Change in the sensitivity of transmitter release to calcium at β2-laminin deficient nerve terminals

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Neurotransmission at a mature neuromuscular junction requires the precise alignment of pre- and postsynaptic elements across the synaptic cleft. One prominent synaptic organiser and mediator of presynaptic nerve terminal differentiation is β2-laminin. β2-laminin deficient neuromuscular junctions exhibit severe presynaptic aberrations (Noakes *et al.*, 1995) and reduced neural transmission (Knight *et al.*, 2003). As a consequence of these studies we investigated the calcium cooperativity of transmitter release in β2-laminin deficient mice. The power relationship between transmitter release and extracellular calcium concentration is a fourth order of cooperativity in mammalian and amphibian neuromuscular junctions (Dodge & Rahamimoff, 1967; Hubbard *et al.*, 1968). We examined transmitter release using extracellular recording techniques, confirming the previously reported reduction in release at mutant terminals and found no difference in the cooperativity relationship between wild-type (3.20 ± 0.085, n=11) and mutant (3.54 ± 0.20, n=17) release sites. We also found that mutant nerve terminals show no significant differences in the paired-pulse facilitation ratios at low frequency of stimulation, even though evoked transmitter release was reduced by over 50%. However, we did observe a rightward parallel shift of the extracellular calcium *vs* transmitter release relationship, indicating a change in the calcium sensitivity in β2-laminin deficient terminals. The results suggest that the reduction in calcium sensitivity leads to a drop in transmitter release, resulting from a possible drop in the density of presynaptic voltage-gated calcium channels (P/Q type) at release sites (active zones). Our hypothesis is supported by the observation that β2-laminin binds to presynaptic voltage-gated calcium channels (VGCCs), which results in VGCC clustering and subsequent synaptic vesicle accumulation, i.e. formation of the active zone (Nishimune *et al.*, 2004).

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