decerebration (present study) may also account for some of the apparent differences.
Alternatively, phasic activity could have been dependent on the rostro-caudal location of
DSCT cells therefore we extended our recordings to DSCT neurons located in the L1 to L5
spinal segments. We found that DSCT neurons from all segments could exhibit rhythmic
activity during fictive locomotion as well as during fictive scratch (but our sample size during
scratch is relatively small). There was no relation between the phasic activity during fictive locomotion and the conduction velocity or the types of excitatory afferent input of a DSCT neuron (see Table 1). There was no relation between the phasic activity during fictive scratch and the types of excitatory afferent input of a DSCT neuron. It is noteworthy that DSCT neurons active during the extension phase of fictive locomotion could be activated by group I afferents from either extensors or flexors.

What is driving tonic and rhythmic activity of the DSCT cells?

Tonic background (or “resting” activity) activity of DSCT cells in unanaesthetized decerebrate preparations without motor activity has been well documented (Arshavskii et al. 1972a; Holmqvist et al. 1956). In principle there could be at least three sources for this activity; firstly, a tonic drive from sensory afferents, secondly a tonic excitatory input from spinal interneurons and/or descending pathways, or, thirdly, a spontaneous activity maintained by intrinsic properties in the DSCT neurons.

It is known that sensory afferents exert monosynaptic excitation of VSCT neurons to a smaller extent than that of DSCT neurons (Arshavsky et al. 1986; Lundberg 1971; Oscarsson 1965). This is also supported by the different distribution of vesicular glutamatergic transporters (VGlut1 and 2) in the glutamatergic terminals on VSCT and DSCT cells (Shrestha et al. 2012). Myelinated primary afferent terminals contain the VGlut1 (Alvarez et al. 2004; Todd et al. 2003; Varoqui et al. 2002), while spinal excitatory interneurons and most descending tracts neurons on the other hand express the VGlut2 (Shrestha et al. 2012; Todd et al. 2003; Varoqui et al. 2002). In labeled VSCT cells it is seen that VGlut2 terminals are dominating, while the opposite is true for DSCT cells in Clarke’s
column (Shrestha et al 2012). If the tonic activity of the DSCT neurons in our present preparation is indeed maintained by tonic excitatory synaptic drive that would either be maintained by activity in the sensory afferents in nerves that were not sectioned during the preparation or originate from the relatively sparse innervations from segmental excitatory interneurons. The most obvious explanation for rhythmic excitatory drive from the spinal CPG circuit of locomotion and scratch would be the activation of spinocerebellar cells via excitatory interneurons which could activate motoneurons in parallel. However, as the overall evidence on the excitation of DSCT neurons belonging to Clarke’s column by spinal excitatory interneurons is sparse (see review by Mann 1973 for electrophysiological data, also see Krutki et al. 2011 and Shrestha et al. 2012 for primarily anatomical evidence) a prominent role of inhibitory spinal interneurons should be considered in “sculpting” the tonic background activity.

Populations of inhibitory interneurons that project to VSCT cells include reciprocal Ia inhibitory interneurons (Lindstrom and Schomburg 1974), non-reciprocal “Ib” inhibitory interneurons (Jankowska et al. 2010; Lundberg and Weight 1971) and group “Ib/group II” inhibitory interneurons (Jankowska et al., 2010). No disynaptic inhibitory input from reciprocal Ia inhibitory interneurons was found in DSCT cells (Hongo et al. 1983a; Lindstrom and Takata 1977), but ”Ib” inhibitory interneurons (Hongo et al. 1983a; b) as well as group Ib/II inhibitory interneurons (Jankowska and Puczynska 2008) contact DSCT cells. The Ib inhibitory interneurons are known to be silenced (or inhibited) during fictive locomotion (McCrea et al. 1995) and fictive scratch (Perreault et al. 1999), while the Ia inhibitory (Geertsen et al. 2011) and sub-populations of the Ib/II inhibitory interneurons (Shefchyk et al. 1990; Stecina 2006) are rhythmically active during both fictive locomotion
and scratch. Thus there is certainly the possibility of phasic inhibitory inputs to both DSCT (from the phasically active Ib/II inhibitory interneurons) and VSCT cells (from the Ia inhibitory interneurons) during these motor tasks.

Holmqvist et al. (1956) reported that the background firing following sectioning of all the lumbar and sacral dorsal roots remains unchanged in DSCT cells. Later Arshavskii and colleagues (1972c) described that the background firing was reduced on average from a firing frequency of 12 Hz to 9 Hz following hindlimb deafferentation. The maintenance of some tonic activity following de-afferentation would then suggest that there is a tonic excitation from central sources. The VSCT cells receive direct excitation from several descending tracts (see Arshavsky et al 1986) but no monosynaptic input from the medial longitudinal fasciculus to DSCT cells have been found (Hammar et al. 2011; Krutki et al. 2011). Thus the longer latency inputs could be mediated by the segmental excitatory interneurones even though there may be only few interneurones involved in this loop. Inhibitory input from supraspinal centers to both DSCT and VSCT cells is likely to be mediated by the same spinal interneurons as those used for sensory-evoked inhibition (Baldissera et al. 1981; Hammar et al. 2011).

There is no direct evidence for the sustained (or the rhythmic) activity of DSCT cells to be caused by intrinsic cellular properties. However, the Clarke’s column neurons are labeled strongly for the voltage gated CaV1.3 Ca²⁺ channels (Zhang et al. 2008) which carry a persistent inward current (PICs) in many neurons. In motoneurons 5-HT is facilitating the PICs (Hounsgaard et al. 1988; Hounsgaard and Kiehn 1989), and it is therefore interesting that the dorsal horn component of the DCST cells (Jankowska et al. 1995) as well as Clarke’s column cells (Pearson et al. 2000) receive rather intense serotonergic innervations.
Serotonergic innervations of VSCT cells are indeed similar to that of DSCT cells and hindlimb motoneurons (Hammar and Maxwell 2002), but there is no electrophysiological studies on how serotonin affects intrinsic properties of VSCT neurons during fictive motor activity. In addition, we would also like to bring up the possibility that VSCT and DSCT neurons may undergo a state-dependent enhancement of their excitability during fictive locomotion and scratch. Lumbar motoneurons have been shown to have their voltage threshold for action potential production lowered (i.e. hyperpolarized) during fictive locomotion (Krawitz et al. 2001) and fictive scratch (Power et al. 2010). In addition the post-spike afterhyperpolarization is reduced in spinal motoneurons during both fictive locomotion (Brownstone et al. 1992) and fictive scratch (Power et al. 2010). These changes facilitate motoneuron recruitment and repetitive firing during these activities, and it is possible that similar changes occur in spinocerebellar tract neurons and contribute to their rhythmic activity during these motor outputs. Further investigation of the state-dependent regulation of DSCT cell excitability (especially in relation to the seemingly sparse excitatory synaptic input to Clarke’s column DSCT cells yet a phasic activation during rhythmic motor activity) is required to address this possibility.

Finally, we would like to address whether the primary afferent depolarization (PAD) and the subsequent antidromic discharges of the primary afferents evoked by the locomotor and/or scratch networks (Bayev and Kostyuk 1982; Bayev and Kostyuk 1981; Bayev et al. 1978; Beloozerova and Rossignol 1999) could contribute significantly to the activity of spinocerebellar tract cells during fictive locomotion and scratch. Firstly, the tonic discharge of afferent fibers in the resting decerebrate state is actually reduced during activation of the locomotor CPG—and even more reduced during fictive scratch when compared to resting i.e.
no motor activity in the same preparation (Cote and Gossard 2003). Secondly, the CPG activity leads to a phase-related modulation of the dorsal root potentials but at the same time it also leads to reduction of transmission in sensory afferent-evoked PAD pathways (Cote and Gossard 2003). All in all, we would have expected a large difference in the modulation of the firing frequency in DSCT cells during the two behaviors i.e. lower changes during fictive scratch than during fictive locomotion. Our results, however, show overlapping frequency modulation (see Fig. 8) therefore we find it unlikely that PAD evoked by the locomotor and scratch CPG is the cause of the firing activity of DSCT cells during fictive motor actions.

**Functional implications of rhythmic firing in the dorsal and ventral spinocerebellar pathways during motor activity**

An extensive discussion on the role of the spinocerebellar pathways with regard to sensory feedback to the cerebellum during motor activity is outside the scope of this study. Our present results emphasize the central -both spinal and descending- inputs to the DSCT cells which have been known (see previous section) but have been underestimated because of the emphasis on the strong sensory input to DSCT cells that has been prevalent since the 1960s.

Our data show that there is an underlying “CPG-driven” activity of DSCT cells during rhythmic motor tasks. Even if the actual recruitment and final firing rate is more strongly influenced by peripheral afferent activity, the underlying locomotor and scratch potentials would provide a fluctuating baseline on which the afferent input is superimposed. The convergence of sensory input with the baseline excitation or inhibition may serve as a gate to allow the selective transmission of sensory input to the cerebellum.
Any hypothesis on the role of the information transmitted by the DSCT (in general, and as compared to that by the VSCT) has to take into account the terminations and interactions at cerebellar level, starting with the convergence at the granule cells e.g. (De Zeeuw et al. 2011; Ekerot and Jorntell 2008) and continuing with the interactions and the convergence from the olivocerebellar projections at Purkinje cell level and at the cerebellar cortical layer (Valle et al. 2012). Several recent reviews focus on the possibility that specific spatiotemporal firing patterns may be of particular significance for information processing in the cerebellum (De Zeeuw et al. 2011; Perciavalle et al. 1995). In a long series of publications Bosco, Poppele and colleagues emphasized the wide, but organized, convergence onto the DSCT as reviewed by (Bosco and Poppele 2001). They argued that the DCST could work as parallel distributed networks that relay information on limb biomechanics and kinematics. In the discussion of the recent report on the convergence of cortico-spinal excitation and inhibition and afferent input to DSCT cells Hantman and Jessell (2010) is referring to internal models of the planned motor activity, and the corollary discharge to distinguish exafference (sensory signals generated from external stimuli in the environment) from re-afference (sensory signals resulting from an animal's own actions) due to the planned movement. Thus they place the DSCT cells in a more central position than previously viewed for motor planning and evaluation. Our present results add to previous evidence demonstrating that the early idea of the DSCT pathway as primarily mediating sensory afferent information cannot be maintained. Even though there are differences between the convergence of sensory afferent input and central excitation versus inhibition for DSCT and VSCT neurons, the overall similarities seem to be dominating during rhythmic motor actions. While the hypothesis of VSCT cells being an input-output comparator stands
yet unchallenged, the role of DSCT cells with respect to sensory-motor integration must be
re-evaluated with an increased appreciation that transmission through the DSCT reflects the
convergence of activity in spinal motor generating circuitry and peripheral sensory afferents.

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Figure Captions

Figure 1. Schematic illustration of methods

A. For the identification of spinocerebellar neurons stimulation of the cerebellar surface (surface stim; indicated by the open arrow) was used in the first series of experiments. The mesencephalic locomotor region was stimulated (MLR stim) as shown by the double arrows in order to evoke fictive locomotion.

B. For the identification of spinocerebellar neurons intra-cerebellar (intra-CB) stimulation was used at approximate sites indicated by the filled arrow in the second series of experiments.

C. Verification of antidromic activation of tract cells by the stimulation of the ipsilateral (ipsi) or the contralateral (contra) dorsolateral funiculus at the level of the first cervical (C1) vertebra was used prior to fictive motor activity (monitored by electroneurogram recordings, ENGs, of hindlimb muscle nerves). Extra-, and/or intracellular recordings in the lumbar segments (L1 – L5) were collected from identified spinocerebellar tract cells.

D. Cord dorsum potential (cdp) recordings after intra-cerebellar stimulation at identified depths with reference to the cerebellar surface. Note that maximal volleys were evoked at depths ranging between 5-7 mm.

E. Single microelectrode recordings extracellularly (e.c.) with antidromic DSCT activation upon stimulation at 6.0 mm depth in the CB. The last trace on the bottom is the cdp recorded at L6 with the dotted line indicating the arrival of the descending volley. Note the collision of the antidromic spike when the cell was firing spontaneously (arrow).
Figure 2. Rhythmic activity of an extracellularly recorded DSCT neuron during fictive locomotion

A. The extracellular microelectrode recording (upper trace) and the rectified and filtered ENG recordings from a variety of hindlimb muscle nerves on the left (L) and right (R) side (Sart, SmAB, PBSt and DP nerves).

B. The step-cycle based average of the normalized (30 bins per cycle) instantaneous firing frequency (IFF) and ENGs of Sart and SmAB.
Figure 3. Intracellular recording from a DSCT neuron during fictive locomotion

A. Intracellular microelectrode recording (DSCT i.c.) and rectified and filtered ENG recordings (same ENG abbreviations as in Fig. 2) during MLR evoked (100 μA, 20 Hz) fictive locomotion. This is the same neuron that was recorded from an extracellular position in Fig. 2. Locomotor related depolarizations in the membrane potential (gray boxes) are apparent.

B. The step-cycle based average of the normalized (30 bin per cycle) membrane potential (DSCT i.c., black line) that was 3.5 mV after action potential generating sodium channels had been inactivated just prior to the intracellular recording period by injection of a depolarizing current overlaid on the IFF (grey line) obtained from recording in an extracellular position (same as in Fig. 2B). The last two lines show average and normalized (30 bin per cycle) ENGs of Sart and SmAB.
Figure 4. Extracellular recording from a VSCT neuron during fictive locomotion

A. Extracellular microelectrode recording (VSCT e.c., top trace) and rectified and filtered ENG recordings during MLR evoked (150 μA, 20 Hz) fictive locomotion. See ENG abbreviations as in Fig. 2 and an additional ankle extensor, gastrocnemius, GS, is shown.

B. The step-cycle based average of the normalized (30 bin per cycle) IFF ENGs of Sart and SmAB.
Figure 5. Intracellular recording from a VSCT neuron during fictive locomotion

A. Intracellular microelectrode (VSCT i.c.) recording of the activity of the same neuron as in Fig. 4 and hindlimb ENG activity (ENG abbreviations as in Fig. 4). Locomotor related depolarizations in the membrane potential (gray boxes) are apparent.

B. The step-cycle based average of the normalized (30 bins per cycle) membrane potential (VSCT i.c., black line) that was 7.1 mV after action potential generating sodium channels had been inactivated just prior to the intracellular recording period by injection of a depolarizing current overlaid on the IFF of this cell (grey line same as in Fig. 4).
Figure 6. Extracellular recording from a DSCT neuron during fictive scratch

A. The extracellular microelectrode recording (DSCT e.c., top trace) the discriminated unit after spike-sorting (second trace from top) and the rectified and filtered; ENG recordings from a variety of hindlimb muscle nerves with abbreviations as in Fig. 2 and in addition, peroneus longus (PerL) is illustrated.

B. The step-cycle based average of the normalized (30 bins per cycle) IFF (upper trace) and ENGs from Sart, PerL and GS.
Figure 7. Intracellular recording from a DSCT neuron during fictive scratch

A. Intracellular microelectrode recording (DSCT i.c.) and rectified and filtered ENG recordings (of plantaris, PI and TA) during fictive scratch. Scratch-cycle related depolarizations in the membrane potential (gray boxes) are apparent.

B. The scratch-cycle based average of the normalized (30 bins per cycle) membrane potential (DSCT i.c., black line) that was 5 mV after action potential generating sodium channels had been inactivated just prior to the intracellular recording period by injection of depolarizing current overlaid on the IFF of this cell (grey line) obtained from recording in an extracellular position in another bout of fictive scratch.
Figure 8. Comparison of DSCT and VSCT firing frequency modulation during fictive locomotion and scratch

Histograms (with 10 Hz binning) showing the number of cells and the change in their instantaneous firing frequency (IFF) during fictive locomotion (A) and during fictive scratch (B). The DSCT cells (filled bars) showed significantly lower modulation of the firing rates then the VSCT cells (open bars). Note that similar firing frequencies were observed during fictive locomotion and scratch.
Table 1. Summary of DSCT activity during fictive locomotion and excitatory input from peripheral afferents

Excitatory input from extensor group I (Extensor gr I), flexor group I (Flexor gr I), cutaneous and group II muscle afferents (Group II & Cutaneous), Group II muscle afferents only, or no input from any of the tested nerves was used for grouping DSCT cells. The percent of cells in each group per total DSCT tested is shown as “% of this category” with the percentage of total summarized in the “Total” column. The number of cells with phase-related modulation of firing frequency are shown in the first row. The number of cells with higher peak firing frequency in the flexion (F) and the extension (E) phase is shown as labeled accordingly. The spinal segments that the neurons were recorded from (L1-L5) are indicated for each group, and summarized in the respective columns. The second row shows the segmental location of those cells that had no step-cycle related modulation of firing frequency. The third row summarized the overall distribution of cells in the groups based on excitatory input (sample size and % of total).
C1 stimulation
contra / ipsi
recording microelectrode in lumbar segments
curare/bicuculline application
hindlimb ENGs
intra-CB stim
surface stim
MLR stim
4 mm
5 mm
6 mm
7 mm
8 mm
cdp at L6
intra-CB stim depth = 6.0 mm
e.c.
10 mV
2 ms
2 ms
A

DSCT e.c.

L Sart

L SmAB

L DP

R Sart

R PBSt

1 s

B

Instantaneous Firing Frequency Hz

78 Hz

IFF at rest = 6.4 Hz

L Sart

L SmAB

averaged step cycle
Em at rest = -52.8 mV

IFF From Figure 2B

Averaged step cycle

Em (mV)
A. VSCT e.c.

L Sart

L SmAB

L GS

R Sart

R SmAB

1 s

B. Instantaneous Firing Frequency

Instantaneous Firing Frequency

IFF at rest = 0 Hz

L Sart

L SmAB

averaged step cycle

2
Averaged step cycle IFF from Figure 3B

Em at rest = -53.7 mV
A

DSCT e.c.

GS

PerL

PBSt

Sart

B

Instantaneous Firing Frequency

100

65 Hz

IFF at rest = 37 Hz

Sart

PerL

GS

averaged scratch cycle
A  DSCT i.c.

-75 mV
-80

PI

TA

B  DSCT i.c.

Em at rest -78.6 mV

IFF 44 Hz

averaged scratch cycle
A  Fictive Locomotion

![Graph](image)

- Black bars represent DSCT.
- White bars represent VSCT.

B  Fictive Scratch

![Graph](image)

- Number of cells vs. change in IFF (Hz)

- Graphs show the distribution of cells with changes in IFF for both DSCT and VSCT conditions.
<table>
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<th>Source of excitatory synaptic input</th>
<th>Group I from Extensors</th>
<th>Group I from Other Nerves</th>
<th>Group II &amp; Cutaneous</th>
<th>Group II Only</th>
<th>No excitatory inputs</th>
<th>Total</th>
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