Degradation of mouse locomotor pattern in the absence of proprioceptive sensory feedback

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Mammalian locomotor programs are thought to be directed by the actions of spinal interneuron circuits collectively referred to as "central pattern generators." The contribution of proprioceptive sensory feedback to the coordination of locomotor activity remains less clear. We have analyzed changes in mouse locomotor pattern under conditions in which proprioceptive feedback is attenuated genetically and biomechanically. We find that locomotor pattern degrades upon elimination of proprioceptive feedback from muscle spindles and Golgi tendon organs. The degradation of locomotor pattern is manifest as the loss of interjoint coordination and alternation of flexor and extensor muscles. Group Ia/II sensory feedback from muscle spindles has a predominant influence in patterning the activity of flexor muscles, whereas the redundant activities of group Ia/II and group Ib afferents appear to determine the pattern of extensor muscle firing. These findings establish a role for proprioceptive feedback in the control of fundamental aspects of mammalian locomotor behavior.

locomotion | proprioception | sensory feedback | pattern generation

In mammals, walking and swimming represent favored terrestrial and aquatic solutions to the general challenge of locomotion. Both forms of movement depend on the temporal coordination of limb muscles at specific joints, driven by stereotypic and individualized patterns of flexor and extensor muscle activation (1–4). At a spinal level, locomotor programs are thought to emerge through the integrated actions of interneuronal circuits that function as central pattern generators (CPGs) and potentially through sensory feedback mediated by cutaneous and proprioceptive inputs (5–7). Advances in defining functional spinal motor circuitry in mammals (8) have nevertheless left unresolved the respective contributions of local interneuronal and sensory feedback systems to the coordination of locomotor activities in vivo. In part, this uncertainty stems from the inability to assess the impact of inactivating defined populations of sensory neurons with anatomical precision in vivo, under conditions in which locomotor output can be evaluated.

Mammalian locomotion has traditionally been analyzed in cats by kinematic and electromyographic (EMG) evaluation of the walking step cycle, with a focus on the hindlimb (1, 2, 4, 9). These studies have shown that individual extensor and flexor muscles controlling the hip, knee, and ankle joints exhibit distinct and stereotypic onset and offset timing, as well as a pronounced alternation in flexor–extensor phasing that accompanies the transition from stance to swing, or swing to stance (1, 2, 4). To address the contribution of proprioceptive feedback to locomotor pattern generation, comparisons have been made between locomotor pattern in normal walking cats and fictive locomotion in the absence of phasic proprioceptive feedback (10–13). Under certain experimental conditions, normal and fictive motor output patterns are similar (10, 11), whereas other conditions reveal striking differences between the two motor programs (11, 13), often restricted to particular muscles (12). These observations suggested that the CPG may not be sufficient to reproduce normal locomotor output. Thus, the degree to which the spinal CPG directs functional locomotor patterns in the absence of proprioceptive sensory feedback remains uncertain.

Prior studies in cat have, nevertheless, provided evidence that proprioceptive feedback modifies stance and swing phase transitions during walking to accommodate changes in task and terrain (14–18), but have not resolved the extent to which proprioceptive sensory feedback contributes to core elements of mammalian locomotor pattern. Nor have the individual contributions of the two main functional classes of proprioceptors—group Ia/II muscle spindle (MS) and group Ib Golgi tendon organ (GTO) afferents been examined. In this study we assessed the role of proprioceptive sensory feedback in mammalian locomotor pattern through an examination of mice in which a mutation in the *Egr3* (early growth response 3) gene selectively impairs group Ia/II muscle spindle activation, eliminating one class of proprioceptive feedback. We assayed *Egr3* mutants in two locomotor tasks—walking and swimming—which differ in the contribution of input from group Ib sensory afferents supplying GTOs (19, 20). Our studies, probe the role of sensory feedback in locomotor control under closed loop conditions and address the role of proprioceptive sensory feedback in assigning patterned motor output in vivo.

Our analysis reveals that normal walking locomotor pattern in mice requires ongoing proprioceptive feedback to generate coordinated stepping movements. The absence of proprioceptive feedback from muscle spindles impairs locomotor pattern by perturbing the precise timing of ankle flexor muscle activity offset during swing phase. In addition, feedback from muscle

Significance

Terrestrial locomotion is thought to be generated by the actions of a circuit of interconnected interneurons (central pattern generator) in the spinal cord that drive the patterned activity of pools of motor neurons, causing sequential contraction of dozens of leg muscles. Sensory feedback exerts a strong modulatory influence on this pattern; nevertheless, it remains unclear whether sensory feedback also plays a role in the generation of the normal locomotor pattern. Through the use of a combination of electrophysiology, behavior, and mouse genetics, we provide evidence that the absence of proprioceptive sensory feedback degrades locomotor pattern, indicating that proprioceptive feedback is required for the construction of locomotor pattern.

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spindles plays a more critical role in pattern generation when feedback from GTOs is absent—in the absence of feedback from muscle spindles and GTOs, coordinated stepping movements fail. These findings show that muscle spindle and GTO afferents provide essential and, in some instances, distinct functions in the patterning of locomotor output.

Results

Genetic and Biomechanical Strategies for Impairment of Proprioceptive Feedback. We assessed the state of locomotor activity in mice by analyzing EMG activity patterns from flexor and extensor muscles controlling hip [flexor: iliopsoas (Ip); extensor: gluteus maximus (GM)], knee [flexor: semitendinosus (St); extensor: vastus lateralis (VL)], and ankle [flexor: tibialis anterior (TA); extensor: gastrocnemius (Gs)] joints during walking and swimming (Fig. S1 *A* and *B*). Kinematic correlates of stepping movements were obtained during walking (Fig. S1*C*), although not during swimming because of inaccuracy in marker tracking. Comparison of walking in wild type and *Egr3* mutants permitted us to address the role of proprioceptive feedback from MSs under conditions in which feedback from GTO afferents is preserved. Under the reduced weight-bearing conditions achieved during swimming (20), locomotor pattern in *Egr3* mutants should reveal the impact of attenuation of group Ib/GTO, as well as group Ia/muscle spindle, feedback signaling.

Proprioceptive Feedback from Muscle Spindles Selectively Controls Ankle Flexor Offset Timing. We first analyzed the duration of the step cycle, swing, and stance phases in mutant and wild-type animals during walking at constant speed (0.2 m/s) to address the role of feedback from the muscle spindles in regulating stepping behavior. Our data indicate that all temporal parameters of the stepping movements were significantly shorter in absence of proprioceptive feedback from muscle spindles than in wild-type animals (Fig. 1 *A*, *ii* and Fig. S2). This observation indicates that feedback from muscle spindles regulates stepping during normal walking.

To address the role of proprioceptive feedback from muscle spindles in the generation of locomotor pattern we compared the EMG activity profiles from flexor and extensor muscles that regulate hip, knee, and ankle joints in wild type and *Egr3* mutant mice (21, 22). We analyzed EMG activity patterns, aligning muscle burst activity to phases of the step cycle defined by kinematic parameters (Fig. 1 and Movies S1 and S2). The onset of Ip and St muscle activities occurred at 80% and 83% of stance phase in wild-type and 84% and 77% through stance phase in *Egr3* mutant mice, respectively (Fig. 1 *A* and *B*). The onset of TA muscle activity occurred at 95% and 93% progression through stance phase in wild-type and *Egr3* mutant animals, respectively (Fig. 1 *A* and *C*). In wild-type animals, Ip burst offset was detected at 97% of swing phase, St burst offset at 33%, and TA burst offset at 67%. In mutant animals, Ip offset occurred at 95%, St offset at 42%, and TA offset at 100% through swing phase (Fig. 1*A*). The difference between wild type and mutant was statistically significant for Ip onset (4% difference, $P < 0.05$) and TA offsets (33% difference, $P < 0.001$). Thus, the absence of sensory feedback from muscle spindles elicits a selective change in the temporal features of ankle flexor muscle activity. This finding implies that group Ia/II afferent feedback from muscle spindles selectively controls the offset of TA activity, but not that of other joint flexors. The preservation of Ip and St burst phase firing presumably reflects other neural control mechanisms, possibly group Ib feedback from GTOs, as discussed below.

We also compared the activity profiles of extensor muscles in relation to their corresponding flexor antagonists in wild-type and *Egr3* mutant mice. In wild-type animals, GM muscle activity was initiated at 88% progression through stance phase and terminated precisely at the end of swing phase, displaying considerable overlap with Ip activity (Fig. 1*D*). The GM onset in *Egr3* mutant mice occurred at 85% through stance phase and offset

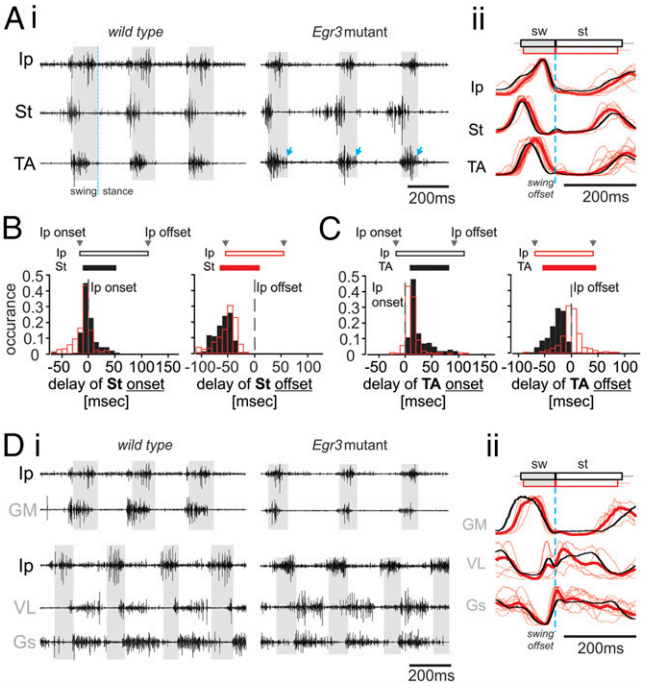

Fig. 1. EMG pattern during wild-type and *Egr3* mutant walking. (*A*, *i*) Raw EMG data from flexor muscles during a walking sequence that includes three swing phases (shaded background) and two complete stance phases (white background) in wild-type (*Left*) and a *Egr3* mutant (*Right*) mice. Blue arrows indicate the persisting activity in tibialis anterior (TA) muscle activity until the end of swing phase. (*ii*) Averaged flexor EMG activities triggered around swing offset (blue dashed lines). Bold red lines represent the pooled average recordings from all *Egr3* mutant animals ($N = 14$ for Ip, 6 for St, and 14 for TA animals), and light thin red lines are averages from individual recordings. Bold black lines represent pooled average from all wild-type mice ($N = 13$ for Ip, 6 for St, and 16 for TA animals). Horizontal black (wild type) and red (*Egr3* mutant) bars on *Top* indicates the average duration (+SD) of swing (sw) and stance (st) phases. (*B and C*) Histograms illustrating the delay of on- and offsets (*Left* and *Right* histograms, respectively) of St (*B,* 6 wild-type and 5 mutant animals) and TA (*C,* 13 wild-type and 10 mutant animals) activity relative to the Ip activity during walking. Black bars represent data from wild-type and open red bars from *Egr3* mutant animals. (*D*, *i*) Two sets of raw EMG recordings, one showing Ip and GM activities (*Top*) and one showing Ip, VL, and Gs activities. (*ii*) Averaged extensor EMG activities triggered around swing offsets (blue dashed lines) ($N = 3$ for GM, 7 for VL, and 10 for Gs wild-type animals and 5 for GM, 5 for VL, and 10 for Gs mutant animals). Horizontal bars on *Top* of *A*, *ii* and *B*, *ii* indicate the average duration (+SD) of sw and st phases in wild-type (black bars) and in *Egr3* mutant (red bars) mice.

occurred at 80% through swing phase. The offset of GM in the mutants occurred ~20% earlier in swing phase compared with wild types, displaying overlap with Ip hip flexor muscle activity. VL bursts in wild-type mice commenced 63% through swing phase, and terminated 81% through stance phase, exhibiting a clear phasic alternation with St activity (Fig. 1*D*). In *Egr3* mutants, the VL onset occurred at 55% through the swing phase and the VL offset occurred at 84% through the stance phase, similar to that of wild-type animals. Gs bursts in wild-type mice were activated 87% through swing phase and persisted 20% into the swing phase of the next step cycle (Fig. 1*D*). Gs activity exhibited no overlap with TA burst activity at the TA–Gs transition point, but did overlap at the Gs–TA transition point (Fig. 1 *A* and *D*). Similar Gs onset and offset timing was observed in *Egr3* mutants, at 81% through swing for offset and 32% through stance for offset, displaying considerable overlap at the Gs–TA and TA–Gs transitions, due to the extended TA burst during swing phase. Together, these findings indicate that elimination of

sensory feedback from muscle spindles selectively degrades the offset phasing of ankle flexor activity during swing phase.

Feedback from Muscle Spindles Ensures Precise Foot Placement During Walking. To assess how EMG changes observed upon deprivation of sensory feedback from muscle spindles influence locomotor behavior, we compared kinematic parameters of hindlimb movement in *Egr3* mutant and wild-type mice. At the onset of swing phase, hip joint angles were similar in *Egr3* mutant and wild-type mice, although at the end of swing phase, hip joint angles were 7.6° more flexed in *Egr3* mutants ($P < 0.001$, Fig. 2 *A* and *B*). Similarly, knee joint flexion was ∼10° greater in *Egr3* mutant mice ($P < 0.001$) throughout the entire swing phase. Ankle joint angles were similar at the beginning of the swing phase, but exhibited ∼22° greater flexion during the middle of swing phase ($P < 0.001$, Fig. 2*A*, green arrow). The enhanced ankle flexion observed in *Egr3* mutants is likely to reflect the persistence of TA muscle burst activity. However, the kinematic changes observed in hip and knee joint angles cannot easily be attributed to changes in TA burst duration, and may reflect changes in the pattern of EMG activities of muscles not analyzed here. Alternatively, the enhanced flexion of the knee joint could reflect a decreased amplitude of VL activation (Fig. 1 *D*, *ii*). Comparing amplitudes of EMG activities from multiple animals is unreliable due to the variability in recording qualities. Kinematic reconstruction of hindlimb placement during swing phase in *Egr3* mutants revealed that foot trajectories attained a higher toe position and pursued an abnormally steep downward trajectory to touchdown (Fig. 2 *C* and *D* and Movie S2), consistent with enhanced flexion of the knee and ankle joints.

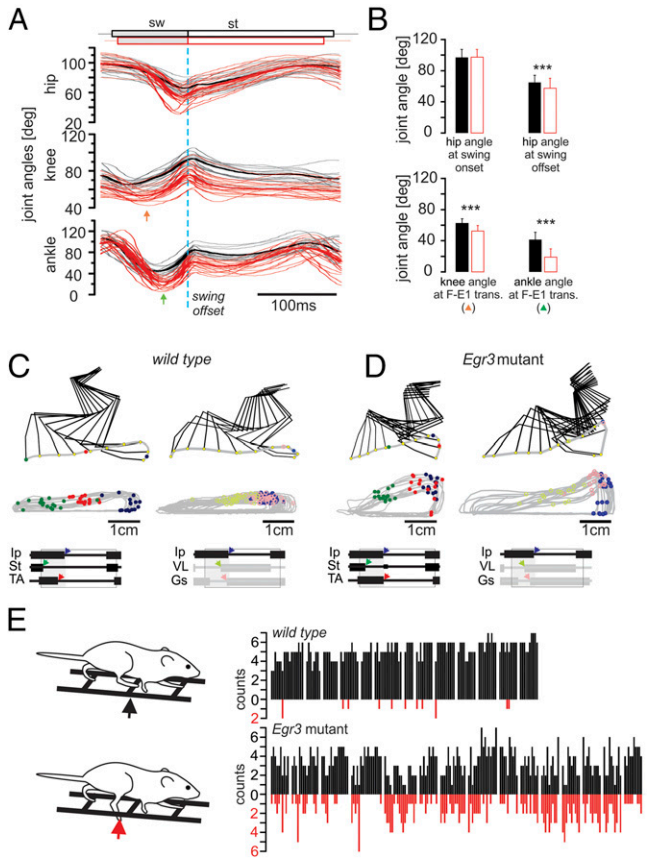

Fig. 2. Kinematic and functional consequences of the absence of early TA offset. (*A*) Average angular movement of three leg joints during walking of wild types (black lines, $n = 16$ animals) and *Egr3* mutant mice (red lines, $n = 18$ animal). Bold lines are pooled averages from all mice and thin lines are averages from individual animals. Horizontal red bars on *Top* indicate the average duration (+SD) of swing (sw) and stance (st) phases, together with the similar data from wild types (black bars). (*B*) Comparison of mean (+SD) of maximal and minimal hip joint angles (*Top* graphs) and minimal joint angles of knee and ankle joints during swing phase (*Bottom* graphs). ***$P < 0.001$. (*C and D*) Stick reconstruction of swing phases on *Top* and toe trajectories of multiple steps overlapped with EMG events from two steps from a wild-type (*C*) and an *Egr3* mutant (*D*) mouse. Sketches on the *Bottom* indicate the color-coded EMG events in *Upper* diagrams. Black and gray bars indicate flexor and extensor activity, respectively. The large rectangle in the background is the average step cycle, shaded area indicating the swing phase. (*E*) The *Egr3* mutant mice make more errors during walking on a horizontal ladder, determined as more frequent foot droppings between rungs than in wild types. Each bar indicates number of steps that landed safely on a rung (black bars) or dropped between the rungs (red bars) counted during one run ($N = 13$ for wild type, 15 for mutant).

The alterations in TA burst offset timing, ankle joint angle, and foot trajectory observed during walking in *Egr3* mutants were associated with a reduced precision in foot placement in a horizontal ladder walking task (23). Wild-type mice achieved a high incidence of rear foot placement, when walking on the rungs of a horizontal ladder, with a foot-drop error rate of only 2% (±2% SD, 13 animals; Fig. 2*E* and Movie S3). In contrast, *Egr3* mutants exhibited a 30% foot-drop error rate (±13% SD, 15 animals; two tailed *t* test, $P < 0.001$ vs. wild type; Fig. 2*E* and Movie S4). Thus, sensory feedback from muscle spindles ensures limb placement accuracy in a skilled motor behavioral task.

Coordinated Activation of Muscles Requires Feedback from Muscle Spindle and GTO Afferents. Why does removal of proprioceptive feedback from muscle spindles affect walking locomotor pattern in such a selective manner, largely limited to the termination of ankle flexor muscle activity? A possible explanation is that feedback from group Ib/GTO attenuates in part for the loss of muscle spindle feedback. To assess the contribution of feedback signaling from group Ib/GTO proprioceptors, we compared the locomotor pattern of wild-type and *Egr3* mutant mice during a swimming task, reasoning that a reduction in gravitational load under conditions of enhanced buoyancy should result in a drastic reduction in joint loading, and thus in the extent of group Ib/GTO sensory activation (*SI Text*) (20).

Initially, we observed that the activity patterns of hindlimb flexor and extensor muscles during wild-type swimming were markedly different from those observed during walking (Fig. 3 and Movie S5). Analysis of flexor EMG activities in wild-type mice revealed that the Ip, St, and TA muscles are activated in a sequential manner, commencing with Ip hip flexor bursts and propagating distally to the TA ankle flexor. Termination of St burst activity occurred before that of the TA and Ip bursts (Fig. 3 *A*–*C*). Thus, at least one flexor muscle is active throughout the entire swimming cycle. This pattern is qualitatively distinct from that seen during walking, where all three flexor muscles are inactive during stance phase. Analysis of extensor EMG activities revealed considerable coactivation of hip, knee, and ankle extensor muscles. Burst activity commenced with the VL knee extensor, followed soon after by synchronous activation of GM hip and Gs ankle extensors (Fig. 3*D*). The offset of VL burst activity occurred earlier than the offset of the GM and Gs bursts, which occurred simultaneously (Fig. 3*D*). These data show that locomotion through water and on solid terrain recruit qualitatively distinct locomotor programs, possibly a consequence of different supraspinal command programs and/or distinct patterns of sensory feedback.

To address the role of proprioceptive feedback from muscle spindles under conditions of reduced group Ib signaling, we compared swimming behavior in wild-type and *Egr3* mutant mice. *Egr3* mutants were able to swim, albeit in a profoundly ataxic manner (Movies S6 and S7). Comparative EMG analysis during swimming

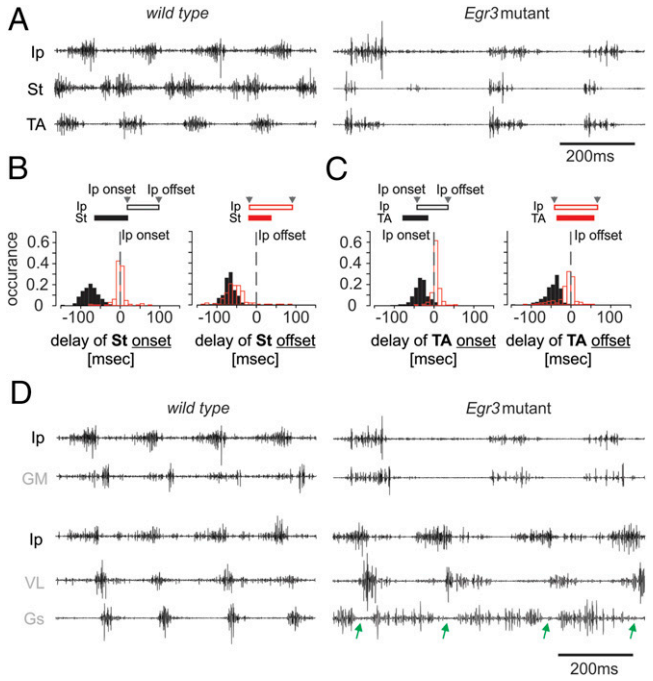

Fig. 3. EMG pattern during wild-type and *Egr3* mutant swimming. (*A*) Examples of EMG recordings from flexor muscles moving the hip (*iliopsoas*, Ip), knee (*semitendinosus*, St), and ankle (*tibialis anterior*, TA) during wild-type (*Left*) and *Egr3* mutant (*Right*) mice swimming. (*B* and *C*) Histograms illustrating the delay of on- and offsets (*Left* and *Right* histograms, respectively) of St (*B*, 6 wild-type and 3 mutant animals) and TA (*C*, 11 wild-type and 10 mutant animals) muscle relative to the activity of on- and offsets of the Ip muscle during wild-type (black bars) *Egr3* mutant (red bars) swimming. (*D*) Two examples of EMG recordings the hip flexor Ip and the most proximal extensor, the *gluteus maximus* (GM) on the *Top* and the Ip and two extensors for knee (*vastus lateralis*, VL) and ankle (*gastrocnemius*, Gs) during wild-type (*Left*) and *Egr3* mutant (*Right*) mice swimming. Green arrows indicate rhythmic off periods of Gs activity during *Egr3* mutant swimming.

in *Egr3* mutant and wild-type mice revealed marked changes in flexor onset timing—Ip, St, and TA onsets were active synchronously rather than in the staggered manner seen in wild-type mice (Fig. 3 *A*–*C*). Moreover, TA burst offset occurred coincidentally with Ip offset (Fig. 3*C*). In contrast, St burst offset still preceded Ip offset, as in wild-type mice (Fig. 3*B*). In addition, GM muscle activity in *Egr3* mutants coincided with that of flexor muscles, a feature observed during normal wild-type walking but not during swimming (Fig. 3*D*). Thus, extensor muscle activation in swimming *Egr3* mutant mice propagates in a proximodistal direction from GM, to VL, to Gs—in contrast to wild-type mice where extensor muscles are active simultaneously.

This comparative analysis of muscle phasing during locomotion in *Egr3* mutant and wild-type mice implies that the offset of ankle flexor muscles is controlled selectively by proprioceptive feedback from muscle spindle afferents. In contrast, the onset of flexor muscles, together with extensor muscle activities, appears to be controlled in a redundant manner by proprioceptive feedback from both muscle spindle and GTO afferents. The persistence of early St burst offset, relative to Ip offset, during both walking and swimming *Egr3* mutant mice suggests that St offset is controlled by spinal interneuronal networks.

Impaired Locomotor Pattern in the Absence of Proprioceptive Feedback. We considered whether the aberrant locomotor pattern observed in *Egr3* mutant mice during a swimming task does indeed reflect the absence of muscle spindle and GTO sensory feedback. To assess this question, locomotor pattern was analyzed in transgenic mice in which all proprioceptive afferents had been eliminated. Proprioceptors were killed in a selective manner through targeted expression of diphtheria toxin A chain in *Pv::cre; Isl2::DTA* (P^{kill}) compound mice (24). We reasoned that if *Egr3* mutant swimming reflects a condition with neither muscle spindle nor GTO feedback, synchronous flexor EMG activities should be evident regardless of whether the P^{kill} mouse swims (Movie S8) or walks (Movie S9).

In P^{kill} mice Ip, St, and TA muscles exhibited synchronous burst onset and offset phasing during both walking and swimming (Fig. 4 *A* and *B* and Fig. S3). This aberrant motor program was similar to that seen in *Egr3* mutants during swimming (Fig. 3*A*), with the exception that in the P^{kill} mouse St offset was synchronized with Ip and TA offsets. These observations support the view that many aspects of locomotor pattern in swimming *Egr3* mutants reflect the absence of functional proprioceptive feedback from both muscle spindle and GTO afferents.

Thus, spinal locomotor circuits without proprioceptive feedback directs synchronous activation of flexor muscle, regardless of precise locomotor behavior. We infer that intrinsic spinal circuits are not sufficient to direct normal locomotor pattern in the absence of proprioceptive sensory feedback.

Discussion

The primary goal of this study was to address the role of proprioceptive feedback from muscle spindles and the GTOs in the generation of motor pattern during natural locomotion in mice. Our data reveal that proprioceptive sensory input is crucial for regulating the temporal parameters of rhythmic movements during walking and swimming, to the emergence of appropriate alternation in the phasing of selected antagonist muscles at individual joints, as well as for the cross-joint coordination of limb muscle activity. Group Ia/II sensory feedback from muscle spindles appears to have a predominant influence in patterning the output of flexor muscles, whereas the joint and redundant activities of group Ia/II and group Ib afferents from GTOs determine the pattern of extensor muscle firing. Together these findings provide evidence that feedback from these two classes of proprioceptive sensory afferents serve both distinct and redundant roles in

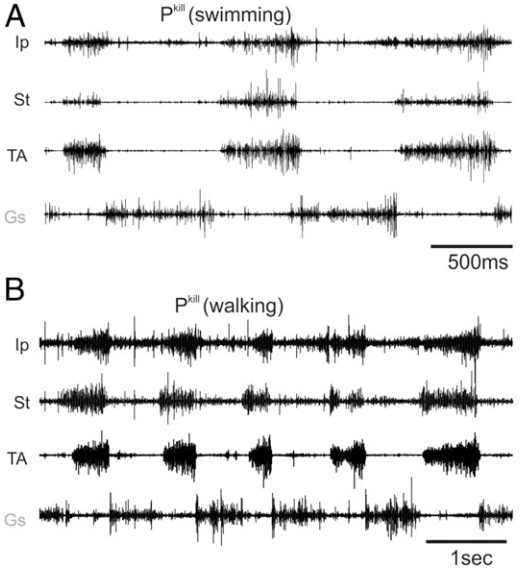

Fig. 4. Flexors are synchronous in a mice line that lacks all proprioceptors. An example of the EMG patterns of three flexors moving three joints and the ankle extensor Gs during swimming (*A*) and walking (*B*) of a *Pv::cre; Isl2::DTA* (P^{kill}) mouse.

assigning elemental aspects of mammalian locomotor pattern (Fig. S4).

Proprioceptive Feedback Regulates Alternating Muscle Activity at Individual Joints. Proprioceptive sensory feedback from muscle spindles contributes to the emergence of an alternating pattern of flexor and extensor muscle activity. *Egr3* mutant mice confronted with a walking task exhibited a pronounced extension in the duration of TA muscle burst activity during swing phase, whereas the onset of Gs burst activity was unchanged. The persistence of TA activity beyond the onset of Gs muscle activation elicits a cocontraction of TA and Gs muscles at the end of swing phase, possibly stiffening the joint at the end of swing phase. Therefore, despite the presence of GTO sensory feedback, proprioceptive information from muscle spindles is required for the generation of alternating flexor and extensor muscle activity at the ankle joint through a selective impact on the timing of flexor burst activity.

Why does loss of group Ia/II sensory feedback from the muscle spindles alter the offset timing of flexor muscles in a selective manner? Such specificity may reflect the potential of the motor system to adapt to the removal of the muscle spindles at early ages. Alternatively, the specificity may have its basis in the fact that initiation of flexor activity is controlled in part by group Ib feedback signal initiated by the unloading of GTOs at the end of stance phase (17, 25). Support for this idea comes from EMG recordings in swimming *Egr3* mutants—a task that reduces or eliminates GTO afferent feedback. In swimming *Egr3* mutants the onset of all flexor muscles is synchronized, leaving open the possibility that the onset of flexor activity is controlled by group Ib sensory feedback from the GTOs. Consistent with this view, in mice in which all proprioceptors are absent the onset of all flexor muscles is synchronized during walking and swimming. Thus, proprioceptive feedback from muscle spindles appears to control selectively the coordinated movement of leg joints at the end of swing phase (14, 18, 26). In contrast, feedback from muscle spindles and GTOs redundantly controls joint movements at the end of stance phase, as the transition from the stance to swing phases requires extension of the hip joint that is signaled by muscle spindles, as well as reduction of load, signaled by GTOs (15–17, 27).

The perturbation of ankle flexor–extensor alternation in *Egr3* mutant mice during walking appears to be selective, because burst patterns of knee flexor and extensor muscles are not appreciably changed (Fig. S4*B*). The lack of an equivalent knee flexor burst extension during swing phase in *Egr3* mutants implies that the activation of St motor neurons is controlled either by spinal CPG network activity or through the redundant involvement of GTO activation. Some support for the latter possibility comes from prior studies of fictive locomotion, showing that St burst duration during fictive locomotion is prolonged when the leg is mechanically extended and is shortened when flexed (13). Our results extend these studies by monitoring the St burst duration during real locomotion and eliminating proprioceptive feedback by genetic methods. In intact P^{kill} mice that lack proprioceptive afferents from both muscle spindles and GTOs, the activity of the St muscle is prolonged, with burst onset and offset synchronized with other flexor muscles during walking and swimming. Together, these observations suggest that proprioceptive feedback from GTOs acts in conjunction with muscle spindle feedback to control knee flexor activity.

Our findings have also uncovered an influence of group Ia/II sensory feedback from muscle spindles on the coordination of muscle activity at the hip joint during swimming but not walking. In wild-type mice, Ip and GM activities are synchronous during walking but alternate during swimming. In *Egr3* mutant animals, the synchronous activation of the Ip and GM muscles is not affected during walking. In contrast, during swimming, Ip and GM muscles are also active in synchrony, as opposed to their alternating activation during wild-type swimming. We interpret this finding to indicate that in the absence of proprioceptive feedback from muscle spindles, Ip and GM burst activities are affected in a context-dependent manner. Our data do not resolve whether the group Ia/II-dependent reversal of Ip–GM coordination is controlled by descending, or by group Ib/GTO feedback, signals. Nevertheless, we conclude that group Ia/II feedback from the muscle spindles is a necessary component of this reversal.

Proprioceptive Feedback Regulates Cross-Joint Muscle Coordination. Our findings provide evidence that proprioceptive sensory feedback from muscle spindles plays an important role in coordinating interjoint muscle activity patterns during a walking task in mice. Interestingly, sensory feedback plays an important role in interjoint coordination in insects (28–30). In walking *Egr3* mutants, the normal precision in timing of TA burst offset at midswing phase erodes, with the consequence that burst offset now occurs at the end of swing phase, synchronous with Ip offset. In contrast, the offset timing of the St muscle relative to Ip offset is similar in wild-type and *Egr3* mutant mice. These observations suggest that coordinated ankle and hip joint movement is achieved by proprioceptive feedback from muscle spindles.

In contrast, the coordination of knee and hip joint movement requires additional proprioceptive feedback from GTO afferents—as evident from our observation that St activity offset in proprioceptor-deficient P^{kill} mice occurs simultaneously with Ip offset. The observation of synchronous flexor bursting in P^{kill} mice walking or swimming is in accordance with fictive locomotor pattern recorded in early-spinal cats (13). Taken together these findings indicate that the coordinated movement of hip and ankle joints is controlled preferentially by proprioceptive feedback from muscle spindles, whereas coordination of hip and knee movements requires conjoint sensory feedback from muscle spindles and GTOs. These findings are supported by earlier studies in cat, suggesting that the end of swing phase is determined by proprioceptive sensory feedback from muscle spindles (14, 18, 26).

What is the functional relevance of coordinating ankle and hip joint movements through group Ia/II proprioceptive feedback from muscle spindles? The prolongation of ankle flexor activity results in overflexion of the ankle joint and causes elevation of the foot during swing phase. Mice exhibit severe difficulties in placing their feet on the rungs of a horizontal ladder in the absence of sensory feedback from muscle spindles. Thus, the accuracy of timing of TA offset during an ongoing protraction movement during swing phase is needed to achieve accurate foot placement. We infer that sensory feedback from muscles is necessary for this skilled motor act. More generally, the ability to manipulate defined neural elements involved in the construction of locomotor pattern, under conditions in which motor behaviors can be analyzed in vivo, may help to resolve functional details about the workings of sensory feedback and local circuits in motor control.

Thus, our data provide evidence that elemental aspects of normal locomotor pattern, such as the precise onset and offset of different muscles, in mice require ongoing proprioceptive feedback. In absence of proprioceptive feedback from muscle spindles, locomotor pattern exhibits a selective perturbation in the timing of ankle flexor activity offset during swing phase. However, in the absence of proprioceptive feedback from both muscle spindles and GTOs, locomotor pattern is more drastically degraded. This observation suggests that group Ia/II feedback from the muscle spindles controls swing phase selectively, whereas group Ia/II and group Ib feedback from the muscle spindles are GTOs and collectively control the stance phase similar to that in insects (31). Thus, locomotion in wild-type mice appears to require ongoing proprioceptive feedback from both muscle spindle and GTO afferents.

Materials and Methods

Mice Lines. We examined the locomotor pattern during walking and swimming in *Egr3* knockout mice (22), in which muscle spindles regress after birth (32), to address the role of proprioceptive feedback from muscle spindles on the locomotor behavior. To address the influence of proprioceptive feedback from both muscle spindles and GTOs, we made use of a mutant mouse, which is an offspring of an intersectional breeding of *Pv::cre* (*Pv,* parvalbumin) (33) and *Isl2::DTA* (*Isl2*: islet-2) (34) mice. Previously, it has been shown that in these offspring, all proprioceptive afferents die selectively (24).

Surgeries. Adult mice were implanted with bipolar EMG recording electrodes (19, 35). Briefly, mice were anesthetized with isoflurane and custom-built EMG recording electrode sets, consisting of four pairs of wire electrodes, were implanted as follows: The neck region and the hind legs were shaved. Small incisions were made into the skin at the neck area and at the hind legs just above the muscles from which the recordings were made. The bipolar electrodes were led under the skin from the neck incision to the leg incisions and implanted into different flexor and extensor muscles that move different leg joints. Finally, the incisions were closed with sutures and the mice were left in their cages for recovery for at least 48 h.

Behavioral Recordings. After recovery, the recording sessions started for which the electrodes were attached to an amplifier (model: MA 102; custom built in the workshop of the Zoological Institute, University of Cologne) via the headpiece connector at their neck (Fig. S1 *A* and *B*). Simultaneously, movement of the hind legs during locomotor behavior was described in a detail by using motion analysis techniques combined with high-speed video recordings of the behavior (Fig. S1*C*) (19, 35). None of the animals were trained for the experiments to avoid the possible complication of different learning capabilities.

EMG activities and movies were recorded during walking on a mouse treadmill (custom built in the workshop of the Zoological Institute, University of Cologne) 0.2 m/s speed. Determination of onsets and offsets of bursts in EMG recordings are described in *SI Determination of Burst Onsets and Offsets*. After walking trials, mice were placed in a tank with ~24 °C water for ~2 min and EMG activity was collected using Power1401 and Spike 2 (version 6.02; CED) software and analyzed by Spike 2, Excel 2003, and StatistiXL (version 1.8). The walking or swimming behavior (Fig. S1*A*) was captured with a high-speed camera, set with the capture rate at 250 frames per second by using a Photron R2 PCI high-speed camera. The video images were stored for later data analysis. The kinematic parameters were calculated automatically by using the motion analysis software Motus (Vicon) and the data were analyzed with Excel and StatistiXL. Data are reported as mean ± SD and differences in distributions were tested by using the Student *t* test (StatistiXL). Values of $P < 0.05$ were considered significant.

To assess the precise foot placement ability during walking, the mice were recorded walking on a horizontal ladder. Mice were placed on a horizontal ladder (rung distance: 2 cm; custom built as described above) and the animals stepping from rung to rung were videotaped from the side. Later the steps were counted as foot securely landing and holding on a rung or foot slipping or dropping down in between the rungs (Fig. 2*E*).

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Supporting Information

Akay et al. 10.1073/pnas.1419045111

SI Text

In this article, we have investigated the locomotor pattern during walking and swimming of wild-type and *Egr3* mutant animals to address the role of proprioceptive sensory feedback muscle spindles in locomotor pattern generation in the presence and absence of proprioceptive feedback from the GTOs, respectively. During stance phase of the stepping cycle, muscles moving the distal joints of the leg generate an isometric contraction to generate the necessary force to keep the body upright, whereas the more proximal hip joint muscle would provide the main part of the extension (1, 2). During swing, however, the muscles of all joints generate an isotonic contraction leading to movement of the leg in the air. Due to this isometric/isotonic muscle contraction during stance vs. isotonic contractions during swing phase, we infer that muscle spindles and the GTOs would be activated during the stance phase, whereas only muscle spindles are active during swing phase. During swimming, however, due to the lack of weight bearing, due to the buoyancy of the water, the GTO activation would be significantly reduced. Therefore, we took advantage of this to investigate the role of muscle spindle feedback in the generation of the locomotor pattern in the presence and absence of GTO feedback, by measuring locomotor pattern during walking and swimming in wild-type and *Egr3* mutant mice.

SI Determination of Burst Onsets and Offsets

The burst onsets and offsets in the EMG recordings were determined manually. The onsets and offsets of bursts of activities in the EMG recordings were determined by identifying the smallest EMG spiking unit during behavior that would occur consistently within a barrage of activity during the rhythmic behavior. Then the first or last occurrence of this smallest or larger unit within one barrage was taken as the beginning or end of a burst.

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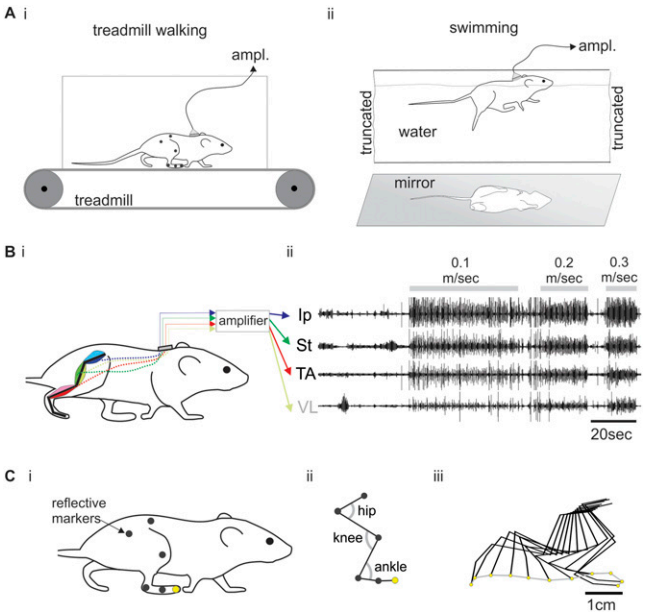


Fig. S1. Kinematic and electromyographic techniques to record locomotor pattern. (*A*) Two different locomotor behaviors, walking on a treadmill (*i*) and swimming (*ii*), were investigated. (*B*) Electromyographic (EMG) recordings from multiple leg muscles were performed with chronically implanted EMG recording electrodes (*i*). Following chronic implantation of the electrodes, the EMG activities could be recorded during free behavior, such as walking on a treadmill at different speeds (*ii*). (*C*) Kinematic data were obtained by reconstruction of the hind leg by means of detecting the coordinates of markers attached on the skin above leg segments (*i*). By connecting the marker coordinates, the leg was reconstructed (*ii*). Frame-by-frame reconstruction of the leg allowed the investigation of the movement (*iii*).

Fig. S2. Average (±SD) step cycle, swing, and stance durations during walking at 0.2 m/s is consistently lower in mutant animals (red bars, $n = 14$ animals) compared with wild-type animals (black bars, $n = 16$ animals). In contrast the cycle duration during swimming is significantly longer in mutant animals compared with wild-type animals. ***$P < 0.001$.

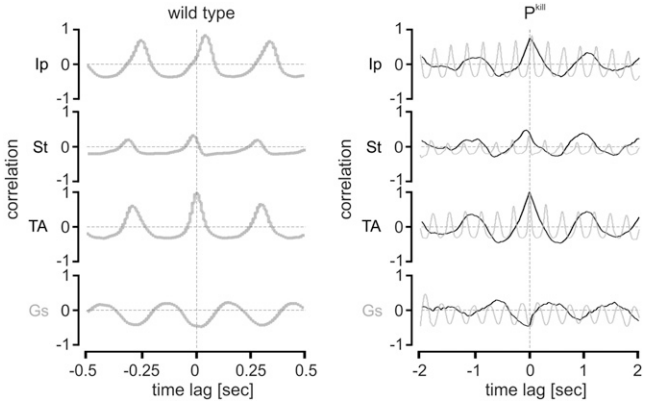


Fig. S3. Correlograms of EMG activity during free-walking wild-type (*Left*) and P^{kill} (*Right*) mice. The reference activity for all graphs is the TA activity. Notice that the *x* axis is differentially scaled in the *Left* and *Right* graphs. Therefore, the same data plotted in the *Left* graphs are also overlapped in the *Right* graphs (light gray lines) to ease the comparison. Notice that the peak of Ip and St correlations are more closely aligned to the zero time lag with higher correlation indicating that flexor muscles are more synchronous in the P^{kill} mice than in wild-type mice.

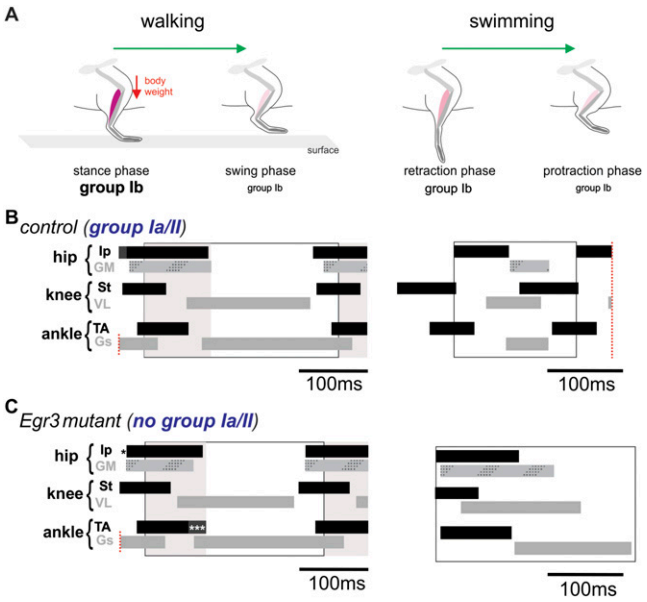


Fig. S4. Locomotor pattern gradually degrades with removal of proprioceptive feedback. (*A*) During walking, group Ib signaling is strong during stance phase due to the body weight, whereas the group Ib signaling is reduced during swing phase when the leg does not carry the body weight. In contrast during swimming, the group Ib signaling is reduced due to the reduced gravitational influence and is similar regardless of whether the foot is moved forward or backward. (*B*) Bar diagram illustrating the activity of all recorded flexor (black) and extensor muscles (gray) moving the three different leg joints during a step cycle (rectangle) during walking (*Left*) and swimming (*Right*, rectangle here indicates swim cycle) in wild-type mice ($n = 16$ for walking and $n = 14$ for swimming). Shaded area on the *Left* indicates swing. (*C*) Same as in *A*, but the graphs illustrate data from *Egr3* mutant mice ($n = 15$ for walking and $n = 15$ for swimming). Asterisks in the black bars in *Egr3* mutant walking indicate that the difference of this parameter compared with wild-type walking is statistically significant after Student *t* test (*$P < 0.05$ and ***$P < 0.001$). No asterisk means differences are not statistically different.

Movie S1. A wild-type mouse walking on a treadmill at 0.2 m/s.

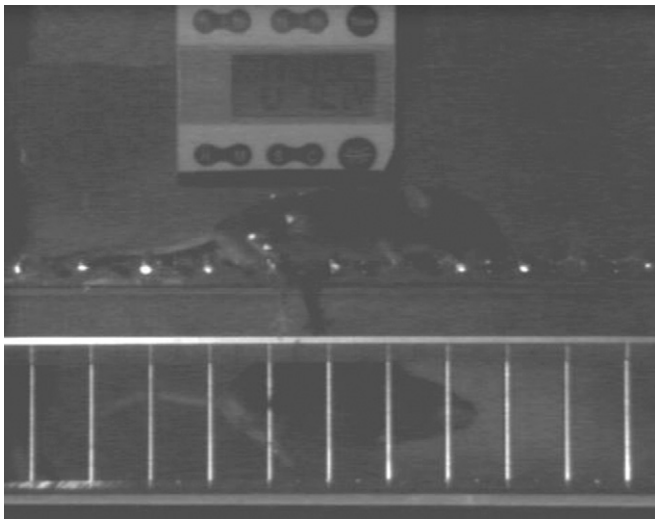
Movie S1

Movie S2. An Egr3$^{-/-}$ mouse walking on a treadmill at 0.2 m/s. Notice the exaggerated foot lifting during swing phase.

Movie S2

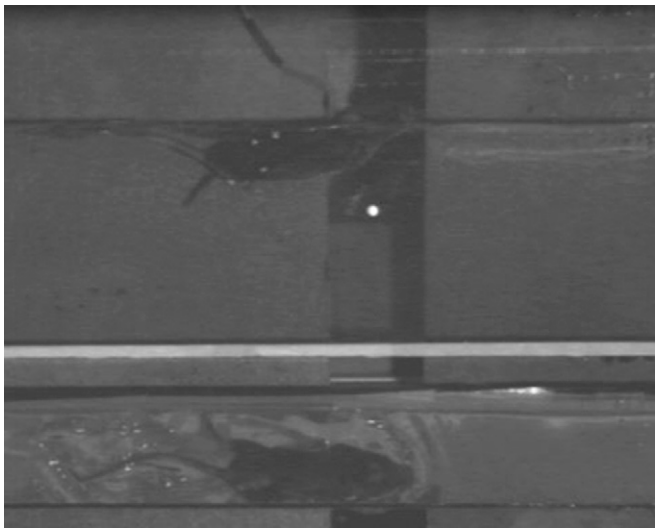
Movie S3. A wild-type mouse walking on a horizontal ladder. Wild-type mice can easily perform this task.

Movie S3



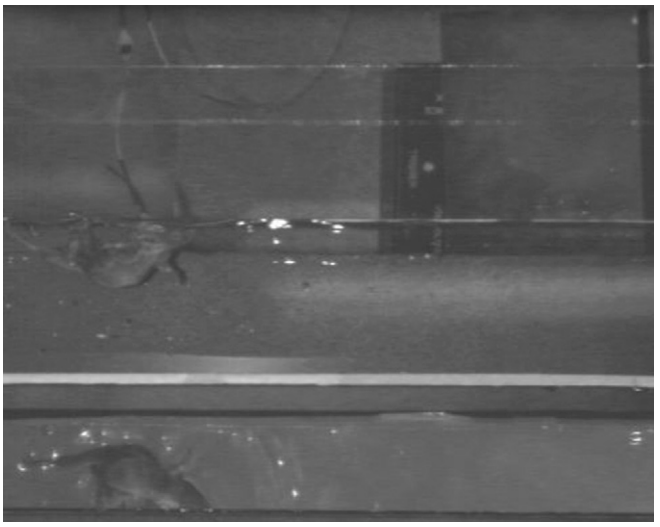
Movie S4. An Egr3$^{-/-}$ mouse walking on a horizontal ladder. The Egr3$^{-/-}$ mice have severe deficits in performing this task.

Movie S4



Movie S5. A wild-type mouse swimming.

Movie S5



Movie S6. Egr3$^{-/-}$ mice are very poor swimmers. Notice the extreme ataxic movements of the mice.

Movie S6

Movie S7. Some Egr3$^{-/-}$ mice (2 of 15 mice) can manage to swim, although still in an ataxic fashion.

Movie S7

Movie S8. P^{kill} mice are very ataxic when place in water similar to the $Egr3^{-/-}$ mice.

Movie S8

Movie S9. P^{kill} mice are very ataxic during walking as they are during swimming (Movie S8).

Movie S9