BMB Reports - Manuscript Submission

Manuscript Draft

**Manuscript Number**: BMB-21-052

**Title**: Proprioception, the regulator of motor function

**Article Type**: Mini Review

**Keywords**: proprioception; motor control; proprioceptive receptor; motor function; regulator

**Corresponding Author**: Kyuhyung Kim

**Authors**: Kyeong Min Moon[1], Jimin Kim[1], Yurim Seong[1], Byunchang Suh[1], KyeongJin Kang[1], Han Kyoung Choe[1], Kyuhyung Kim[1,*]

**Institution**: [1]Department of Brain and Cognitive Sciences, DGIST, Daegu, 42988, Republic of Korea,
[2]KBRI (Korea Brain Research Institute, Daegu, 41068, Republic of Korea,

# Proprioception, the regulator of motor function

Kyeong Min Moon[1†], Jimin Kim[1†], Yurim Seong[1], Byunchang Suh[1],

KyeongJin Kang[1,2], Han Kyoung Choe[1] and Kyuhyung Kim[1,2]

[1]Department of Brain and Cognitive Sciences, DGIST, Daegu, 42988,

[2]KBRI (Korea Brain Research Institute), Daegu, 41068,

Republic of Korea.

Corresponding author: K.Ka; kangkj@kbri.re.kr, H.K.C; choehank@dgist.ac.kr,

K.Ki; khkim@dgist.ac.kr

[†]These authors contributed equally to this work.

Keywords: proprioception, motor control, proprioceptive receptor

**Abstract**

In animals, proper locomotion is crucial to find mates and foods and avoid predators or dangers. Multiple sensory systems detect external and internal cues and integrate them to modulate motor outputs. Proprioception is the internal sense of body position, and proprioceptive control of locomotion is essential to generate and maintain precise patterns of movement or gaits. This proprioceptive feedback system is conserved in many animal species and is mediated by stretch-sensitive receptors called proprioceptors. Recent studies have identified multiple proprioceptive neurons and proprioceptors and their roles in the locomotion of various model organisms. In this review we describe molecular and neuronal mechanisms underlying proprioceptive feedback systems in *C. elegans*, *Drosophila*, and mice.

## Introduction

"It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is most adaptable to change". Charles Darwin.

Animals must detect and respond to changes in external and internal environments in order to survive and reproduce. The sensory and motor systems of an animal exhibit remarkable sensitivity and plasticity in their structures and functions as needed to adjust to ever-changing environmental conditions. For example, when animals encounter predators or hazardous conditions, their sensory systems detect spatiotemporal patterns of these harmful stimuli, which are then transduced, integrated, and processed in central nervous systems to control motor systems, leading to change in behavioral programs, including locomotive maneuvers (1). Moreover, this behavioral modification is further modulated by previous experience and internal status.

Humans have a multitude of senses, including vision, audition, olfaction, gustation, and somatosensation, which are traditionally described as five senses. In the 19$^{th}$ century, Scottish anatomist Sir Charles Bell first characterized "muscle sense" and referred to it as the sixth sense (2-4). In the early 20$^{th}$ century, Charles Scott Sherrington studied the peripheral source of sensory afferents and their control on muscle contraction, and introduced the terms "*exteroception*", "*interoception*", and "*proprioception*" (5, 6). The exteroception senses environmental stimuli from outside the body, whereas the interoception detects internal states or signals from internal organs. Moreover, proprioception senses movement of our body, such as limbs or muscles. Early studies of proprioception showed that animal locomotion is mediated by a highly coordinated sensorimotor feedback system including proprioception (7, 8) and that defects in proprioception result in motor defects, such as uncoordinated movement (8-11).

Motor control via the proprioceptive sensory system is well conserved in many motile animals, from vertebrates to invertebrates. In mammals, proprioceptive systems are well described anatomically and functionally (12-14). Invertebrate proprioceptive organs have also been well described in several species such as flies, worms, and cockroaches (15, 16). Despite previous studies, the molecular mechanisms underlying proprioceptive feedback in motor control are still unclear. Here**,** we review recent findings about molecular and neuronal mechanisms underlying proprioception and its motor control in representative model systems, including *C. elegans*, *Drosophila*, and mice.

## Proprioception in *C. elegans*

The nematode *Caenorhabditis elegans* has a relatively simple nervous system, with only 302 neurons, roughly 8,000 synapses, and 95 muscles involved in locomotion (17, 18). However, *C. elegans* displays a broad spectrum of locomotive behaviors, such as crawling, swimming, and head steering. Thus, the *C. elegans* system provides an opportunity to investigate the locomotive behavior mediated by the proprioceptive feedback system at a single-cell circuit level.

### Crawling behavior

*C. elegans* crawls on solid substances such as a plane agar gel and moves forward and backward in the sine wave shape. During forward locomotion, *C. elegans* also exhibits omega turns in which to make a sharp angle turn, allowing the worms to change direction. Crawling is well coordinated to generate dorsoventral sinusoidal waves with constant speed, wavelength, wave width, and bending angle, and is controlled by neural circuits consisting of

multiple proprioceptive neurons and proprioceptive receptors (19-22).

The DVA interneuron has been shown to mediate mechanical sensory integration (23, 24). Xu and coworkers identified the DVA interneuron as a type of proprioceptive neuron that regulates body bending angle (Fig 1A) (25). This group found that *trp-4* mutants exhibit an increased bending angle, referred to as "exaggerated bending" (Fig 1B); this defect is restored by expressing the TRP-4 proteins specifically in the DVA neuron (25). The *trp-4* gene encodes a transient receptor potential channel N subtype (TRPN), and TRP-4 proteins are localized throughout the axon of the DVA neuron (Fig 1B) (25, 26). Moreover, calcium imaging data indicate that the DVA neuron is physically activated by the body stretch via the TRP-4 channel. However, laser ablation of the DVA neuron in *trp-4* mutants suppressed the mutant phenotype and instead decreased the bending angle, suggesting that additional factor(s) in the DVA neuron regulate body bending (25). Together, these results indicate that the DVA neuron acts as a proprioceptive neuron-type that detects body stretch via the TRP-4 proprioceptive receptor to shape waveform in crawling. In addition, Hu et al. found that the DVA neuron secretes neuropeptide NLP-12, which mediates aldicarb-induced potentiation of cholinergic transmission (27). Moreover, aldicarb-induced muscle contraction induced NLP-12 secretion via the TRP-4 stretch receptor in the DVA neuron, supporting that the DVA neuron and TRP-4 receptor play in proprioception to couple muscle contraction to cholinergic transmission.

The next candidates as proprioceptive neurons are the PVD and FLP sensory neurons (Fig 1A). These neurons appear to sense noxious signals in a way similar to how mammalian nociceptive receptor neurons do so (28, 29). Albeg and coworkers identified new roles of the PVD and FLP neurons in modulating crawling (30). The PVD and FLP neurons have characteristic structures that directly detect body stretch (Fig 1A) (17, 30). The dendritic

branches of the PVD and FLP neurons encompass the whole body; PVD branches cover the body region from the pharynx to the tail, whereas FLP branches surround the head region (Fig 1A) (29, 30). Moreover, their terminal branches are positioned between the body-wall muscle and hypodermis (30, 31). This group found that PVD-ablated mutants exhibit a decreased bending angle and a more extended waveform (Fig 1B). Also, mutants lacking functional PVD and FLP neurons exhibited locomotive defects, including reduced speed, increased reversal, and pauses (30). The PVD and FLP neurons express the MEC-10 DEG/ENaC channel, which has been identified in mechanosensation (Fig 1B) (29, 32). The group found that *mec-10* mutants exhibit a decreased bending angle (Fig 1B) and that during locomotion, the PVD neurons are activated through the MEC-10 channel (30). Thus, these results indicate that the MEC-10 channel can function as a proprioceptor in the PVD and FLP neurons, and that proprioceptive feedback from the PVD and FLP neurons may modulate proper crawling. However, direct activation of the PVD and FLP by muscle contraction and their downstream targets needs to be further verified in order to conclude that the PVD and FLP neurons and MEC-10 are bona fide proprioceptive neurons and receptors, respectively.

**Head steering**

During forward movement, *C. elegans* exhibits head steering, moving the head left and right repeatedly. Yeon and coworkers found that the SMDD sensory/inter/motor neurons control the head steering of *C. elegans* (33). The SMD neurons consist of two pairs of neurons (dorsal SMDDs and ventral SMDVs), of which cell bodies are located in the head, and whose synapse-free processes extend along the body (Fig 1A) (17). Moreover, these neurons innervate the head/neck muscles, suggesting a role in head locomotion. Previously, the SMD neurons have been shown to regulate omega turns (34). This group showed that

genetic and physical ablation of the SMDD neurons causes ventral circling locomotion (Fig 1B) and optogenetic activation of all four SMD neurons together, resulting in synchronized activation of all four cells that also results in similar locomotive defects. Moreover, a forced stretch of neck muscle activated the SMDD neurons, which in turn directly regulated dorsal head-muscle contraction. These results indicate that the SMDD neurons are the bona fide proprioceptive cells, and that these cells are both necessary and sufficient to generate head-steering locomotion. This group also showed that the SMDD neurons co-express mechanosensitive TRP-1 and TRP-2 TRPC channels, of which double mutants, but not single mutants, exhibit ventral circling locomotion similar to that of SMDD-ablated animals (Fig 1B) (26, 33). Moreover, in *trp-1 trp-2* double mutants, the $Ca^{2+}$ activity of the SMDD neurons was synchronized with that of the SMDV neurons. Ectopic expression of TRP-1 or TRP-2 in the AWC chemosensory conferred neck bending dependent $Ca^{2+}$ activity. These results indicate that these two TRPC channels are necessary and sufficient for proprioceptive responses, detect dorsal neck-muscle stretch and desynchronize dorsal muscles from body locomotion circuits.

**Wave propagation**

*C. elegans* exhibits distinct locomotive behaviors that are repetitive, rhythmic, and depend on their environment. For example, they show repetitive S-shaped crawling on a solid agar plate and repetitive C-shaped swimming in a liquid (22, 35, 36). This undulatory wave appears to result from coordinating rhythmic muscle activities generated by a central pattern generator (CPG) (37, 38). These rhythmic activities from CPGs are transmitted to the whole body and are properly maintained by proprioceptive feedback (39-43). For example, the cell bodies of B-type cholinergic motor neurons are located in the ventral nerve cord, and their

process extends along the ventral or dorsal region (17). Wen et al. found that these B-type motor neurons are activated by body bending, and that inactivation of the B-type motor neurons by the expression of the $K^+$ channel prevents propagation of the wave from the anterior region to the posterior region (41). Thus, these results suggest that B-type motor neurons respond to body bending and provide proprioceptive feedback to the subsequent motor neurons to generate wave propagation.

**Proprioception in *Drosophila***

The fruit fly *Drosophila melanogaster* is an attractive model system to study proprioceptive feedback. A single set of *Drosophila* molecular genetic tools can be applied to two distinct behavioral arenas, the larva, and the adult fly, which show different behavioral mechanisms in responding to sensory inputs. Moreover, both larvae and adults contain well-identified proprioceptive organs and mechanoreceptors (16, 44, 45).

**Leg control in adult-fly locomotion**

Proprioception of the adult legs is in part mediated by the mechanosensory apparatus such as chordotonal organs (COs) (Fig 2A). Insect legs harbor multiple classes of exteroceptive and interoceptive mechanoreceptors (45). COs house internal mechanoreceptor neurons typically located at and between joints residing in individual limbs and body segments. The fundamental unit of COs is called the scolopidium, consisting of one to three bipolar sensory neurons and two types of accessory cells (neuron-enveloping scolopale and neuron-anchoring cap cells). The femoral chordotonal organ (FeCO) is a well-known proprioceptor in insects and is widely located in the legs (Fig 2A). In *Drosophila*, FeCO is structurally well

conserved, but specific functional studies are still in progress. Mendes et al. suggested that the *Drosophila* leg FeCO is functionally involved in locomotive coordination (46) through genetic manipulations for loss of function studies. Both deficiency of the *nanchung* (*nan*) gene, which encodes a member of Transient Receptor Potential Vanilloid (TRPV) expressed in sensory cilia of COs and inhibition of the leg FeCO sensory neurons led to impaired gaits and decreased speed. These results suggest that the *Drosophila* leg FeCO functions in proprioceptive feedback to generate precise patterns of leg movements during walking (46). Furthermore, *Drosophila* genetics combined with two-photon calcium imaging allowed Mamiya and coworkers to study the anatomy and function of 152 neurons in FeCO while the legs were magnetically controlled (47). Genetically separable groups of FeCO neurons with anatomically differential innervation to the ventral nerve cord (VNC) were necessary for specific functions, such as encoding tibial position, movement direction, bidirectional movement, and vibration frequency. Based on the innervation patterns to the VNC, the three subtypes were named club, claw, and hook, although their cell bodies reside in the same FeCO. Thus, *Drosophila* FeCO neurons are critical for precise leg coordination for gaiting; hence flies use functionally and anatomically distinct mechanosensors within FeCO neurons.

Despite functional studies of proprioceptive organs, the molecular mechanisms of leg proprioception are still unclear. For leg proprioception, Akitake et al. found that the TRPγ ion channel functions in *Drosophila* leg-motor control (48). TRPγ is a Transient Receptor Potential Canonical subfamily member in *Drosophila*; it was previously identified as a cation channel subunit heteromerizing with other TRPC channels, such as TRP and TRPL, for transmission of visual signal transduction. Akitake et al. found that TRPγ functions as a mechanoreceptor in the leg FeCO (48). The *trpγ* reporter is expressed in FeCO (neurons and scolopale cells) and macrochaetes on the dorsal thorax and legs. The *trpγ* alleles show

decreased forward walking speed, decreased average step length, and impaired leg replacement (Fig 2B). These postural defective behavior phenotypes indicate that *trpγ* is essential for proprioception-mediated fine motor control. Patch-clamp recording of TRPγ expressed in HEK293 cells showed that TRPγ was directly activated by membrane stretch (48). Cheng et al. found that TRP channel TRPN1/NompC is required for controlling locomotion of adult flies (49), and that *nompC* mutants display uncoordinated leg/wing twisting movements and reduced walking speed (Fig 2B). The *nompC* is expressed in many neurons, especially proprioceptors in leg joints (50) and ciliary tips of COs and along sensory neuron dendrites (49, 51). The subcellular NompC localizations appear to be associated with ANK repeats in its N terminal cytoplasmic domain and contribute to NompC protein stability (49). These results suggest that TRPN1/NompC mechanosensitive channels may functionally be involved in leg proprioceptors to generate precise locomotive behavior in adult flies.

In summary, three different studies of leg proprioception indicate that the *Drosophila* leg mechanosensory organ FeCO is pivotal for the coordination of fly gaiting. Such joint-moving-dependent FeCO activation can be initiated by stretch-sensitive TRPγ and TRPN1/NompC channels. Further studies will be required to unravel the precise roles of these and other mechanoreceptor molecules in mechanosensitive organs as well as FeCO.

**Visuomotor gain**

The generation of precise motor control is mediated by multisensory integrations, such as in vision, hearing, and touching. In *Drosophila*, flight is controlled by the halteres, which are vestigial wings that function as gyroscopes. Interestingly, motoneurons innervating the haltere muscles were identified as a target of excitatory visual interneurons, detailing a

possible mechanism of visually regulated flight (52). Recently, Bartussek et al. suggested that the integration of visual signals and proprioceptive feedback from wings and halteres generates precise wing steering muscles (WSM) activation (53). Object fixation, optomotor altitude control, and saccadic escape reflexes were examined with wing kinematics under haltere immobilization or wing-nerve treatment, revealing antagonistic signalings between wings and halteres. Decreased wing-steering ranges resulted from haltere immobilization, whereas that range was increased by suppression of wing feedback. Thus, Bartussek et al. suggested that two different proprioceptive feedbacks regulate visuomotor gain to control the muscle spiking phase to enable precise flight (53).

**Larval locomotion**

As in adults, sensory feedback is essential for larval crawling, but the specific roles of neurons and muscles in crawling remain to be fully understood (54, 55). Genetic manipulations on two classes of multidendritic (md) neurons, bipolar dendrites (bd), and the class I mds showed that they are essential in normal larval crawling as proprioceptors (Fig 2A) (56). Further studies revealed morphologies and positions of the diverse multipledendritc (md) neurons (57, 58), suggesting that each of six md cell types may show functional differences in proprioceptive feedback circuits. To identify the functional difference of each md cell type, Vaadia et al. performed *in vivo* 3D imaging of the dendrites in freely moving *Drosophila* larvae to observe their deformation and neuronal calcium dynamics during crawling using high-speed volumetric SCAPE microscopy (59). Six md neurons—ventral posterior dendritic arborization neuron (vpda), dorsal dendritic arborization neuron E (ddaE), dorsal dendritic arborization neuron D (ddaD), dorsal multidendritic neuron 1 (dmd1), and dorsal and ventral bipolar dendrite md neurons (dbd and vbd)—responded at different times

during peristaltic waves. Five neurons (vpda, ddaE, ddaD, dmd1, and vbd) were sequentially activated during segment contraction. In contrast, the dbd neuron was excited during segment stretch. These findings indicate that the six md neurons may function as a proprioceptor during larval locomotion and be functionally synchronized. Moreover, ddaD and ddaE neurons also showed excitation in head turning and retraction. In this work and a previous study of the synaptic connections of these neurons (60), Vaadia et al. hypothesized that in generating larval forward crawling, ddaD, vbd, and dmd1 neurons may activate inhibitory premotor neurons to mediate segment relaxation and anterior wave propagation (59). On the other hand, the vpda neuron provides input into the excitatory premotor neuron A27h to activate GABAergic dorsolateral (GDL) interneurons to inhibit the neighboring contraction of anterior segments from preventing premature wave propagation (61).

Mechanosensitive ion channels have been associated with the coordination of larval crawling. Cheng et al. found that the *Drosophila* mechanosensitive channel TRPN1/NompC plays a role in that function (49). The expression of *nompC* was observed in several neurons, such as class I da neurons (ddaD, ddaE), bd neuron (dbd), chordotonal neurons (lch1, lch5, vchA, and vchB), ventral bd neuron (vbd), and class I da neuron (vpda). Null mutations of *nompC* in larvae showed prolonged stride duration with normal stride size and decreased crawling speed (Fig 2B). These phenotypes have similarly occurred when bd and class I da neurons were silenced (56). The calcium activities of both bd and class I da neurons in *nompC* null mutants were reduced more than were those of wild-type larvae during crawling. These results suggest that NompC is required for peristaltic muscle contraction in larval crawling by bd and class I da neurons (49, 56, 59). He et al. found that the *Drosophila* transmembrane channel-like (TMC) gene functions in larval crawling via class I da neurons (Fig 2B) (62). This group also used high-speed confocal microscopy to observe deformation

and calcium activity of proprioceptors, ddaE, and ddaD during crawling. The ddaE dendrites were deformed by muscle contraction, exhibiting increased calcium activities during forward locomotion. However, the ddaE dendrites showed relatively unmoved and low calcium activities during backward locomotion. On the other hand, the ddaD dendrites were deformed by muscle contraction, showing increased calcium activities during backward locomotion, but were relatively less moved with low calcium activities during forward locomotion. As the molecular mechanism of these relative proprioceptive responses, He et al. presented behavioral genetics data associated with the *Tmc* gene, which is expressed in class I dendrites and is a well-known candidate as a gene encoding a mechanoreceptor (Fig 2B). In *Tmc-1* mutants, larvae showed enhanced head curl behavior and increased backward locomotion (Fig 2B) (63). Moreover, ddaD and ddaE in the mutants had decreased calcium activities and dendrite curvature in both forward and backward locomotion. Thus, *Drosophila* larval forward and backward locomotions are mediated by different mechanosensitive neurons but are likely mediated by a single mechanosensitive channel TMC-1 (62). One recent physiological study of bipolar dendritic (dbd) neurons also presented pharmacological and genetic lines of evidence linking the mechanosensitive Piezo channel to the stretch sensitivity of dbd neurons (64).

**Proprioception in mammals**

As in *C. elegans* and *Drosophila*, proprioception plays a crucial role in the movement regulation of mammals. For fine coordination of movement, proprioception functions through multisensory integration in concert with other sensory modalities, including vision, touch sensation, and vestibular function.

**Anatomical and genetic identity of proprioceptive sensory neurons**

There are two types of major proprioceptive organs in mammals: muscle spindles (MS) and Golgi tendon organs (GTO) (Fig 3A) (8, 65). The MS is located in the middle of the muscle fibers, with its sensory afferent ending innervating the intrafusal muscle fiber. In contrast to extrafusal muscle fibers that contract upon an alpha motor-neuron impulse to produce major muscular power, intrafusal fibers are located inside the fusiform (spindle-like) capsule and are innervated by surrounding type Ia or II proprioceptive sensory afferents. When intrafusal fibers are stretched by movement, the type Ia afferent triggers an action potential corresponding to the change in muscle length and the current length of the muscle, whereas the firing rate of the type II afferent encodes the length of muscle (8, 65). The GTO is located in the junction between tendon and muscle. Type Ib sensory nerve endings innervate the distal ending of the tendon, which is ensheathed in the capsule. The contraction of the muscle elicits a stretch of the tendon linked to the muscle, thereby triggering the action potential of the GTO afferents. The GTO also detects the force imposed upon the tendon, allowing the sensation of isometric exercise. The cell bodies of both MS and GTO reside in the dorsal root ganglion, which contains a cluster of cell bodies enriched with mechanosensitive, chemosensitive, and temperature-sensitive peripheral sensory neurons and bilaterally neighbors the spinal cord (8, 65).

Mammalian joints also contain sensory organs of low-threshold mechanosensitivity, such as Ruffini endings and Pacinian corpuscles (65). However, in contrast to chordotonal neurons in insects, the joint sensation does not seem to play a critical role except in detecting a movement threshold, because joint replacement surgery can spare the proprioceptive control of fine movement (8).

The soma of both MS and GTO resides in the dorsal root ganglion (DRG) with other peripheral sensory neurons, such as mechanosensitive touch-sensing neurons and thermosensory neurons (65, 66). In the DRG, peripheral sensory neurons are not topographically segregated according to their function but are distributed in a salt-and-pepper pattern. It is the molecular composition and projection pattern that distinguish proprioceptors from other peripheral sensory neurons (67-69). These proprioceptive DRG neurons are derived from neural-crest progenitors and characterized by the expression of parvalbumin (PV), TrkC, and Runx3 (67, 68). However, either PV or Runx3 expression does not exclusively coincide with proprioceptors, even among DRG sensory neurons, because their expression is also found in the cutaneous mechanosensitive receptors (67, 69).

Recent progress in single-cell RNA sequencing offers unprecedently detailed genetic insight into the molecular signatures of the proprioceptors. Usoskin et al. investigated the molecular details of 500 DRG sensory neurons (70). Sharma et al. cataloged the developmental landscape of DRG neurons and identified the developmental trajectory of proprioceptive neurons in relation to other DRG sensory neurons (71). Based on intersectional labeling of proprioceptive neurons, Oliver et al. could almost exclusively sort proprioceptive neurons and sequence the proprioceptors with enough depth to cover the subtypes (72). They identified five clusters in adult proprioceptive neurons, which correspond to types Ia, II MS, and GTO.

In line with these molecular signatures, Cre drive lines that specifically expressed Cre DNA recombinase in either PV+ or Runx3+ cells provided efficient genetic accessibility to proprioceptors (72-74). However, these marker genes are expressed not only in proprioceptors but also in certain types of cutaneous mechanosensory neurons, raising cell-type validation issues for Cre driver-based studies. To genetically label proprioceptive

neurons exclusively and efficiently, advanced genetic techniques are required in addition to proprioceptive-specific genetic-profile information. For example, an intersectional double genetic switch using both Cre and Flp driven by PV and Runx3, respectively, is reportedly efficient and exclusive in specifically labeling proprioceptors (72). Since the genetic toolbox controlled by Flp is rather limited compared to Cre-based toolbox, further development of intersectional switches would facilitate the specific genetic manipulation of proprioceptors. Because the proprioceptive identity is postnatally established (71), inducible systems such as CreERT2 would be required to finely delineate the cell-type specificity of proprioceptive neurons (75).

**Physiological properties of proprioceptors and their molecular principles**

Proprioceptors respond to mechanical deformation of afferent endings by eliciting action potentials with notably high fidelity and low adaptation. This property makes proprioceptors exceptionally well adapted to ceaselessly monitor the position and movement of our body, where the cognate sensory stimuli are constantly present within a relatively limited range, in contrast to the evanescent sensory stimuli that stimulate our "five senses". Accordingly, the physiological response of the proprioceptive system provides classic evidence supporting a fundamental concept of neuroscience, that the sensory stimuli generate the action potential of a fixed intensity in the sensory neuron and that the frequency of the generated action potential correlates with the strength of given stimuli (76).

Recent experimental approaches have used the fact that the DRG proprioceptors can also be excited by mechanical stimulation of the proprioceptor soma by micrometer-level indentation by a blunt-end glass needle (72-74, 77). Although the subcellular distribution of

molecular receptors that transduce mechanical stimuli into a change in membrane potential is not clearly understood, the membrane-potential changes of proprioceptors in response to mechanical force imposed upon the soma of these neurons suggest that the distribution of the mechanosensitive proprioceptive channels is not limited to the sensory afferent endings. As we will explore later, although proprioception provides important information in feedback control of motor regulation, defective motor control is not necessarily attributable to the deficit of proprioception. Therefore, electrophysiological analyses of proprioceptors in response to mechanical stimuli are vital in identifying proprioceptive ion channels. A recent key discovery of mammalian proprioceptors is firmly rooted in the defective mechanosensitive current response in the DRG neurons. For example, electrophysiological properties of DRG PV neurons that genetically lack Piezo2 did not exhibit a mechanically activated rapid inactivating current, which represents the main response type of DRG PV neurons (73). A physiological study of mechanosensory neurons located in the mesenphalic trigeminal nucleus (MTN), responsible for the proprioception of the head, found them to be dependent on Piezo2 (74). In addition to a rapidly adapting current, DRG proprioceptive neurons also exhibit an intermediate and slow adapting current dependent on Tentonin3 (77). Notably, the intermediate adapting current is not impaired in either Piezo2-/- or Tentonin3 -/- DRG, indicating that other mechanosensitive channels may function as a novel yet unappreciated molecular receptor of proprioception. Because current knowledge about the mechanosensitive proprioceptive channels cannot explain the *in vivo* physiology of proprioceptive neurons, such as the different mechanisms differentiating types Ia, Ib, and II fibers, advances in genetic handles to proprioceptive neurons, combined with electrophysiological analysis, will provide detailed insight into the differential transduction mechanisms of proprioceptor subtypes.

**Implications of proprioception for mammalian behavior**

The most well-known and most widely exercised example of a motor program that depends on proprioception is the spinal monosynaptic stretch reflex, also known as a knee-jerk reflex. Brief hitting of the patellar ligament stretches the quadricep muscle and the muscle spindle therein. The stretch of the muscle spindle, in turn, induces the firing of the proprioceptive neurons, which then communicate with downstream spinal motor neurons to extend the leg. Normal proprioception is a crucial component in successful knee jerk reflexes, whereas the deficit in the reflex indicates not only abnormal proprioception but also damage in the reflex arc, either interneuron or motor neuron or muscle function. Specific examination of proprioception requires a more explicit experimental design.

More than 30 different tests have been suggested to exam the proprioceptive functions of humans (78). There is no single gold-standard test to evaluate all the proprioceptive functions of the subject. Instead, each test evaluates a specific proprioceptive function of each location: perception of the static position of a body part or of a body movement. The degradation of proprioception results in the loss of acuity in movement control, which is worsened by the deprivation of complementary sensory modality and by poor novel motor learning. In the mouse genetic model, genome sequence analysis identified PIEZO2 as a molecular cause that leads to proprioception deficits (79, 80). The patients carrying mutant PIEZO2 suffered from lack of proprioception, impaired motor coordination, electrophysiological phenotypes, and various degrees of joint malformation, without any compromised cognitive functions. Retarded initiation of walking is reported in infants (79, 80).

Mouse models have been a preferred mammalian model for studying proprioceptive

functions in mammals because of their genetic accessibility. Early studies investigating the development of proprioceptive neurons from their progenitors produced several mutants with developmentally abnormal proprioceptors who later lacked proper proprioceptive functions as adults. Genetic models with problems in synaptic connection between proprioceptive neurons and motor neurons or in the functional development of the muscle spindle further validate that proprioception crucially coordinates posture as well as walking and swimming in mice (Fig 3B) (81, 82).

Another line of evidence about how proprioception contributes to motor coordination in mice originates from mutants that lack the mechanosensitive ion channels responsible for proprioception. Piezo2 is abundantly expressed in the DRG PV neurons, a predominant marker of proprioceptive sensory neurons. Patapoutian and colleagues reported that conditional knockout of Piezo2 in the DRG PV neurons ablated the proprioceptive function of the mouse (73). Atypical limb coordination in tail-suspended posture was observed in mutants, along with abnormal and less-fluent walking. These results were recapitulated in the HoxB8-Cre-dependent conditional knockout of Piezo2. PV neuron-specific knockout of Piezo2 also elicited behavioral deficits in several balance and movement tests, including gait analysis, balance-beam walking, a two-limb wire-hanging test, and a rotarod test (74). Similar deficits were also reported in Tentonin3 knockouts (77).

**Summary**

Thus, locomotion in model animals requires further studies for us to gain a more wholistic understanding of how proprioceptive computation accurately accommodates mechanosensory inputs from components of locomotion, as we start to glimpse a mechanistic insight into the

molecular and neuronal substrates for the sensory levels of proprioception. Considering that human proprioception contributes to both conscious and unconscious perception of limb and trunk position and movement, the current behavioral assays in model animals are limited, in that they do not require conscious processing of proprioceptive information. Whereas most behavioral assays focus on motor coordination, the engagement of proprioception in motor learning and rehabilitation may provide an additional layer of insight (83). Recent progress in machine-vision technologies, such as DeepLabCut and MoSeq, will aid a better understanding of proprioception in motor control by facilitating machine-vision-based kinesthetic analysis (84, 85). Furthermore, there is currently no study investigating the causal relationships of proprioceptive neurons in motor control at millisecond precision with reversibility, warranting an optogenetic study to understand the dynamic contribution of proprioception to motor control.

**Funding**

This study was supported by the National Research Foundation of Korea (NRF-2020R1A4A1019436).

**Figure Legends**

**Figure 1.** Proprioceptive neurons and proprioceptors in *C. elegans*. (A) The morphology and position of the DVA interneuron, PVD and FLP sensory neurons, and SMDD sensory/inter/motor neurons. (B) Locomotive phenotypes of putative proprioceptor gene mutants, including *trp-4, mec-10*, and *trp-1/2*.

**Figure 2**. Proprioceptive neurons and proprioceptors in *Drosophila* and larvae. (A) The structure of individual scolopidia in *Drosophila* leg FeCO, showing neuron cell body (ncb), cilia (ci), cap cell (cc), scolopale cell (sc) (Green marks indicate the location of the proprioceptive organ in *Drosophila*) (left). The structure of neurons in proprioceptive organs and expressed location in larvae (right). (B) Locomotive phenotypes of putative proprioceptor gene mutants, including TRPγ, NompC (adult and larva), and TMC1.

**Figure 3.** Morphology of rodent proprioceptors and their implications in motor control. (A) The anatomical structure of rodent proprioceptors. Two main proprioceptive neurons, Muscle spindle (MS) and Golgi tendon organ (GTO) have their sensory afferent endings in the middle of the muscle and the tendon, respectively. (B) Behavioral deficits observed in the mutant mice lacking proper proprioception. Top panel: abnormal limb postures in hanging. Bottom panel: Error-prone stepping in walking test.

## Reference

1. Maniere G and Coureaud G (2019) Editorial: From Stimulus to Behavioral Decision-Making. Front Behav Neurosci 13, 274
2. Singh AK (1991) The Comprehensive History of Psychology, Motilal Banarsidass, 66
3. Dickinson MH, Farley CT, Full RJ, Koehl MA, Kram R and Lehman S (2000) How animals move: an integrative view. Science 288, 100-106
4. Bell C (1834) The hand; its mechanism and vital endowments, as evincing design, W. Pickering, London, 348
5. Sherrington CS (1906) The integrative action of the nervous system, Yale University Press, New Haven, CT, US, 411
6. Sherrington CS (1913) Reflex inhibition as a factor in the co-ordination of movements and postures. Quarterly Journal of Experimental Physiology 6, 251-310
7. Dietz V (2013) Gait disorders. Handb Clin Neurol 110, 133-143
8. Proske U and Gandevia SC (2012) The proprioceptive senses: their roles in signaling body shape, body position and movement, and muscle force. Physiol Rev 92, 1651-1697
9. Dietz V (2002) Proprioception and locomotor disorders. Nat Rev Neurosci 3, 781-790
10. Pearson KG (1995) Reflex Reversal in the Walking Systems of Mammals and Arthropods; in 135-141, Springer US,
11. Ruffini A (1898) On the Minute Anatomy of the Neuromuscular Spindles of the Cat, and on their Physiological Significance. J Physiol 23, 190-208 193
12. Gandevia SC and McCloskey DI (1976) Joint sense, muscle sense, and their combination as position sense, measured at the distal interphalangeal joint of the middle finger. J Physiol 260, 387-407
13. Moore JC (1984) The Golgi tendon organ: a review and update. Am J Occup Ther 38, 227-236
14. Windhorst U (2007) Muscle proprioceptive feedback and spinal networks. Brain Res Bull 73, 155-202
15. Burke W (1954) An Organ for Proprioception and Vibration Sense in *Carcinus Maenas*. Journal of Experimental Biology 31, 127-138
16. Pringle JWS (1938) Proprioception in insects I. A new type of mechanical receptor from the palps of the cockroach. Journal of Experimental Biology 15, 101-113
17. White JG, Southgate E, Thomson JN and Brenner S (1986) The Structure of the Nervous-System of the Nematode Caenorhabditis-Elegans. Philosophical Transactions of the Royal Society B-Biological Sciences 314, 1-340
18. Jarrell TA, Wang Y, Bloniarz AE et al (2012) The connectome of a decision-making neural network. Science 337, 437-444
19. Butler VJ, Branicky R, Yemini E et al (2015) A consistent muscle activation strategy underlies crawling and swimming in Caenorhabditis elegans. J R Soc Interface 12, 20140963

20. Fang-Yen C, Wyart M, Xie J et al (2010) Biomechanical analysis of gait adaptation in the nematode Caenorhabditis elegans. Proceedings of the National Academy of Sciences of the United States of America 107, 20323-20328
21. Karbowski J, Cronin CJ, Seah A, Mendel JE, Cleary D and Sternberg PW (2006) Conservation rules, their breakdown, and optimality in Caenorhabditis sinusoidal locomotion. Journal of Theoretical Biology 242, 652-669
22. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77, 71-94
23. Puckett Robinson C, Schwarz EM and Sternberg PW (2013) Identification of DVA interneuron regulatory sequences in Caenorhabditis elegans. PLoS One 8, e54971
24. Wicks SR, Roehrig CJ and Rankin CH (1996) A dynamic network simulation of the nematode tap withdrawal circuit: Predictions concerning synaptic function using behavioral criteria. Journal of Neuroscience 16, 4017-4031
25. Li W, Feng ZY, Sternberg PW and Xu XZS (2006) A C-elegans stretch receptor neuron revealed by a mechanosensitive TRP channel homologue. Nature 440, 684-687
26. Xiao R and Xu XZS (2011) C. elegans TRP Channels. Transient Receptor Potential Channels 704, 323-339
27. Hu ZT, Pym ECG, Babu K, Murray ABV and Kaplan JM (2011) A Neuropeptide-Mediated Stretch Response Links Muscle Contraction to Changes in Neurotransmitter Release. Neuron 71, 92-102
28. Way JC and Chalfie M (1989) The Mec-3 Gene of Caenorhabditis-Elegans Requires Its Own Product for Maintained Expression and Is Expressed in 3 Neuronal Cell-Types. Genes & Development 3, 1823-1833
29. Chatzigeorgiou M, Yoo S, Watson JD et al (2010) Specific roles for DEG/ENaC and TRP channels in touch and thermosensation in C. elegans nociceptors. Nature Neuroscience 13, 861-U106
30. Albeg A, Smith CJ, Chatzigeorgiou M et al (2011) C-elegans multi-dendritic sensory neurons: Morphology and function. Molecular and Cellular Neuroscience 46, 308-317
31. Liang X, Dong XT, Moerman DG, Shen K and Wang XM (2015) Sarcomeres Pattern Proprioceptive Sensory Dendritic Endings through UNC-52/Perlecan in C. elegans. Developmental Cell 33, 388-400
32. Huang MX and Chalfie M (1994) Gene Interactions Affecting Mechanosensory Transduction in Caenorhabditis-Elegans. Nature 367, 467-470
33. Yeon J, Kim J, Kim DY et al (2018) A sensory-motor neuron type mediates proprioceptive coordination of steering in C. elegans via two TRPC channels. PLoS Biol 16, e2004929
34. Gray JM, Hill JJ and Bargmann CI (2005) A circuit for navigation in Caenorhabditis elegans. Proc Natl Acad Sci U S A 102, 3184-3191
35. Korta J, Clark DA, Gabel CV, Mahadevan L and Samuel ADT (2007) Mechanosensation and mechanical load modulate the locomotory gait of swimming C-elegans. Journal of Experimental Biology 210, 2383-2389

36. Pierce-Shimomura JT, Chen BL, Mun JJ, Ho R, Sarkis R and McIntire SL (2008) Genetic analysis of crawling and swimming locomotory patterns in C. elegans. Proceedings of the National Academy of Sciences of the United States of America 105, 20982-20987
37. Grillner S (2003) The motor infrastructure: From ion channels to neuronal networks. Nature Reviews Neuroscience 4, 573-586
38. Marder E and Calabrese RL (1996) Principles of rhythmic motor pattern generation. Physiological Reviews 76, 687-717
39. Denham JE, Ranner T and Cohen N (2018) Signatures of proprioceptive control in Caenorhabditis elegans locomotion. Philos Trans R Soc Lond B Biol Sci 373
40. Fieseler C, Kunert-Graf J and Kutz JN (2018) The control structure of the nematode Caenorhabditis elegans: Neuro-sensory integration and proprioceptive feedback. J Biomech 74, 1-8
41. Wen Q, Po MD, Hulme E et al (2012) Proprioceptive Coupling within Motor Neurons Drives C. elegans Forward Locomotion. Neuron 76, 750-761
42. Fouad AD, Teng S, Mark JR et al (2018) Distributed rhythm generators underlie Caenorhabditis elegans forward locomotion. Elife 7
43. Gao S, Guan SA, Fouad AD et al (2018) Excitatory motor neurons are local oscillators for backward locomotion. Elife 7
44. Shanbhag SR, Singh K and Naresh Singh R (1992) Ultrastructure of the femoral chordotonal organs and their novel synaptic organization in the legs of Drosophila melanogaster Meigen (Diptera : Drosophilidae). 21, 311-322
45. Tuthill JC and Wilson RI (2016) Mechanosensation and Adaptive Motor Control in Insects. Current Biology 26, R1022-R1038
46. Mendes CS, Bartos I, Akay T, Márka S and Mann RS (2013) Quantification of gait parameters in freely walking wild type and sensory deprived Drosophila melanogaster. eLife 2
47. Mamiya A, Gurung P and Tuthill JC (2018) Neural Coding of Leg Proprioception in Drosophila. Neuron 100, 636-+
48. Akitake B, Ren Q, Boiko N et al (2015) Coordination and fine motor control depend on Drosophila TRPgamma. Nat Commun 6, 7288
49. Cheng LE, Song W, Looger LL, Jan LY and Jan YN (2010) The Role of the TRP Channel NompC in Drosophila Larval and Adult Locomotion. Neuron 67, 373-380
50. Walker RG, Willingham AT and Zuker CS (2000) A Drosophila mechanosensory transduction channel. Science 287, 2229-2234
51. Lee E, Sivan-Loukianova E, Eberl DF and Kernan MJ (2008) An IFT-A protein is required to delimit functionally distinct zones in mechanosensory cilia. Curr Biol 18, 1899-1906
52. Chan WP, Prete F and Dickinson MH (1998) Visual input to the efferent control system of a fly's "gyroscope". Science 280, 289-292
53. Bartussek J and Lehmann FO (2016) Proprioceptive feedback determines visuomotor gain in Drosophila. R Soc Open Sci 3, 150562

54. Fox LE, Soll DR and Wu CF (2006) Coordination and modulation of locomotion pattern generators in Drosophila larvae: effects of altered biogenic amine levels by the tyramine beta hydroxlyase mutation. J Neurosci 26, 1486-1498
55. Suster ML and Bate M (2002) Embryonic assembly of a central pattern generator without sensory input. 416, 174-178
56. Hughes CL and Thomas JB (2007) A sensory feedback circuit coordinates muscle activity in Drosophila. Molecular and Cellular Neuroscience 35, 383-396
57. Corty MM, Tam J and Grueber WB (2016) Dendritic diversification through transcription factor-mediated suppression of alternative morphologies. Development 143, 1351-1362
58. Grueber WB, Jan LY and Jan YN (2002) Tiling of the Drosophila epidermis by multidendritic sensory neurons. Development 129, 2867-2878
59. Vaadia RD, Li WZ, Voleti V, Singhania A, Hillman EMC and Grueber WB (2019) Characterization of Proprioceptive System Dynamics in Behaving Drosophila Larvae Using High-Speed Volumetric Microscopy. Current Biology 29, 935-+
60. Schneider-Mizell CM, Gerhard S, Longair M et al (2016) Quantitative neuroanatomy for connectomics in Drosophila. Elife 5
61. Fushiki A, Zwart MF, Kohsaka H, Fetter RD, Cardona A and Nose A (2016) A circuit mechanism for the propagation of waves of muscle contraction in Drosophila. Elife 5
62. He L, Gulyanon S, Mihovilovic Skanata M et al (2019) Direction Selectivity in Drosophila Proprioceptors Requires the Mechanosensory Channel Tmc. Curr Biol 29, 945-956 e943
63. Guo Y, Wang Y, Zhang W et al (2016) Transmembrane channel-like (tmc) gene regulates Drosophila larval locomotion. Proc Natl Acad Sci U S A 113, 7243-7248
64. Suslak TJ, Watson S, Thompson KJ et al (2015) Piezo Is Essential for Amiloride-Sensitive Stretch-Activated Mechanotransduction in Larval Drosophila Dorsal Bipolar Dendritic Sensory Neurons. Plos One 10
65. Tuthill JC and Azim E (2018) Proprioception. Curr Biol 28, R194-R203
66. Wang F, Bélanger E, Côté SL et al (2018) Sensory Afferents Use Different Coding Strategies for Heat and Cold. Cell Reports 23, 2001-2013
67. de Nooij JC, Doobar S and Jessell TM (2013) Etv1 inactivation reveals proprioceptor subclasses that reflect the level of NT3 expression in muscle targets. Neuron 77, 1055-1068
68. Levanon D, Bettoun D, Harris-Cerruti C et al (2002) The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. EMBO J 21, 3454-3463
69. Abraira VE and Ginty DD (2013) The sensory neurons of touch. Neuron 79, 618-639
70. Usoskin D, Furlan A, Islam S et al (2015) Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci 18, 145-153
71. Sharma N, Flaherty K, Lezgiyeva K, Wagner DE, Klein AM and Ginty DD (2020) The emergence of transcriptional identity in somatosensory neurons. Nature 577, 392-398
72. Oliver KM, Florez-Paz DM, Badea TC, Mentis GZ, Menon V and de Nooij JC (2020) Molecular development of muscle spindle and Golgi tendon organ sensory afferents revealed by single

proprioceptor transcriptome analysis. bioRxiv, 2020.2004.2003.023986

73. Woo SH, Lukacs V, de Nooij JC et al (2015) Piezo2 is the principal mechanotransduction channel for proprioception. Nat Neurosci 18, 1756-1762
74. Florez-Paz D, Bali KK, Kuner R and Gomis A (2016) A critical role for Piezo2 channels in the mechanotransduction of mouse proprioceptive neurons. Sci Rep 6, 25923
75. Daigle TL, Madisen L, Hage TA et al (2018) A Suite of Transgenic Driver and Reporter Mouse Lines with Enhanced Brain-Cell-Type Targeting and Functionality. Cell 174, 465-480 e422
76. Adrian ED and Zotterman Y (1926) The impulses produced by sensory nerve-endings: Part II. The response of a Single End-Organ. J Physiol 61, 151-171
77. Hong GS, Lee B, Wee J et al (2016) Tentonin 3/TMEM150c Confers Distinct Mechanosensitive Currents in Dorsal-Root Ganglion Neurons with Proprioceptive Function. Neuron 91, 107-118
78. Hillier S, Immink M and Thewlis D (2015) Assessing Proprioception: A Systematic Review of Possibilities. Neurorehabil Neural Repair 29, 933-949
79. Chesler AT, Szczot M, Bharucha-Goebel D et al (2016) The Role of PIEZO2 in Human Mechanosensation. N Engl J Med 375, 1355-1364
80. Masingue M, Faure J, Sole G, Stojkovic T and Leonard-Louis S (2019) A novel nonsense PIEZO2 mutation in a family with scoliosis and proprioceptive defect. Neuromuscul Disord 29, 75-79
81. Akay T, Tourtellotte WG, Arber S and Jessell TM (2014) Degradation of mouse locomotor pattern in the absence of proprioceptive sensory feedback. Proc Natl Acad Sci U S A 111, 16877-16882
82. Arber S, Ladle DR, Lin JH, Frank E and Jessell TM (2000) ETS gene Er81 controls the formation of functional connections between group Ia sensory afferents and motor neurons. Cell 101, 485-498
83. Takeoka A and Arber S (2019) Functional Local Proprioceptive Feedback Circuits Initiate and Maintain Locomotor Recovery after Spinal Cord Injury. Cell Rep 27, 71-85 e73
84. Mathis A, Mamidanna P, Cury KM et al (2018) DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. Nat Neurosci 21, 1281-1289
85. Wiltschko AB, Tsukahara T, Zeine A et al (2020) Revealing the structure of pharmacobehavioral space through motion sequencing. Nat Neurosci 23, 1433-1443

Table 1.

| Model | Proprioceptor | Function | Location | Channels | References |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| ***C. elegans*** | DVA neuron | Mechanical sensory integration, Control body bending angle | Dorsorectal ganglion | TRP-4 | 23, 24, 25, 26, 27 |
| | PVD & FLP neurons | Sensing noxious signals, Control crawling behavior | Lumbar ganglion (Tail) / Head | MEC-10 | 17, 28, 29, 30, 31, 32 |
| | SMDD neurons | Regulation of omega turn, Control head steering | Ventral ganglion in the head | TRP-1, TRP-2 | 17, 26, 33, 34 |
| | B type cholinergic motor neurons | Undulatory locomotion, Control wave propagation | Ventral nerve cord | - | 17, 41 |
| ***Drosophila*** | dbd, ddaE, ddaD neuron (Larvae) | Peristaltic muscle contraction, Control stride size and crawling speed | Chordotonal organ | NompC | 49, 56, 59 |
| | ddaE, ddaD neuron (Larvae) | Peristaltic muscle contraction, Control forward/ backward locomotion | Chordotonal organ | TMC | 56, 59, 62, 63 |
| | FeCO (Femoral Chordotonal organ) (Adult fly) | Membrane stretch sensing, Control walking speed and leg replacement | Neurons, scolopale cells, macrochaetes, dorsal thorax and leg | TRPγ | 46, 47, 48 |
| | FeCO (Femoral Chordotonal organ) (Adult fly) | Mechanosensing, Control walking speed and leg/ wing twisting movements | Neurons, leg joints and ciliary tips of COs | NompC | 46, 47, 49, 50, 51 |
| **Mouse** | Type Ia and II sensory afferents | Sensing movement stretching, Control muscle length | Muscle spindle and soma reside in dorsal root ganglion | PIEZO2 | 8, 65, 72, 73, 80, 81 |
| | Type Ib sensory afferent | Sensing muscle contraction, Sense isometric exercise | Golgi tendon organ and soma reside in dorsal root ganglion | PIEZO2 | 8, 65, 72, 73, 80, 81 |

**Figure 1.**

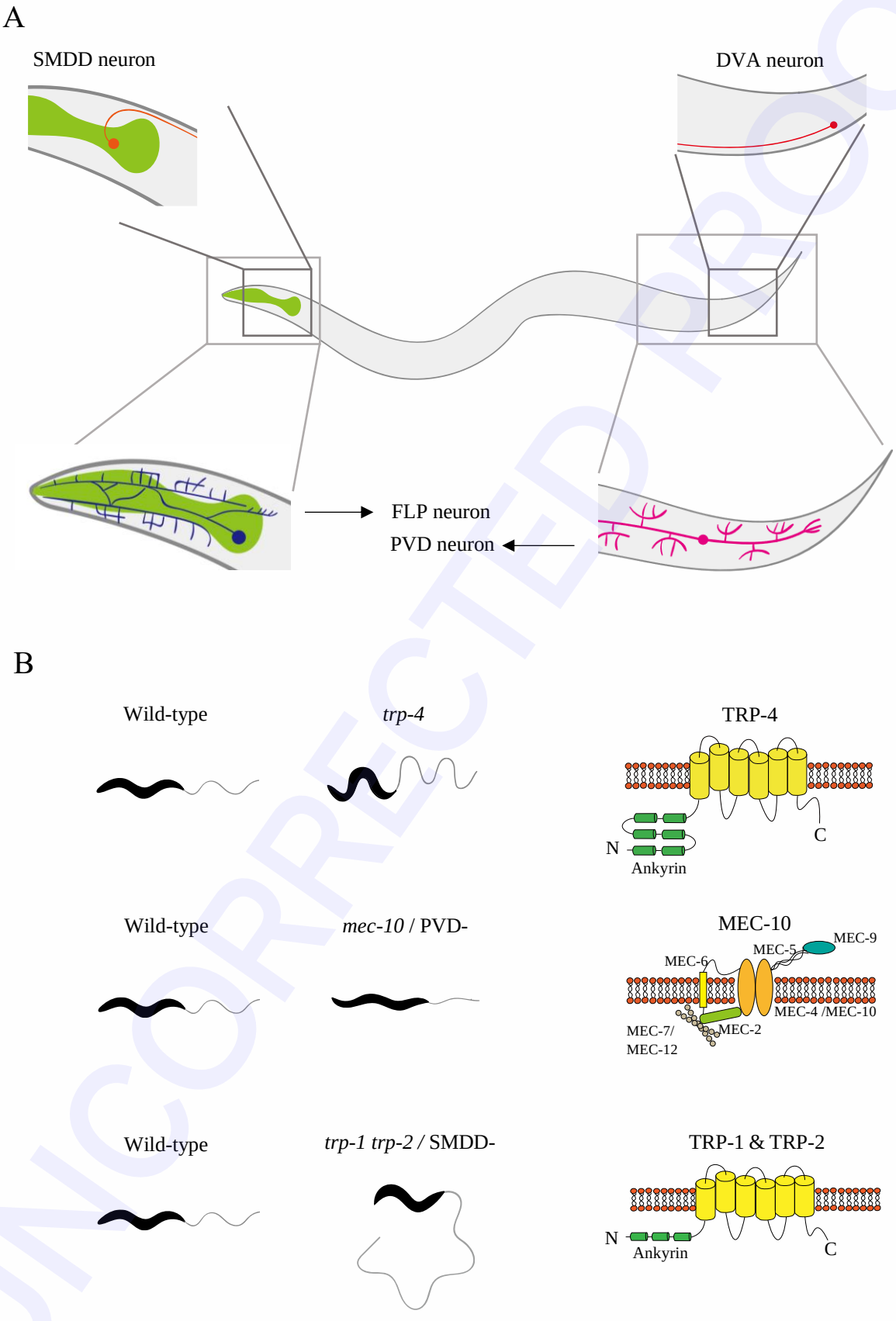

**Figure 2.**

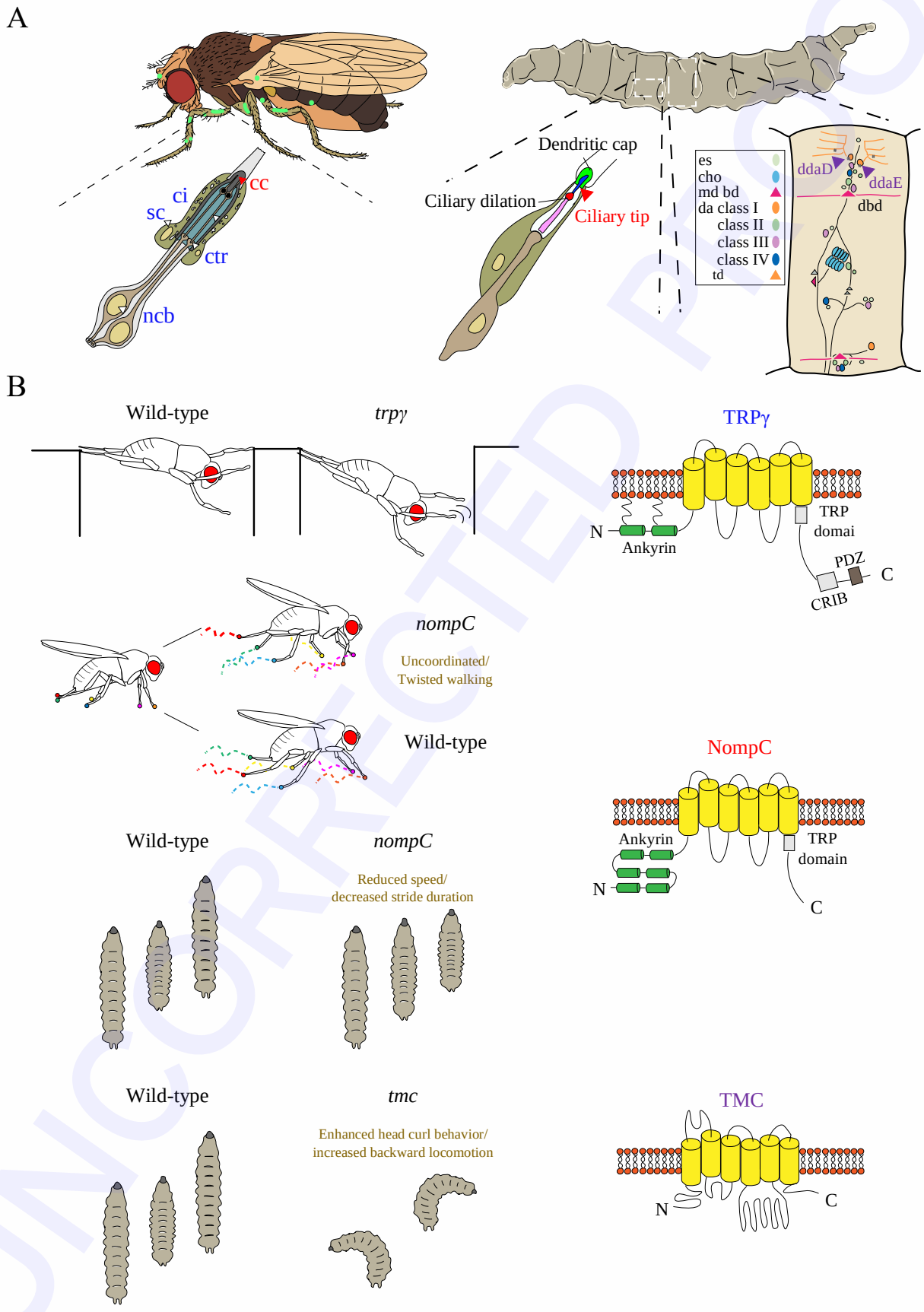

**Figure 3.**