

The neuronal mechanisms underlying locomotion termination

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Abstract

It is critical for animals to be able to stop locomotion according to their external and internal needs. Limited studies show there are dedicated neuronal pathways responding to either sensory inputs or commands from higher brain areas to inhibit the locomotor circuit and actively terminate locomotion. Such neuronal mechanisms are clearly demonstrated by the quick ending of ongoing locomotor rhythms following the activation of “stop” cells in the brainstem. In contrast, some activity-dependent mechanisms intrinsic to the locomotor neuronal network are responsible for the spontaneous ending of locomotor episodes. Known autonomous mechanisms involve the release/accumulative production of adenosine or the mediation of long-lasting hyperpolarisation by Na⁺ pumps following locomotion. We discuss some recent studies that have led to these findings.

Keywords

Locomotion; CPG; Brainstem; Stop Cells

Introduction

The locomotor rhythms that produce a multitude of behaviours - including crawling, walking, swimming and flying - are generated by neuronal networks called central pattern generators (CPGs) [1,2]. The CPG networks are located in the ventral nerve cord of invertebrates and the spinal cord of vertebrates [1,3–5]. It is essential for animals to be able to halt ongoing locomotor activities, either after they have arrived at their destination, to avoid clashes and predation or simply to have a rest. The ability to end locomotion is also necessary when animals switch from one locomotion mode to another due to terrain changes, e.g. from flying in air to walking on grounds in insects [6] and birds. Although we have gained many crucial insights into how locomotor rhythms are generated - for review see [1] - few studies have been devoted to understanding how locomotor activity ends.

The brainstem (subdivided into the midbrain, pons and medulla) is a key integrative structure involved in many ascending and descending pathways [7]. Vertebrate spinal CPG networks receive inputs from critical brainstem neurons to influence the initiation, maintenance and cessation of locomotion [8,9]. More specifically, reticulospinal excitatory pathways and the mesencephalic locomotor region (MLR) have been shown to be critical in locomotion initiation and motor pattern choice in multiple species [4,9]. Key to this review is that some descending brainstem neurons have been shown to inhibit locomotion and have been termed “stop” cells. The firing of “stop” cells is closely correlated with locomotion ending, suggesting that they may initiate the locomotion termination. “Stop” cell candidates have been found in vertebrates - *Xenopus* tadpoles [10], lamprey [11] and mice [12] - along with invertebrates - *Drosophila* [13], crayfish [14], crickets [15]. Their apparent conservation suggests an evolutionary advantage [5]. In tadpoles, lamprey and mice, the specific activation of these “stop” cells has been shown to halt locomotion. Alternative run-down/wind-up

mechanisms (e.g. Na⁺ pump activity[16] and adenosine accumulation [17]) intrinsic to the spinal CPG network that are directly controlled by the cellular and biochemical repercussions of ongoing locomotion can also progressively act to end a locomotion episode.

Tadpole Mechanosensory Stopping Response

The simple, developing *Xenopus laevis* tadpole has been used to study vertebrate locomotor control for decades [18]. The neuronal circuitry consists of columns of swimming CPG neurons which extend continuously from the spinal cord into the hindbrain. Among them, excitatory descending neurons (dINs) in the caudal hindbrain/rostral spinal cord are crucial in tadpole swimming rhythm generation [19–21]. Exciting a single dIN using intracellular depolarising current injections can occasionally initiate swimming [20,22], and hyperpolarising current injections into a single dIN can end ongoing swimming in many cases [23].

Tadpoles stop swimming when they swim into solid objects or hit the water surface. The activation of two sensory pathways has been shown to mediate their stopping response. The first pathway involves mechanosensory neurons in the trigeminal ganglia, which sense slow pressure on the cement gland or the pulling of the cement gland mucus [24]. These sensory neurons make glutamatergic synapses onto the GABAergic mid-hindbrain reticulospinal (MHR) neurons. Remarkably, current injections evoking repetitive firing of a single MHR neuron could terminate swimming within one swimming cycle [10] (Fig.1A, B). The termination is mediated by the fast monosynaptic GABAergic inhibitory post-synaptic potentials (IPSPs) that MHRs produce on swimming CPG neurons [10,24,25] (Fig.1B). The second stopping pathway is activated when the tadpole swims into obstacles head-on. The head-on clashes excite the rapid-transient detectors in the head skin, the peripheral neurites of

some mechanosensory neurons within the trigeminal ganglia [26,27]. This, in turn, recruits some unidentified cholinergic neurons in the brainstem, producing long-lasting hyperpolarisation of swimming CPG neurons located in the mid-hindbrain region and thus, terminating swimming [27] (Fig.1C). Pharmacological and voltage-clamp experiments show G protein-coupled inward-rectifying potassium channels (GIRKs) mediate the inhibition after the postsynaptic M₂ acetylcholine receptors are activated [27,28]. Unlike the brief GABAergic IPSPs, the GIRK-mediated inhibition lasts up to a couple of minutes, reducing tadpole motor responses in a manner resembling some acute symptoms for concussion. It is unclear if the termination of swimming is just the consequence of a concussion response [27].

“Stop” Cells in the Lamprey Brainstem

The spinal CPG responsible for generating swimming rhythms in lamprey has also been extensively studied [29]. The MLR, a key structure for locomotor control between the midbrain and hindbrain, activates reticulospinal cells to initiate and influence swimming rhythms generated by the spinal CPG [30,31]. The MLR projections have been shown to make both glutamatergic and cholinergic synapses with the reticulospinal neurons [11,32,33].

A discrete population of reticulospinal neurons in lamprey have recently been identified as “stop” cells [11,34]. The middle rhombencephalic reticular nucleus (MRRN), previously recognised as essential for locomotor initiation and maintenance [32,35], are also critical to locomotor termination. More specifically, the “stop” cells are located in the caudal area of MRRN (figure 2A). They show high frequency firing at the end of swimming bouts, evoked by stimulating the MLR, a ‘tail pinch’ or at the offset of a spontaneous swimming episode [11] (Fig.2B). Depending on the neuronal activity pattern during a swimming episode, two other sub-populations of neuron have also been identified within the MRRN. These are

“start” and “maintain” cells and are active at the initiation of and throughout swimming, respectively. Pharmacologically activating the “stop” cells using D-glutamate terminates swimming, although the application of glutamatergic blockers only managed to slow down swimming towards the end of swimming episodes. It is still unclear which upstream brain areas activate these “stop” cells, what the postsynaptic targets of the “stop” cells are and what neurotransmitter the lamprey “stop” cells use [11].

“Stop” Cells in the Mouse Brainstem

As in lamprey and tadpoles, mammalian spinal locomotor CPG circuits are also critically controlled by brainstem motor centres [36,37]. The complicated nature of the organisation of the brainstem reticular formation in mammals, where inhibitory and excitatory descending brainstem neurons are mostly intertwined [37,38], presents huge challenges in the classification and studying of neurons in this region using conventional anatomical and physiological methods [12]. Previously, it was shown in cats that stimulating the rostral medullary and caudal pontine reticular formation electrically can lead to the general inhibition of the motor system [39,40]. The recent application of developmental genetics and optogenetics in neonatal mice has enabled the revelation of “stop” neurons in this region - a population of V2a CHX10-expressing neurons responsible for locomotion termination. The combination of fast optogenetic activation and sequential removal of the rostral brainstem by slicing has located these V2a “stop” cells in the rostral medulla and caudal pons area (more specifically, in the Rostral Gigantocellularis/rGi, and Caudal Pontine Reticular Nuclei/PnC, Fig.3A) [12]. Activating the “stop” cells quickly stops locomotor activities (Fig.3B). The V2a “stop” cells are glutamatergic, and their axons mostly terminate in lamina VII of the lumbar spinal cord. It has been proposed that they indirectly terminate locomotion by

activating the local inhibitory interneurons although their postsynaptic targets also include some excitatory interneurons [12].

An additional study has noted further potential “stop” cell populations within the mouse brainstem’s reticular formation. The optogenetic activation of specific glycinergic neurons could crucially lead to locomotor arrest and these cells are present in areas adjacent to where the V2a CHX10-expressing “stop” cells are located [9]. More specifically, these neurons were present in the lateral paragigantocellular nucleus (LPGi), the alpha part of the gigantocellular nucleus (GiA) and the ventral part of the gigantocellular nucleus, along with the more dorsally located gigantocellular nucleus (Gi) (Fig.3C). These neurons receive inputs from multiple sources including glutamatergic neurons in MLR and their ipsilateral axons terminate in the ventral spinal cord innervating motoneurons and potentially interneurons. Interestingly, activating glycinergic neurons in these areas appeared to halt locomotion with variable effects on muscle tones, i.e. with maintained body muscle tone for LPGi and GiA, muscle tone loss for GiV and spasm for Gi.

Like the lamprey counterparts, it is unknown how the mouse “stop” cells are physiologically activated and what their cellular activity is during locomotion and when locomotion ends. It is also unclear if there are synaptic connections between the V2a “stop cells” and their adjacent glycinergic locomotor-halting neurons. The existence of diverse populations of inhibitory and excitatory neurons in close proximity responsible for terminating locomotion highlights the difficulty in studying the functional organization of the reticular formation.

Run-Down/ Wind-Up Stopping Mechanisms

Unlike the active “stop” cell-mediated responses which are activated on demand by sensory activation or descending commands, some run-down/wind-up mechanisms have been identified to mediate spontaneous stopping responses of locomotion. These run-down/wind-up processes do not involve supra-spinal brain areas and are intrinsic to the spinal locomotor CPG circuitry (Fig.4).

One such intrinsic mechanism relates to the progressive breakdown of ATP to adenosine during *Xenopus* tadpole swimming. At the beginning of a swimming episode, ATP released in the spinal cord inhibits voltage-gated K^+ channels by activating P2Y receptors and keeps the excitability of swimming CPG neurons high. With the progression of swimming, ATP is broken down to adenosine, which then accumulates to inhibit voltage-dependent Ca^{2+} channels by activating P1 receptors, reducing the excitability of CPG neurons and gradually drawing the swimming to a halt [17]. Adenosine, generally produced by the extracellular hydrolysis of ATP, can also be released from astrocytes [41]. A recent study in mice suggests that adenosine release by astrocytes during ongoing locomotor can inhibit locomotion rhythm generation by enhancing the D_1 -like dopamine receptor signalling pathway. The astrocytically-derived adenosine acts on neuronal A_1 adenosine receptors and reduces the frequency of fictive locomotor rhythms induced by NMDA application in neonatal mouse spinal cords [42].

A recently highlighted activity-dependent mechanism that can modulate locomotor episode length and affect when locomotion ends is mediated by the activation of the ubiquitously expressed Na^+/K^+ -ATPase (Na^+ pump). The $\alpha 3$ -containing Na^+ pump has been known to play critical roles in maintaining ionic gradients across the membrane, and the resting membrane potential in neurons and they are sensitive to low-level increases in intracellular Na^+

concentration. However, the $\alpha 1$ -containing Na^+ pump is mostly responsive to higher increases in intracellular Na^+ concentrations. In drosophila larvae motoneurons [43], tadpole swimming CPG neurons [44,45] and neonatal mouse spinal neurons [19], prolonged neuronal spiking evoked by intracellular current injections can increase intracellular Na^+ concentration. The elevated Na^+ concentration then activates the $\alpha 1$ -containing Na^+ pump, gives rise to an ultraslow afterhyperpolarisation (usAHP) lasting for up to one minute. This Na^+ pump activity, therefore, is dynamically linked with neuronal activity intensity, consequently affecting the locomotor frequency and episode duration [19,43,45] (Fig. 4A). Interestingly, Na^+ pump activity can be subject to regulations by some neuromodulators [46,47], e.g. enhancement by dopamine [48] and depression by serotonin [49] and nitric oxide [50].

Conclusive remarks

Generally, there are very few studies directly addressing how locomotor activity ends, either actively or spontaneously. The mechanisms terminating a locomotor episode fall in two broad categories: mechanisms activated by sensory stimulation or commands from higher brain areas, or some autonomous run-down/windup processes in the CPG circuits. The pathways upstream to the “stop” cells are unclear in most examples. In the case of spontaneous ending of locomotor activities, the source of autonomous modulators - like ATP and adenosine - still needs further investigation. From the rather limited mechanisms we have reviewed, we can perhaps summarise a couple of general rules governing the termination of locomotion. Firstly, multiple mechanisms can co-exist in the same locomotor circuit. This is clearly demonstrated in the tadpole swimming circuit where intrinsic purinergic and Na^+ pump mechanisms can lead to spontaneous ending of swimming whereas both GABA-ergic and cholinergic brainstem pathways can be activated by specific sensory inputs to terminate

swimming. Multiple stopping mechanisms in lamprey and mice are also implied since locomotion can still end after the identified “stop” cells are disabled [11,12]. Secondly, the understanding of cellular mechanisms leading to the inhibition of locomotion is still limited. Based on the mechanisms already revealed for activity-dependent and active termination in *Xenopus* tadpoles, it appears that the active termination of locomotor activity is a distinct mechanism and not achieved by an acceleration or upregulation of the intrinsic activity-dependent run-down/wind-up mechanisms. The presence of dedicated neuronal pathways to locomotor termination, including the “stop” cells, only manifests the importance of stopping mechanisms in motor control.

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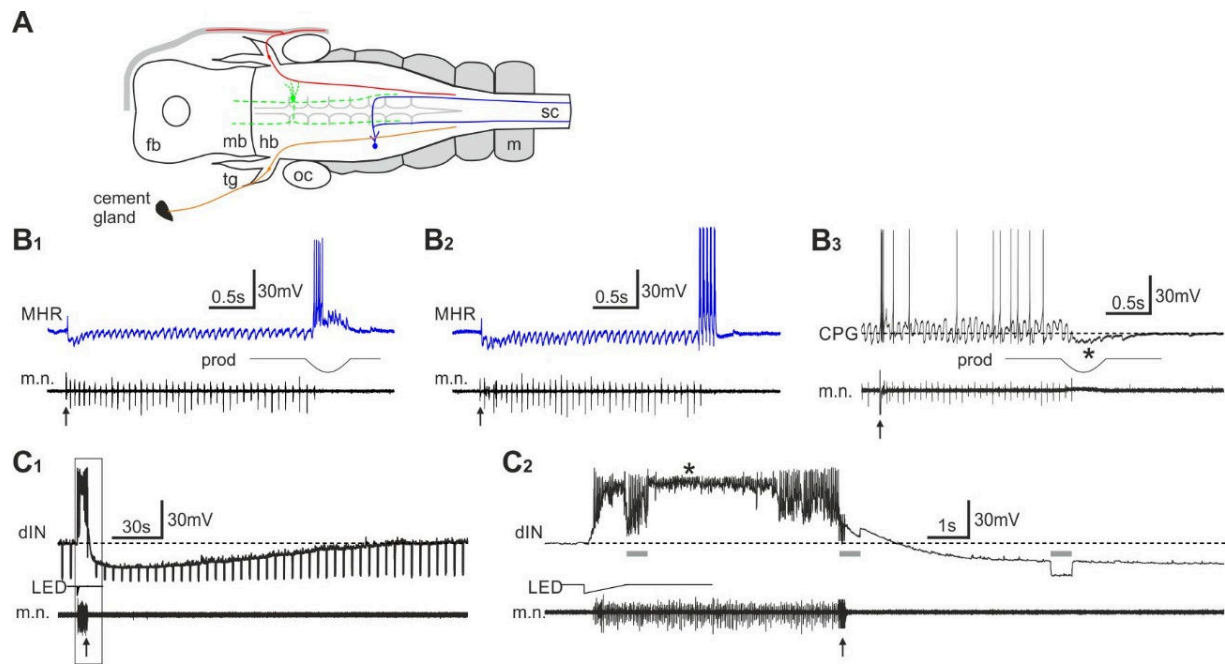


Figure 1. Swimming termination induced by mechanosensory inputs in stage 37/38 *Xenopus* tadpoles.

A. A dorsal-view diagram of tadpole brain showing two tadpole swimming stopping pathways. The activation of slow movement sensors innervating the cement gland (orange) activates GABAergic MHR cells (blue), to inhibit the swimming CPG primarily in the spinal cord (sc). Activating rapid-transient detectors innervating the head skin (red) excites some unidentified cholinergic neurons in the brainstem (dotted green) to inhibit swimming CPG neurons in the hindbrain. Other structures: trigeminal ganglion (tg); forebrain (fb); midbrain (mb); hindbrain (hb); otic capsule (oc); and myotomes (m).

B1. The activity of an MHR neuron during swimming (m.n. is motor nerve activity) and following the stimulation (indicated by “prod”) of the cement gland. **B2.** Five short step current injections evoking two spikes each in an MHR lead to the stopping of swimming. **B3.** A CPG neuron receives brief GABAergic inhibition (*) when the cement gland is prodded. **C1.** Long-lasting inhibition mediated by GIRK channels in a hindbrain dIN following head skin stimulation (arrow, five 0.2 ms pulses at 30 Hz). Swimming was induced by dimming an LED close to the tadpole head. The boxed area is stretched in **C2.** Regular downward

deflections in **C**₁ are caused by – 40 pA step current injections at 0.2 Hz (grey bars in **C**₂). *
in **C**₂ marks a period of lack of dIN spiking due to depolarisation block.

Dashed lines in **B**₃, **C**₁ and **C**₂ indicate resting membrane potential. **A** and **C** are adapted from [27], **B**₁₋₂ from [10]. Arrows in **B** and **C** indicate the time of electrical skin stimulation.

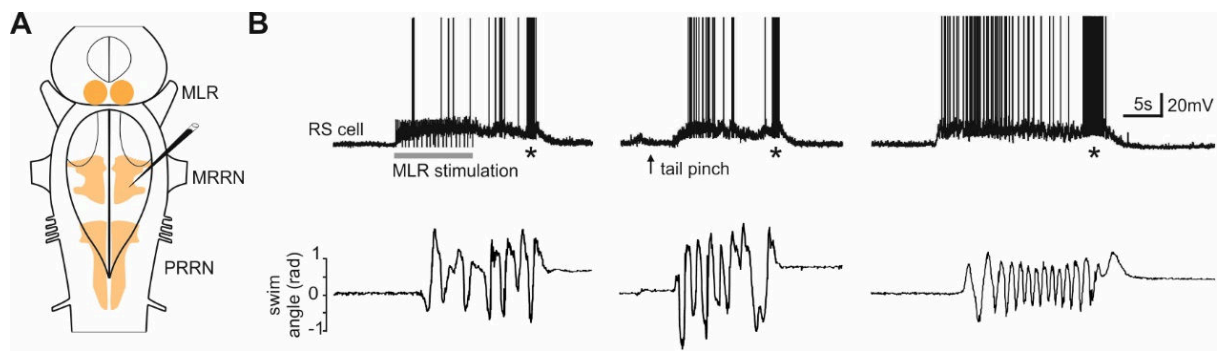


Figure 2. “Stop” cells in the termination of lamprey swimming.

A. Diagram showing the location of critical motor control centres in the brainstem (orange): MLR, MRRN and posterior rhombencephalic reticular nucleus (PRRN). The electrode indicates the region where the majority of “stop” cells are found.

B. One “stop” cell (RS) shows enhanced spiking (*) at the end of swimming evoked by MLR stimulation, tail pinching (arrow) and spontaneous swimming (far right). Swimming in this semi-intact preparation is monitored by measuring the angle of tail bends (swim angle) in a video at 30 fps.

A-B are adapted from [11]

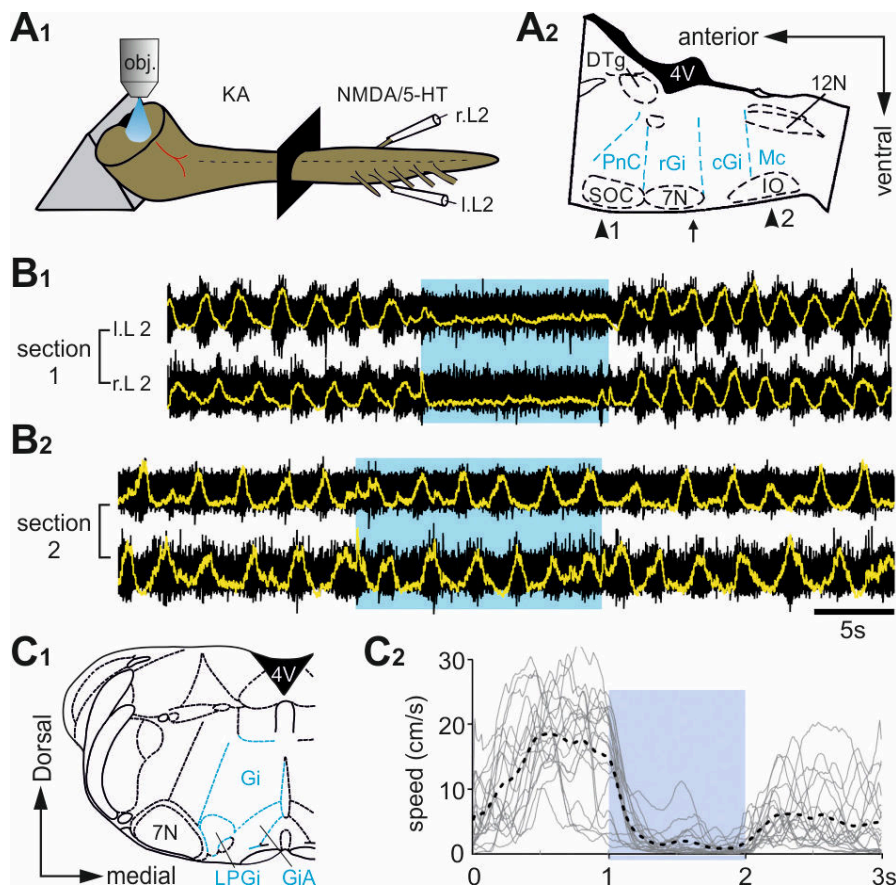


Figure 3. Light activation of glutamatergic V2a “stop” neurons and glycinergic neurons expressing Channelrhodopsin in the mouse brainstem reticular formation terminates locomotion.

A1. Diagram showing a split-bath experimental set-up. The brainstem is transversely sectioned at different longitudinal positions successively (between arrow heads in **A2**) to determine the “stop” cell location. The anterior end and lumbar end of the spinal cord is separated by a Vaseline barrier (black square), to allow kynurenic acid (KA) application to block glutamatergic transmission rostral to the barrier and NMDA/5-HT induction of locomotion rhythms in the posterior. Motoneuron recordings were taken from the 2nd lumbar roots on the left and right sides (l.L2, r.L2).

A2. Sagittal section of a brainstem segment showing the location of “stop” cells in the reticular formation (blue text and lines). Abbreviations: 4V - 4th ventricle; 7N - facial

nucleus; Amb:12N - hypoglossal nucleus; SOC - Superior olivary complex; IO - Inferior olive; DTg - laterodorsal tegmental nucleus; rGi, cGi - rostral and caudal gigantocellular reticular nucleus; Mc - magnocellular reticular nucleus; PnC - caudal pontine reticular nucleus.

B1. Light activation (blue shading) of a preparation sectioned at position 1 in **A2** stops fictive locomotor rhythms. **B2.** Light activation of the same preparation sectioned at a more caudal position (position 2 in **A2**) fails to stop locomotor rhythms. **A-B** are adapted from [12].

C1. A coronal section of mouse brainstem (arrow in **A2**) showing main areas containing locomotion-stopping glycinergic neurons in the reticular formation (blue text and outlines).

Gi – gigantocellular nucleus; LPGi – lateral paragigantocellular nucleus; GiA – alpha part of the gigantocellular nucleus. **C2.** Light activation of glycinergic neurons in Gi in a mouse slows down/stops locomotion (grey lines are individual trials, black dashed line is average).

C is adapted from [9].

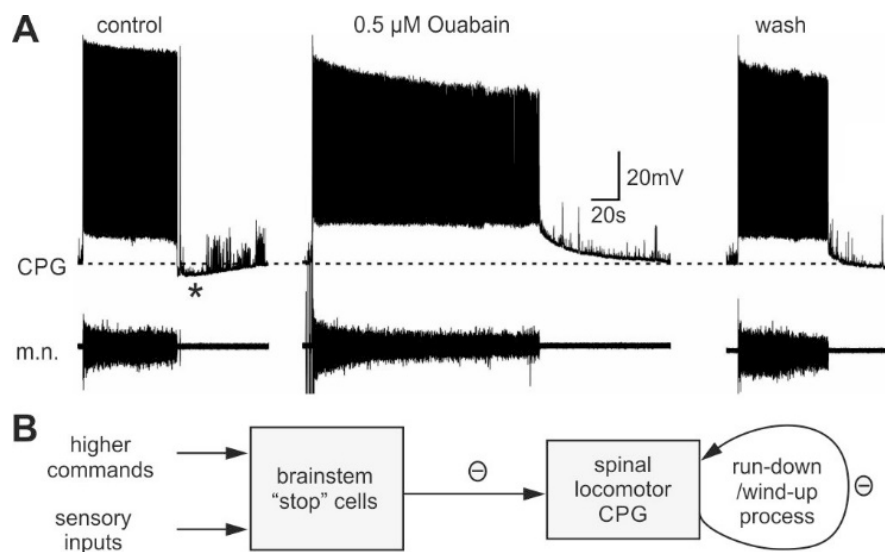


Figure 4. The control of tadpole swimming episode length by Na^+ pump activity and a general summary of locomotion termination mechanisms.

A. The usAHP (*) and its abolishment by the Na^+ pump blocker (ouabain) immediately after swimming episodes in a stage 42 *Xenopus laevis* tadpole. The dashed line indicates the resting membrane potential.

B. Schematic illustrating both brainstem "stop" cells and run-down/wind-up processes intrinsic to the spinal locomotor CPG can terminate locomotion. **A** is adapted from [45].