Developmental Switching of Nicotinic Acetylcholine Receptor Subunits Supports Central Cholinergic Synapse Maturation

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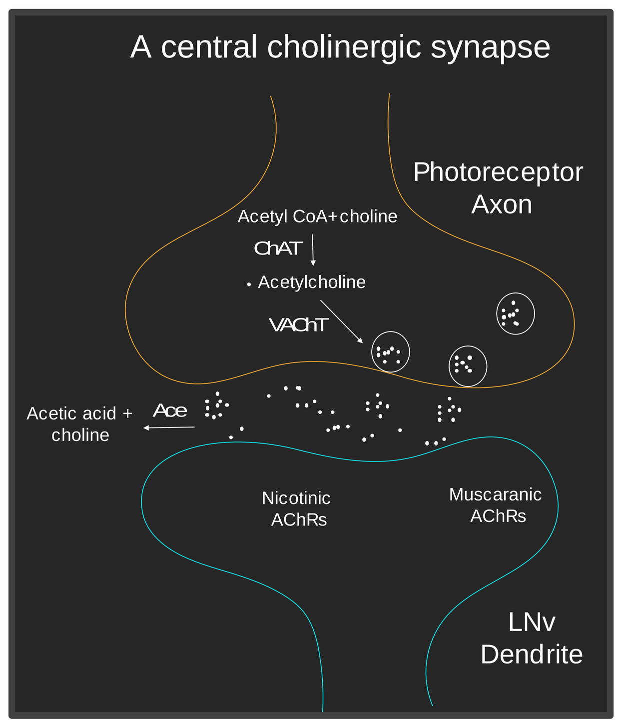
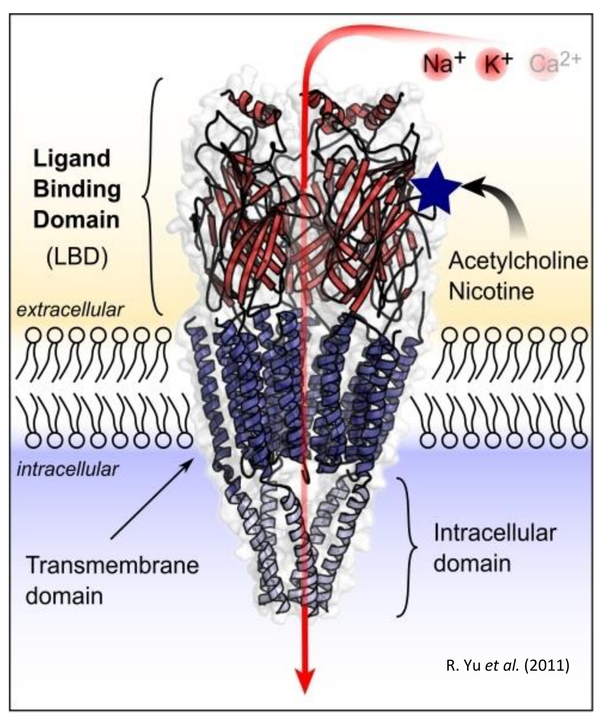
Introduction

Construction and maturation of the postsynaptic apparatus are crucial for synapse and dendrite development. The fundamental mechanisms underlying these processes are most often studied in glutamatergic central synapses in vertebrates. Whether the same principles apply to excitatory cholinergic synapses, such as the ones found in the insect central nervous system (CNS) is not known. To address this question, we investigated *Drosophila* ventral lateral neurons (LNvs) and identified nAchRα1 (Dα1) and nAchRa6 (Dα6) as the main functional nicotinic acetylcholine receptor (nAchR) subunits in these cells. With morphological and calcium imaging studies, we demonstrated distinct roles of these two subunits in supporting dendrite morphogenesis and synaptic transmission. Furthermore, our analyses revealed a transcriptional upregulation of Dα1 and downregulation of Dα6 during larval development, indicating a close association between the temporal regulation of nAchR subunits and synapse maturation. Together, our findings show transcriptional regulation of nAchR composition is a core element of developmental and activity-dependent regulation of central cholinergic synapses.



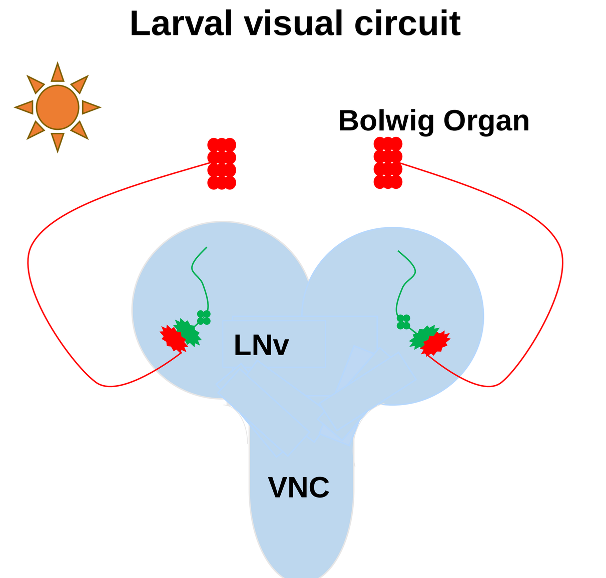
nAchR subunits form a pentameric channel



Methods

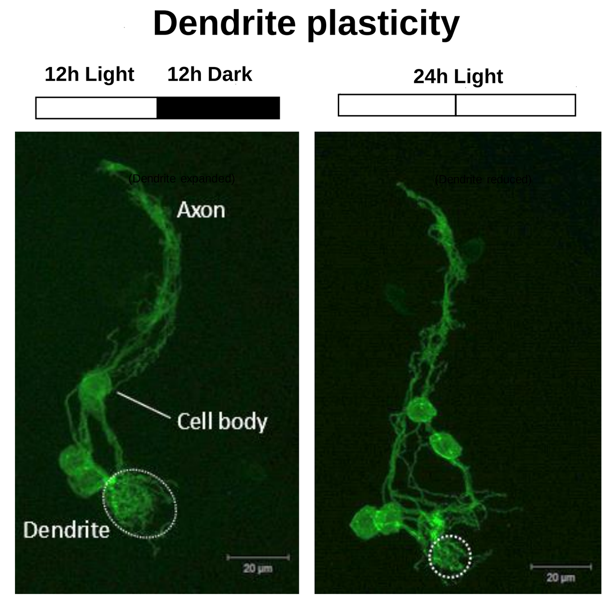
1. Morphological studies on LNv dendrites

Larval visual circuit



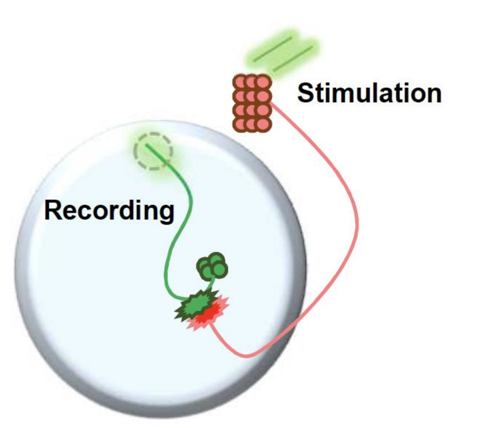
LNvs are projection neurons in the larval visual circuit, receiving inputs from photoreceptors.

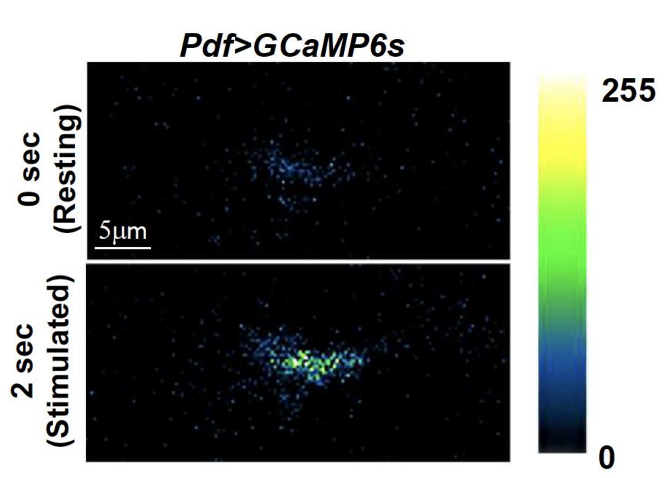
Dendrite plasticity



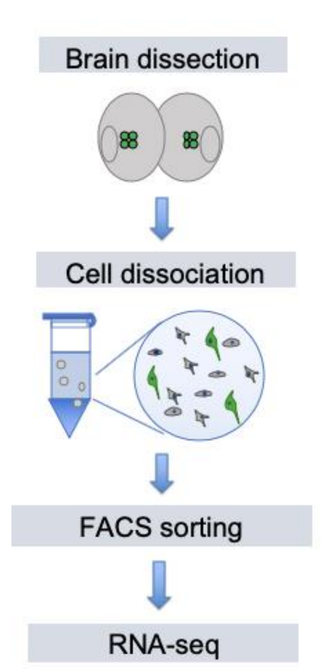
LNvs display experience-dependent dendritic plasticity. LL conditions reduce the size of LNv dendrites.

2. Physiological studies using calcium imaging





3. RNA-seq analyses and genetic screens



			Number	Subunit Gene Name
Acetylcholine receptors	Nicotinic	α	1	nAchRα1(CG5610)
			2	nAchRα2(CG6844)
			3	nAchRα3(CG2302)
			4	nAchRα4(CG12414)
			5	nAchRα5(CG32975)
			6	nAchRα6(CG4128)
			7	nAchRα7(CG32538)
		β	1	nAchRβ1(CG11348)
			2	nAchRβ2(CG6798)
			3	nAchRβ3(CG11822)
	Muscarinic		1	mAchR-A(CG4356)
			2	mAchR-B(CG7918)

Results

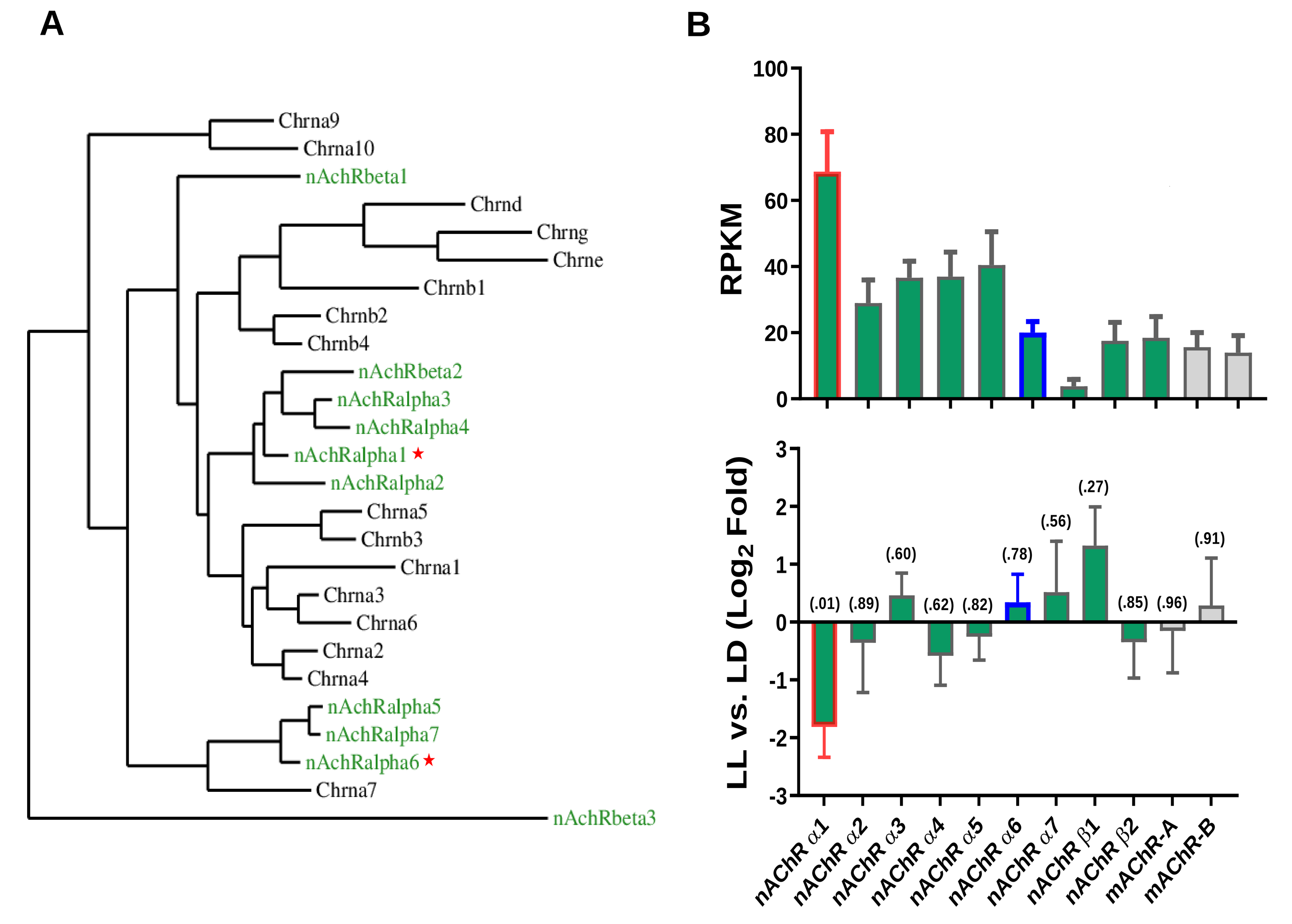


Figure 1. Phylogenetic relationship between *Drosophila* nAchR subunits and their expression within the larval LNv. (A) 10 nAchR subunits in the *Drosophila* genome (green) along with their human homologs (black). (B) LNv-specific RNA-seq analyses revealed the expression of both nicotinic and muscarinic Ach receptor subunits. Top: transcript levels from larvae raised in the LD condition; Bottom: Activity-dependent changes of nAchRa1 revealed by comparing LL vs. LD conditions.

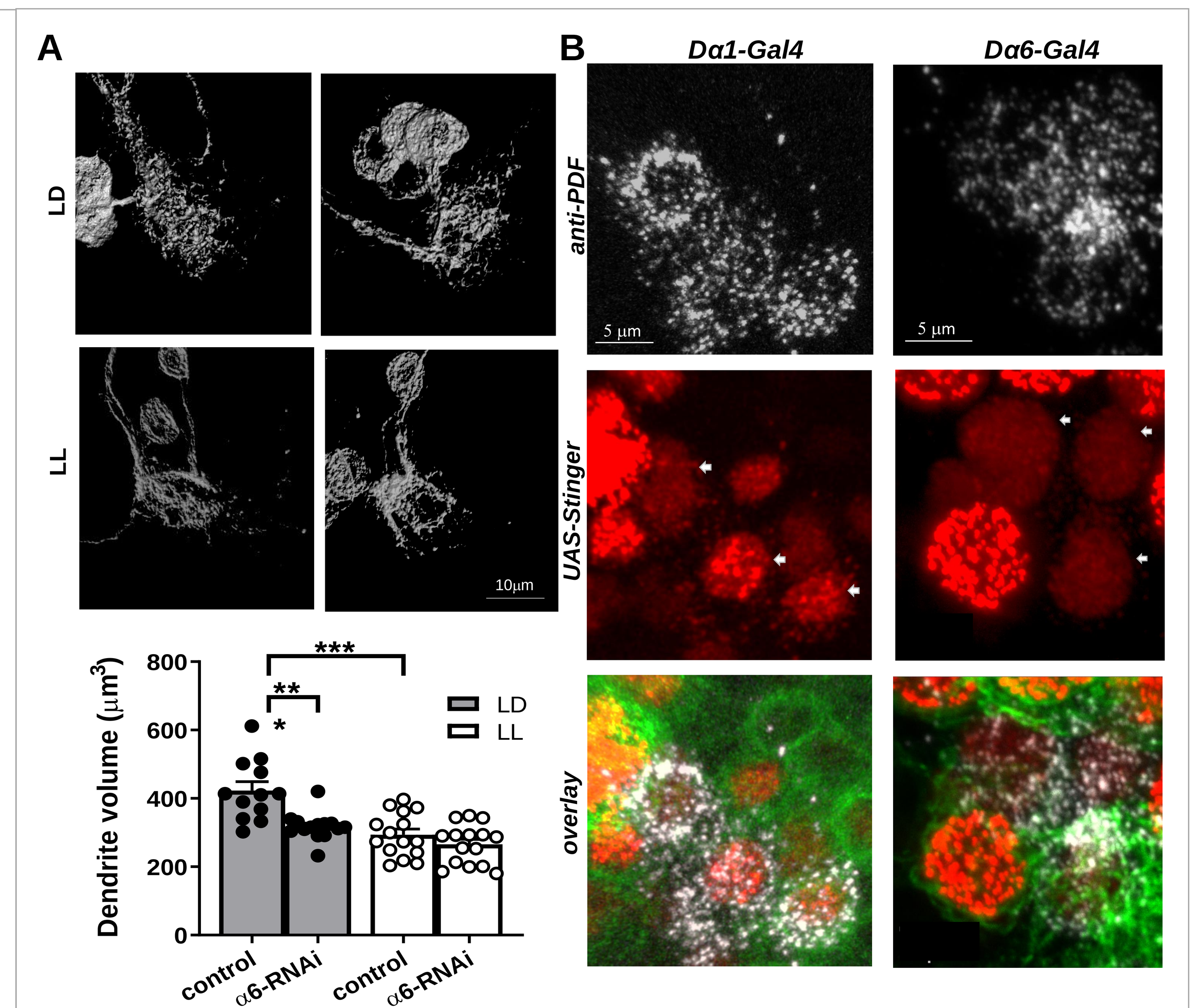


Figure 2. The role of Dα6 in the structural development of the larval LNv dendritic arbor. (A) RNAi knockdown of Dα6 in the LNv reduces the dendrite volume in the LD condition, and eliminates the structural plasticity induced by the LL condition. (B) Expression of both Dα1 and Dα6 is confirmed in the LNv using the Trojan-Gal4 gene trap technique.

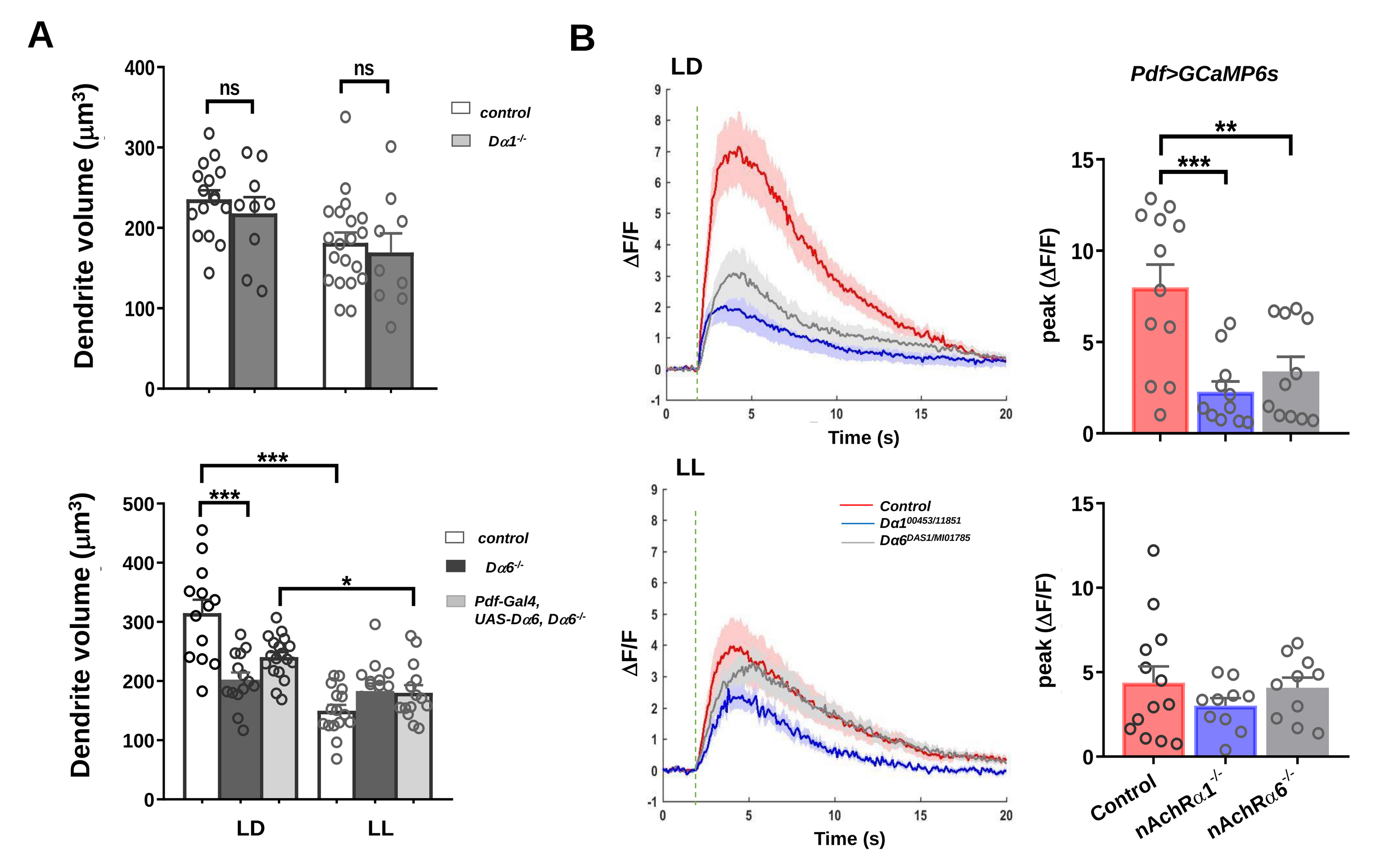


Figure 3. Distinct functions of Dα1 and Dα6 in the developing LNvs. (A) Morphological analysis of nAchR mutants reveals that Dα6, but not Dα1, is critical for development of the LNv dendritic arbor. Compared to the control group, Dα6 mutants showed a significant reduction of LNv dendrite volume. (B) Both Dα1 and Dα6 are essential for proper LNv neurophysiology. Light evoked calcium responses in LNvs are severely dampened in the mutants of both subunits. Left: Average traces of calcium transients induced by light pulses (green) are show. Right: quantifications of the amplitude of the calcium responses.

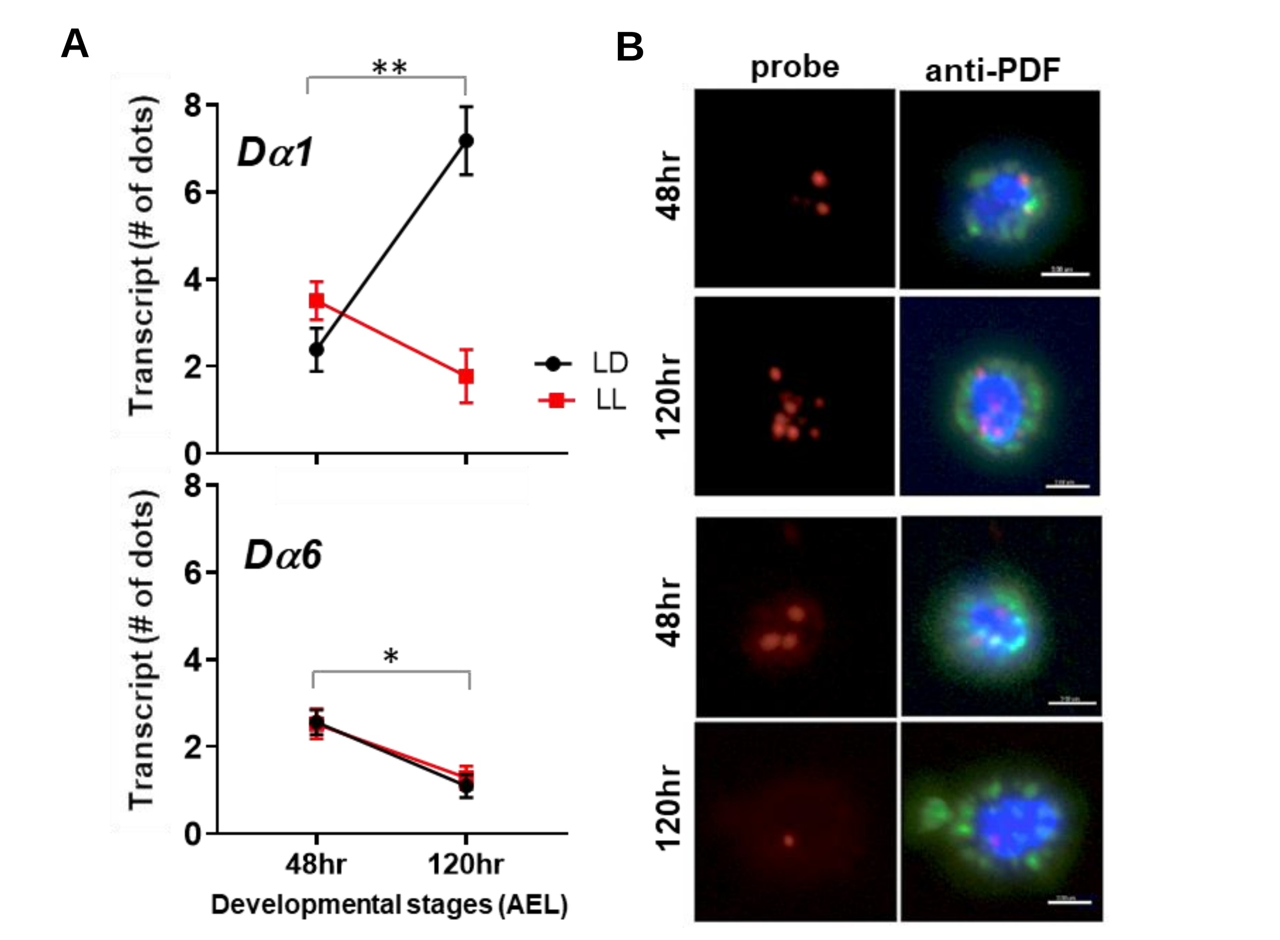


Figure 4. Expression of Dα1 and Dα6 is regulated by developmental stage and neuronal activity. (A) Quantitative *in situ* hybridization in dissociated larval LNvs reveal distinct temporal profiles of Dα1 and Dα6 transcripts. Dα1(top) is upregulated during development whereas Dα6(bottom) is reduced. Moreover, LL conditions eliminate the upregulation of Dα1 transcripts whereas the downregulation of Dα6 transcripts is not affected by light conditions. (B) Representative images from the qFISH experiments.

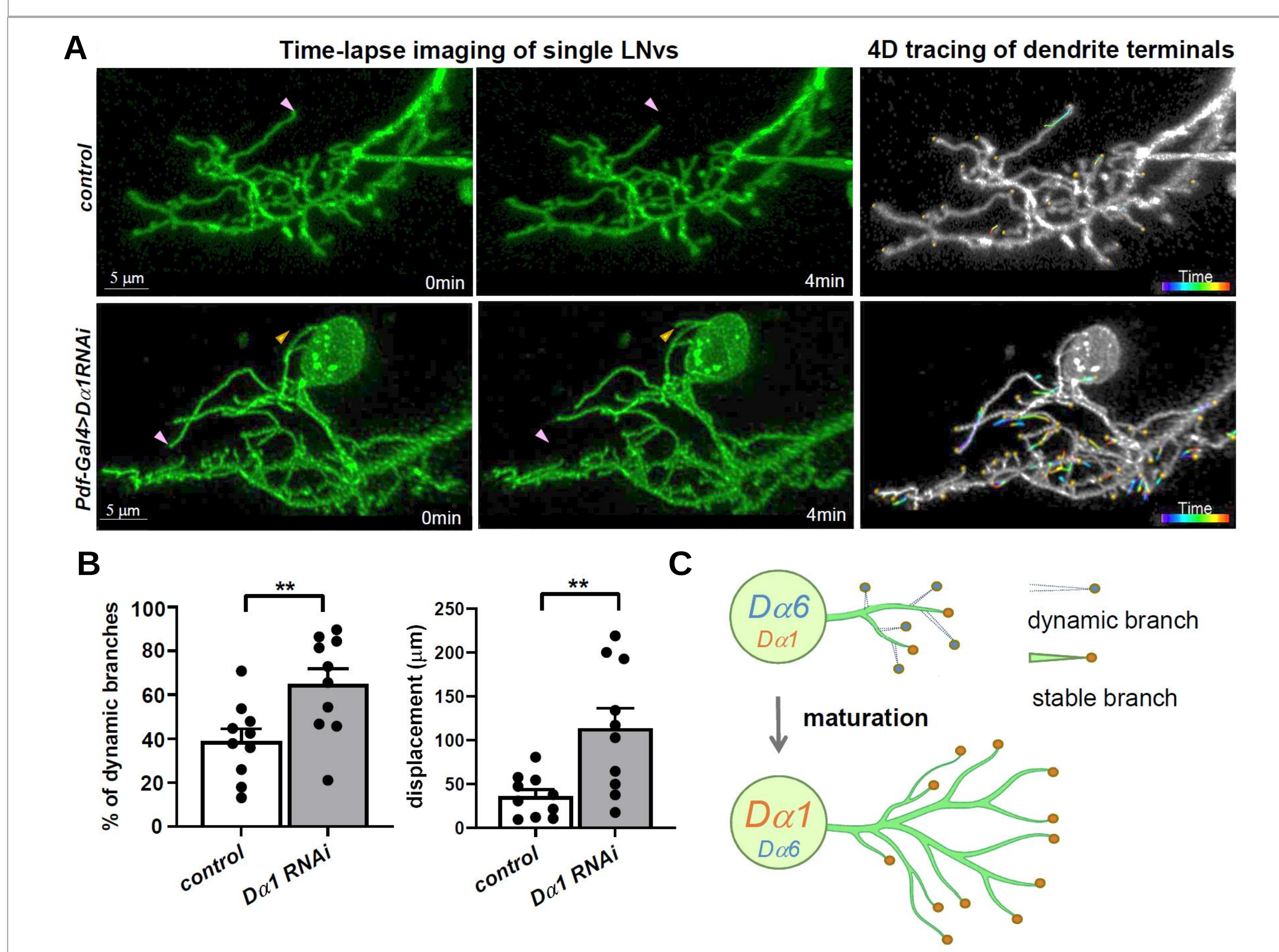
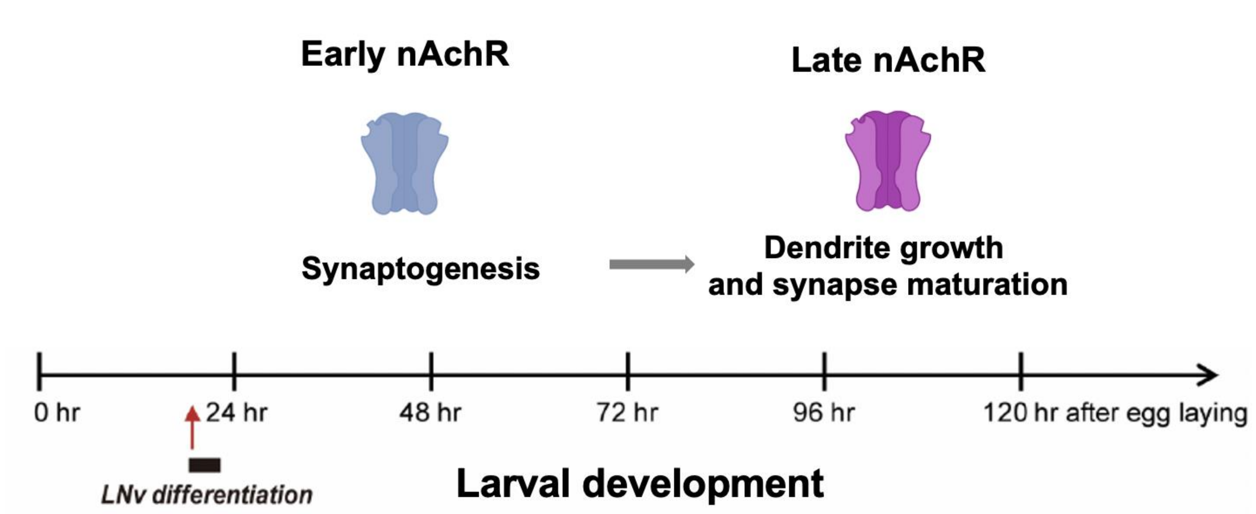


Figure 5. Dα1 is required for the stabilization of LNv dendrites during larval development. (A) LNvs of Dα1-deficient animals display highly dynamic dendritic filopodia associated with immature synapses. (B) Quantification of dendrite dynamics: a significant increase is seen in the percentage of dynamic branches (left) as well as total displacement of branches (right). (C) A diagram illustrating the role for developmental switching of nAchR subunits in dendrite dynamics and synapse maturation.

Conclusions

- Both Dα1 and Dα6 are expressed in the developing larval LNvs.
- Dα6 is required for LNv dendrite morphogenesis and physiology, whereas Dα1 is only necessary for LNv's synaptic transmission.
- The developmental regulation of Dα6 and Dα1 transcripts generates a coordinated subunit switch that is critical for central cholinergic synapse maturation and dendrite development.



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