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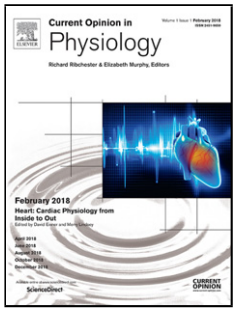

Title: Motor task-selective spinal sensorimotor interneurons in mammalian circuits

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PII: S2468-8673(19)30014-8
DOI: https://doi.org/10.1016/j.cophys.2019.01.014
Reference: COPHYS 140

To appear in:

Please cite this article as: Koch SC, Motor task-selective spinal sensorimotor interneurons in mammalian circuits, *Current Opinion in Physiology* (2019), https://doi.org/10.1016/j.cophys.2019.01.014

Motor task-selective spinal sensorimotor interneurons in mammalian circuits

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Highlights

- Proprioceptive and cutaneous input is necessary for an adaptive locomotor gait
- Sensory input during locomotion can recruit protective, or corrective reflexes
- Specialised INs in the spinal cord integrate sensory input with motor activity
- Recent genetic advances have allowed identification of these INs and their networks
- Spinal INs adjust motor activity to sensory input according to the motor task

Abstract

Spinal sensorimotor networks integrate sensory information into the ongoing locomotor program, allowing adaptation of motor behaviour to the external world. This review summarises sensorimotor research to date, and highlights how recently identified spinal sensorimotor interneurons have unveiled the task-specificity of local spinal networks in mammalian systems.

Keywords: Spinal cord; proprioception; dorsal horn; locomotion; presynaptic inhibition; stumbling corrective reaction; flexion reflex

Abbreviations: CPG: central pattern generator; IN: interneuron; GTO: Golgi Tendon Organ; Ptf1α: pancreas associated transcription factor α; GAD65/GAD2: glutamic acid decarboxylase isoform 65/2; GAD67/GAD1: glutamic acid decarboxylase isoform 67/1; RORβ: RAR related orphan receptor β; RORα: RAR related orphan receptor α; Satb2: special AT-rich sequence binding protein 2; GABA: gamma aminobutyric acid; PAD: primary afferent depolarization; LI-V: spinal dorsal horn laminae I-V; Ctip: CtBP-interacting protein Atoh: atonal bhlh transcription factor homolog 1; vGluT2: vesicular glutamate transporter 2.

Introduction

During movement, spinal motor circuits receive afferent sensory input from proprioceptors, which signal muscle/tendon length and tension and cutaneous mechanoreceptors, which convey touch and pressure. While basic rhythmic locomotor patterns can be established through spinal central pattern generator (CPG) networks, sensory input allows adaptation to the environment [1-3]. The synchronization of sensory-motor adjustments to ongoing movement occurs through the recruitment of specialised spinal sensorimotor interneurons, which are activated in a task-specific manner. This review will summarise sensorimotor research to date, and highlight genetically identified spinal sensorimotor circuits in the mouse that enable sensory-driven modification of motor behavior.

Spinal modulation of sensory input into motor centers- *Electrophysiological identification of spinal sensorimotor networks*

Until recently, the effect of sensory afferent input on locomotion was characterized by electrically stimulating, or surgically removing the influence of proprioceptive or cutaneous afferents from cat hindlimb or paw and detailing their effects using kinematic analyses [2,4-10]. These experiments, and subsequent rodent studies, revealed a striking phase-dependent interplay between afferent input and stepping behaviour, whereby the onset and offset of step cycles were defined by patterned peripheral activity (Figure 1). Protective motor reflexes were equally found to be phase-specific: peripheral stimulation during the swing phase recruits flexion to avoid a stimulus, whereas stimulation during the stance phase prolongs the extensor phase to stabilise the limb [11-13]. The adaptability of reflexes to the locomotor phase suggested the possibility of task-specific sensorimotor networks, which would allow sensory information to be integrated with ongoing activity from locomotor centers to modulate motor output.

Direct evidence for spinal sensorimotor networks arose from intracellular electrophysiological recordings from motoneurons in the decerebrate cat. Primary afferent stimulation of cutaneous or proprioceptive afferents revealed disynaptic inhibitory and excitatory pathways onto motoneurons, which could be differentially recruited following the induction of a fictive locomotor rhythm [11,12,14]. These experiments were the first demonstration of spinal excitatory and inhibitory interneurons located at the interface of sensory afferent input and motor output, which could be modulated by fictive locomotor patterns. In order to understand the role of these sensorimotor circuits in normal reflexive or stepping behaviour however, sensorimotor interneurons needed to be identified and manipulated in the behaving animal.

Genetic identification of sensorimotor interneurons

Tracing studies from the periphery and extracellular recordings from within the spinal cord revealed sensorimotor neurons were most likely located in intermediate spinal cord, in the afferent termination zone of proprioceptors, and in the deep dorsal horn, the termination zone of cutaneous afferents [11,15-17]. These target locations have subsequently been used as the basis for the identification of interneurons involved in sensorimotor integration. Developmental studies in the mouse and chicken have outlined progenitor domains and transcription factors necessary for ascribing neuronal dorso-ventral and medio-lateral positioning within the dorsal horn, as well as specifying expression of molecular markers, neurotransmitters and anatomical connectivity (as

reviewed previously by [18]). These expression profiles combined with the ever-increasing toolkit of recombinases and viruses have offered unprecedented access to sensorimotor circuits [19,20]. This review will focus upon identified spinal interneurons within the sensorimotor circuit, which have been shown to affect motor behaviour in a task-selective manner. These have fallen broadly into two categories: inhibitory neurons which prevent protective reflex recruitment during gross motor behavior (locomotion), and excitatory neurons activated by cutaneous signals in order to fine-tune ongoing movement (grasping and ladder beam walking; Table 1).

It should be noted that to date there are no identified inhibitory sensorimotor interneurons associated with the cutaneous motor pathway or excitatory spinal interneurons at the interface of sensory input and motor output, although a third class of identified neurons, excitatory *Atoh 1* interneurons, may recruit proprioceptive motor circuits indirectly through recruitment of supraspinal pathways [21].

Inhibitory sensorimotor circuits regulating locomotion- the role of presynaptic inhibition

Proprioceptive afferent input is necessary for locomotor phase transition, muscle contraction, and stepping frequency through the recruitment of phasically-active spinal circuits (Figure 1;[3]). Inhibitory circuits recruited by sensory afferents can act by dampening motor output postsynaptically, or dampening primary afferent input presynaptically, thereby enabling fiber- and circuit-selective filtering of sensory input into defined motor pools (See Box 1; [8,22-26]). In this manner, input from ankle proprioceptors can be selectively dampened to prevent activation of extensor motoneurons during swing, and so prevent the recruitment of the flexion reflex. The actions of presynaptic inhibitory circuits are widespread, and can be recorded during active and fictive locomotion, by primary afferent depolarizations (PAD) in primary afferents, in the spinal cord, and in motoneurons [24,27], yet the neuronal source had been unknown until recently. Cellular classification from electrophysiological studies largely classified neurons by activity pattern within the locomotor phase, primary afferent input, and posthoc analysis of neurotransmitter phenotype, revealing a population of inhibitory interneurons with a range of activity patterns and molecular profiles [28].

Genetically identified presynaptic inhibitory sensorimotor interneurons- sensory specificity for reflex control

The genetic characterization of these circuits led to the identification of a subpopulation of Ptf1α-derived neurons, a transcription factor necessary for the expression inhibitory interneuronal phenotype in the spinal dorsal horn. Inhibitory Ptf1α-derived interneurons make presynaptic inhibitory contacts onto primary afferent terminals in the dorsal and intermediate dorsal horn [29,30] and express GAD2, one of the enzymes necessary for the production of the inhibitory neurotransmitter GABA [8,29-33]. Genetically targeting GAD2-expressing interneurons (GAD2 INs) therefore offers access to presynaptic inhibitory networks, and a behavioral correlate of the role of these circuits in the mouse. Ablation of GAD2 INs in the cervical dorsal horn, and loss of presynaptic inhibitory control, selectively impairs smooth movement during reaching behavior [8], emphasizing the precision of presynaptic inhibitory control and its critical role in sensory filtering during ongoing movement. Due to the broad expression pattern of GAD2 INs in the dorsal and intermediate dorsal horn, ablation of GAD2 INs in the cervical spinal cord not only impairs smooth

movement in reaching, but also results in increased spontaneous scratch behavior in mice [8]. This secondary effect is likely due to a loss of inhibitory control of cutaneous fibers in the dorsal horn [8,32,33], which offers the prospect of discrete subpopulations of Ptf1α/GAD2 INs neurons may be selectively recruited to inhibit sensory transmission pathways (cutaneous or proprioceptive) in a task-dependent manner [29,30,32,34].

Evidence for this arises from a subpopulation of GAD2 INs in intermediate dorsal horn which are identified by the expression of the RAR related orphan receptor β (RORβ; RORβ/GAD2 INs) [29,34]. RORβ/GAD2 INs receive input from proprioceptive and low threshold myelinated afferents and preferentially form presynaptic inhibitory contacts onto flexor afferents. Interfering with the function or targeting of *RORβ* inhibitory synapses, consequently results in a loss of flexor afferent filtering during the swing phase of locomotion, leading to a "duck gait" hyperflexion phenotype without the interruption of other behaviors [34]. The RORβ/GAD2 circuit is therefore likely recruited to prevent flexion reflex activation during locomotion, highlighting the modular specificity of spinal circuits [8,22-26]. Contrary to the broader GAD2 IN population, the RORβ/GAD2 phenotype is predominantly restricted to the hindlimb despite broad genetic targeting of the RORβ population, which could suggest differential recruitment patterns are needed to activate lumbar locomotor interneurons versus cervical sensorimotor neurons involved in reaching. There is evidence for phasic PAD to be driven by central pattern generators (which show peak PAD at flexion), as well as demonstration that fluctuation of PAD is locomotion is predominantly the result of patterned primary afferent activity [7,35-38]. It is therefore likely that the recruitment of sensorimotor circuits under normal stepping conditions is the summation of central, descending and peripheral influences, and that it is through the integration of these diverse inputs that circuits achieve their motor phase selectivity.

Diversity in inhibitory sensorimotor interneurons- neuronal localization and sensorimotor task selectivity

The encoding of such diversity of input and how this affects motor activity can be examined within a single molecular class of neurons, such as Satb2 inhibitory interneurons (Satb2 INs). Satb2 INs were identified as a component of the sensorimotor network by transcriptional screening of the intermediate dorsal horn [39,40]. A subset of Satb2 INs are derived from the Ptf1α INs and express GAD2, but Satb2 INs are otherwise diverse their lineage and expression patterns. They receive nociceptive and proprioceptive input, and make postsynaptic contacts onto premotor and motoneurons, which is their proposed mode of action. Conditional deletion of *Satb2* gene from the spinal cord leads a change in Satb2 IN fate and migration, disrupting their projection to motor centers and leading to two distinct sensorimotor phenotypes: prolonged hyperflexion in response to noxious stimulation (associated with nociceptive input), and hyperflexion of the ankle during early swing phase of locomotion (associated with proprioceptive input) [39]. Anatomical evidence suggests that the nociceptive and proprioceptive phenotypes could arise from two distinct *Satb2* subpopulations: a lateral subpopulation, which receives nociceptive afferent input, and a medial subpopulation, which co-express *Ctip* and receive input from proprioceptive and cutaneous afferents. These studies provide evidence for the key influence of neuronal positioning on the recruitment and functional activity of a spinal circuit, although the change in molecular profile of Satb2 INs after gene deletion leaves the full interpretation of results unclear.

Genetically identified excitatory sensorimotor networks regulating fine motor control

Cutaneous afferent input can be spared for locomotor behavior, but is necessary for fine motor control and the recruitment of reflexes [5,41-44]. Much of the cutaneous input into motoneurons arises via a disynaptic (or oligosynaptic) excitatory circuit [45], suggesting detailed signals from the periphery are amplified by spinal relays to facilitate precise motor adjustments. The first genetically identified population arose from targeting the dorsal progenitor domain dorsal interneuron 3 (dI3), which give rise to Islet 1-expressing dI3 interneurons (dI3 INs) in the intermediate dorsal horn [43]. dI3 INs receive myelinated afferent input and drive fine motor control via a phasically-driven disynaptic excitatory pathway. As such, interfering with dI3 IN excitatory synaptic output, by selective genetic deletion of glutamatergic transporter vGluT2, results in a behavioural deficiency in hindpaw grip strength and reduced performance of refined locomotor tasks [43,46]. Although dI3 INs are phase-selective, allowing integration of input into locomotor patterns, their role in grasp suggests they may be involved in multiple cutaneous circuits. Despite a lack of a detectable sensory phenotype, dI3- silenced mice develop a spontaneous scratching phenotype similar to that seen in GAD2-ablated mice [8,29,32], which could be the consequence of a loss of cutaneous-evoked PAD. A described example of this circuit is thought to underlie basis of cutaneous reflex excitability in the stumbling corrective reflex, whereby cutaneous activity can excite or dampen presynaptic inhibition of proprioceptive input in a phase-dependent manner [45]. Spontaneous scratching following dI3-ablation could suggest two populations of dI3 INs: a population to generate fine motor activity, and one to dampen the cutaneous scratch reflex via GAD2 INs.

Genetically identified excitatory sensorimotor classes- supraspinal inputs

Afferent input into sensorimotor interneurons does not only arise from primary afferents and spinal CPG. In order to allow corrective movements to be adjusted precisely, descending motor centers are likely needed to drive excitatory sensorimotor pathways in the spinal cord during locomotion [11]. A transcriptional screening of dorsal populations identified RORα excitatory interneurons (RORα INs), located predominantly in the deep dorsal horn, as prime candidates for cutaneous sensorimotor modulators of fine corrective movement. RORα INs receive low threshold myelinated afferent input from a wide range of peripheral receptors and project directly onto both ankle flexor and extensor premotor and motoneurons [42,47]. Presynaptic tracing additionally revealed that they receive direct input from the motor cortex and cerebellar neurons, suggesting they may integrate cutaneous afferent input with descending motor commands to modulate motor output. Targeted ablation of RORα INs in the lumbar spinal cord, achieved through a combination of Cre and FlpO recombinases, results in a selective deficit in light touch sensation and an inability to perform fine motor corrective movements [42]. This touch-insensitive phenotype emphasizes the role of touch in movement, and the afferent convergence needed convey environmental cues to motor centers. Further dissection of the RORα INs population could shed light onto sensorimotor integration between afferent subtypes and spinal segmental areas.

Conclusion

In recent years, the merging of developmental biology, genetics and electrophysiology has revealed a high degree of functional specialisation within spinal sensorimotor circuits which allows

task-specific recruitment of dorsal interneurons (Figure 2). The sensory selectivity of interneurons has also uncovered the specificity of primary afferent input in driving motor behavior: loss of touch leads to impaired grasp and corrective movements; increased proprioception leads to ataxia. Although we are beginning to understand the behavioural significance of these neuronal networks, an important question moving forward is how defined circuits are engaged during locomotion to gate and relay sensory inputs to motor centres. Many of the populations described above have been shown to receive descending input from brainstem nuclei or higher cortical centres, and be dependent on this input for their maturation and function [48]. Subpopulations of cervical spinal interneurons are differentially controlled by sensory or motor cortices [49], allowing for an added level of complexity in the integrative capabilities and functionality of sensorimotor networks. Taking a more integrated approach to investigating sensorimotor circuits by utilizing a variety of naturalized behavioural tests, interrogating intact circuits, and examining the developmental of these circuits, may offer some insight into how sensorimotor circuits function and the integrative capabilities of spinal interneurons [50].

Acknowledgments: I would like to thank Maria Fitzgerald, Martyn Goulding, Graziana Gatto, Lorenzo Fabrizi, and Sandrine Géranton for helpful discussions and comments on the manuscript. S.C.K. is supported by a Marie Curie Actions Fellowship from the European Union's Seventh Framework Programme for research, technological development and demonstration (624861).

AUTHOR DECLARATION

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.
We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.
We confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing we confirm that we have followed the regulations of our institutions concerning intellectual property.
We understand that the Corresponding Author is the sole contact for the Editorial process (including Editorial Manager and direct communications with the office). He/she is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs. We confirm that we have provided a current, correct email address which is accessible by the Corresponding Author and which has been configured to accept email from s.koch@ucl.ac.uk
Signed by all authors as follows:
Stephanie C Koch 15/10/18

Box 1: Presynaptic inhibition of primary afferent terminals. Presynaptic inhibition of proprioceptive afferents is mediated by GABAergic interneurons in the intermediate deep dorsal horn, receiving strong cutaneous and proprioceptive input in a trisynaptic or disynaptic pathway [8,22-26]. These neurons form monosynaptic contacts onto proprioceptive and/or cutaneous primary afferent terminals in the dorsal horn, express inhibitory GABA receptors. Due to the depolarized chloride reversal potential of primary afferents, activation of these receptors by spinal inhibitory INs leads to an increased chloride conductance, leading to a primary afferent depolarisation (PAD). PAD is associated with selective presynaptic inhibition of terminal activity, and decreased neurotransmitter release [10].

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Using a combination of genetics, anatomical characterization, sequencing and behavioral analysis, Abraira et al. functionally dissects interneurons within the “low threshold afferent termination zone”. Their detailed analysis reveals functional and anatomical heterogeneity within the neuronal population, despite these neurons being located within the same laminar location, and within the same afferent termination zone. This highlights the importance of characterizing neuronal populations by multiple methods of analysis.

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Using a combination of single nuclear sequencing and novel functional analysis, this study dissects interneuronal populations according to molecular identity and functional activity patterns. Through careful and detailed analysis, the authors found surprising functional and molecular heterogeneity within neuronal populations within the same laminar location, and provide open access to new molecular markers.

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Using RNA sequencing, Haring et al. dissect dorsal horn populations into thirty individual subgroups distinguishable by receptor expression, neurotransmitter phenotype and localization within the dorsal horn. This study reveals an impressive molecular architecture in the dorsal horn, in which neurons within a single parental class are represented within the same dorsal laminar layer. Many of the subgroups are identifiable are by novel neuronal markers, combinations or markers, which the researchers have made freely accessible on a published archive.

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Figure 1: Summary of proprioceptive recruitment during locomotor stepping phases. During locomotion, the hindlimb moves from stance, whereby the limb is on the ground (right, highlighted limb in red), to swing, whereby the limb is in the air (left). Transition of stance to swing is enabled through the coordinated activity of groups of proprioceptors: muscle spindle afferents Golgi tendon organs (GTO), and through muscle activity of flexors and extensors [3-5]

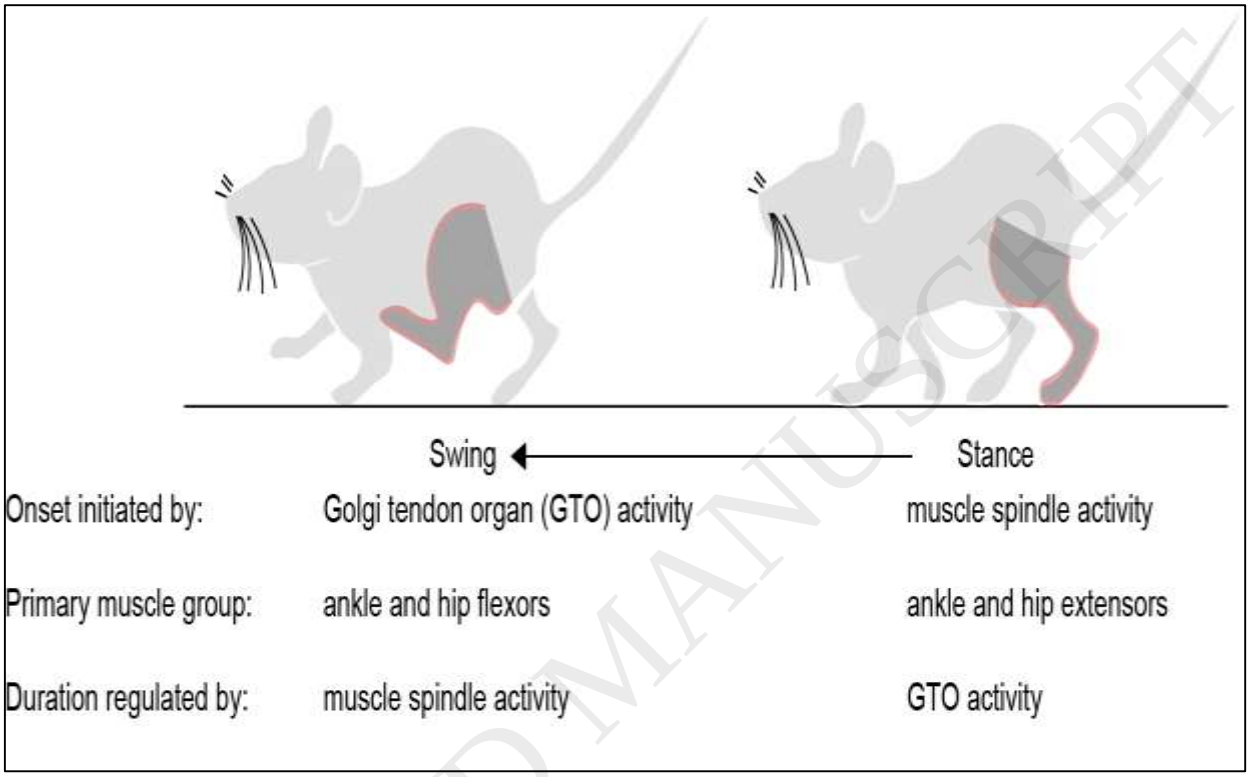

Fig. 2. Schematic of INs recruited during motor behavior. (A) Gross motor behaviour- locomotion. Sensorimotor INs in the intermediate dorsal horn are located in the terminal afferent zone of proprioceptive afferents (dark grey triangles) which arise from the hindlimb. During stepping, activity in proprioceptive afferents and spinal motor circuits activate sensorimotor interneurons, which filter proprioceptive input by presynaptic inhibition (open triangles) as it approaches motoneurons (light grey circles), or by postsynaptic inhibition of motoneurons or premotor neurons (closed triangles). Two inhibitory subpopulations of sensorimotor interneurons have been identified acting within locomotor networks, RORβ/GAD2 INS (pale red), and Satb2 INs (dark red). See text for details (B) Fine motor behaviour- eg. beam walking. Sensorimotor INs in the deep dorsal horn are located in the terminal afferent zone of cutaneous afferents (yellow triangles) which arise from the hindpaw. During fine motor behavior, activity in cutaneous afferents, spinal motor circuits (dotted grey line), and descending circuits from the brain (green triangle) activate sensorimotor interneurons, which amplify cutaneous input (closed purple triangles) onto motor and/or premotor neurons, thereby allowing fine adaptation to motor behavior. Dotted line indicates possible polysynaptic connectivity. Two excitatory subpopulations of sensorimotor interneurons have been identified acting within fine motor behavior, RORα INS (dark purple), and dI3 INs (pale purple). See text for details.

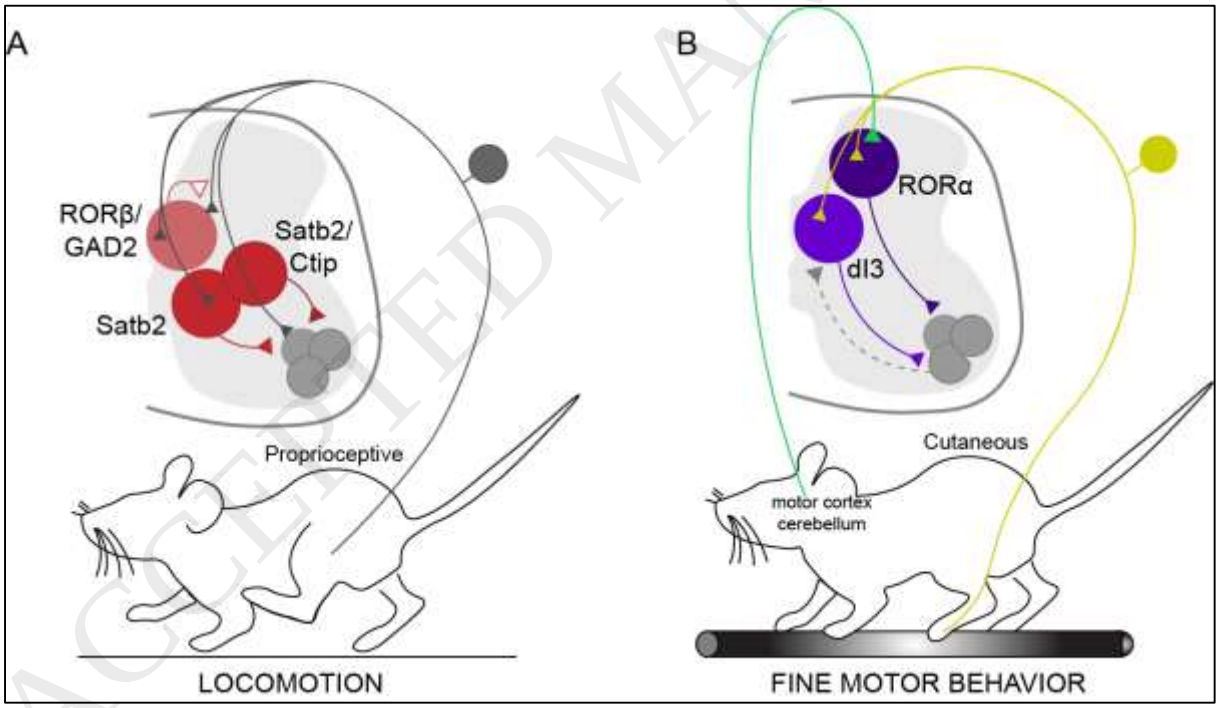

Table 1. Summary table of genetically identified sensorimotor interneurons and their known properties within sensorimotor circuits. See text for details.

Sensorimotor subpopulation	Neuronal position in dorsal horn	Primary Afferent Input	Associated markers	Neuronal phenotype	Sensorimotor behavioral phenotype
GAD2 INs [8,33]	Deep dorsal horn LIII-IV Intermediate dorsal horn LV-VII	Low threshold/ proprioceptive	Derived from Ptf1α A subset express RORβ	Inhibitory	Loss of fluid movement during forelimb reaching Spontaneous scratch phenotype
RORβ/GAD2 INs [34]	Intermediate dorsal horn LV-VI	Low threshold/ proprioceptive	87% overlap with GAD2	Inhibitory	Hyperflexion in the swing phase of locomotion
Satb2 INs [39]	Intermediate dorsal LV	Low threshold/ Proprioceptive Nociceptive	Ctip	Inhibitory (95.2%)	Hyperflexion of ankle during early swing phase of locomotion Prolonged flexion during the noxious withdrawal reflex
dI3 INS [43,46]	Intermediate dorsal LV-VII	Low threshold	Islet 1	Excitatory	Loss of hindlimb grip Loss of fine corrective motor skills on ladder Spontaneous scratch phenotype
RORα INs [42]	Deep dorsal LII_i-III	Low threshold	CCK, cMaf, MafA, PKCγ	Excitatory (92%)	Loss of corrective motor skills on ladder