Comment on "Reelin Promotes Peripheral Synapse Elimination and Maturation"

Individual synaptic sites on rodent skeletal muscle fibers are innervated by two or more axons at birth. All inputs but one are then eliminated during the first two postnatal weeks, leaving each muscle fiber singly innervated. Quattrocchi et al. (2) reported that polyneuronal innervation of neuromuscular synapses persists well beyond this period in reeler mice (mutant mice lacking Reelin, a protein important for brain development). Their evidence was in large part histological: Axons were labeled with antibodies to neurofilaments and synaptic vesicle proteins, and postsynaptic sites were labeled with α-bungarotoxin, which binds specifically to acetylcholine receptors in the postsynaptic membrane. Neuromuscular junctions were described as showing multiple sites. The finding that Reelin, a protein important for brain development, appeared to be innervated by two axons in a few cases (<10%), single endplates appeared to be innervated by two axons of the mutants were studied at ages after synapse elimination is complete in control animals [postnatal day (P)15–39]. We found no evidence of extensive polyneuronal innervation in any of these reeler muscles. That is, ≤2% of neuromuscular junctions per muscle were polyneuronally innervated in both mutants and controls. In contrast, Quattrocchi et al. reported an incidence of 42.7% polyneuronal innervation at P30 (2). In a few cases (<10%), single endplates appeared to be innervated by two axons.

We assessed polyneuronal innervation in muscles from a total of 13 reeler mutants. We imaged neuromuscular junctions in the diaphragm, triangularis sterni, sternomastoid, soleus, tibialis anterior, and extensor digitorum longus muscles from at least two mutant animals. This set includes most of the muscles studied in (2). Ten of the mutants were studied at ages after synapse elimination is complete in control animals [postnatal day (P)15–39]. We found no evidence of extensive polyneuronal innervation in any of these reeler muscles. That is, ≤2% of neuromuscular junctions per muscle were polyneuronally innervated in both mutants and controls. In contrast, Quattrocchi et al. reported an incidence of 42.7% polyneuronal innervation at P30 (2). In a few cases (<10%), single endplates appeared to be innervated by two axons; per hemidiaphragm did not differ between reeler mice (mean ± SD: 3640 ± 693, n = 3) and littermate controls (3344 ± 463, n = 3, P = 0.57, Student’s t test). We also stained diaphragms for acetylcholinerase as an alternate means of marking synaptic sites (5), and then teased fibers from the muscles. Of 134 fibers examined, none had more than one synaptic site (Fig. 3).

Quattrocchi et al. (2) further stated that synaptic maturation is impaired in the absence of Reelin, reporting a 31 to 57.2% decrease in neuromuscular junction area at P7–P30 (table 1 in (2)]. They argued that this decrease could not be accounted for by the muscle atrophy that occurs in reeler mutants. We also noted that muscles were atrophic in older reeler mice and found a decreased synaptic size (acetylcholine receptor-rich area at P28 in diaphragm; control mean ± SEM: 347 ± 13.5 μm², n = 24; reeler, 291 ± 13.0 μm², n = 23; P < 0.01, Student’s t test). However, we believe that atrophy could account for the decreased synaptic size; when we measured synaptic area in the diaphragm at P21, before atrophy is severe, we found no significant difference between control (mean ± SEM: 187 ± 8.4 μm², n = 28) and reeler mice (177 ± 7.2 μm², n = 29, P = 0.39).

As a second measure of maturation, Quattrocchi et al. (2) took advantage of the fact that the initially plaque-shaped postsynaptic apparatus becomes perforated and branched postnatally (1). They determined the percentage of synaptic sites that had >1 perforation in hindlimb muscle and reported that this fraction was 82.8% in controls, but only 35.8% in mutants at P30. We made similar measurements at P26 in triangularis sterni muscle, but found no difference in perforation (mean number of perforations or openings: 4.84 in reeler, 4.97 in littermate control; n = 150 synapses measured in each animal; P = 0.54, Student’s t test).

Although the same mutant allele sometimes has different phenotypes in different genetic backgrounds, we do not believe that strain differences explain our inability to...
replicate the results of Quattrocchi et al. (2). Weinzierl and Feng (Durham, NC) used reeler mice from Jackson Laboratory (Bar Harbor, ME), as did Quattrocchi et al. Bidoia, Buffelli, and Cangiano (Verona, Italy) used reeler mice originally obtained from Jackson Laboratories (gift of F. Keller, Universita Campus Bio-Medico, Rome) and subsequently crossed to YFP-16 mice (4); reeler,YFP-16 mice and littermate unaffected YFP-I mice were analyzed, as well as reeler mice from the same litters that were GFP-negative. The St. Louis group also examined a reeler mouse from the colony used in (2), kindly provided by G. D’Arcangelo (Baylor College of Medicine, Houston, TX) and illustrated in Fig. 2.

In summary, three groups of investigators, using animals from four different colonies, have been unable to replicate the main findings of Quattrocchi et al. (2). The Verona and St. Louis groups both assessed polynemal innervation. The Durham and Verona groups both assessed synapse number. The Durham and St. Louis groups both assessed synaptic maturity. We have no ready explanation for the difference between our results and those of Quattrocchi et al. (2).

C. Bidoia
Dipartimento di Scienze Neurologiche e della Visione
Universita’ di Verona
Strada Le Grazie 8, Verona, Italy

T. Misgeld
Department of Anatomy and Neurobiology
Washington University School of Medicine
660 S Euclid Street
St. Louis, MO 63110, USA

E. Weinzierl
Department of Neurobiology
Duke University Medical School
Durham, NC 27710, USA

M. Buffelli
Dipartimento di Scienze Neurologiche e della Visione
Universita’ di Verona

G. Feng
Department of Neurobiology
Duke University Medical School

A. Cangiano
Dipartimento di Scienze Neurologiche e della Visione
Universita’ di Verona

J. W. Lichtman
J. R. Sanes
Department of Anatomy and Neurobiology
Washington University School of Medicine
E-mail: sanesj@pcg.wustl.edu

References

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