Behavioral and Electromyographic Characterization of Mice Lacking EphA4 Receptors

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Akay, Turgay, Hernish J. Acharya, Karim Fouad, and Keir G. Pearson. Behavioral and electromyographic characterization of mice lacking EphA4 receptors. J Neurophysiol 96: 642–651, 2006. First published April 26, 2006; doi:10.1152/jn.00174.2006. EphA4 receptors play an important role in axon guidance during development. Disrupting the expression of these receptors in mice has been shown to modify neuronal connections in the spinal cord and results in the production of a characteristic hopping gait. The EphA4-null mouse has been used in numerous investigations aimed at establishing mechanisms responsible for patterning motor activity during walking. However, there have been few detailed behavioral or electrophysiological studies on adult EphA4-null mice. We used high-speed video recordings to determine the coordination of leg movements during locomotion in adult EphA4-null mice. Our data show that the hopping movements of the hind legs are not always associated with synchronous movements of forelegs. The coupling between the forelegs is weak, resulting in changes in their phase relationship from step to step. The synchronous coordination of the hind legs can switch to an alternating pattern for a short period of time during recovery from isoflurane anesthesia. Comparison of the kinematics of hind leg movements in EphA4-null mice and wild-type animals shows that besides the synchronous coordination in EphA4-null mice, the swing durations and the swing amplitude are shorter. Electromyographic recordings from a knee extensor muscle show double bursting in the EphA4-null animals but single bursts in wild types. This double burst changes to single-burst activity during swimming and when hind legs are stepping in alternation. These observations suggest an influence of sensory feedback in shaping the pattern of muscle activity during locomotion in the mutant animals. Our data give the first detailed description of the locomotor behavior of an adult mouse with genetically manipulated spinal networks.

INTRODUCTION

Recent investigations in mice have provided valuable information on the neurobiology of walking (Bonnot et al. 2002; Butt et al. 2005; Goldshmit et al. 2004; Leblond et al. 2003; Whelan 2003). The increased use of mice to study walking has been fueled by three important factors: first, the possibility of eliciting sequences of fictive locomotion in reduced preparations of neonatal mice, allowing the recording of single cells within central pattern generator (CPG) networks (Bonnot et al. 1998; Nishimaru et al. 2000; Whelan et al. 2000); second, the possibility of genetically modifying neuronal networks in the spinal cord that pattern motor output (Dottori et al. 1998; Kullander et al. 2003; Lanuza et al. 2004); and third, the ability to identify groups of spinal neurons based on their patterns of gene expression during development (Edlund and Jessell 1999; Goulding et al. 2002; Jessell and Sanes 2000; Shirasaki and Pfaff 2002). Mutant mice with genetically modified locomotor networks where particular neurons are visible by fluorescent protein expression are now available (Kiehn and Butt 2003; Kiehn and Kullander 2004). Furthermore, quantitative analysis of the motor output of mutant mice in which specific interneuron types are absent (Dottori et al. 1998; Gosgnach et al. 2006; Goulding and Pfaff 2005; Leighton et al. 2001) or silenced (Gosgnach et al. 2006) has become a promising approach to understand the neuronal mechanism of locomotion. Despite the increased use of mice, surprisingly little effort has been made to describe the walking behavior of adult mice. Only a few studies have described either leg kinematics (Fortier et al. 1987; Leblond et al. 2003) or interleg coordination during walking (Clarke and Still 1999; Kullander et al. 2001a; Starkey et al. 2005), and only one of these studies combined the analysis of kinematic and electromyographic (EMG) data (Leblond et al. 2003). To examine interleg coordination all but one investigation (Clarke and Still 1999) used techniques that provided only spatial parameters such as paw prints. However, understanding the neuronal mechanism underlying leg coordination additionally requires the examination of temporal patterns of movement and motor activity. Some information on motor patterns has been gained from recordings of EMG activity during walking (Leblond et al. 2003; Pearson et al. 2005; Scholle et al. 2005). If the potential of using molecular-genetic techniques in mice for gaining an understanding of the neurobiology of mammalian walking is to be fully realized, then it is essential that the behavior and motor patterns in genetically modified and normal animals must be characterized. In this study we have carried out such an analysis in EphA4-null mutant mice because neonatal EphA4-null mutants have been used extensively to gain information about the locomotor network (Butt et al. 2005; Kullander et al. 2003). In this mutant mouse the EphA4 receptors are not expressed (Dottori et al. 1998). This results in numerous abnormalities in axonal projections in the CNS, including a higher number of ipsilateral projections of corticospinal neurons and contralateral projections of putative local excitatory interneurons in the spinal cord (Dottori et al. 1998; Kullander et al. 2001b, 2003). The latter projections are considered to be primarily responsible for the characteristic hopping gait in this mutant that involves synchronous hind legs movements. It has been found that in vitro preparations this synchronous pattern can be changed to an alternating pattern by blocking the reuptake of

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the transmitters glycine and γ-aminobutyric acid (GABA) (Kullander et al. 2003). This finding indicates that the inhibitory connections responsible for the normal left–right alternation still exist in EphA4-null mice and that the abnormal excitatory connections are dominating the coordination of activity in the hind legs.

In this investigation we compared leg movements of EphA4-null and wild-type mice during walking using high-speed video analysis, and recorded EMG activity from flexor and extensor muscles of the hind legs to assess possible abnormalities in the pattern-generating networks coordinating rhythmic movements of these legs. EMG recordings were also performed during swimming and scratching in mutant and wild-type animals to establish the extent to which coordination of hind leg movements is altered in behaviors other than walking. A preliminary report of our findings was previously published (Akay et al. 2005).

METHODS

Experiments were performed on eight homozygous EphA4-null mice from our breeding colony [breeders provided by M. Goulding from a colony initially established using a gene-trap method by Leighton et al. (2001)] and six wild-type C57BL/6 (WT) mice (Charles River). The experiments were approved by the University of Alberta animal welfare committee and conducted in accordance with the guidelines set by the Canadian Council on Animal Care.

Behavioral experiments

The procedure for high-speed videorecording during walking was previously described in Pearson et al. (2005). Except for two EphA4-null mice, animals were briefly anesthetized with Forane (Isoflurane, Baxter, Toronto, Ontario, Canada) and the custom-made three-dimensional reflective markers (2 mm diameters) were glued onto the shaved skin at the level of the iliac crest, hip, knee, ankle, paw, and tip of the fourth digit (toe) of the left hind leg, and one on the wrist of the left foreleg (Fig. 1A). Because of slippage of the skin above the knee joint during walking, the knee joint marker was placed only to estimate the knee position from the videorecordings. The actual knee position was calculated by triangulation from the position of hip and ankle joint markers, using the measured lengths of the femur and tibia. For the video recordings during free walking, the mice where placed into a custom-made Plexiglas walkway [90 × 5 × 13 cm (length × width × height)]. After recovery from anesthesia, the mice walked back and forth in the walkway and a section of 40 cm length in the center of the walkway was viewed with a high-speed camera (Photron Fastcam) set with the capture rate at 250 frames/s (Fig. 1A). Video data were stored directly to computer memory for later analysis. Coordination between the legs was determined from video images captured by a mirror placed underneath the walkway set at about 45° from vertical. These images allowed measurement of the time of swing onset of each leg, defined as time of the onset of forward movement of the paw.

EMG implantation

The fabrication and implantation of the EMG electrodes is described in Pearson et al. (2005). The animals were either anesthetized with intraperitoneal (ip) injection of a mixture of 0.2 ml Hypnorm (fentanyl citrate 0.315 mg/ml and fluanisone 10 mg/ml; Janssen-Cilag, Buckinghamshire, UK) and 0.2 ml Versed (Midazolam hydrochloride 5 mg/ml; SABEX 2002, Boucherville, Quebec, Canada) in 1 ml of sterile water, or with Forane. For the injectible anesthetic an initial dose of 0.2 ml was given ip and was supplemented as needed by subcutaneous injections.

After the mice were deeply anesthetized, the hind- and forelegs and the neck were shaved. Small incisions were made on the shaved areas and each bipolar electrode was led under the skin from the neck incision to the leg incisions. The needles on the distal end of each electrode were used to draw the pair of electrode wires through the muscle until the knot proximal to the bared regions was placed firmly against the surface of the muscle. The distal end of the pair of electrodes was loosely knotted and the knot moved to the muscle surface where it was tightened. The wires distal to the second knot were removed by cutting them close to the knot. The incisions on the legs were closed and the headpiece was stitched to the skin near the neck incision by 4–0 silk suture (Ethicon). After each surgery, 0.024 mg of Buprenex (buprenorphine hydrochloride; Reckitt Benkiser Healthcare, Hull, UK) was injected subcutaneously for analgesia. The mice were left at least 2 days in their cages to recover before any handling or experiments were performed.

In total, four wild-type mice and five EphA4-null mice were implanted with EMG electrodes. The remaining animals (two wild-type and three EphA4-null) were used only for videorecordings. In four wild-type mice and four EphA4-null mice, extensor muscles in all four legs (triceps: elbow extensor, Tr- in forelegs; and vastus lateralis: knee extensor, VL- in hind legs) were implanted. In one EphA4-null mouse, the ankle flexor muscles [tibialis anterior (TA)] of the left and right hind legs were recorded instead of foreleg extensor muscles. EMG activity was recorded during free walking, swimming, and scratching. The amplified EMG signals were stored on a magnetic tape (Vetter 4000A PCM recorder) for later analysis. Swimming behavior was elicited by placing the mice in a 33 cm-diameter plastic basin filled with lukewarm water. Scratching behavior occurred spontaneously in all mice.
Data analysis

All EMG recordings were digitized off-line using the Axotape (Axon Instruments) analog-to-digital conversion system (1 kHz). The digitized records were analyzed using custom-written software (Matlab) designed to analyze timing of various events in the EMG records. The video data were analyzed using Peak Motus 8.2 motion analysis software (ViconPeak, Denver, CO). Kinematic parameters of the stepping movements, such as swing and stance durations, were measured from data files created by the Peak Motus system. We defined the cycle period as the duration between one swing onset to the next swing onset. Swing amplitude was defined as the distance between the start of the swing onset (paw lift off) and the swing offset (after paw touch down).

Statistics

The differences in means were tested with the Student’s t-test (for unpaired data). The significance level in the figures are shown as: *P < 0.05, **P < 0.01, or ***P < 0.001. In the text and figures N indicates the number of mice and n is the number of trials.

RESULTS

Comparison of the gait patterns in wild-type and EphA4-null mice

The primary focus of this investigation was to describe the characteristics of hopping in EphA4-null mice and compare these with characteristics of walking in wild-type mice. We first investigated the coordination of all four legs during walking. In Fig. 2, each histogram shows the distribution of swing onsets in a chosen leg (as indicated by the arrow tip) relative to the step cycle of a reference leg (as indicated by the origin of the arrows). The histograms with black bars represent data measured from walking sequences obtained from wild-type mice and the histograms with the white bars represent data measured from EphA4-null mice. The two histograms at the bottom of Fig. 2 illustrate the most obvious differences between wild-type and EphA4-null: alternating and synchronous stepping of the hind legs, respectively.

The situation is different for the forelegs. The two histograms on the top of Fig. 2 show that the swing movements alternate in wild-type animals (phase values clustered around 0.5), but in the EphA4-null mice there is no preferred phase relationship in swing movements of the two forelegs. The latter is indicated by the broad distribution of phases (white bars). The reason for the different number of steps (n) in all four histograms is that the forelegs often disappeared from the screen before the hind legs, thus leading to higher numbers in hind leg steps. Additionally, in EphA4-null mice, hind legs sometimes skip steps of the forelimbs leading to a higher number of steps in the forelegs. The increased number of steps and weaker coordination between forelegs is also illustrated in Fig. 3. In this figure, the phase of swing onset of the right hind

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Phase histograms showing the coupling between the hind legs (bottom histograms) and the forelegs (top histograms), and between the left and right hind and forelegs (left and right histograms, respectively), in 3 wild-type (black bars) and 3 EphA4-null mice (white bars). Phase values are the times of the occurrence of swing onset of a leg relative within the interval from swing onset to the next swing onset in a reference leg. Origin of each arrow indicates the reference leg, and the arrows point to the leg for which the phases were measured.

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Phase values of swing onset vs. time of one hind leg relative to the opposite hind leg (open circles) and one foreleg relative to the other foreleg (open triangles) during 4 walking trials of 2 different EphA4-null mice.
Comparison of kinematics of stepping in hind legs of wild-type and EphA4-null mice

Our next objective was to examine the kinematics of movements in the hind legs of EphA4-null mice to establish whether there are significant differences in movements of individual legs compared with wild-type animals. In Fig. 4A, the durations of the swing (open circles) and stance (closed circle) phases were plotted versus the cycle periods of each step in the wild-type (left, $N = 6$ mice, $n = 151$ steps) and the EphA4-null (right, $N = 6$, $n = 184$) mice. These figures show that the EphA4-null mice generally tend to step with longer cycle periods indicated by the broader distribution of the data points toward the right side of the $x$-axis. Both graphs in Fig. 4A indicate a significant relationship between the stance durations and cycle period and no correlation between the swing durations and cycle period, whereas in the EphA4-null mice swing durations are shorter. The shorter swing durations in EphA4-null mice are also illustrated in Fig. 4B, where the mean and SDs of the swing durations from wild-type ($N = 6$, $n = 151$) and EphA4-null mice ($N = 6$, $n = 184$) are shown.

We next examined the relationships between cycle period, stance duration, swing duration, and walking speed. Figure 5 shows plots of the average cycle periods (top graph), stance durations (middle graph), and swing durations (bottom graph) within one walking sequence (defined as one crossing of the viewed 40-cm section of the walkway; see Fig. 1B) plotted versus the average speed of that particular walking sequence. The top graph demonstrates that the EphA4-null mice (open circles) performed stepping with shorter cycle periods to achieve the same walking speed compared with wild-type mice (closed circles). The shorter cycle periods were accompanied by significantly shorter swing durations (Fig. 5, bottom graph). The graph in the middle of Fig. 5 shows that the stance durations were similar for comparable walking speeds for wild-type and EphA4-null mice. In addition, from Fig. 5 it is clear that EphA4-null mice tend to walk with lower walking speeds than those of the wild-types.

Decreased swing duration in EphA4-null mice raised the question whether this was attributable to decreased stride length. Indeed, Fig. 6 illustrates that the kinematics of hind leg movement during a single swing phase was noticeably different in EphA4-null mice compared with wild-type mice. The stick figures in Fig. 6A show the swing phase from a wild-type (WT, left) and from an EphA4-null (right) mouse during

FIG. 4. Comparison of cycle periods and stance and swing durations in wild-type ($N = 6$, $n = 151$) and EphA4-null mice ($N = 6$, $n = 184$). A: scatterplots showing the relations between swing (open circles) and stance (closed circles) durations and cycle periods in both groups of mice. Notice that in all mice the stance durations were significantly correlated with the cycle period (wild-type: $R^2 = 0.97$; EphA4-null: $R^2 = 0.99$). B: bar graphs showing the means and SD of swing durations in wild-type mice (black bars) and EphA4-null mice (white bars). Average swing duration is significantly lower in EphA4-null mice ($P < 0.001$).
comparable step cycle durations (0.34 s for WT and 0.35 s for EphA4-null mouse). These examples show that the EphA4-null mouse swing amplitude was shorter than that in the wild-type mouse. This is also shown as bar graphs on the bottom graph in Fig. 6, also showing that EphA4-null mice had significantly shorter at the same walking speed compared with that of the wild-type mice. Third, the EphA4-null mice swing durations and amplitudes were significantly shorter than those in wild-type mice. Furthermore, during each swing movement, the forward propulsion of the body measured by the zero crossing of the crest position reversed to negative values. Fifth, changes in the hip joint during swing were considerably greater in EphA4-null mice. Sixth, the three leg joints (hip, knee, and ankle) have reduced angular excursions (i.e., move in more flexed ranges during each step in EphA4-null mice), indicating the leg is kept more underneath the body. In conclusion the hind leg movements associated with hopping in EphA4-null mice are qualitatively different from those for walking in wild-type mice.

Comparison of EMG activity during walking, swimming, and scratching in wild-type and EphA4-null mice

Given the differences in kinematics during walking/hopping we were interested to know whether these differences arose from major differences in the underlying motor pattern generated by spinal neuronal networks. We recorded the EMG activity of the VL muscle from the left and right hind legs because the VL muscle is easily accessible and is one of the main muscles extending the knee. Interestingly, we found that in EphA4-null mice the EMG showed two prominent bursts of activity with a low level of activity between (Fig. 8A, right). We refer to this pattern as double bursting. In contrast, the extensor phase consisted of only one fairly uniform burst in wild-type animals (Fig. 8A, left). The double burst in VL is also shown in Fig. 8B in recordings of another mouse, in which we also recorded EMG from a flexor muscle [Tibialis anterior (TA)] showing the flexor phases of the steps (gray area). The missing TA burst between the two VL bursts (arrows) shows that the double bursts are part of a single step cycle. In Fig. 8C, averaged EMG activity over the normalized cycle period from four mice is illustrated, showing that in all four animals there were two peaks of activity (double bursts) of VL activity during a step cycle. Finally, we never observed double bursting in the forelimb extensor muscles (Triceps) or in TA muscles in wild-type or in EphA4-null mice (not shown).

The occurrence of double burst in VL muscles raises the question of whether the same abnormality of the motor pattern occurs during other rhythmic behaviors. To address this question...
we recorded the EMG activity of the same muscles during swimming. EMG recordings from extensor muscles of all four legs during swimming in wild-type (left) and EphA4-null (right) mice are presented in Fig. 9A. Burst activity in the VL muscles of the two hind legs alternated in wild-type animals and occurred almost synchronously in EphA4-null mutants. This corresponds to patterns of coordination in hind leg movements during walking. However, a significant difference with walking was that only single bursts of activity occurred in the VL muscles during each cycle during swimming in EphA4-null animals. Another major difference was that forelegs moved synchronously during swimming in EphA4-null animals, as indicated by the almost synchronous bursts of activity in the Triceps (elbow extensor) muscles of the two forelegs (Fig. 9A, right).

The occurrence of synchronous movements of the two hind legs during walking and swimming in EphA4-null mice raises the question of whether the coordination in the two legs can ever be uncoupled so that rhythmic movements can occur in one leg but not the other. The most likely behavior for this to occur is scratching where normal mice make repetitive rhythmic movements of a single hind leg to scratch the ipsilateral ear, head, or trunk. EMG recordings from the left and right TA and VL muscles in EphA4-null mice clearly revealed that during scratching similar synchronous bursts occurred in both of these muscles in both hind legs (Fig. 9B, right). By contrast, in wild-type animals, rhythmic bursts occurred only in the muscles of the scratching leg (Fig. 9B, left).

**Hind leg stepping in EphA4-null mice can alternate in certain circumstances**

Synchrony in the hind legs in three behavioral situations in EphA4-null mice raises the issue of whether any circumstances exist where the hind legs step in alternation rather than in synchrony. If this can occur then it would indicate that the neuronal system responsible for the alternating left–right coordination still exists in adult EphA4-null mice, as has been demonstrated in neonates (Kullander et al. 2003). One situation in which the stepping in the hind legs of some adult EphA4-null mice alternated was for a short period of time (1 to 2 min) during recovery from isoflurane anesthesia. We observed this phenomenon in three of eight adult EphA4-null mice. This is illustrated in Fig. 10A, where the phase histograms of stepping in different pairs are presented for two EphA4-null mice when hind legs alternated during recovery from isoflurane anesthesia (gray bars) and in the same mice when the hind legs moved synchronously during walking. When stepping in the hind legs alternated, the forelegs also stepped in a coordinated, alternating manner (top gray histogram), and the coupling between ipsilateral fore- and hind legs was stronger (histograms on the left and right of Fig. 10A).

Of special interest was that when stepping of the hind legs in the EphA4 mice alternated, the burst activity in the VL muscles was similar to that recorded from wild-type animals (i.e., a single burst during the stance phase), which is illustrated in Fig. 10B. The records on the left show raw EMG activity of the
two VL muscles in an EphA4-null mouse when the stepping of hind legs alternated, whereas the records on the right show the EMG pattern in the same animal when it was fully recovered from the anesthetic and stepping synchronously with the hind legs. Note the double bursts of activity in the latter situation (see also Fig. 8).

DISCUSSION

In this investigation we have extended previous observations on the behavior of adult EphA4-null mice. Apart from observing the well-known synchronous stepping of the hind legs during walking in these animals, we found that the coordination of stepping in the forelegs is more variable and that movement kinematics of the hind legs during walking are qualitatively different in EphA4-null mice compared with wild-type animals. EMG recordings from the hind leg extensor muscle, vastus lateralis (VL), showed that this difference in movement kinematics is associated with different patterns of activity in the VL muscle. In the EphA4-null mice VL had double bursts during each step cycle instead of a single burst in wild-type mice. We also found that alternating stepping movements of the hind legs could be produced in some EphA4-null mice during recovery from isoflurane anesthesia, and during alternating stepping VL activity reverted to a pattern similar to the wild-type pattern. We also examined the coordination of hind leg movements during swimming and scratching in EphA4-null mice using EMG techniques and found that synchronous movements of the hind legs also occur during these behaviors. The implications of these finding are discussed in the following sections.

Interleg coordination during rhythmic behaviors in EphA4-null mice

Until now the walking behavior of the adult EphA4-null mice has been described only anecdotally and documented only by means of footprint patterns (Dottori et al. 1998; Kullander et al. 2001b). All previous studies have noted the synchronous, hopping movements of the hind legs in these animals, but none has described movements of the forelegs. By using high-speed video recordings, we found that coupling between the forelegs was extremely variable, ranging from synchrony to alternation. There are a number of possible explanations for the loose coordination of the forelegs. First, the coupling of the central neuronal networks producing rhythmic foreleg movements might not be abnormal. In this case, these networks would tend to continue producing alternating stepping commands, although coupling might be modified by abnormal patterns of input from the hind leg pattern-generating networks and/or by abnormal sensory feedback arising from the modified movements of the body resulting from the hop-
stepping in the legs was similar to that of wild-type animals. Those that it did, it was clear that the pattern of coordination of isoflurane anesthetic. This did not occur in all animals, but in adult EphA4-null mice as the animals are recovering from recovery from isoflurane anesthetic are the result of an enhancement of transmission in the normal inhibitory GABAergic commissural pathways and that this enhancement outweighs transmission in the abnormal excitatory commissural pathways.

We demonstrated that the synchronous hind leg stepping is also maintained in behaviors other than walking. This suggests that the abnormal commissural excitation in these mice is functional in different behaviors. A striking result is that the synchronous rhythmic movements of hind legs can also be observed in scratching behavior. Notice that during wild-type scratching the contralateral hind leg is not active at all. However, in EphA4-null mice the commissural innervation is apparently strong enough to initiate synchronous leg movement of the contralateral side.

**Kinematics of hind leg movement in EphA4-null mice**

An interesting issue is whether the individual pattern-generating networks controlling movements of each of the legs in EphA4-null mice develop abnormally, in addition to the obvious abnormal development of pathways coupling the two hind leg pattern-generating networks. Indications that EphA4 receptors also play a role in the development of the pattern-generating network controlling movements of one leg come from in vitro experiments, showing that abnormal EphA4 receptor function during development can lead to impaired flexor–extensor alternation (Egea et al. 2005). Therefore abnormal development and functioning of individual pattern-generating networks must be considered as a real possibility given the obvious differences in the kinematics of the hind leg movements during walking. Our observations have identified the following differences. First, the EphA4-null mice step with a shorter cycle period compared with wild-type mice at the same walking speed. The shorter cycle periods are produced by shortening the swing durations. Second, the swing amplitude is significantly smaller than normal in the EphA4-null mice, which explains the shorter cycle periods at a given walking speed. Third, the EphA4-null mice show larger ventral–dorsal undulation of the crest and much larger changes in forward velocity during a single step cycle resulting in the characteristic hopping movements of the hind quarters. Finally, movements at the hip, knee, and ankle joints occur at much more flexed positions in the EphA4-null mice, resulting in the hind legs being positioned more under the body compared with wild-type animals (Fig. 6).

Despite all these differences in the kinematics of movements in the hind legs, it is not necessarily the case that any of them...
reflect abnormal functioning of the pattern-generating network controlling a single leg. It remains possible that the changes in the kinematics of leg movement in EphA4-null mice are simply a secondary consequence of the abnormal intersegmental coupling between the two hind leg pattern-generating networks and perhaps between the two foreleg pattern-generating networks (see previous section). Indeed, we favor this possibility based on our findings from EMG recordings from hind leg muscles (see following text).

EMG pattern in extensor muscles during walking, swimming, and scratching

The EMG recordings from hind leg muscles revealed that the knee extensor muscle VL in EphA4-null generated two bursts of activity during the stance phase, compared with a single burst of activity in wild-type mice (Figs. 8 and 10B). One interpretation of this observation is that the individual pattern-generating networks do develop abnormally in the EphA4-null mice. An alternative interpretation is that the double bursting in the VL muscle results from a change in how activity in the VL motoneurons is regulated by sensory feedback signals. The dynamics of loading on the legs during walking and hopping would probably be different, and we know that load signals have a profound effect on patterning the locomotor output in different preparations, including vertebrates and invertebrates (reviewed in Duysens et al. 2000). The double bursts that occur in VL during one step cycle could be explained by differences in kinematics, such as the sudden load of the leg on touch down. This would generate larger than normal forces in leg extensor muscles, which would transiently inhibit the activity in the VL motoneurons, thus leading to the silencing of activity in VL early in the stance phase. The afferents responsible for the inhibition could arise from high-threshold force-sensitive free nerve endings in the VL muscle or other extensor muscles (Cleland and Rymer 1993), assuming these exist in mice as they do in cats. The second burst of activity in VL would emerge as this inhibitory signal wanes.

Evidence in support of the notion that changes in sensory feedback are primarily responsible for the abnormal pattern of activity in the VL muscles in EphA4-null mice comes from our observations on the pattern of activity in swimming mice and during recovery from isoflurane anesthetic. During swimming the VL muscles generated only a single burst per cycle that...
resembled the pattern during walking in wild-type animals (Fig. 9). Presumably during swimming the load variations during the extension phase of leg movement are much smaller than those during hopping. Similarly, when stepping of the hind legs of EphA4-null mice alternated during recovery from isoflurane, only single bursts of activity occurred in the VL muscles (Fig. 10B). This is strong evidence in support of the notion that the individual pattern-generating networks develop normally in EphA4-null mice. Interestingly, in the same animals exhibiting normal patterns of activity in VL during alternating stepping, the VL muscles began to generate double bursts per cycle as soon as the animals started hopping (Fig. 10B).

Obviously, the observations we have made in this study cannot conclusively answer the question of whether individual pattern-generating networks develop abnormally in EphA4-null mice (this will require recording fictive motor patterns in adult animals). Nevertheless, our data indicate that major differences in the motor patterns in the mutant and normal animals could be a secondary consequence of the altered mechanics associated with the synchronous hopping behavior.

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