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An explant muscle model to examine the refinement of the synaptic landscape

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HIGHLIGHTS

- Use of innovative whole muscle explant system examining the dynamics of AChR cluster prepatterning.
- RyR1 activity and DHPR activity repress Wnt3a ability to eliminate aneural AChRs.
- RyR1 counteracts the ability of Agrin to aggregate AChR clusters.
- RyR1 is required for proper distribution of aneural and neuronal AChR clusters.
- PKA and CDK5 are molecular players within the muscle that control regionalization of aneural AChR clusters.

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ABSTRACT

Signals from nerve and muscle regulate the formation of synapses. Transgenic mouse models and muscle cell cultures have elucidated the molecular mechanisms required for aggregation and stabilization of synaptic structures. However, far less is known about the molecular pathways involved in redistribution of muscle synaptic components. Here we established a physiologically viable whole-muscle embryonic explant system, in the presence or absence of the nerve, which demonstrates the synaptic landscape is dynamic and malleable. Manipulations of factors intrinsic to the muscle or extrinsically provided by the nerve illustrate vital functions during formation, redistribution and elimination of acetylcholine receptor (AChR) clusters. In particular, RyR1 activity is an important mediator of these functions. This physiologically relevant and readily accessible explant system provides a new approach to genetically uncouple nerve-derived signals and for manipulation via signaling molecules, drugs, and electrical stimulation to examine early formation of the neuromuscular circuit.

1. Introduction

In the nervous system, patterns of electrical activity induce pre-synaptic molecular mechanisms involved in axon guidance and fine-tuning of connections to target tissues (Hanson and Landmesser, 2006, 2004; Hubel and Wiesel, 1962). The motor axon connection with the muscle results in the formation of large synapses, known as neuromuscular junctions (NMJ), and the postsynaptic NMJ in postnatal animals has been well studied. During embryogenesis and development of the axon-muscle connection, the muscle assembles the synaptic components that will eventually comprise the NMJ postsynaptic apparatus. The main synaptic components assembled and clustered in the embryonic muscle are acetylcholine receptors (AChR). Transgenic studies provide evidence that AChR clusters can assemble independently of the nerve (Lin et al., 2001). Axons of the phrenic nerve innervate the mouse diaphragm at embryonic day 13.5 (E13.5). As the nerve grows on the muscle at E14.5, aneural AChR clusters distribute near but not directly apposed to the nerve (Lin et al., 2001), which has been called "prepatterning" (Fig. 1a) (Lin et al., 2001; Lomo, 2003; Witzemann, 2006; Yang et al., 2001). After E16.5, AChR clusters are juxtaposed to the nerve (Lin et al., 2001). The change of AChR clusters from broad prepattern to redistribution at the nerve endplates suggests that the embryonic nerve influences the organization of AChR clusters (Misgeld et al., 2002). Communication from the nerve onto the muscle induces muscle-intrinsic pathways that function in redistribution of AChR clusters (Brandon et al., 2003; Lin et al., 2005; Misgeld et al., 2002, 2005; Pacifici

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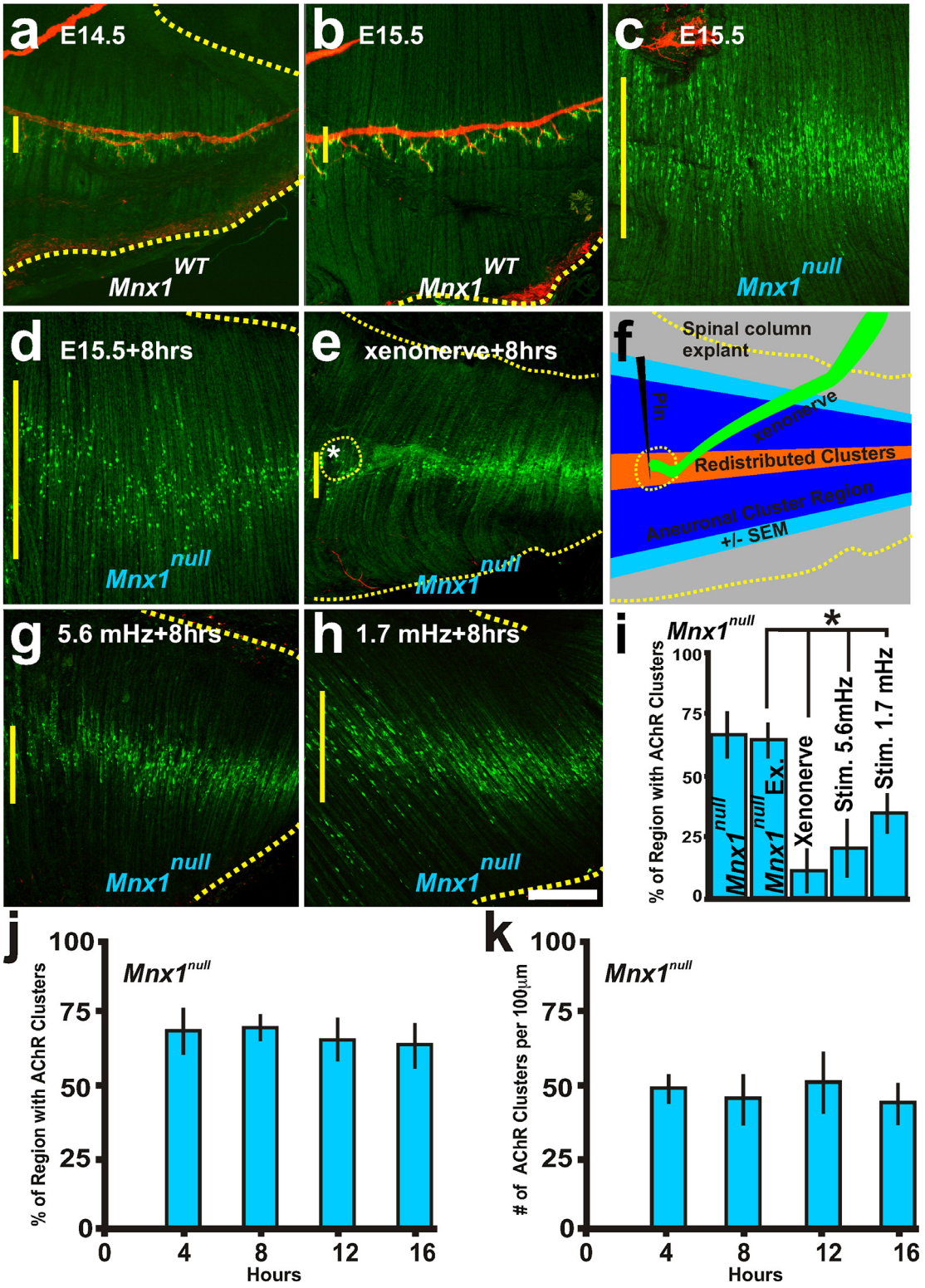

Fig. 1. Neuronal and electrical activity cues control the distribution of AChR clusters. Representative examples of AChR clusters (α-bungarotoxin conjugated Alexa-488; green) and the phrenic nerve (neurofilament-160; red). (a and b) E14.5 and E15.5 diaphragm from wildtype mice with AChR clusters apposed to the phrenic nerve. (c) E15.5 diaphragms from *Mnx1*^{null} mice without a phrenic nerve. (d) E15.5 *Mnx1*^{null} diaphragm explant cultured for 8 h. (e) Littermate *Mnx1*^{null} diaphragm with *Mnx1*$^{GFP/+}$ spinal cord-phrenic nerve explant pinned during 8 h culture (asterisk and dotted yellow line indicate xenonerve placement, the xenonerve detached during fixation) shows a narrowed AChR cluster region compared to (d). (f) Schematic of explant showing the region of aneural AChR clusters at the start of the experiment (blue) and the redistribution and centralization of AChR clusters in the presence of the xenonerve (orange). (g and h) E15.5 *Mnx1*^{null} diaphragms stimulated every 3 min (5.6 mHz) or 10 min (1.7 mHz) for 8 h induces redistribution and elimination of AChR clusters. (i) Quantification of the distribution of AChR clusters within the *Mnx1*^{null} explant diaphragms before and after culture, with xenonerve, and following electrical stimulation. (j and k) Percent regionalization (j) and number (k) of AChR clusters at 4, 8, 12, and 16 h of culture of *Mnx1*^{null} diaphragm explants. Scale bar = 200 μm; yellow vertical line on the left of each panel represents the largest width of the AChR cluster region within ventral quadrant of diaphragm shown. Each condition was examined in 5 diaphragms.