Long-term potentiation in mice lacking the neural cell adhesion molecule L1

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Genetic evidence indicates that cell adhesion molecules of the immunoglobulin superfamily (IgCAMs) are critical for activity-dependent synapse formation at the neuromuscular junction in *Drosophila* and have also been implicated in synaptic remodelling during learning in *Aplysia* (see [1] for review). In mammals, a widely adopted model for the process of learning at the cellular level is long-term potentiation (LTP) in the hippocampal formation. Studies *in vitro* have shown that antibodies to the IgCAMs L1 and NCAM reduce LTP in CA1 neurons of rat hippocampus, suggesting a role for these molecules in the modulation of synaptic efficacy, perhaps by regulating synaptic remodelling [2]. A role for NCAM in LTP has been confirmed in mice lacking NCAM [3] (but see [4]), but similar studies have not been reported for L1. Here we examine LTP in the hippocampus of mice lacking L1 [5,6], using different experimental protocols in three different laboratories. In tests of LTP *in vitro* and *in vivo* we found no significant differences between mutant animals and controls. Thus, contrary to expectation, our data suggest that L1 function is not necessary for the establishment or maintenance of LTP in the hippocampus. Impaired performance in spatial learning exhibited by L1 mutants may therefore not be due to hippocampal dysfunction [6].

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Results and discussion

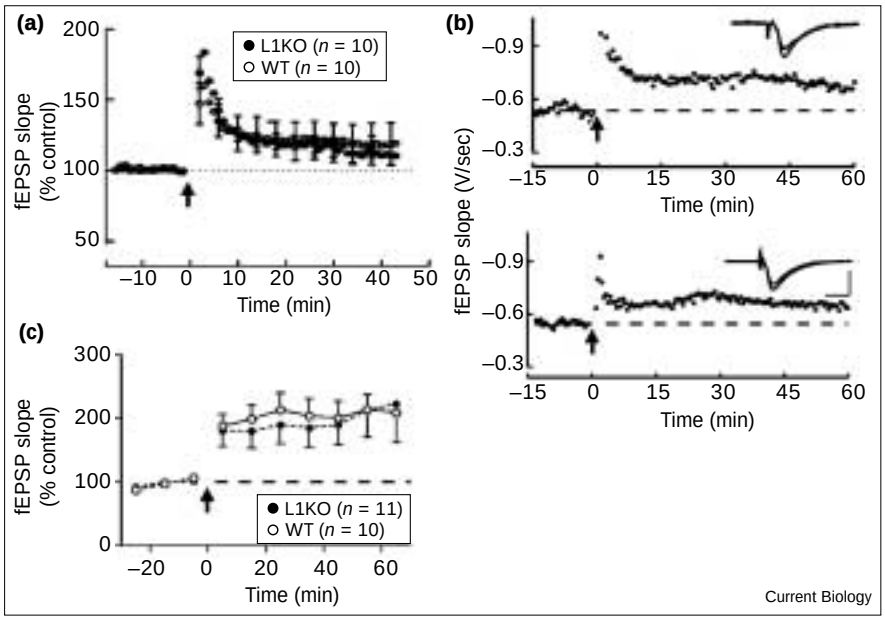
Protein immunoblotting and quantitative reverse transcription polymerase chain reaction (RT–PCR) indicated that L1 hemizygous mutant male mice (L1KO mice) do not make detectable L1 protein or mRNA [5,6]. To assess the sensitivity of the RT-PCR analysis performed previously [6], total mRNA preparations from wild-type littermates were diluted into similar preparations from L1 mutant mice and then subjected to nested RT–PCR. We were able to detect L1 mRNA when present at as little as 0.5% of normal levels (data not shown). Thus we conclude that if mRNA is being synthesised from the L1 gene in L1KO mice, it must be present at < 0.5% of normal levels.

Although a number of specific defects have been detected in the nervous system of our L1 mutant mouse [5–7] and an independently constructed L1 mutant mouse [8], the overall architecture of much of the nervous system, including the hippocampal formation, appeared normal. On specific strain backgrounds, a reduction of the volume of the hippocampus has been noted and aberrations were noted in a subpopulation of granule cells and pyramidal cells, but even in these mice "all types of neurons and interneuronal connections found in the wild type hippocampus were present in the hippocampus of L1KO mice" [7].

L1 protein is normally found distributed throughout the adult hippocampus and dentate gyrus, particularly in the dendritic layers containing synapses in which LTP can be induced [9]. We studied two of the three major excitatory pathways — the perforant path input to granule cells of the dentate gyrus and the Schaffer-commissural input to pyramidal cells of area CA1, the latter being the subject of the L1 antibody study [2]. Experiments were performed both *in vitro* and in anaesthetised mice.

In vitro recordings from acutely dissected hippocampal slices from wild-type control and L1KO males were made independently in two laboratories using distinct methodologies [10,11]. In both cases, induction of LTP was measured in the CA1 subfield after high-frequency stimulation of CA3 axons. In total, recordings were made in 21 hippocampal slices from 11 L1KO mice and from 21 slices from 11 wild-type controls (the results from the two laboratories are shown separately in Figure 1). Although the different protocols for inducing LTP led to a greater degree of LTP in the Antwerp experiments (Figure 1c) than in the Chapel Hill experiments (Figure 1a,b), it is clear that the magnitude of LTP for up to 60 minutes post-tetanus was the same in L1KO and wild-type animals in both laboratories. There was no significant difference between the two groups of mice in either study (ANOVA).

Figure 1



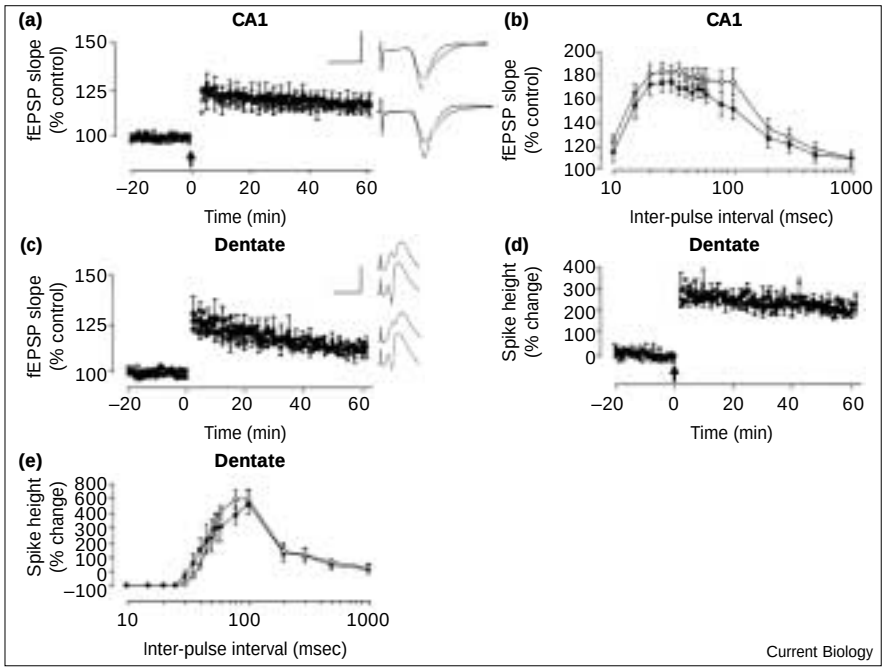
LTP *in vitro* is similar in area CA1 of wild-type and L1KO mice. Normalised mean slope (± SEM) of fEPSPs evoked in area CA1, as a function of time. **(a)** Data from 10 slices from three L1KO mice and 11 slices from three control wild-type (WT) littermates prepared as described [10]. **(b)** Data from an individual L1KO mouse and a wild-type littermate. Inset, sample responses. Calibration, 0.5 mV, 10 msec. **(c)** Normalised fEPSP slope averaged over 10 min intervals for each animal, and presented as group means ± SEM for control (n = 10 slices from 8 mice) and L1KO mice (n = 11 slices from 8 mice), prepared as described [11]. At 60 min post-tetanus, the fEPSP slope was increased by 119 ± 61% in L1KO compared to 111 ± 49% in the control. Arrows in (a–c) indicate application of high-frequency stimulation. In (a) and (b) (Chapel Hill data) this consisted of five stimulus pulses at 100 Hz delivered every 200 msec, repeated 10 times. This entire sequence was repeated four times, 20 sec apart. In (c) (Antwerp data) high-frequency stimuli consisted of 20 stimuli at 50 Hz, grouped in three trains separated by 10 sec intervals.

As an additional test of synaptic transmission, the field evoked postsynaptic potential (fEPSP) was recorded as a function of stimulus intensity. The input-output curves in the wild-type and L1KO slices were not significantly different (Antwerp group, data not shown). These results together indicate that baseline synaptic function, and the induction and early expression of LTP *in vitro*, are normal in CA3–CA1 synapses in mice lacking L1.

LTP induction was also tested in L1KO mice *in vivo*, as previously described [12]. In separate experiments, we measured LTP of the fEPSP in area CA1 (Figure 2a), and of the fEPSP and population spike in the dentate gyrus (Figure 2c,d), following tetanic stimulation. There were no significant differences between L1KO mice and wild-type animals in the degree of LTP produced in either region for up to 60 minutes post-tetanus. These data indicate that LTP in CA1 and the dentate gyrus is not affected in mice lacking L1 protein. In addition, we measured paired-pulse facilitation of the fEPSP, a measure of presynaptic short-term plasticity, in area CA1 and found no difference between the two groups (Figure 2b). Finally, we measured paired pulse inhibition and facilitation of the population spike in the dentate gyrus, a measure of recurrent inhibition and disinhibition respectively, and found this to be similarly unaffected (Figure 2e).

We have presented data that indicate that the L1 protein is not necessary for the generation of LTP in the mouse hippocampus for at least the first 60 minutes post-tetanus. The apparent absence of any effect on hippocampal LTP in mice lacking L1 over this period conflicts with the *in vitro* studies of Lüthi *et al.* [2]. In those studies, anti-L1 polyclonal antibodies and fragments of the L1 protein, specifically fragments containing the immunoglobulin domains, induced a robust reduction in LTP directly after tetanus. How can these apparently conflicting data be reconciled? One possibility that is always hard to rule out in genetic loss-of-function experiments is that other molecular or cellular systems may compensate for the missing protein. Thus, while we have been unable to detect major changes in the expression of other L1-like IgCAMs (particularly NrCAM and neurofascin) in our L1 mutant mice, it remains a formal possibility that redundant mechanisms may account for the differences between the studies. There are, however, important differences in the experimental paradigms used in each case which must also be considered. Most important is whether the addition of antibodies (or protein fragments) to slice cultures can be thought of as equivalent to the genetic removal of L1 from cells. As acknowledged by Lüthi *et al.*, although antibodies may block L1 homophilic adhesion, they may also trigger intracellular signalling. Indeed, polyclonal antibodies against L1 have been shown to inhibit phosphorylation of tubulin [13] or stimulate phosphatase activity [14], depending on the particular set of antibodies used. Addition of L1 protein fragments to cells in culture has also been shown to stimulate various intracellular signalling pathways [13–15]. Thus, the effects seen by Lüthi *et al.* may reflect the stimulation of L1 signalling during LTP induction.

Figure 2



LTP is similar in area CA1 and in the dentate gyrus of anaesthetised wild-type and L1KO mice. (a) Mean change in the slope of the fEPSP in area CA1 as a function of time, recorded from six L1KO and seven wild-type control (WT) littermates, expressed as a percentage of the mean value in the 10 min before the high-frequency train (arrow). At 60 min post-tetanus, the EPSP slope was increased by 19.5 ± 5.2% in the mutants compared to 17.7 ± 4.3% in wild-type mice. (b) Paired pulse facilitation in area CA1. The graph plots the percentage change in the slope of the fEPSP evoked by the second of a pair of stimuli, relative to that evoked by the first, as a function of the interval between the pair of stimuli. (c,d) Mean change in (c) the slope of the fEPSP slope and (d) population spike height from six L1KO and six wild-type littermates. EPSP potentiation was 14.5 ± 5.1% (L1KO) and 13.0 ± 3.6% (WT), and spike potentiation 209 ± 35.6% (L1KO) compared to 219 ± 31.4% for WT (not significant) measured 55–60 min after HFS. (e) Paired pulse depression followed by facilitation of the population spike in L1KO and WT mice. (a–e) Mill Hill data. Filled circles, data from mutant mice; open circles, data from wild-type mice. High-frequency stimulation consisted of 50 stimuli at 100 Hz for CA1, and six series of six trains of six stimuli at 400 Hz, 200 msec between trains and 20 sec between series, for the dentate gyrus. Sample responses recorded before and 60 min after high-frequency stimulation are shown for CA1 and dentate; upper pairs WT, lower pairs L1KO. Calibration, 3 mV, 10 msec.

Whether such signalling is relevant in normal synaptic function remains to be seen. Similar arguments might be applied to the experiments using antibodies to NCAM [2], although anti-NCAM antibodies affected LTP at relatively low concentrations (50 μg/ml) compared with those used to perturb L1 (2 mg/ml). Importantly, however, genetic experiments confirm a role for NCAM in LTP [3,16].

The mutant L1 allele present in our mice could, in principle, give rise to mRNA that would encode an aberrant L1 protein lacking immunoglobulin domain VI (the exons encoding this domain are missing in our mutant). Indeed, Dahme *et al.* [8], who have generated similar, but not identical (different exons were deleted) L1 mutant mice, were able to detect a small amount (≤ 1% of normal) of an abnormal protein product in their null mice, which they presumed to be the product of an aberrantly spliced mRNA. In our L1 mutant, however, neither L1 protein nor RNA was detectable by western blotting [5] or RT–PCR [6] respectively. Our data indicate that the sensitivity of the RT–PCR experiments was such that we would have seen L1 RNA species in the mutant mice had they been present at ≥ 0.5% of normal levels. Even if such low levels of RNA were able to produce levels of aberrant L1 protein similar to that seen by Dahme *et al.*, it seems unlikely that this would be sufficient to rescue LTP function. Studies of Fasciclin II (Fas II; an NCAM-like IgCAM) in *Drosophila* suggest that even wild-type protein must be present at > 10% of normal to rescue the synapse stabilisation found in Fas II null mutants [17].

Thus, our data suggest that L1 function is not necessary for the establishment or maintenance of LTP in the hippocampus. This finding is interesting with regard to the impaired performance in the Morris water maze test of the L1 mutant mice [6]. It was suggested that the poor performance of the L1 mutants could be due to a cerebellar rather than to hippocampal dysfunction, as there were abnormalities in cerebellar cytoarchitecture, particularly in regions involved in eye movement. The results presented here are compatible with this hypothesis. In the L1 mutant mouse, up to 30% of pyramidal and granule cell neurons are missing, presumably because of a requirement for L1 during their development [7]. Despite this, our results indicate that the remaining neurons are able to support normal levels of LTP.

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