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

Olechowski-Bessagueta, A., Cardolet, L., Thoby-Brisson M., Lambert F.M.

Introduction

In vertebrates, central vestibular neurons (2° VN) are subdivided in distinct populations according to their properties, projection patterns and developmental history. In amphibians, vestibular neurons are organized in transversal reticular slices exhibiting a laminar organization, as in the rhombencephalon of adult vertebrates (Beraneck et al., 2007). In fact, intrinsic membrane properties between larval and adult central vestibular neurons (LVST) differ in sequence and Amacrine (Nagata et al., 1987) and intrinsic membrane properties probably reflect functional and phylogenetic differences (Beraneck et al., 2007). However, the proportion was inverted from histological examination of vestibular neurons from Straka et al. (2001). Hence, we focused on the LVST neurons, larval and adult stages, which could be related to specific vestibular functional pathways. In summary, our preliminary results suggest some 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. This study aims to investigate intrinsic membrane properties of vestibular neurons between larval and adult stages.

Materials and methods

Experiments were performed on post-metamorphic 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 (Newkirk and Faber, 1956). Briefly, successive to anesthesia in a 0.05% MS-222 water solution and after forebrain removal, the CNS was dissected in cold Ringer’s solution (0.1 M NaCl, 3 mM KCl, 30 mM NaHCO3, 0.5 mM NaHPO4, 2.6 mM CaCl2, 1 mM MgSO4, and 11 mM glucose, pH 7.4). Posterior double (RDA) crystals were applied to a binary injection performed at the ventral surface of the rostral hindbrain. CNS in vitro preparation was incubated in circulating ringer’s saline at 18°C for at least 3 h to allow membrane labeling of vestibular neurons.

Patch-clamp recordings of RDA-LVST neurons were performed in whole-cell configuration on 35μm transversal or horizontal transverse slices continuously perfused with oxygenated Ringer’s solution at 18°C. The neurons were filled with intracellular solution (in mM: 115-Kgluconate, 2-MgCl2, 2-ESCA, 10 HEPES, 2-MgATP, 0.2-MgEGTA and 2-mM NaH2PO4). Different protocols were used to characterize intrinsic membrane properties of RDA-LVST neurons.

Discussion

Phasic and tonic neurons were found in the LVST at larval and adult stages. Adult neurons exhibit a higher proportion of phasic and tonic neurons than in larval stage, with similar intrinsic membrane properties (Beraneck et al., 2007). However, the proportion was inverted from histological examination of vestibular neurons from Straka et al., 2001.

Intrinsic membrane properties of intermediate neurons: Larval Vs adult xenopus

Our preliminary results suggest some 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.

Conclusion

Preliminary results show that LVST larval and adult neurons (phasic/tonic) offer by the type of ANK the discharge dynamic (mean and firing rates), the expression of hyperpolarization-activated conductance (H). Furthermore, adult LVST neurons (larval phases) exhibit a higher proportion of intrinsically firing ANK than in larval phases, suggesting some ongoing maturation process during metamorphosis. Both 2° VN curve and presence of 2° VN in some larval LVST neurons suggest the expression of dendro-specific INK conductances that are known to be involved in regulating membrane properties (Beraneck et al., 2007). The present study showed that LVST ANK and INK conductances involved in the setting of 2° VN dynamic and dendrites dynamics. These differences observed in intrinsic membrane properties between larval and adult stages could reflect a developmental adaptation in LVST pathway related to the metamorphosis-induced remodeling of the posturo-locomotor system.

To be continued...

Patch-clamp recordings of RDA-LVST neurons were performed in whole-cell configuration on 35μm transversal or horizontal transverse slices continuously perfused with oxygenated Ringer’s solution at 18°C. The neurons were filled with intracellular solution (in mM: 115-Kgluconate, 2-MgCl2, 2-ESCA, 10 HEPES, 2-MgATP, 0.2-MgEGTA and 2-mM NaH2PO4). Different protocols were used to characterize intrinsic membrane properties of RDA-LVST neurons.

FIGURE 1: Intrinsic membrane properties of phasic neurons: Larval Vs adult xenopus

A) Phasic neurons were more encountered in adult than in larval LVST neurons. As depicted on diagrams, adult phasic neurons exhibit higher amplitude of voltage-gated current (I-V curves) than larval phasic neurons. Bar current were present at both larval and adult stages. Stages were identified according to external body criteria (Newkirk and Faber, 1956). Briefly, successive to anesthesia in a 0.05% MS-222 water solution and after forebrain removal, the CNS was dissected in cold Ringer’s solution (0.1 M NaCl, 3 mM KCl, 30 mM NaHCO3, 0.5 mM NaHPO4, 2.6 mM CaCl2, 1 mM MgSO4, and 11 mM glucose, pH 7.4). Posterior double (RDA) crystals were applied to a binary injection performed at the ventral surface of the rostral hindbrain. CNS in vitro preparation was incubated in circulating ringer’s saline at 18°C for at least 3 h to allow membrane labeling of vestibular neurons.

B) Intrinsic membrane properties of tonic neurons: Larval Vs adult xenopus

Phasic and tonic neurons were found in the LVST at both larval and adult stages. Adult LVST exhibited some proportion of phasic and tonic neurons that was called to adult stage, with similar intrinsic membrane properties (Beraneck et al., 2007). However, the proportion was inverted from histological examination of vestibular neurons from Straka et al. (2001). Hence, we focused on the LVST neurons, larval and adult stages, which could be related to specific vestibular functional pathways. In summary, our preliminary results suggest some 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.

This study aims to investigate intrinsic membrane properties exhibited by LVST neurons from identified LVST pathway at both larval and adult stages. Underlying developmental plasticity mechanisms related to the posturo-locomotor system are re-modeling.