Signals from the ventrolateral thalamus to the motor cortex during locomotion

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25 **ABSTRACT**

26   The activity of the motor cortex during locomotion is profoundly modulated in the

27 rhythm of strides. The source of modulation is not known. In this study we examined the

28 activity of one of the major sources of afferent input to the motor cortex, the ventrolateral

29 thalamus (VL). Experiments were conducted in chronically implanted cats using an

30 extracellular single neuron recording technique. VL neurons projecting to the motor

31 cortex were identified by antidromic responses. During locomotion, the activity of 92%

32 of neurons was modulated in the rhythm of strides. 67% of cells discharged one activity

33 burst per stride, a pattern typical for the motor cortex. The characteristics of these

34 discharges in most VL neurons appeared to be well suited to contribute to the

35 locomotion-related activity of the motor cortex. In addition to simple locomotion, we

36 examined the VL activity during walking on a horizontal ladder, a task that requires

37 vision for correct foot placement. Upon transition from simple to ladder locomotion, the

38 activity of most VL neurons exhibited the same changes that have been reported for the

39 motor cortex, i.e. an increase in the strength of stride-related modulation and shortening

40 of the discharge duration. Five modes of integration of simple and ladder locomotion-

41 related information were recognized in the VL. We suggest that, in addition to

42 contributing to the locomotion-related activity in the motor cortex during simple

43 locomotion, the VL integrates and transmits signals needed for correct foot placement on

44 a complex terrain to the motor cortex.

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46 **KEY WORDS:** cat, activity, walking

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49   **INTRODUCTION**

50   This study on the activity of the ventro-lateral thalamus (VL) in the walking cat

51   was prompted by the fact that during locomotion the activity of the target of VL

52   projection – the motor cortex – changes periodically in the rhythm of locomotor

53   movements (Armstrong and Drew 1984; Beloozerova and Sirota 1985, 1993a, 1993b;

54   Drew 1993). The origin of this modulation is unclear. In decerebrated cats, it was shown

55   that integrity of the cerebellum is required for all subcortical descending tracts: vestibulo-

56   , reticulo-, and rubro-spinal, to exhibit locomotion-related modulation of their activity

57   (Orlovsky 1970, 1972a, 1972b). Neurons of those tracts receive direct synaptic

58   projections from the cerebellum. The motor cortex also gives rise to a major descending

59   tract – the pyramidal tract. The motor cortex does not receive direct input from the

60   cerebellum, but receives input from the VL, one synapse away from it. We hypothesized

61   that during locomotion the VL passes locomotion-related information from the

62   cerebellum to the motor cortex. The first goal of this study was to elucidate whether the

63   pattern of activity of VL neurons during locomotion in an uncomplicated environment

64   (simple locomotion) is suitable to contribute to the locomotion-related activity in the

65   motor cortex. We found that the majority of VL neurons were well fit for such a role.

66   It is known that VL neurons change their activity prior to voluntary movements

67   (Evarts 1971; Kurata 2005; Neafsea et al. 1978; Schmied et al. 1979; Strick 1976; van

68   Donkelaar et al. 1999). We hypothesized that VL is also involved in voluntary

69   modifications of locomotion. The second goal of this study was to explore whether VL

70   neurons contribute to an increase in modulation of the motor cortex activity that is

71   observed during locomotion over a complex terrain (complex locomotion). On complex

72   terrain, vision is required to adjust steps to irregularities of the walking path. During

73   stepping under visual control the activity of the motor cortex is dramatically different

74   from that during walking on the flat surface, and these changes are crucial for correct

75   stepping on a complex terrain (Beloozerova and Sirota 1988, 1993a; Beloozerova et al.

76   2010; Drew 1988, 1993). The inputs responsible for the changes of the activity in the

77   motor cortex during complex locomotion as compared to simple walking are not clear.

78   We have previously explored whether area 5 of parietal cortex, which is known to

79   integrate visual and motor information for control of limb movements (e.g. Buneo and

80   Andersen 2006; Mountcastle 1995), could be responsible for modulation of the motor

81   cortex activity during complex locomotion (Beloozerova and Sirota 2003). We found,

82   however, that the pattern of activity of area 5 neurons was quite different from that of the

83   motor cortex and thus seemed unlikely to be the primary source of modulation. Here we

84   consider another possibility that during complex locomotion the input from the VL

85   changes. We hypothesized that, in addition to basic locomotion-related information, the

86   VL transmits information that is needed to control landing positions of feet during

87   walking on a complex terrain to the motor cortex. We have found strong evidence

88   supporting this hypothesis.

89   Brief account of a part of this study was published in abstract form (Beloozerova

90   and Sirota 2002).

91

92   **METHODS**

93   Extracellular recordings from single VL neurons were obtained during chronic

94   experiments in cats. One adult female (cat A) and two adult males (cats B and C) were used.

95   Methods of surgical preparation and recording technique have been previously described in

96   detail and will be briefly reported here (Beloozerova and Sirota 1993a; Prilutsky et al. 2005;

97   Sirota et al. 2005; Zelenin et al. 2010). The experimental protocol was in compliance with

98   NIH guidelines for the care and use of animals in research, and was approved by the Barrow

99   Neurological Institute Animal Care and Use Committee.

100   *Locomotion tasks*

101   Two locomotion tasks were used: 1) simple locomotion on a flat surface, and 2)

102   complex locomotion on crosspieces of a horizontal ladder. It has been demonstrated in

103   several studies that simple locomotion does not require vision, while complex locomotion

104   does (Beloozerova and Sirota 2003; Liddell and Phillips 1944; Marigold and Patla 2008;

105   Sherk and Fowler 200; Trendelenburg 1911). During ladder locomotion cats step on the

106   support surface with substantially less spatial variability and more accuracy as compared to

107   simple locomotion (Beloozerova et al. 2010).

108   Cats were habituated to the experimental environment and trained to walk in an

109   experimental chamber over a period of one month (Pryor 1975; Skinner 1938). The walking

110   chamber was a rectangular enclosure with two connected parallel corridors (2.5 x 0.5 m

111   each): one had flat walking surface, the other contained a horizontal ladder (Beloozerova and

112   Sirota 1993a,b; Beloozerova et al. 2010). The centers of the ladder crosspieces were spaced

113   25 cm apart, equal to one half of a cat's average stride length during locomotion in the

114   chamber with flat floor. The crosspieces had flat tops, and were 5 cm wide, which was

115   slightly greater than the 3 cm diameter support area of the cat paw. While walking in the

116   chamber, cats passed through the two corridors sequentially, occasionally changing direction

117   from clockwise to counterclockwise. After each round, food was dispensed into a feeding

118   dish in one of the corners. Cats were trained, upon arrival, to stand in front of the feeding

119   dish quietly during 3-5 sec. One second in the middle of this period was considered as

120   "standing".

121   Cats were accustomed to wear a cotton jacket, a light backpack with connectors, and a

122 sock with a small metal plate on the sole of the foot for recording foot contact with the floor.

123 The floor in the chamber and the crosspieces of the ladder were covered with an electrically

124 conductive rubberized material. During locomotion, the duration of the swing and stance

125 phases of the forelimb contralateral to the side of recording in the VL was monitored by

126 measuring the electrical resistance between the plate and the floor (Steps trace in Fig. 1C)

127 (Beloozerova and Sirota 1993a,b; Sirota et al. 2005).

128 *Surgical procedures*

129   Surgery was performed under Isoflourane anesthesia in aseptic conditions. The skin

130 and fascia were retracted from the dorsal surface of the skull. At ten points around the

131 circumference of the skull, stainless steel screws were implanted. The screw heads were then

132 embedded into a plastic cast that formed a circular base. Later this base was used for fixation

133 of connectors, electrode microdrive, preamplifier, and to rigidly hold the cat's head while

134 searching for neurons. An arrangement of seven or nineteen 28 gauge hypodermic guide

135 tubes was implanted above the VL. The outer diameter of the 7-tube arrangement was 1.08

136 mm, and that of 19-tube one was 1.8 mm. Cat A received the 7-tube implant in both left and

137 right hemispheres, cat B received the 19-tube implant in both left and right hemispheres, and

138 cat C received one 7-tube implant in the left hemisphere. The tip of the arrangement was

139 lowered to the vertical Horsley and Clarke coordinate V +7.0. In two cats (A and B), on the

140 left side of the head, the region of the motor cortex was exposed by removing overlaying

141 tissues (Fig. 5A). The exposure was covered with a 1 mm thick acrylic plate. The plate was

142 pre-perforated with holes of 0.36 mm in diameter spaced by 0.5 mm, and the holes were

143 filled with bone wax. The plate allowed for later implantation of stimulation electrodes in the

144 motor cortex for identification of thalamo-cortical projection neurons.

145   *Single-unit recording*

146   Several days after the surgery the motor cortex was mapped in an awake animal using

147   multiple-unit recording and microstimulation techniques. During microstimulation, trains of

148   ten 25µA 0.2 ms cathodal pulses at 350 Hz were applied using a monopolar platinum-

149   tungsten quartz insulated microelectrode with impedance of 200-500 kOm. Stimulating

150   electrodes were implanted into the motor cortex (MC) representation of the distal (MCd) and

151   proximal (MCp) forelimb at approximate coordinates A 25-26, L 7-9 and L 5-6, respectively

152   (Fig. 5A,B). Electrodes were made of platinum-iridium Teflon insulated wire with outer

153   diameter 140 µm (A-M Systems Inc, Cartsborg, WA). The 0.4 mm tip of the wire was freed

154   from isolation and tapered. Wires were individually inserted into the cortex 1 mm apart

155   through perforations in the acrylic plate implanted above it, and fixed. A detailed description

156   of the identification of the motor cortex was given earlier (Beloozerova et al. 2005).

157   Extracellular recordings from the VL were obtained using tungsten varnish-insulated

158   microelectrodes (120 µm OD; FHC Inc., Bowdoin, ME) or platinum-tungsten quartz

159   insulated microelectrodes (40 µm OD) pulled to a fine tip and mechanically sharpened using

160   a diamond grinding wheel (Reitboeck 1983). The impedance of both types of electrodes was

161   1-3 MΩ at 1000 Hz. A custom made light-weight (2.5 g) manual single-axis micro-

162   manipulator chronically mounted on the cat's skull was used to advance the microelectrode

163   (Fig. 1B). Signals from the micro-electrode were pre-amplified with a miniature, custom

164   made preamplifier positioned on the cat's head, and then further amplified and filtered (0.3-

165   10 kHz band pass) with the CyberAmp 380 (Axon Instruments, Union City CA). After

166   amplification, signals were digitized with a sampling frequency of 30 kHz and recorded

167   using a computerized data acquisition package (Power-1401/Spike-2 System, Cambridge

168   Electronic Design, Cambridge, UK) (Figure 1C).

169   *Identification of neurons*

170   The ventrolateral thalamus is subdivided into two parts by its afferent connections

171   (Asanuma et al. 1983; Ilinsky and Kultas-Ilinsky 1984). The major projections to the

172   posterior part originate in cerebellar nuclei while the anterior part receives its primary input

173   from the basal ganglia (Grofová and Rinvik 1974; Hendry et al. 1979; Larsen and McBride

174   1979; Rinvik and Grofová 1974; Sakai et al. 1996). In this paper, we identify divisions of the

175   thalamic ventrolateral nuclear complex in accordance with nuclear delineation of the cat

176   brain atlas of Reinoso-Suarez (1961). Thus, we denote the anterior division of the complex as

177   the ventral anterior nucleus (VA). This area is analogous to the anterior part of the ventral

178   lateral nucleus (VLa) in primates. The posterior division of the ventrolateral complex we

179   name the ventral lateral nucleus (VL). This region is analogous to the posterior part of

180   primate's ventral lateral nucleus (VLp). All neurons whose activity is reported in this paper

181   were collected in the VL.

182   The somatic receptive fields of neurons were examined in animals resting with their

183   head restrained. Somatosensory stimulation was produced by lightly stroking fur, palpation

184   of the muscle bellies and tendons, as well as by passive joint movements. In the left VL of

185   two cats, A and B, in which stimulation electrodes were implanted in the left motor cortex,

186   neurons were tested for antidromic activation from the motor cortex. The motor cortex was

187   stimulated with 0.2 ms single rectangular pulses of 0.1-1.0 mA intensity applied 2-3 s apart.

188   Current was passed between each pair of implanted wires where individual wires were within

189   1-1.5 mm from each other. The principal criterion for identification of antidromic activation

190   of thalamo-cortical projection cells (TCs) was the test for collision of spikes (Bishop et al.

191   1962; Fuller and Schlag 1976) (Fig. 5C,D). For calculation of the conduction velocity, the

192   distance between recording electrodes in the VL and stimulation electrodes in the motor

193   cortex was estimated as 31.5 mm. Occasional orthodromic responses were not considered in

194   this study.

195   *Processing of neuronal activity*

196   To compare the activity of neurons during two locomotion tasks we used only the

197   strides for which the average duration in the two tasks differed by less than 10%. The onset

198   of swing of the forelimb contralateral to the VL recording site was taken as the beginning of

199   the step cycle. The duration of each step cycle was divided into 20 equal bins, and a phase

200   histogram of the discharge rate of the neuron in the cycle was generated and averaged over

201   all selected cycles (e.g., Beloozerova et al 2010).

202   The coefficient of stride-related frequency modulation (M) was calculated using a

203   histogram of cell activity throughout the step cycle. It was defined as $M = (1 - F_{min}/F_{max}) \times$

204   100%, where F_{min} and F_{max} are the minimal and the maximal frequencies of discharge in

205   the histogram. Neurons with $M > 50\%$ were judged to be stride-related. This was based on an

206   analysis of fluctuation in the activity of neurons in the resting animal. For this analysis, the

207   activity of 40 neurons recorded while the cat was sitting in the head-restraining device was

208   processed as if the cat was walking. The timing of steps made by the same cat during

209   preceding walking test was used to construct the histogram. This analysis showed that at rest,

210   the values of M never exceed 50%. In addition to M, the "depth" of modulation (dM) was

211   also calculated using the histogram. It was defined as $dM = (N_{max} - N_{min})/N \times 100\%$, where

212   N_{max} and N_{min} are the number of spikes in the maximal and the minimal histogram bin,

213   respectively, and N is the total number of spikes in the histogram.

214   In stride-related neurons the portion of the cycle, in which the discharge rate

215   exceeded the value of the minimal rate plus 25% of the difference between the maximal and

216   minimal rates in the histogram, was defined as a "period of elevated firing (PEF)" (as

217 illustrated in Fig. 6B; Sirota et al. 2005). PEFs were smoothed by renouncing all one-bin

218 peaks and troughs (a total of 1% of bins were altered throughout the database). Using PEFs

219 we separated cells whose activity was concentrated in one phase of the stride from those

220 active in different phases. In neurons with a single PEF, the "preferred phase" of discharge

221 was assessed using circular statistics (Batshelet 1981, Drew and Doucet 1991, Fisher 1993,

222 see also Beloozerova et al. 2003, Sirota et al. 2005).

223   To determine what differences in the modulation parameters of individual neurons

224 during simple and complex locomotion could not be explained by spontaneous fluctuation in

225 the discharge, we compared the activity of single neurons in sets of randomly selected steps

226 of the same task. Recordings from 50 neurons that were long enough to enable selection of at

227 least two non-overlapping sets of 25-40 steps per task were used. From each record the first

228 step was assigned to set 1, second – to set 2, third – to set 3, etc. For each neuron, the M, the

229 dM, the preferred phase, and the duration of the PEF were calculated for each set of steps and

230 compared. When three or more sets were available, all possible pair-wise comparisons were

231 considered; across all neurons, over 100 comparisons were made. Values of differences

232 occurring in less than 5% of these "sham" comparisons across all neurons were determined.

233 During analysis, the differences in activity characteristics of a neuron during simple and

234 complex locomotion that exceeded these values were considered, with 95% confidence, to be

235 caused by the difference in the locomotion tasks, but not by spontaneous background

236 fluctuation in the discharge rate.

237   For comparisons of activities of individual neurons in different tasks and between

238 groups of neurons two-tailed t-test was used. Non-parametric χ^2 test was used for comparison

239 of categorical data. For all the tests, the significance level was set at p=0.05. Unless indicated

240 otherwise, for all mean values the standard deviation (SD) is given.

241 *Histological procedures*

242   In cat A, 8 nL of 1% WGA-HRP (Sigma) were injected by pressure in the center of

243 the area of recording in VL, 48 hours prior to the animal's sacrifice. The injection was made

244 through a flexible capillary tube made of fused silica (147 μm OD, 50 μm ID; Polymicro

245 Technologies, Phoenix, AZ) that was directly connected to the output of pressure micro-

246 injector PMI-200 (Dagan, Minneapolis, MN). On the day of termination the animal was

247 deeply anesthetized with pentobarbital sodium, and reference electrolytic lesions were made

248 in the areas of recording and stimulation. The cat was then perfused with 3%

249 paraformaldehyde, and a series of 10, 20, and 30% sucrose/phosphate buffer solutions (0.1

250 M, pH 7.4). The left half of the brain was frozen and sectioned at 40 μm in the parasagittal

251 plane. Every fourth section was processed with a modified tetramethyl benzidine (TMB)

252 reaction (Gibson et al. 1984; Mesulam 1982) and then lightly counterstained with thionine.

253 Adjacent sections were stained with cresyl violet. In addition, the right cerebellum was

254 sectioned in the coronal plane and processed in the same manner. Brain sections were

255 inspected under polarized light illumination. Locations of retrogradely labeled neurons were

256 marked on digital images of brain sections using a computerized plotting system (Image

257 Tracer, Translational Technology). Positions of electrode tracks were estimated using the

258 reference lesions.

259   In cat B, 1.5 μL of 10% red fluorescent microspheres (Lumafluor Inc., Naples FL)

260 were injected into the most anterior part of the explored area in the VL, six months prior to

261 animal's sacrifice. On the day of termination, the cat was deeply anesthetized with

262 pentobarbital sodium, and reference electrolytic lesions were made in the areas of recording

263 and stimulation. That cat was perfused with 10% paraformaldehyde. The brain was blocked

264 into 3 blocks: frontal (rostral to A14), middle (between A14 and P2), and caudal (caudal to

265   P2). The middle block containing thalamus was sectioned in the coronal plane. The frontal

266   cortex ipsilateral to the recorded VL and the posterior part of the brainstem with the

267   cerebellum contralateral to the recorded VL were sectioned in the parasagittal plane. Frozen

268   50 μm sections were made. Every fourth section was mounted on slides, cleared with acetone

269   and xylene, and coverslipped. Observation and digital imaging of cells retrogradely labeled

270   with red fluorescent beads were done with Neurolucida 8, Zeiss Axioscope and

271   AxioCamMR3 (Carl Zeiss Int.) using a rhodamine filter. For identification of brain structures

272   the adjacent sections were stained with cresyl violet.

273   Cat C received no tracers. The brain sections were obtained and stained with cresyl

274   violet using same techniques as in cat B.

275

276   **RESULTS**

277   ***Location of neurons***

278   The activity of 238 VL neurons was recorded during walking both on the flat floor

279   and along the horizontal ladder. The majority of neurons were recorded in two cats: 94 in cat

280   A and 99 in cat B. In cat A, all but 10 cells were recorded from the left VL. In cat B, 60

281   neurons were recorded from the left VL, and 39 from the right VL. The remaining neurons

282   (n=45) were recorded from cat C.

283   ANATOMICAL RECONSTRUCTION. Histological examination of recording sites

284   showed that, in cat A, recordings were obtained from the anterior portion of the VL at

285   coordinates A 10.5-11.0, L 4.7-5.3, and V +0.5-4.0. Here and further, the vertical coordinate

286   is given after subtraction 10 mm from the coordinate of the atlas of Reinoso-Suarez (1961) to

287   align it with coordinates of other commonly used atlases of the cat brain. The recording site

288   is shown on a parasagittal section of the brain in Figure 2A. It was marked with an

289   electrolytic lesion and injection of WGA-HRP. The site is situated approximately 2 mm

290   caudally to the Nucleus caudatus - a landmark for identification of the anterior-posterior

291   position of the section. A reconstruction of the locations of neurons that were recorded in this

292   cat is given in Figure 2D, plates 11.0-10.5.

293   In cat B, nearly symmetrically on the left and right sides of the thalamus, recorded cells

294   were located in the central portion of the VL at coordinates A 9.5-10.75, L/R 3.0-5.0, and V -

295   0.5 -+3.5. The recording site in the left thalamus is shown on a coronal section in Figure 2B. It

296   was labeled with an electrolytic lesion and injection of red fluorescent beads (Fig. 4A). A

297   landmark for identification of the anterior-posterior position of the section was the caudal

298   putamen that at this level has a striped appearance in the cat. A reconstruction of locations of

299   neurons, combined from the left and right VL, is shown in Figure 2D, plates 10.75-9.5.

300   In cat C, recordings were made from the most caudal aspect of the VL at coordinates

301   A 8.75-9.25, L 4.0-4.3, and V 0.5-1.5. A reference lesion that was made approximately 200

302   μm caudally to the most caudal recording track in this cat is shown on a coronal section of

303   the thalamus in Figure 2C. A landmark for identification of the anterior-posterior position of

304   the section was the most rostral aspect of the lateral geniculate body (LG) that is visible in

305   this section. A reconstruction of locations of neurons recorded in this cat is given in Figure

306   2D, plates 9.25-8.75.

307   RECEPTIVE FIELDS. The somatosensory receptive fields of 168 neurons were

308   tested. All receptive fields were found on the contralateral side of the body. One third of

309   neurons (32%, 53/168) responded to passive movements of the shoulder joint and/or

310   palpation of muscles on the back or neck. Slightly more than a half of these cells showed

311   directional preference to shoulder movement, and responded better either to flexion (17/168)

312   or to extension and/or abduction of the joint (13/168). 84% of tested cells were TCs. Fewer

313 neurons (18%, 30/168) responded to movements in the elbow joint. Almost all of these

314 neurons had a directional preference: half of them responded to flexion and another half to

315 extension of elbow. 95% of tested cells were TCs. The number of neurons with receptive

316 fields on the paw or wrist was relatively small (10%, 17/168). Typically, these neurons

317 responded to pressure on the paw or to the wrist ventral flexion. 67% of tested cells were

318 TCs. In addition, 15% (25/168) of neurons responded to stimulation of most of the forelimb,

319 typically to flexion in the shoulder and extension in the elbow, or to synergistic movements

320 in both joints. This group also included the only two neurons for which activity diminished

321 during somatosensory stimulation. Among all cells tested only one had a cutaneous receptive

322 field responding to brushing of fur on the medial surface of the paw. We found only six cells

323 that had somatosensory receptive fields on the hind limb. Overall, characteristics of

324 somatosensory receptive fields were in agreement with previously reported data (e.g.,

325 Asanuma and Hunsperger 1975).

326   Neurons that responded to stimulation of different parts of the forelimb were

327 distributed randomly in the VL: there were no clear clusters of shoulder-, elbow- or wrist-

328 related cells (Fig. 2D). There were also 23% (38/168) of neurons that did not respond to any

329 somatosensory stimulation. They were intermingled with the responsive ones. Five of these

330 cells responded to vestibular stimulation produced by the whole animal translations or

331 rotations; they were found laterally and caudally in the VL (Fig. 2D). Two other neurons

332 without somatosensory receptive fields responded to visual stimulation.

333   AFFERENT CONNECTIONS. In cat A, WGA-HRP was injected in the center of

334 the area of recordings. The injection had a core approximately 1.5 mm in diameter (dark

335 area in Figure 2A). Numerous retrogradely labeled neurons were found in the anterior half

336 of the lateral (dentate) nucleus and in the anterior interposed nucleus of the right

337   (contralateral) cerebellum (Fig. 3A). In the dentate nucleus, labeled cells were found in its

338   rostral part, with the highest density at P 8.0-8.5, and were not seen caudal to P 9.0. The

339   most ventral part of the nucleus was free from labeled cells. In the anterior interposed

340   nucleus, labeled neurons filled its entire rostro-caudal extent, but were confined to the

341   lateral half of the nucleus. Ipsilaterally to the injection site, labeled neurons were found in

342   the lateral half of the entopeduncular nucleus (from L 7.0 to L 5.5). Most laterally, labeled

343   cells filled the entire nucleus, but more medially cells were concentrated only in the

344   anterior and posterior poles of it.

345   In cat B, red fluorescent beads were injected into the most anterior part of the explored

346   area. Injected beads spread vertically by approximately 3 mm and filled the entire dorso-

347   ventral extent of the VL; medio-laterally, they covered 0.6-0.7 mm of the medial part of the

348   nucleus (L 3.5-4.2, Figure 4A). Labeled cells were found throughout the entire rostro-caudal

349   extent of the contralateral dentate nucleus (Fig. 3B). In the lateral half of the nucleus labeled

350   cells were located mostly ventrally; in the medial half they were also present more dorsally,

351   where two clusters of cells were evident: the rostral and caudal ones (Fig. 4D). Labeled cells

352   were also found in the contralateral posterior interposed nucleus. They were distributed

353   throughout its entire rostro-caudal extent, most intensively at the laterality of L 3.0-4.0 mm

354   (Fig. 3C, 4E). A number of labeled neurons were seen in the contralateral anterior interposed

355   nucleus (Fig. 3C). Also, several cells were found throughout the contralateral fastigial nucleus

356   (not shown), and a few were seen in the contralateral inferior vestibular nucleus (Fig. 3C and

357   Fig. 4G). In addition, a longitudinally extended group of labeled cells was found in the

358   contralateral dorsal hypothalamic area. The ipsilateral entopeduncular nucleus was free from

359   label. Numerous labeled cells were found in the ipsilateral frontal cortex, throughout layer VI

360   of the anterior and posterior sigmoid gyri, at the laterality of L 4.0-9.0 (Fig. 4H), as well as

361   more caudally, across the lateral ansate sulcus into suprasylvian gyrus up to the caudal area 5

362   (Fig. 4I). In the area 4γ, labeled cells densely filled the entire layer VI. In other cortical areas

363   labeled cells were sparser and formed a thinner stretch through layer VI (Fig. 4C). In all

364   cortical areas that contained labeled neurons in layer VI, medium-size labeled pyramidal cells

365   were occasionally found also in the adjacent part of layer V.

366

367   ***Neurons projecting to the motor cortex***

368   Among the 238 neurons that were recorded during locomotion, 116 were identified as

369   projecting to the motor cortex (thalamo-cortical neurons, TCs). Positions of stimulating

370   electrodes in the left precruciate cortex of cat B are schematically shown in Figure 5A. The

371   electrodes were placed in the distal forelimb (paw) representation of the motor cortex (MCd)

372   and in the proximal forelimb (elbow, shoulder) representation (MCp) (see Methods). In

373   Figure 5B, a track and electrolytic lesion made by one of the stimulation electrodes are

374   shown on a photomicrograph. The lesion is visible in the cortical layer VI adjacent to layer V

375   that is populated with giant pyramidal cells characteristic for area 4γ. In both cat A and cat B,

376   all stimulating electrodes were placed in the layer VI of area 4γ in the precruciate sigmoid

377   gyrus. An example of an antidromic response of a TC neuron to stimulation of MCd is given

378   in Figures 5C, D.

379   The TCs were distributed fairly evenly throughout the area of recording. In Figure

380   2D, they are represented by filled shapes. Most TC neurons responded either to stimulation

381   of MCd or MCp, and only few responded to stimulation of both sites. Interestingly, the vast

382   majority (72%) of neurons projecting to MCd had receptive fields on proximal parts of the

383   forelimb, shoulder or elbow, and only 9% had receptive field on the wrist or paw. Neurons

384   projecting to the more medial cortical areas that are related to elbow and shoulder, MCp, had

385   various receptive fields. Latencies of antidromic responses of different TCs varied in the

386   range of 0.5-5.5 ms (Fig. 5E). Estimated conduction velocities ranged from 5 to 70 m/s (Fig.

387   5F). We have arbitrarily divided the TC neurons into two subgroups: responding with a latent

388   period of 1.0 ms or faster ("fast" TCs) and responding with longer delays ("slow" TCs). Two

389   thirds of slow TCs were collected in cat A.

390

391   ***Activity during standing***

392   The discharge rate of VL neurons during standing varied in the range of 0.6-63

393   spikes/s, and was 20.2±12.5 spikes/s on average. Neurons with somatosensory receptive

394   fields were more active than neurons without them (24.3±1.5 *vs.* 15.4±2.1 spikes/s (mean ±

395   SEM), respectively; p<0.05, t-test). Neurons located laterally with coordinates L 4.5-5.3

396   (mostly collected from cat A, Fig. 2A,D) were less active than those located medially at L

397   3.1-4.4 (mostly collected from cat B, Fig. 2B,D) (14.8±1.2 *vs.* 25.6±1.9 spikes/s (mean ±

398   SEM), p<0.05, t-test). Fast conducting TCs were more active on average than slow

399   conducting ones (23.7±2.2 *vs.* 15.4±1.2 spikes/s (mean ± SEM); p<0.05, t-test; Fig. 5G).

400   Moreover, there was a linear relationship between conduction velocity and the discharge rate

401   in the standing animal (Fig. 5H).

402

403   ***Characteristics of locomotion***

404   During recording from each neuron, cats ran between 15 and 100 (typically 25-50)

405   times down each of the corridors. From these trials, 15–150 strides (50±30) of each

406   locomotion task were selected for the analysis according to criteria outlined in the Methods.

407   For different neurons, the average duration of selected strides was between 600–750 ms. The

408   ratio of stance duration to the duration of the cycle (the stride duty factor) was 0.57–0.60.

409   The gait that cats used during walking both on flat surface and along the ladder was a walk

410   with the support formula 2-3-2-3-2-3-2-3 (Hildebrand 1965). Further details of biomechanics

411   and muscle activities in cats walking on the flat surface and along the horizontal ladder in

412   this experimental setup can be found in Beloozerova et al. (2010).

413

414   ***Population activity during simple and complex locomotion***

415   Upon transition from standing to walking the discharge rate of many neurons changed.

416   It increased, up to ten fold, in 31% of neurons, or decreased, down to less than a half, in 27%

417   of them. Because changes in the activity were opposite in different neurons, the average

418   discharge rate of the whole population did not change. We found no correlation between

419   receptive fields of the neurons and changes in the discharge rate with the start of locomotion.

420   The average activity of neurons without somatosensory receptive fields, however, was lower

421   than that of somatosensory responsive cells ($p<0.05$, t-test). Neurons with receptive fields on

422   different parts of the forelimb had similar average activity. The average discharge rate of

423   neurons during walking along the horizontal ladder was similar to that observed during

424   standing and walking on the flat surface. Fast-conducting TCs were more active than slow

425   ones during all tasks, however ($p<0.05$, t-test).

426   During simple locomotion, the activity of 92% of neurons (220/238), including 91%

427   of TCs (105/116) was modulated in the rhythm of strides: it increased in one phase of the

428   stride and decreased in another phase. Two basic patterns of modulation were recognized:

429   one or two "periods of elevated firing" (PEFs, see Methods). Most common was the one PEF

430   pattern seen in 67% (148/220) of neurons including 63% (66/105) TCs. Two PEFs were

431   observed in 31% (69/220) of cells, including 35% (37/105) TCs. During locomotion along

432   the ladder, the activity of 96% of neurons (229/238) was also modulated: the one-PEF pattern

433   was seen in 63% (145/229) of neurons, and the two-PEF pattern in 34% (78/229) of them. In

434   addition, there were three neurons with three PEFs during simple locomotion and seven such

435   neurons during ladder locomotion; activity of these cells we did not consider further. Most

436   neurons had a similar modulation pattern during both locomotor tasks, but in some neurons

437   the pattern changed. We will first compare activities of one- and two-PEF populations during

438   simple and complex locomotion and then look into activities of individual cells.

439   NEURONS WITH ONE PEF PER STRIDE. During simple locomotion a

440   representative neuron shown in Figure 6 discharged throughout all phases of the stride except

441   for the middle of stance when it was practically silent (Fig. 6A,B). The discharge within the

442   PEF varied in intensity, forming three small sub-peaks; the maximum discharge rate was 80

443   spikes/s. In Figure 6B the PEF is indicated by a solid black horizontal line, and the preferred

444   phase of the activity is shown by a circle. During ladder locomotion, rather than discharging

445   throughout most of the stride cycle, the neuron was active almost exclusively around the

446   swing-stance transition (Fig. 6G,H), but peaked near the same preferred phase as during simple

447   locomotion. Its firing rate reached 118 spikes/s, significantly higher than during simple

448   locomotion (p<0.05, t-test), whereas the activity in the trough during stance remained low.

449   Consequently, the strength of modulation was larger during ladder than during simple

450   locomotion. The duration of the PEF shortened by one half.

451   Figures 6F,L show phase positions within the step cycle of PEFs and preferred phases

452   of all neurons during simple (F) and ladder locomotion (L). One can see that, during both tasks,

453   PEFs of different neurons were distributed fairly evenly over the cycle. Duration of PEFs

454   differed between neurons in the range of 20-90% of the cycle, and lasted about 65% of the

455   cycle on average during either task. In the vast majority of neurons, the discharge rate within

456   the PEF varied. In 27% of neurons, at least two sub-peaks with activity above the average

457   discharge rate were observed; this level is twice as high as the threshold discharge rate for PEF

458   recognition (Fig. 6B). The strength of the stride-related modulation of the discharge varied

459   between neurons. About 20% of neurons were completely silent for a part of the step cycle; the

460   majority, however, were active throughout the cycle, while their discharge rate was modulated.

461   During simple locomotion, the coefficient of modulation, M, was 81±1%, and the coefficient

462   dM was 8±0.2%. During ladder locomotion the strength of modulation was larger: the M was

463   87±1% and the dM was 9.6±0.3% (mean ± SEM); the increase in both M and dM was highly

464   significant (p<0.0001, t-test). Because of an even distribution of PEFs throughout the cycle and

465   their relatively long duration, however, PEFs overlapped each other, and 60-70% of neurons

466   were simultaneously active at any time of the cycle during either task (Fig. 6D,J). As a result,

467   despite the substantial modulation of the activity in most of individual neurons, the averaged

468   discharge rate of the population was around 20-25 spikes/s throughout the cycle during both

469   simple and ladder locomotion (Fig. 6E,K).

470   We did not find any simple correlation between neuronal responses to somatosensory

471   stimulation in the quiescent animal and preferred phases of their activity during locomotion.

472   During simple locomotion, the preferred phases of most neurons responsive to passive extension

473   of the shoulder or elbow were timed to the periods of extension of the shoulder or elbow,

474   respectively. In contrast, most neurons responsive to passive flexion of the shoulder or ventral

475   flexion of the wrist had preferred phases not timed to periods of flexion in these joints.

476   Furthermore, an overwhelming majority of neurons without somatosensory receptive fields had

477   profoundly modulated activity. Similar inconsistency was found during ladder locomotion.

478   In contrast to this inconsistency, neurons with receptive fields involving different joints

479   tended to have their PEF in specific phases of the step cycle (Fig. 7). During simple

480   locomotion, neurons responsive to passive movements in the shoulder joint and/or palpation of

481   back, chest, or neck muscles more often had PEF during the swing and early stance phases

482   (Fig. 7A). During ladder locomotion, however, these neurons more often had their PEF during

483   stance, and their population activity during stance was higher than that during simple

484   locomotion (Fig. 7D). Neurons responsive to passive movement of the elbow more often had

485   their PEF in the late stance and early swing during both tasks (Fig. 7B; E). Most neurons

486   responsive to stimulation of the paw or movement in the wrist joint had their PEF in the

487   beginning and middle of stance (Fig. 7C; F). PEFs and preferred phases of neurons without

488   receptive fields were evenly distributed over the cycle.

489   The one-PEF neurons were distributed over the entire area of recording in the VL

490   without apparent clustering; they projected to both distal and proximal forelimb representations

491   in the motor cortex via both fast and slow conducting TCs. When grouped according to the site

492   of projection in the motor cortex (distal *vs.* proximal), or the conduction velocity (fast *vs.*

493   slow), during simple locomotion the neuronal populations did not show any differences in the

494   strength of modulation, or duration of PEF. However, the neurons projecting to the cortical

495   paw representation, as well as slow-conducting TCs had subtle but statistically significant

496   higher activity during swing as compared to stance, while their counterparts did not.

497   Furthermore, the neurons with preferred phases in the first half of swing (SW1) or in the first

498   half of stance (ST1) had higher activity during their respective preferred periods, as compared

499   to the neurons with preferred phases in the second half of swing (SW2) or in the second half of

500   stance (ST2) (36.8±2.2 vs. 27.3±1.5 spikes/s (mean ± SEM); $p<0.05$, t-test).

501   During ladder locomotion, fast TCs were more active during swing as compared to

502   stance ($p<0.05$, t-test), whereas slow TCs tended to be more active in the opposite phase. The

503   step-related modulation of neurons with shoulder or elbow receptive fields was stronger than

504   that of neurons with wrist/paw receptive fields: the coefficient M was 87-90% *vs.* 75%, and

505   dM was 9.4-9.8% *vs.* 6.4% (p<0.005, t-test). In addition, neurons with preferred phases in the

506   first half of swing (SW1) became still more active during swing (p<0.05, t-test), while the

507   activity of neurons with preferred phases in the second half of swing (SW2) did not change,

508   and their number decreased by 35%.

509   NEURONS WITH TWO PEFs PER STRIDE. An example of activity of a

510   representative neuron with two PEFs is shown in Figure 8. During simple locomotion this

511   neuron was active during both swing-stance and stance-swing transitions (Fig. 8A). The

512   discharge rate reached 25 and 30 spikes/s in two different peaks (Fig. 8B). On the ladder, the

513   neuron was still active during swing-stance and stance-swing transitions, but its two PEFs

514   deviated from each other in both amplitude and duration. The PEF during swing-stance

515   transition became markedly longer and had higher discharge rate than the other PEF. In

516   addition, one of the troughs deepened. As a result, the strength of modulation was larger

517   during ladder locomotion, while the total duration of PEFs was shorter.

518   Figures 8E,J show the distributions of PEFs in all two-PEF neurons. By the phase and

519   duration of their PEFs, neurons could be loosely sub-divided into three groups. The first group

520   included cells that had a long PEF during swing and a short PEF during stance – the "swing"

521   neurons (33% of cells). PEFs of these neurons are concentrated in the upper third of the

522   graphs. Second group consisted of cells that had a long PEF during stance and a short PEF

523   during swing – the "stance" neurons (25% of cells). PEFs of these neurons are concentrated in

524   the lower part of the graphs. In both swing and stance neurons, during simple locomotion the

525   longer PEF was typically (in 70% of cells) also the stronger one, as the discharge rate in it

526   exceeded that in the shorter PEF by 20±15 spikes/s. The duration of the longer PEF ranged

527   from 25 to 70% of the cycle, and the duration of the shorter one ranged from 10 to 30%.

528   During ladder locomotion, the activity of stance neurons differed in the discharge intensity

529 during swing: in many of these neurons the discharge rate during the shorter PEF in swing

530 was as high as during the longer PEF in stance. By contrast, in the majority (73%) of swing

531 neurons during ladder locomotion the activity during the longer PEF in swing was still higher.

532   The third and most populous group included neurons whose two PEFs were of

533 approximately similar duration ranging from 15 to 40% of the cycle each (40% of cells). In

534 these "transition" neurons, the first PEF typically started in mid-swing and lasted into stance,

535 while the second PEF started in the mid-stance and continued into swing. In Figures 8E,J,

536 PEFs of these neurons are concentrated in the middle of the graphs. The average duration of

537 each PEF was $30\pm7\%$ of the step cycle, approximately half as long as in the one-PEF group.

538 Although in transition neurons the two PEFs were of a similar duration, in 68% of them the

539 discharge in one of the PEFs was more intense, by 15 ± 12 spikes/s on average.

540 Because of a rather even distribution of PEFs of different neurons throughout the

541 cycle, and their long duration, the PEFs overlapped each other, and 50-70% of neurons were

542 simultaneously active in any phase of the cycle (Fig. 8C,H). The discharge rate of the

543 population of two-PEF neurons was slightly modulated around the value of 20 spikes/s, with

544 two small peaks during the swing-stance and stance-swing transition periods (Fig. 8D,I).

545 During simple locomotion, the strength of stride-related frequency modulation, M,

546 was $79\pm12\%$, and dM was $8.2\pm3.4\%$. Both values were similar to those in the one-PEF

547 group. Upon transition to ladder locomotion, however, in sharp contrast with the one-PEF

548 group, the strength of modulation in the two-PEF population did not increase.

549 In two-PEF cells the active phase during locomotion usually differed from the phase

550 that could be expected based on somatosensory responses in the resting animal, similar to

551 that in one-PEF neurons. Unlike one-PEF neurons, however, the activities of two-PEF cells

552 with receptive fields involving different joints were similarly distributed across the stride: all

553 groups had two subtle maxima at swing-stance and stance-swing transitions.

554   Two-PEF neurons were distributed over the entire area of sampling in the VL and

555 intermingled with one-PEF cells. Both fast and slow conducting TCs with two-PEFs

556 projected to both the distal and proximal forelimb representations in the motor cortex.

557 Groups of neurons assembled according either to the site of projection to the motor cortex

558 (distal *vs.* proximal) or to conduction velocity (fast *vs.* slow) during simple locomotion were

559 similar in their activity phases, strength of modulation, and duration of PEFs. During ladder

560 locomotion, however, the step-related modulation of neurons projecting to the paw area was

561 stronger than that of neurons projecting to the elbow or shoulder areas: the coefficient M was

562 $84\pm2\%$ *vs.* $77\pm3\%$, and dM was $9.3\pm0.6\%$ *vs.* $7.0\pm0.7\%$ (mean $\pm$ SEM) ($p<0.05$, t-test). Also,

563 the PEFs of neurons projecting to the paw/wrist area were slightly shorter, lasting for $55\pm2\%$

564 rather than $65\pm3\%$ of the cycle ($p<0.05$, t-test). During either task, swing, stance, and

565 transition neurons were similar in their average and peak discharge rates, strength of

566 modulation, and durations of PEF. Swing neurons, however, were more active during swing

567 than stance neurons during stance (30 ± 2.7 *vs.* 20 ± 1.2 spikes/s, $p<0.05$, t-test).

568

569 ***Individual neurons discharge differently during two locomotion tasks***

570   Upon transition from simple locomotion to walking along the horizontal ladder,

571 individual neurons changed their activity even to a greater extent than the populations did as

572 a whole: 79% of neurons changed at least one characteristic of the activity. *First*, the mean

573 discharge rate changed in 42% (100/238) of neurons, increasing in 19% (46/238) of neurons

574 and decreasing in 23% (54/238) of them (Fig. 9A). The increase was on average $63\pm55\%$,

575 and the decrease was $34\pm16\%$. The peak discharge rates averaged over one histogram bin

576   (1/20th of the cycle) also changed in many neurons upon transition from simple to ladder

577   locomotion. They increased in 30% of cells (68 out of 230 neurons whose activity was step-

578   related in at least one of the tasks) and decreased in 14% (33/230). The average increase was

579   20.5±9.0 (73±53%) and the decrease was 16.5±6.5 (32±15%) spikes/s, respectively.

580   *Second*, the strength of frequency modulation changed in an even larger fraction of

581   cells than did the mean or peak rates: it increased in 44% of cells (96 out of 217 neurons

582   modulated in both tasks, p<0.01, χ^2-test), and decreased in 11% (24/217). The dM increases

583   ranged from 20% to 200% of the value observed during simple locomotion and were

584   57±37% on average, while the decreases were 33±10%. In contrast to the scatter plot of

585   mean frequencies (Fig. 9A), data points in the plot for dM (Fig. 9B) were more widely

586   distributed along the vertical axis. The increase in M was 15±8% on average.

587   *Third*, the duration of the PEF changed in 44% of neurons (96/217), decreasing in

588   24% (52/217) and increasing in 20% (44/217) of them by 15-50% of the cycle. In many

589   neurons, the duration of the PEF considerably differed between simple and ladder

590   locomotion, and there was no correlation between these two values (r=0.2, Fig. 9C).

591   *Fourth*, the preferred phase of activity changed in 26% of one-PEF neurons (in 38 out

592   of 145 cells modulated with one PEF in both tasks, Fig. 11A), and the phase positions of

593   individual PEFs changed in some two-PEF cells. In addition, the number of PEFs per cycle

594   changed in many neurons. Close to a half (43%, 30/69) of neurons with two-PEF pattern

595   during simple locomotion had one PEF during ladder locomotion (Fig 12A,B). Also, 15%

596   (22/148) of neurons that had one PEF during simple locomotion had two PEFs on the ladder

597   (Fig. 12C,D).

598   Finally, 10 neurons were involved in the locomotion-related activity during the ladder

599   task only, and three neurons lost their modulation during walking on the ladder. We will

600 consider further the changes in the discharges of individual neurons separately for the cells

601 active in the same phase during both locomotion tasks, and for the cells that were active in

602 different phases.

603   NEURONS ACTIVE IN THE SAME PHASE DURING BOTH TASKS. The

604 preferred phases of activity in a half of one-PEF neurons (80/148, 54%) did not change upon

605 transition from simple to ladder locomotion, whereas other parameters of the discharge could

606 change (Fig. 10). The most striking was the change in the strength of stride-related

607 modulation. In 24 (30%) neurons its value increased by 50±30%. Along with this increase,

608 either the average discharge rate or the duration of PEF often changed. Both tended to

609 decrease as the depth of modulation increased. At the same time, the peak discharge rate and

610 the average activity within the PEF typically increased (by 10-35 spikes/s) or did not change.

611 There were also five cells, in which the duration of the PEF decreased without a change in

612 the strength of modulation. Thus, during complex locomotion, the activity of 36% (29/80)

613 neurons with one PEF during both locomotion tasks became more task-related as well as

614 more time-restricted, better tuned to a particular phase of the step cycle, and more precise

615 (compare Figures 6 A,B and G,H); we have termed these cells "group I" neurons. Group I

616 neurons projected to both distal and proximal forelimb representations in the motor cortex,

617 and had a variety of conduction velocities and receptive fields. Two thirds of them were

618 located medially in the VL. The preferred phases of activity of group I neurons were

619 distributed evenly across the stride. Another 10% (8/80) of neurons decreased their

620 modulation depth during ladder locomotion by 30±8% on average. Besides these, 24%

621 (19/80) of cells changed their discharge rate without altering the depth of modulation or PEF

622 duration, and another 24% (19/80) discharged similarly during two locomotion tasks.

623   Half of two-PEF neurons (38/69, 55%) also did not change their phases of activity upon

624   transition from simple to complex locomotion (e.g. Fig. 8A,B and F,G). Discharges of 36%

625   (25/69) of them were different during the two tasks in other aspects, however. Similar to one-

626   PEF cells, 16 (23%) of these neurons had stronger modulation, or shorter PEFs, or both during

627   ladder locomotion, while mean activity could either increase or decrease. We have named

628   them "group II" neurons. All but one of these neurons projected to the distal forelimb

629   representation in the motor cortex. Group II neurons had a variety of axonal conduction

630   velocities, and their receptive fields could be either on distal or proximal segments of the

631   forelimb. Swing, stance, and transition neurons were all represented in this group.

632   NEURONS ACTIVE IN DIFFERENT PHASES DURING TWO TASKS. A change

633   in the phase of activity of a neuron upon transition from simple to complex locomotion could

634   occur either because of a phase shift of the same discharge pattern within the cycle, or

635   because of re-formation of the pattern, so that the neuron had a one-PEF pattern in one

636   locomotion task and a two-PEF pattern during another task.

637   Phase shifts of the discharge pattern were seen almost exclusively in one-PEF

638   neurons. The preferred phase in 38 (26%, 38/148) of one-PEF neurons changed between two

639   tasks in this manner (Fig. 11). We have named them "group III" neurons. The average phase

640   shift in group III neurons was 20±9% of the cycle (Fig. 11A). Approximately similar

641   numbers of neurons discharged earlier and later in the cycle during ladder versus simple

642   locomotion (Fig. 11A). Figure 11C-F illustrates two most common types of phase shifts.

643   Aside from the preferred phase shift, the most striking modification in the activity of group

644   III neurons was a change in the strength of stride-related modulation. It increased in 50%

645   (19/38) of neurons by 65±30% on average. This was similar to the behavior of group I

646   neurons. In group III, however, the average discharge rate was typically similar during both

647   locomotion tasks, while the duration of PEF was also shorter in more than a half of the cells

648   (Fig.11B). Thus, during complex locomotion, the activity of very many group III neurons

649   became also more strongly stride-related and often more focused on a specific phase, precise,

650   as compared to simple locomotion. Group III neurons had a variety of receptive fields, but

651   elbow extension was disproportionally represented. The great majority of group III neurons

652   projected to the distal forelimb area of the motor cortex; 75% of group III TCs were "slow",

653   and two thirds of group III cells were located laterally within the nucleus.

654   Some neurons with one PEF during simple locomotion changed the preferred phase of

655   activity on the ladder due to acquisition of a second PEF (22/148, 15%). We have named them

656   "group IV" neurons. Figure 12A-D illustrates two most common types of phase shifts. There

657   was no particular phase in which the new PEF would tend to appear, and during ladder

658   locomotion the number of group IV cells active in each phase of the cycle remained the same

659   as during simple locomotion. And once again, besides the change in the pattern of modulation,

660   the other major modification in the activity of these neurons was an increase of modulation: in

661   45% of group IV neurons, it increased by 74±43% on average. Group IV neurons had a variety

662   of receptive fields, projected to both distal and proximal forelimb representations in the motor

663   cortex, and nearly all group IV TCs were "slow" ones. Two thirds of group IV cells were

664   located laterally in the VL.

665   In contrast to one-PEF neurons that only infrequently acquired a second PEF, the two-

666   PEF cells often changed their discharge pattern ($p<0.0001$, χ^2-test). Nearly half of neurons

667   (30/69, 43%) with two PEFs during simple locomotion had one PEF during walking on the

668   ladder (Fig. 12E-H). We have named them "group V" neurons. In 18 of these neurons the

669   reduction in PEF number occurred because one of the PEFs seen during simple locomotion

670   was absent on the ladder (Fig. 12E,F). In the overwhelming majority of the neurons (n=14) this

671   was accompanied by an increase in the discharge rate within the remaining PEF. In 12 other

672   cells the transition from two-PEF to one-PEF pattern was accomplished by an increase in the

673   activity during one of the inter-PEF intervals, joining the previously distinct PEFs (Fig.

674   12G,H). There was no particular phase preference for which PEF tended to disappear in

675   different neurons, and on the ladder the number of group V cells active in each phase of the

676   cycle remained about the same as during simple locomotion, despite the fact that on the ladder

677   they had only one PEF instead of two. The other major modification in the activity of group V

678   neurons was the change in the depth of modulation. It increased in one third of the cells by an

679   average 72±56%. Group V neurons projected to both distal and proximal forelimb

680   representations in the motor cortex, had a variety of conduction velocities, were scattered

681   across the nucleus, but almost all had receptive fields on the shoulder.

682

683   **DISCUSSION**

684   ***VL signals conveyed during simple locomotion***

685   The first goal of this study was to elucidate whether during locomotion VL neurons

686   discharge in a manner that is suitable to contribute to the locomotion-related activity in the

687   motor cortex. We found that they do. The activity of 92% of VL neurons was modulated in

688   the rhythm of strides, and 67% of neurons, including 63% of TCs, discharged with a single

689   period of elevated firing (PEF) per cycle. This discharge pattern is similar to that observed in

690   the majority of neurons in the motor cortex (Armstrong and Drew 1984; Beloozerova and

691   Sirota 1985, 1993ab; Drew 1993). Thus, the one-PEF TCs can directly contribute to the

692   activity in the motor cortex during locomotion. All phases of the step cycle are covered with a

693   nearly equal neuronal representation (Fig. 6C,D,F) and similar intensity (Fig. 6E). A

694   substantial number of VL neurons discharges with two PEFs per stride, including 35% of

695   TCs. Since a limb CPG (central patter generator) discharges only one time per cycle (rev. in

696   McCrea and Rybak, 2008) if a neuron discharges more than once, this means that it receives

697   input from more than one limb CPG or that there are several peripheral inputs that

698   significantly influence its discharge. The two-PEF pattern is less frequently observed in the

699   motor cortex (Armstrong and Drew 1984; Beloozerova and Sirota 1985, 1993a,b; Drew

700   1993). However, in most two-PEF VL neurons one of the PEFs is typically shorter and

701   weaker (Fig. 8E) and might lack a sufficient effect on the activity of the target cortical

702   neurons.

703   We found two notable differences in the activity of VL neurons and efferent neurons

704   of motor cortex layer V. The average depth of modulation (dM) is lower in the VL: 8±0.2%

705   *vs*.10±0.7% (Sirota et al. 2005; $p<0.05$, t-test), and the discharge within the activity bursts is

706   more variable. That is, stride-related responses of VL neurons are less phase-specific as

707   compared to those of motor cortex layer V neurons. This agrees with previous findings of a

708   weaker directional specificity of VL neurons discharges during arm and wrist movements as

709   compared to that of neurons in the motor cortex (Kurata 2005; Strick 1976), and is in line

710   with analogous observation in the visual system, where responses of neurons in the lateral

711   geniculate nucleus are less specific to visual stimuli than those of cells in the visual cortex

712   (e.g., Tsao and Livingstone 2008).

713   The locomotion-related modulation of activity in the VL during simple locomotion

714   may arise from two main sources: the spino-thalamic projection (Craig 2008; Mackel et al.

715   1992; Yen et al. 1991) and the cerebello-thalamic projection (Evrard and Craig 2008; Ilinsky

716   and Kultas-Ilinsky 1984; Nakano et al. 1980; Steriade 1995). In addition, influence from the

717   motor cortex may partially contribute. In decerebrated cats, it was found that the cerebellum

718   plays the pivotal role in driving locomotion-related discharges in neurons of subcortical

719 motor centers, including neurons of the red and vestibular nuclei, and neurons of reticular

720 formation giving rise to the reticulo-spinal tract (Orlovsky 1970, 1972a, 1972b; rev in

721 Arshavsky et al. 1986). It can be suggested that the VL, another subcortical motor nucleus

722 receiving direct connections from the cerebellum, does not differ in this respect. Indeed, the

723 cerebellum receives information from both the spinal locomotor CPG and from

724 somatosensory receptors (Arshavsky et al. 1986; Lundberg, Oscarsson 1962; Oscarsson

725 1965), and can pass both types of information to the VL. All deep cerebellar nuclei project to

726 the area of VL that we have explored (e.g. Angaut 1979, Evrard and Craig 2008; Ilinsky and

727 Kultas-Ilinsky 1984; Nakano et al. 1980; Rinvik and Grofová 1974, Rispal-Padel and

728 Grangetto 1977). And it was shown that all these nuclei house neurons whose activity is

729 strongly step-related during locomotion (Orlovskii 1972, Armstrong and Edgley 1984,

730 Nilaweera and Beloozerova 2009). Interposed nucleus neurons more often (57% of cells)

731 discharge one burst of spikes per stride, while 39% discharge two bursts (Armstrong and

732 Edgley 1984). These proportions are similar to those that we found in the VL (p>0.05, χ^2

733 test). Also, similarly to VL neurons, periods of elevated firing in interposed nucleus neurons

734 are widely distributed through the stride; and as a population they too have a slightly elevated

735 activity during swing phase. Lateral nucleus neurons usually have a single PEF, and their

736 preferred phases are also distributed across the step cycle (Beloozerova and Sirota 1998;

737 Nilaweera and Beloozerova 2009).

738

739 ***VL signals conveyed during complex locomotion***

740   The second goal of the present study was to explore whether VL neurons discharge in a

741 manner that is suitable to contribute to the additional modulation of the activity in the motor

742 cortex that occurs during locomotion over complex terrain. Do VL neurons transmit to the

743   motor cortex signals that are needed to control the landing positions of feet during walking on

744   a complex terrain? We found that the activity of VL neurons with one PEF was modulated

745   more strongly on the ladder than during simple locomotion. The fast-conducting TCs and

746   neurons with preferred phases in the first half of swing during simple locomotion increased

747   their activity during swing, while the slow-conducting TCs and shoulder-related cells became

748   more active during stance (Fig. 7). The overwhelming majority of individual one-PEF and two-

749   PEF neurons changed their discharges upon transition from simple to complex locomotion. The

750   dominant change, similar to that in the motor cortex, was an increase in the depth and temporal

751   precision of the modulation (Fig. 9B,C). In contrast to simple walking, during complex

752   locomotion the depth of modulation in VL neurons was as high as in the motor cortex efferent

753   population of layer V: 9.6±0.3% *vs*. 11±0.8% (Sirota et al. 2005; p>0.05, t-test). The further

754   increase in the similarity between VL and motor cortex discharges during complex locomotion

755   suggests that the VL may have a significant contribution to the modulation of the motor cortex

756   activity during this task.

757   The VL appears to be more than a simple relay for signals passing to the motor cortex

758   during complex locomotion. Many of VL neurons discharged in different phases of the cycle

759   during simple and complex locomotion. This shows that the information related to the

760   complex environment changes the basic locomotion-related discharge pattern of VL neurons.

761   Five major modes of integration can be recognized.

762   The first two modes were represented in the activity of neurons of groups I and II,

763   respectively (Fig. 6A,B and G,F; Fig. 8A,B and F,G; Fig. 13). These were adjustments of the

764   modulation (with one PEF or two PEFs, respectively) with regard to magnitude only. In the

765   activity of group I and II neurons the simple locomotion-related pattern was dominant, and

766   the role of complex locomotion-related information was to increase the activity level and the

767   efficacy of the stride-related modulation. These modes of integration could either occur in the

768   cerebellum, with results transmitted to the VL, or take place in the VL itself. Having large

769   numbers of neurons whose activity patterns retain their phase position within the step cycle

770   might be beneficial for smooth performance of complex movements such as locomotion in

771   challenging environments.

772   The third mode of integration was "fine-tuning" of timing of the activity, as seen in

773   group III neurons (Figs. 11, 13). The simple locomotion-related information also dominated

774   the activity of these neurons but, on the ladder, its timing was slightly adjusted by complex

775   locomotion-related input. We hypothesize that this assisted in proper timing of feet

776   placements on the crosspieces of the ladder.

777   The fourth mode of integration was the conversion of two-PEF neurons into one-PEF

778   ones (Figs. 12E-H, 13). In this mode, the simple and complex locomotion-related information

779   had approximately equal roles, with the simple locomotion-related information still having a

780   significant contribution to determining the discharge timing of the neuron, and with the

781   complex locomotion-related information substantially altering it. Conversion of two-PEF

782   neurons into one-PEF ones aligned discharge pattern of VL neurons with that of cells in the

783   motor cortex and presumably increased efficacy of VL influences upon the motor cortex.

784   The fifth mode of integration was a summation of simple and complex locomotion-

785   related inputs coming in different phases of the stride (Figs. 11A-D, 13). Here the simple and

786   complex locomotion-related information also had approximately equal roles, and the

787   complex locomotion-based information simply supplemented the existing simple

788   locomotion-based pattern. This changed phasing of neurons activity; and we hypothesize that

789   it assisted in accurate timing of feet placements during complex locomotion.

790

791 ***The nature of signals conveyed by the VL during complex locomotion***

792   What is the content of the complex locomotion-related information conveyed by the

793 VL to the motor cortex during complex locomotion? We have previously demonstrated that

794 the mechanics of simple locomotion on the flat surface and complex locomotion along the

795 horizontal ladder used in our studies are rather similar (Beloozerova et al. 2010). The

796 crosspieces of the ladder are positioned at comfortable distances, and their tops are flat and

797 wide enough to easily accommodate the foot. Under these conditions, in the forelimbs, there

798 are only slight differences in the wrist movements and in the activity of some wrist-related

799 muscles between simple and complex locomotion. One can hypothesize however, that largely

800 similar movements of simple and ladder locomotion still produce different proprioceptive

801 afferentation, which is then transmitted to the motor cortex via the VL. It was shown that the

802 level of fusimotor activity is often higher during difficult motor tasks, especially those that

803 are novel, strenuous, or are associated with high degree of uncertainty (Hulliger et al. 1989;

804 Prochazka et al. 1988). Our ladder locomotion task was well practiced, however, entirely

805 predictable, and, judging from levels of EMG activity (Beloozerova et al. 2010) not at all

806 strenuous. Thus, it does not seem very likely that a potential difference in the proprioceptive

807 afferentation between simple and ladder locomotion can be responsible for the entire volume

808 and spectrum of discharge differences of VL neurons during these two tasks.

809   In addition to moving the wrist slightly differently during simple and ladder

810 locomotion, on the ladder cats assume a more bent-forward posture by lowering the center of

811 mass and rotating the neck down. These differences in the activity of very proximal as well as

812 very distal limb muscles, albeit small, can be sensed by neurons in the VL and conveyed to the

813 motor cortex. Indeed, we found that during ladder locomotion the population of neurons

814 responsive to passive movements in the shoulder joint and/or palpation of back, chest, or neck

815 muscles reorganizes its activity as compared to simple walking (Fig. 7A,D). It could be then

816 expected that, similarly, the activity of the wrist-related population will too reflect the

817 difference in kinematics in this joint during two locomotion tasks. We did not see this,

818 however. Although many individual wrist-related neurons changed their activity between the

819 tasks, as a group, the activity of this population was similar during simple and complex

820 locomotion (Fig. 7C,F). This can be explained by the too small difference in the kinematics

821 between the tasks. Alternatively, it can be suggested that the activity of some neurons in the

822 VL is not directly related to mechanics of the movements and somatosensation.

823   While differences in mechanics of locomotion between simple and ladder tasks are on

824 the small side, and the relationship of the activity of VL neurons to those differences is

825 inconsistent, cats move their eyes and look at the walking pathway in a very different manner

826 during simple and ladder locomotion (Beloozerova et al. 2010; Rivers et al. 2009). The cats

827 only infrequently look at the surface of the pathway when it is flat, but intensively inspect the

828 surface on every single run when walking along the ladder. Moreover, on the ladder there is a

829 correlation between timing of gaze shifts and stride phase (Rivers et al. 2010). Similar data

830 have been reported for humans walking on stepping-stones (Hollands et al. 1995; Hollands

831 and Marple-Horvat 2001). Considering rather similar limb motor patterns in the two

832 locomotion tasks but dramatically different gaze behaviors, we want to suggest that at least a

833 part of the differences in discharges of VL neurons during simple and ladder locomotion

834 reflects differences in processing of visual information during these two tasks, as well as the

835 changes in motor commands made on the basis of visual information. During locomotion in

836 complex environment visual information about the position of the stepping target is first

837 processed through visual networks and then at some point is incorporated into the basic

838 locomotion rhythm in order to guide the limb. From this point on it becomes integrated

839 "visuo-motor" information that, in the afferent sense, is "[processed] visual information",

840 while in the efferent sense it is a "limb control signal" reflecting preparation of the

841 movement. It has been suggested that visual information about the environment is integrated

842 with movement-related information in the cerebellum, and then funneled to the motor cortex

843 via the VL for control of limb movements (Glickstein 2000; Glickstein and Gibson 1976;

844 Stein and Glickstein 1992). Martin and Ghez (1985) have earlier found that the onset of

845 elbow movement-related activity in many motor cortical neurons, although indeed well time-

846 related to the movement, is even better time-locked to the visual trigger stimulus. Based on

847 anatomical evidence, the authors suggested that the most likely pathway to convey such

848 processed visual information to the motor cortex is the VL.

849

850 ***Relative contribution of signals from the VL and associative cortex to motor cortex activity***

851 ***during visually guided locomotion***

852   Two sources of signals that modify the stride-related modulation of neuronal activity

853 in the motor cortex during locomotion over a complex terrain can be suggested: cortical and

854 subcortical. While the VL appears to be well suited to supply the motor cortex throughout the

855 entire step cycle with synthesized vision-related information thus determining the motor

856 cortex's overall activity outline, *direct* projections from associative cortical areas are likely to

857 provide a more specific shaping.

858   Research in non-human primates showed that neurons in the cortical area 6, the

859 premotor cortex, discharge in close relationship with reaching movements to visual targets

860 (e.g., Crammond and Kalaska 1994, Wise et al. 1992). Area 6 has substantial inputs from

861 parietal visual areas as well as direct cortico-cortical projections to the motor cortex (Ghosh

862 1997, Jones et al. 1978, Marconi et al. 2001). So far only one study has examined the activity

863   of area 6 during treadmill locomotion (Criado et al. 1997). It was found that 59% of neurons in

864   area 6 change their discharge rate with the start of locomotion, typically by increasing it. The

865   discharge of only ~30% of these cells, however, is stride-related during locomotion. In

866   addition, nearly all of step-related area 6 cells discharge predominantly during the stance phase

867   of the stride. Thus, although area 6 neurons may influence the motor cortex and VL during

868   stance, when it is believed that planning of the next limb transfer takes place (Laurent and

869   Thomson 1988; Hollands and Marple-Horvat 1996), they cannot be responsible for the

870   majority of swing and transition phase-related activity in either the motor cortex or VL.

871   Regarding direct cortico-cortical projections from parietal area 5 to the motor cortex,

872   although they are numerous (e.g., Andujar and Drew 2007; Babb et al. 1984, Caria et al. 1997,

873   Mori et al. 1989), at least those that come from layer III of area 5b carry rather low activity

874   during both simple and complex locomotion, both in terms of proportion of neurons involved

875   (~50%) as well as their average discharge rates (~1 spikes/s, Beloozerova et al. 2011). The

876   potential for the parietal area 5 to supply the motor cortex with vision-based information during

877   complex locomotion has been considered in detail in our previous publication (Beloozerova and

878   Sirota 2003). We have concluded that the activity of this area is not well suited to directly

879   determine the great majority of the locomotion-related responses in the motor cortex. Based on

880   data obtained in experiments with cats overstepping a moving obstacle on the treadmill, a

881   suggestion that area 5 is most likely to insert its influence on locomotion mechanism via

882   subcortical motor centers such as basal ganglia or lateral cerebellum has been also made by

883   Andujar and colleagues (2010). The role of the sparse direct projection from area 5 to the motor

884   cortex during complex locomotion is yet to be determined.

885   Overall, available data suggest that subcortical routes that link visual and motor

886   cortex areas participate very importantly in transmission of vision-based signals that modify

887   stride-related modulation of neuronal activity in the motor cortex during complex

888   locomotion. The pathway that runs via the pons, cerebellum, and VL is the largest of all

889   subcortical links between visual and motor cortices. In this study we investigated signals that

890   are conveyed from the VL to the motor cortex during visually guided locomotion. We

891   suggest that they have significant contribution.

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1145

1146 **LEGENDS TO FIGURES**

1147 **Figure 1.** Experimental paradigm. **A:** A schematic drawing of a parasagittal section of the

1148 brain showing the position of chronically implanted guide tubes for recording electrodes

1149 above the ventrolateral thalamus (VL) and stimulating electrodes in the forelimb

1150 representation of the motor cortex (MC). **B:** The method of insertion and advancement of

1151 electrodes into the VL. (1) A group of 28 gauge cannules is chronically implanted in the

1152 cortex above VL. (2) An electrode is manually inserted into one of the cannulaes and

1153 soldered to an arm (3) of a micromanipulator (4). A wire (5) that leads to a miniature

1154 preamplifier positioned on the head of the animal is also soldered to the arm. In this manually

1155 driven micromanipulator, one revolution of the screw results in 200 μm advancement of the

1156 electrode. **C:** Locomotion tasks: walking in a chamber on a flat surface and along a

1157 horizontal ladder. The trace Gates indicates when cat has passed the beginning and end of

1158 each of the chamber's corridors. The trace Steps shows the swing and stance phases of the

1159 right forelimb recorded with an electro-mechanic sensor. The trace Neuron shows discharge

1160 of a neuron from the VL.

1161

1162 **Figure 2.** Sites of recording in the VL. **A:** The recording site in cat A is shown on a

1163 photomicrograph of a parasagittal section of the thalamus. It was located in the rostral VL.

1164 The arrow points to the electrolytic lesion mark and the darkened area of tissue filled with

1165 WGA-HRP. The site is ~2 mm caudally to the Nucleus caudatus (NC) of the basal ganglia.

1166 **B:** The recording site in cat B is shown on a photomicrograph of a coronal section of the

1167 thalamus. It was positioned in the middle of the VL. The arrow points to the electrolytic

1168 lesion mark and darkened area where fluorescent beads were deposited. The caudal part of

1169 putamen (PU), a landmark for the anterior-posterior position of the section, is seen laterally.

1170 **C:** The recording site in cat C is shown on a photomicrograph of a coronal section of the

1171 thalamus. It was positioned in the caudal VL. The arrows point to a track from a reference

1172 electrode. The most rostral aspect of the lateral geniculate body (LG), a landmark for the

1173 antirior-posterior position of the section, is visible laterally. **D:** A reconstruction of positions

1174 of individual neurons recorded during locomotion in cats A, B, and C.

1175 ■ - Squares show neurons with somatosensory receptive fields on the shoulder (responding to

1176 passive movements in the shoulder joint and/or palpation of muscles on the back or neck);

1177 ♦ - Diamonds show cells that were activated by movements in the elbow;

1178 ▲ - Up-facing triangles represent neurons with receptive fields on the wrist or paw;

1179 ▼ - Down-facing triangles show neurons whose receptive field encompassed the entire

1180 forelimb;

1181 ☼ - Stars indicate neurons responsive to vestibular stimulation;

1182 ● - Circles denote neurons without somatosensory receptive fields and those whose receptive

1183 fields were not identified.

1184 Filled symbols represent neurons with axonal projections to the motor cortex (thalamo-

1185 cortical neurons, TCs); open symbols represent neurons whose projections were not identified.

1186 Abbreviations: AV – nucleus anterio-ventralis thalami, CI – capsula interna, CL – nucleus

1187 centralis lateralis, CLA – claustrum, EPN – nucleus entopeduncularis, LA – nucleus lateralis

1188 anterior, LG – lateral geniculate nucleus, LME – lamina medullaris externa thalami, LP –

1189 nucleus lateralis posterior, NC – nucleus caudatus, OT – optic tract, PC – pedunculus cerebri,

1190 PU – putamen, RE – nucleus reticularis thalami, SUB – nucleus subthalamicus, VA – nucleus

1191 ventralis anterior, VL – nucleus ventralis lateralis, VM – nucleus medialis, VPL – nucleus

1192 ventralis postero-lateralis, VPM – nucleus ventralis postero-medialis.

1193 **Figure 3.** Cerebellar projections to the recording area in the VL. **A:** Neurons in the anterior

1194 interposed (INa) and lateral (dentate, DN) nuclei in cat A, retrogradely labeled with WGA-

1195 HRP. Neurons are depicted with black circles on photomicrographs of coronal sections of the

1196 cerebellum contralateral to the injection site. **B, C:** Neurons in the dentate, anterior and

1197 posterior (INp) interposed nuclei, and inferior vestibular nucleus (IVN) in cat B, retrogradely

1198 labeled with red fluorescent beads. Neurons are shown on photomicrographs of parasagittal

1199 sections of the cerebellum contralateral to the injection site. LVN – lateral vestibular nucleus.

1200 Each circle represents one labeled neuron.

1201

1202 **Figure 4.** Cerebellar and cortical neurons labeled with fluorescent beads in cat B. **A:** A

1203 coronal section of the thalamus containing the electrolytic lesion mark and fluorescent beads

1204 deposit site in the area of recording as seen using a rhodamine filter. **B:** A coronal section of

1205 the brain at the same anterior-posterior level as in A, cresyl violet stain. **C:** Labeled neurons

1206 in the caudal area 5 in the suprasylvian gyrus. **D:** Labeled neurons in the anterior interposed

1207 nucleus (INa). **E:** Labeled neurons in the posterior interposed nucleus (INp). **F:** A

1208 parasagittal section of the brainstem and cerebellum, cresyl violet stain. **G:** Labeled neurons

1209 in the inferior vestibular nucleus (IVN). **H:** A composition of parasagittal sections of the

1210 frontal cortex showing the fold of cruciate sulcus (Cru). Sections contain neurons in layer VI

1211 labeled with red fluorescent beads. The white arrow points to a small depression on the top of

1212 the cortex that was left by one of stimulation electrodes chronically implanted in the motor

1213 cortex (MC). **I:** Labeled neurons in the forelimb representation of the motor cortex in the

1214 anterior sigmoudeus gyrus. In **C**, **D**, **E**, and **G** the scale is as in **I**.

1215

1216

1217 **Figure 5.** Axonal conduction velocities of thalamo-cortical projection neurons (TCs). **A:** A

1218 photograph of the dorsal surface of the left frontal cortex of cat B. Entrance points of

1219 stimulation electrodes into the precruciate sulcus are schematically shown by black dots.

1220 Electrodes were placed in the paw (the motor cortex distal forelimb representation, MCd),

1221 the elbow and shoulder representations (the motor cortex proximal forelimb representation,

1222 MCp) as determined by multiunit recording and micro-stimulation procedures. The position

1223 of the parasagittal section, whose photomicrograph is shown in panel B, is indicated by a

1224 dashed line. Cru- cruciate sulcus, Pcd – post-cruciate dimple, mAns – medial ansate sulcus.

1225 **B:** Photomicrograph of a parasagittal section through the rostral precruciate sulcus stained

1226 with cresyl violet. Layers of cortex are numbered. Layer V, which contains giant pyramidal

1227 cells, is highlighted by dashed lines. One of the clusters of giant cells in layer V that are

1228 characteristic for area 4γ is circled. Arrows point to a track and electrolytic lesion made by a

1229 stimulation electrode that was placed in layer VI. **C:** Stimulation of the motor cortex evoked

1230 a spike in a TC neuron with a latency of 0.8 ms. **D:** To determine whether this spike was

1231 elicited antidromically, on a next trial a spontaneous spike of the neuron was used to trigger

1232 cortical stimulation with 0.4 ms delay. Stimulation delivered with a delay smaller than the

1233 time needed for a spontaneous spike to reach the site of stimulation (that is approximately

1234 equal to the latent time of an antidromic spike) was not followed by a response. This

1235 indicated a collision of ortho- and antidromically conducted spikes and confirmed the

1236 antidromic nature of the evoked spike. **E:** Distribution of latencies of antidromic responses

1237 to stimulation of the motor cortex of all 116 TC neurons whose activity was recorded during

1238 locomotion. **F:** Distribution of estimated conduction velocities. In E,F dashed lines separate

1239 "fast" (latencies 0.4-1.0 ms) and "slow" (latencies 1.1-6.0 ms) conducting neurons. **G:**

1240 Relationship between the mean discharge rate of individual neurons in the resting animal and

1241   their antidromic latency, approximated with a power function. **H:** Relationship between the

1242   mean discharge rate of individual neurons in the resting animal and axonal conduction

1243   velocity, approximated with a linear regression. r - coefficient of correlation.

1244

1245   **Figure 6.** Population characteristics of one-PEF neurons. **A, B** and **G, H:** Example of

1246   activity of a typical neuron (group I neuron, see below) during walking on the flat surface (**A,**

1247   **B**) and along the horizontal ladder (**G, H**). The activity is presented as a raster of 50 step

1248   cycles (**A, G**) and a histogram (**B, H**). In the rasters, the duration of strides is normalized to

1249   100%, and the rasters are rank-ordered according to the duration of the swing phase. The end

1250   of swing and the beginning of the stance in each cycle is indicated by an open square. In

1251   histograms, the horizontal interrupted line indicates the average discharge frequency during

1252   the task. The horizontal black bar shows the period of elevated firing (PEF) and the circle

1253   indicates the preferred phase (as defined in the "Methods" section). **C, I:** Distribution of

1254   preferred phases of activity of all one-PEF neurons during simple (**C**) and ladder (**I**)

1255   locomotion. **D, J:** Proportion of active neurons (neurons in their PEF) in different phases of

1256   the step cycle during simple (**D**) and ladder (**J**) locomotion. **E, K:** The mean discharge rate of

1257   neurons during simple (**E**) and ladder (**K**) locomotion. Thin lines show SEM. **F, L:** Phase

1258   distribution of PEFs during simple (**F**) and ladder (**L**) locomotion. Each horizontal bar

1259   represents the PEF location of one neuron (shown in black in one cycle only) relative to the

1260   step cycle. Neurons are rank ordered so that those active earlier in the cycle are plotted on the

1261   top of the graph. Vertical solid lines highlight one cycle. Vertical interrupted lines denote end

1262   of swing and beginning of stance phase.

1263

1264

1265 **Figure 7.** One-PEF neurons with receptive fields on different joints tend to discharge during

1266 different phases of the stride. **A, D:** Activity of neurons responsive to movements in the

1267 shoulder joint, and/or palpation of back, chest, or neck muscles during simple (**A**) and ladder

1268 (**D**) locomotion. **A1, D1:** Phase distribution of PEFs. **A2, D2:** Corresponding phase

1269 distribution of discharge frequencies. The average discharge frequency in each 1/20th portion

1270 of the cycle is color-coded according to the scale shown at the bottom of the figure. **A3, D3:**

1271 Proportion of active neurons (neurons in their PEFs) in different phases of the step cycle

1272 during simple (**A3**) and ladder (**D3**) locomotion. **A4, D4:** The mean discharge rate during

1273 simple (**A4**) and ladder (**D4**) locomotion. Thin lines show SEM. Vertical interrupted lines

1274 denote end of swing and beginning of stance phase. **B, E:** Activity of neurons responsive to

1275 passive movement of the elbow joint. **C, F:** Activity of neurons responsive to stimulation of

1276 the paw or movement in the wrist joint. Stars indicate significant differences between the

1277 activity of populations during different periods of the cycle.

1278

1279 **Figure 8.** Population characteristics of two-PEF neurons. **A, B** and **F, G:** Example activity

1280 of a typical neuron (group II neuron, see below) during walking on the flat surface (**A, B**) and

1281 along the horizontal ladder (**F,G**). The activity is presented as a raster of 50 step cycles (**A,**

1282 **F**) and as a histogram (**B, G**). In rasters, the end of swing and the beginning of the stance

1283 phase in each cycle is indicated by an open square. In histograms, the horizontal interrupted

1284 line indicates the average discharge frequency during the task; the horizontal black bar shows

1285 periods of elevated firing (PEFs). **C, H:** Proportion of active neurons (neurons in their PEF)

1286 at different phases of step cycle during simple (**C**) and ladder (**H**) locomotion. **D, I:** The

1287 mean discharge rate during simple (**D**) and ladder (**I**) locomotion. Thin lines show SEM. **E,**

1288 **J:** Phase distribution of PEFs during simple (**C**) and ladder (**H**) locomotion. Each trace

1289   shows PEFs of one neuron (shown in black in one cycle only). Vertical solid lines highlight

1290   one cycle. Vertical interrupted lines denote the end of swing and beginning of stance phase.

1291

1292   **Figure 9.** Comparison of activity characteristics of individual neurons during simple and

1293   ladder locomotion. The abscissa and ordinate of each point show the values of a

1294   characteristic of a neuron during simple and ladder locomotion, respectively. Neurons whose

1295   characteristics were statistically significantly different during the two tasks are shown with

1296   filled circles, the other ones are shown with open circles. **A:** Mean discharge frequency

1297   averaged over the stride. **B:** Coefficient of frequency modulation, dM. **C:** Duration of PEF.

1298   For two-PEF neurons, the combined duration of two PEFs is given. The coefficient of

1299   correlation (r) is indicated in **A–C**.

1300
1301
1302   **Figure 10.** Changes in activity characteristics of neurons that were active in similar phases of

1303   the stride and had one PEF during both simple and ladder locomotion. **A:** Negative

1304   correlation between change in the depth of modulation (dM) and mean discharge frequency.

1305   **B:** Negative correlation between the change in the depth of modulation and duration of PEF.

1306   In **A** and **B:** The abscissa and ordinate of each point show the difference in a discharge

1307   characteristic of a neuron between simple and ladder locomotion tasks. The difference is

1308   positive if the value of the parameter was larger during ladder locomotion. Only neurons with

1309   statistically significant difference in the depth of modulation between two tasks are shown.

1310   The coefficient of correlation (r) is indicated.

1311

1312   **Figure 11.** Changes in activity characteristics of neurons that had one PEF during both

1313   simple and ladder locomotion but were active in different phases of the stride (group III

1314 neurons). **A:** Comparison of preferred phases of activity in individual neurons during simple

1315 and ladder locomotion. The abscissa and ordinate of each point show the preferred phase of a

1316 neuron during simple and ladder locomotion, respectively. Neurons whose characteristics

1317 were statistically significantly different during the two tasks are shown with filled circles; the

1318 others are shown with open circles. Areas that correspond to the swing phase during one task

1319 but stance phase during the other task are shaded. **B:** Negative correlation between the

1320 change in the depth of modulation and the duration of PEF. The abscissa and ordinate of each

1321 point show the difference in a discharge characteristic of a neuron between simple and ladder

1322 locomotion. The difference is positive if the value of the parameter was larger during ladder

1323 locomotion. Only neurons with statistically significant differences in the depth of modulation

1324 between two tasks are shown. The coefficient of correlation (r) is indicated. **C:** A schematic

1325 presentation of the most frequently observed type of the change in the discharge pattern of

1326 group III neurons upon transition from simple to ladder locomotion: the shift in the preferred

1327 phase occurs because the activity in a part of the PEF observed during simple locomotion

1328 decreases during ladder locomotion. Solid line shows the activity during simple locomotion,

1329 interrupted line shows the activity during ladder task. Vertical solid lines designate the cycle,

1330 and vertical dotted line shows the transition between swing and stance. **D:** Example of

1331 activity of a typical group III neuron. The area histogram shows the activity of the neuron

1332 during simple locomotion. The bar histogram shows its activity during ladder task. To

1333 promote visualization of the difference between activities in two tasks, the cycle is repeated

1334 twice. **E, F:** Same as C and, D but showing the second most frequent type of the activity

1335 change in group III neurons when the shift in the preferred phase occurs because the activity

1336 in a part of the PEF observed during simple locomotion decreases during ladder locomotion,

1337 and this reduction is accompanied by an increase in the discharge rate during the remaining

1338 part of PEF.

1339

1340 **Figure 12.** Changes in the activity characteristics of neurons that had different number of

1341 PEFs during different locomotion tasks (group IV and group V neurons). **A:** A schematic

1342 presentation of the most frequently observed type of the change in the discharge pattern of

1343 group IV neurons upon transition from simple to ladder locomotion. The change in the

1344 number of PEFs occurs because two small sub-peaks in the PEF during simple locomotion

1345 are shaped into two full PEFs. This occurs most often by deepening the trough between the

1346 sub-peaks, but also through a further increase in the discharge rate within these peaks. The

1347 solid line shows activity during simple locomotion, and the interrupted line shows the

1348 activity during ladder task. Vertical solid lines designate the cycle, and vertical dotted line

1349 shows transition between swing and stance. **B:** An example of the activity of a typical group

1350 IV neuron. The area histogram shows the activity of the neuron during simple locomotion.

1351 The bar histogram shows its activity during the ladder task. To promote visualization of the

1352 difference in activities between two tasks, the cycle is repeated twice. **C, D:** Same as A,B

1353 but showing the second most frequent type of activity change in group IV neurons. The

1354 change in the number of PEFs occurred because the PEF observed during simple locomotion

1355 asymmetrically changed in the amplitude, became shorter, and a new PEF appeared in a

1356 former trough. **E, F:** Same as A, B but for the most frequent type of activity change in group

1357 V neurons. **G, H**: same as A, B but for the second most frequent type of activity change in

1358 group V neurons.

1359

1360   **Figure 13.** Signals conveyed from the VL to the motor cortex during locomotion. See
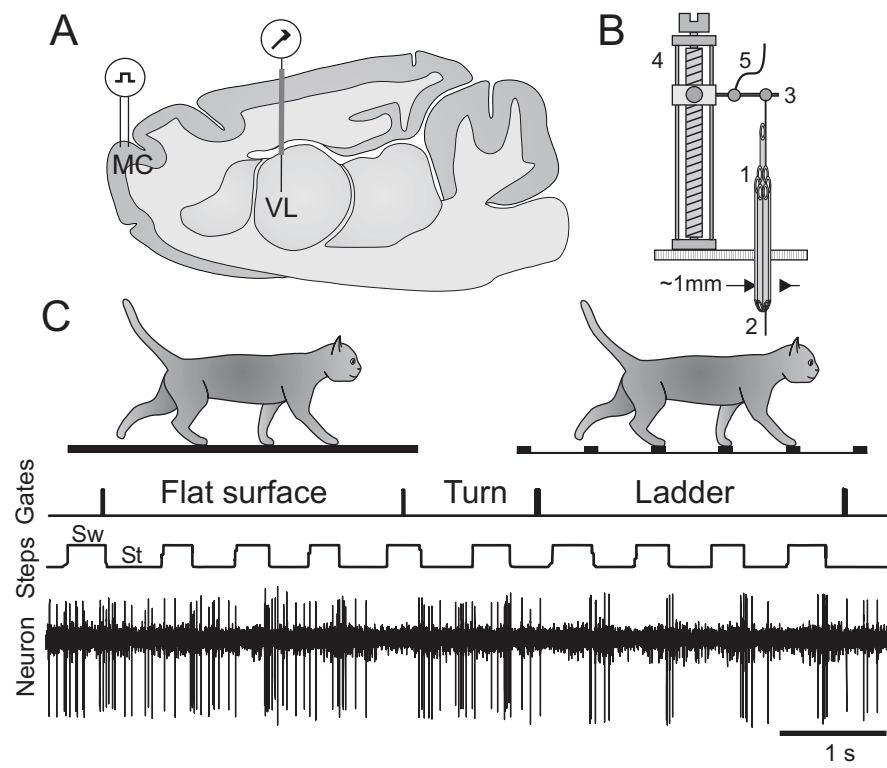
1361   Discussion for details.
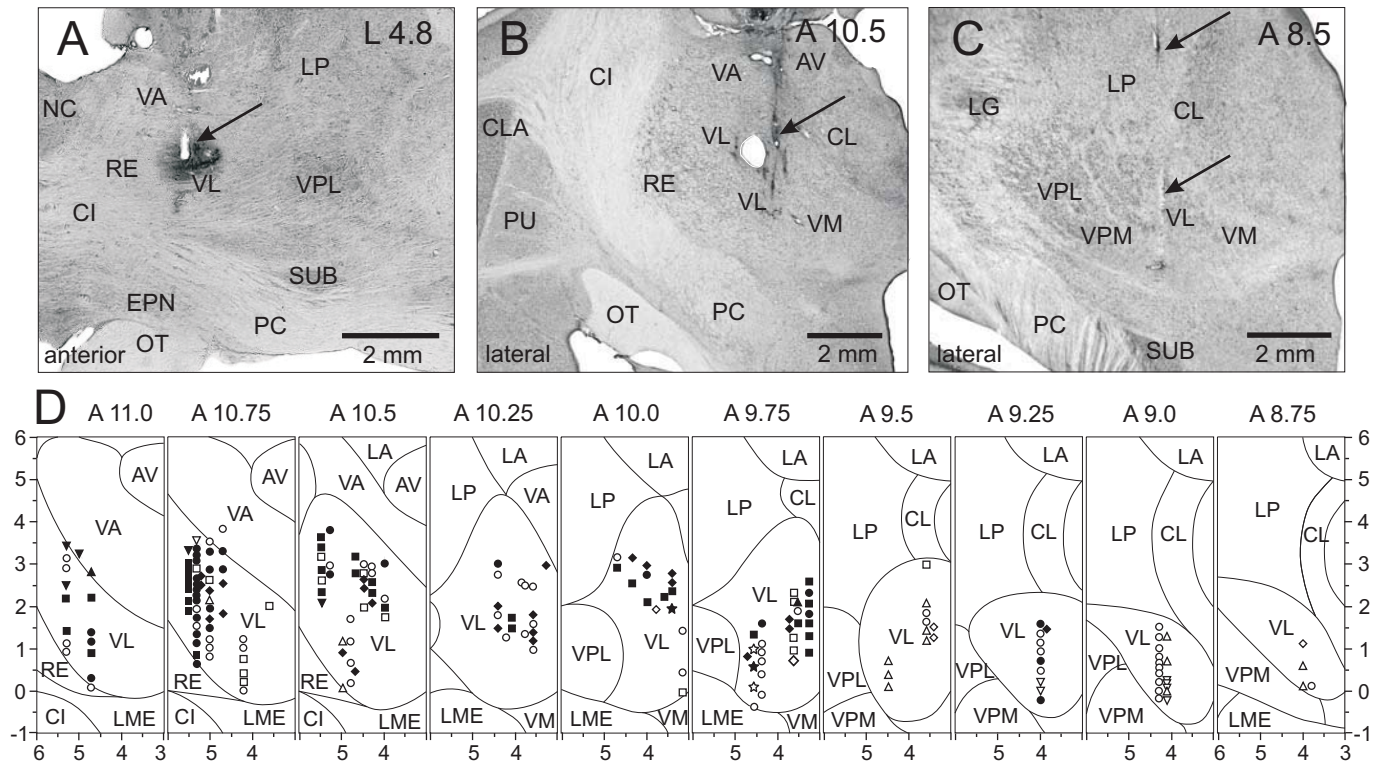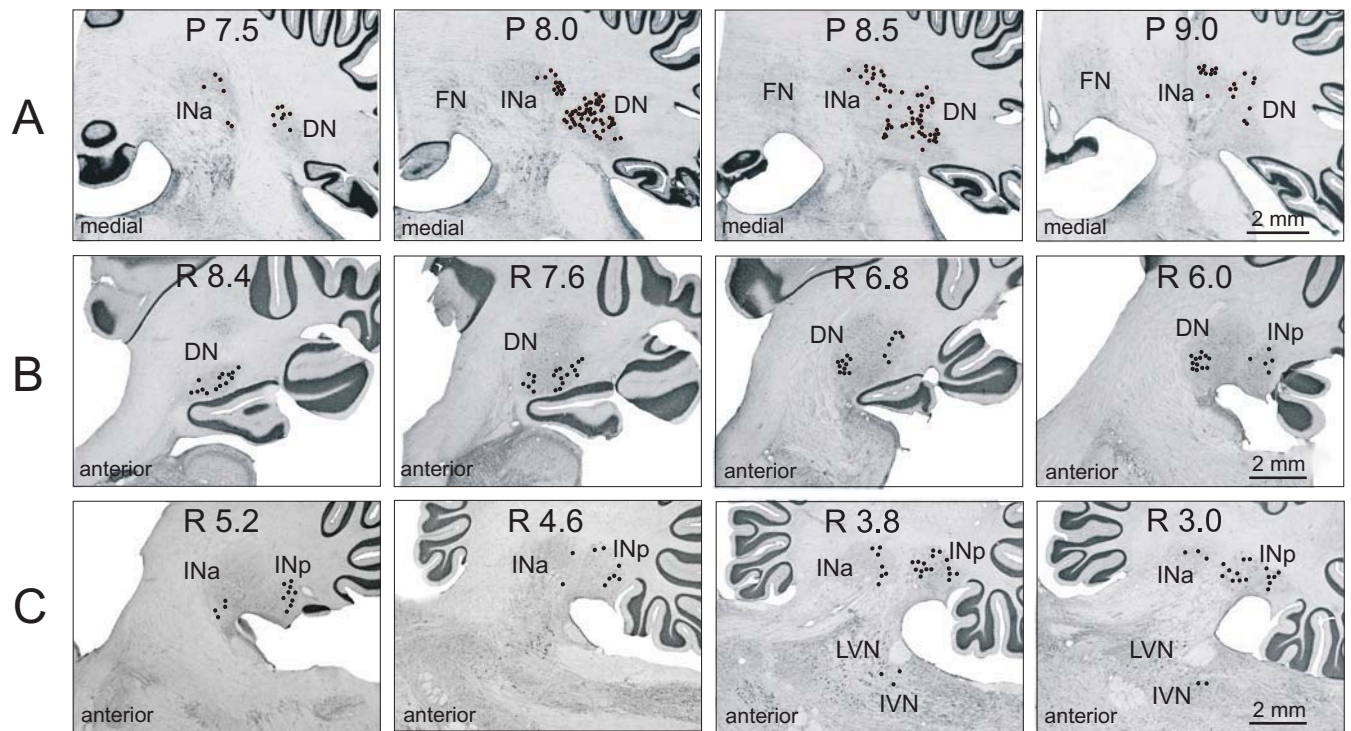
1362

A

MC
VL

B

4
5
3
1
~1mm
2

C

Gates
Flat surface   Turn   Ladder

Steps
Sw
St

Neuron

1 s

Figure 1

Figure 2

Figure 3

Figure 4

A midline ↑ mAns Cru MCp MCd Pcd anterior 2 mm

B I II III V VI Cru anterior 1 mm

C MC stim. Spont. Spike Antidr. Spike 1 ms

D MC stim. Spont. Spike 1 ms

E Number of neurons, % Latency, ms

F Velocity, m/s

G Discharge rate, spikes/s DR=18*Latency$^{-0.69}$ r=0.5 Latency, ms

H DR=0.44*Vel+6.5 r=0.9 Velocity, m/s

Figure 5

Flat

A

B
cell 699
120
spikes/s
0
swing   stance

C
10
Pref. ph., %
0

D
100
neurons, %
0

E
40
spikes/s
0
swing   stance

F
swing stance

Ladder

G

H
cell 699
120
spikes/s
0
swing   stance

I
10
Pref. ph., %
0

J
100
neurons, %
0

K
40
spikes/s
0
swing   stance

L
swing stance

Figure 6

Flat

Ladder

Shoulder

A1　A2　D1　D2

A3　A4 ★　D3　D4 ★

100 neurons,%　40 spikes/s　100 neurons,%　40 spikes/s

0　0　0　0

Elbow

B1　B2　E1　E2

B3　B4 ★　E3　E4 ★

100 neurons, %　40 spikes/s　100 neurons, %　40 spikes/s

0　0　0　0

Wrist

C1　C2　F1　F2

C3　C4 ★　F3　F4

100 neurons, %　40 spikes/s　100 neurons, %　40 spikes/s

0　0　0　0

swing stance　swing stance　swing stance　swing stance

spikes/s 0-20　21-40　41-60　61-80　81-100　101-110　111-120

Figure 7

Flat

Ladder

A

TC 575

B
spikes/s
40
0

C
neurons, %
100
0

D
spikes/s
40
0

E

swing   stance

swing   stance

F

TC 575

G
spikes/s
40
0

H
neurons, %
100
0

I
spikes/s
40
0

J

swing   stance

swing   stance

Figure 8

Figure 9

Figure 10

A Preferred Phase, ladder, %
Preferred Phase, flat, %

B ΔPEFw
ΔdM
r=0.8
p<0.01

C n=12

D spikes/s
VL 11

E swing stance

F spikes/s
TC 168
swing   stance   swing   stance

Figure 11

A   n=12

B 30   TC 521
spikes/s
0

C   n=10

D 25   TC 144
spikes/s
0

E   n=18

F 100   VL 73
spikes/s
0

G   n=12

H 25   TC 222
spikes/s
0

swing stance   swing   stance   swing   stance

Figure 12

Figure 13