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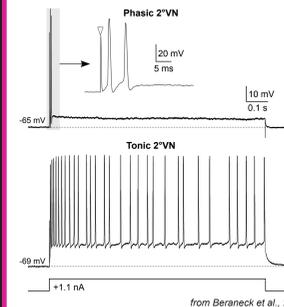
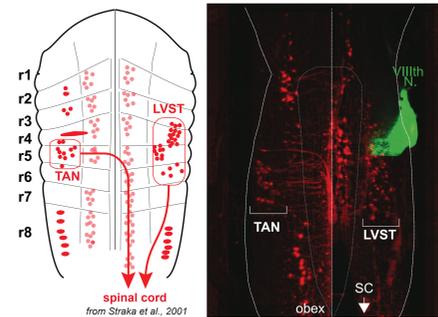
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Characterization of intrinsic membrane properties of vestibulo-spinal neurons through xenopus development

Olechowski-Bessaguet A., Cardoit L., Thoby-Brisson M., Lambert F.M.

Introduction

In vertebrates, central vestibular neurons (2^oOVN) are subdivided in distinct populations according to their projection and their function (vestibulo-ocular/spinal - commissural...). In amphibian, vestibulospinal neurons (VSN), involved in postural control, are organized into lateral vestibulospinal tract (LVST) and tangential (TAN) nuclei projecting to ipsi- and contralateral spinal cord, respectively (Straka et al., 2001). Here, we focused on the LVST neurons, largest group (and common to other vertebrates lineages) localized mostly in rhombomeres 4, at the level of the VIIIth nerve entrance.



In adult terrestrial frog, two groups of 2^oOVN were described according to their discharge dynamic and their intrinsic membrane properties (Beranek et al., 2007). In response to injection of positive current steps phasic neurons exhibit a high-frequency burst of 1-5 spikes, with monophasic AHP, whereas tonic neurons fire continuously spikes with a biphasic AHP. Such membrane properties tune phasic and tonic 2^oOVN in band-pass and low-pass neuronal filters, respectively.

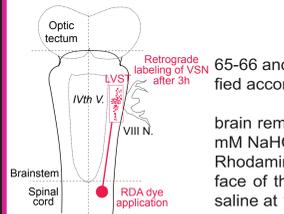
Consequently, phasic neurons could encode motion-related high dynamic sensory-motor signals whereas tonic neurons could encode position-related slow dynamic signals in vestibulo-motor behaviors. Nonetheless this characterization was based on 2^oOVN that were not related to a specific vestibular functional pathway.

The metamorphosis in *Xenopus laevis* toad, a purely aquatic anuran, offers a unique opportunity to unravel integrative properties of central vestibular neurons in relation to a specific vestibular sensory reference frame (aquatic vs terrestrial, otolith vs canal) and to a given locomotor system (tail- vs limb based swimming).

This study aims to investigate:

- Intrinsic membrane properties exhibited by 2^oOVN from identified LVST pathway at both larval and adult-like stages.
- Underlying developmental plasticity mechanisms related to the posturo-locomotor system re-modeling.

Materials and methods

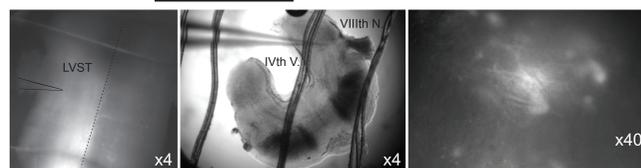
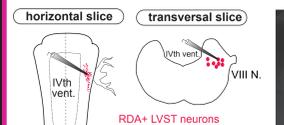


Animals and Tissue preparation

Experiments were performed on post-metamorphosis adult-like (juvenile) stage 65-66 and larval stage 53-56 of south African clawed toad *Xenopus laevis*. Stages were identified according to external body criteria (Nieuwkoop and Faber, 1956).

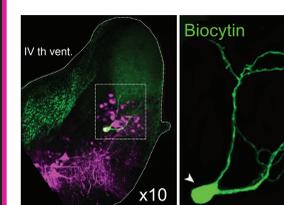
Briefly, consecutive to anesthesia in a 0.05% MS-222 water solution and after fore-brain removal, the CNS was dissected in cold Ringer's saline (93.5 mM NaCl, 3 mM KCl, 30 mM NaHCO₃, 0.5 mM NaH₂PO₄, 2.6 mM CaCl₂, 1 mM MgCl₂, and 11 mM glucose, pH 7.4). Rhodamine dextran (RDA) crystals were applied in a tiny incision performed at the ventral surface of the rostral hemi-cord. CNS in vitro preparation was incubated in circulating Ringer's saline at 16°C for at least 3h to allow retrograde labeling of vestibulospinal neurons.

Patch-clamp recording



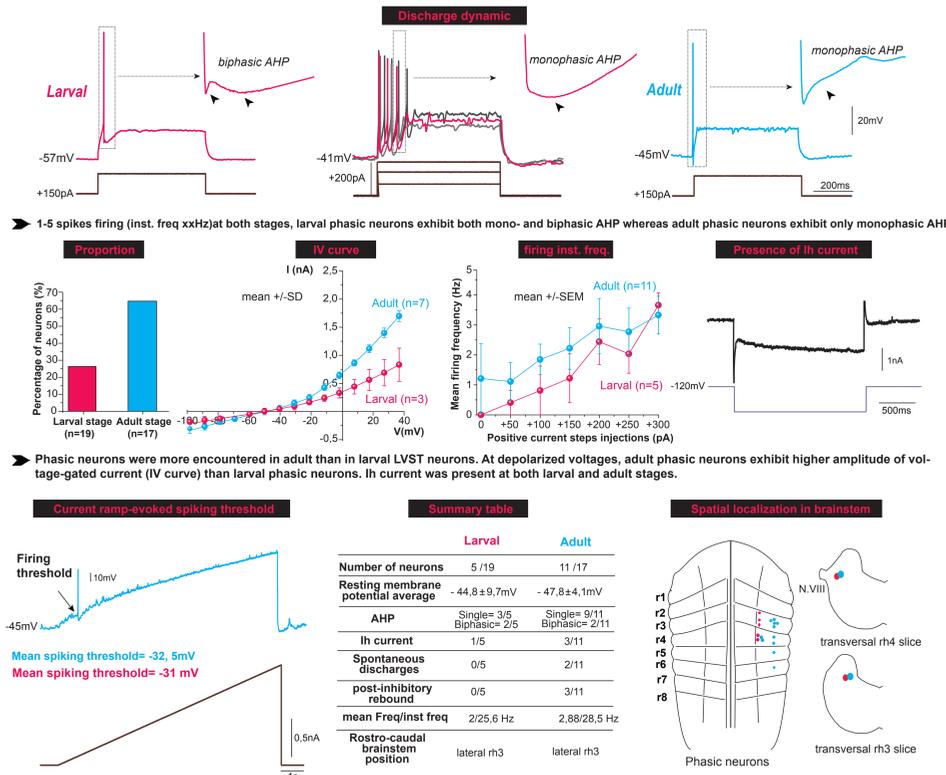
Patch-clamp recordings of RDA+ LVST neurons were performed in whole-cell configuration on 350µm transversal or horizontal brainstem slices continuously perfused with oxygenated Ringer's solution at 18-20°C. Patch electrodes were filled with intracellular solution (in mM= 115 K-gluconate, 2 MgCl₂, 2 EGTA, 10 HEPES, 2 MgATP, 0.2 NaGTP and 0.01% biocytin). Different protocols in current clamp (-200 to +300pA current step serie and current ramp) and voltage clamp configuration (Ih, IV curve) were applied to measure discharge dynamics and basic membrane properties of RDA+ LVST neurons.

Fluorescent confocal imaging after recording

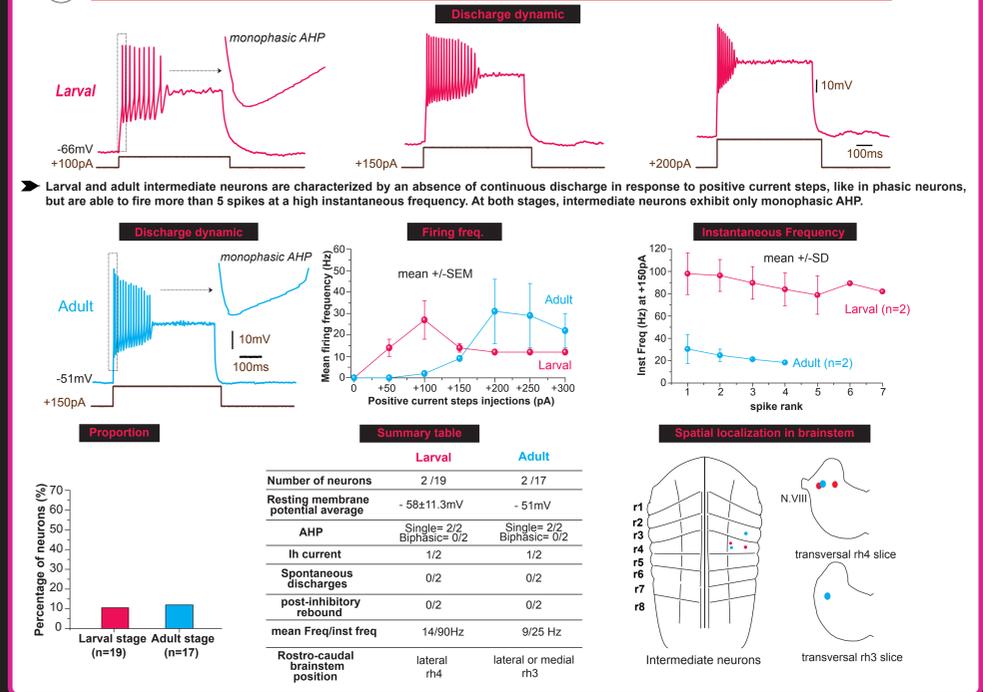


Patch-clamp recorded RDA+ LVST neurons were intracellularly filled with biocytin. After recordings slices were fixed in PFA 4% and revealed with Alexa fluor 488 streptavidin. Slices were imaged with a confocal microscope at 10x and 40x to allow a good medio-lateral and rostro-caudal localization of recorded neurons within the entire LVST population.

A Intrinsic membrane properties of phasic neurons: Larval Vs adult xenopus

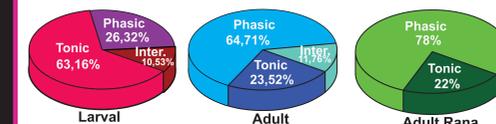


C Intrinsic membrane properties of intermediate neurons: Larval Vs adult xenopus

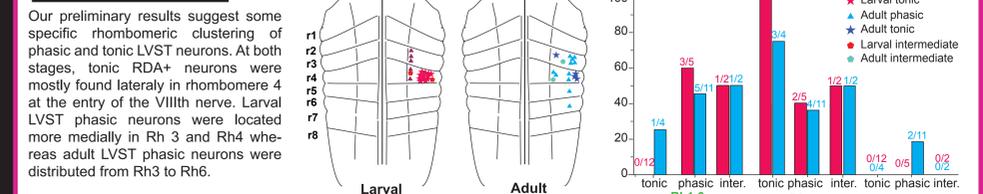


Conclusion

Proportion of tonic and phasic neurons in xenopus compared to adult Rana



Rostro-caudal brainstem position



Our preliminary results suggest some specific rhombomeric clustering of phasic and tonic LVST neurons. At both stages, tonic RDA+ neurons were mostly found laterally in rhombomere 4 at the entry of the VIIIth nerve. Larval LVST phasic neurons were located more medially in Rh3 and Rh4 whereas adult LVST phasic neurons were distributed from Rh3 to Rh6.

► Preliminary results show that LVST larval and adult neurons (phasic/tonic) differ by the type of AHP, the discharge dynamics (mean and inst. firing freq.), the expression of hyperpolarization-activated conductance (Ih). Furthermore, adult LVST neurons (at least phasic) seem to exhibit a higher amplitude of voltage-gated current (IV) than larval neurons that suggests some ongoing maturation processing during metamorphosis. Rectifying IV curve and presence of Ih current in some of LVST neurons suggest the expression of voltage-dependent K⁺ conductances that are known to be involved in filtering properties of vestibular neurons like ID conductance (depending of Kv1.1 channel), previously described to confer band-pass filter characteristic in rana phasic neurons (Beranek et al., 2007). Therefore it will be interesting to investigate in detail the spectrum of Na⁺ and K⁺ conductances involved in the setting of AHP shape and of discharges dynamics. These differences observed in intrinsic membrane properties between larval and adult xenopus could reflect a developmental adaptation in LVST pathway related to the metamorphosis-induced remodeling of the posturo-locomotor system.

To be continued...

► Electrical stimulation of VIIIth nerve branches, with trains of current pulses that occur with instantaneous frequencies following a sinus temporal waveform, will allow investigating neuronal filtering properties of LVST RDA+ neurons.

► Comparison of membrane intrinsic properties with vestibulo-ocular neurons, involved in other vestibulo-motor function and commissural vestibular neurons, involved in the push-pull pathways between left and right vestibular nuclei.