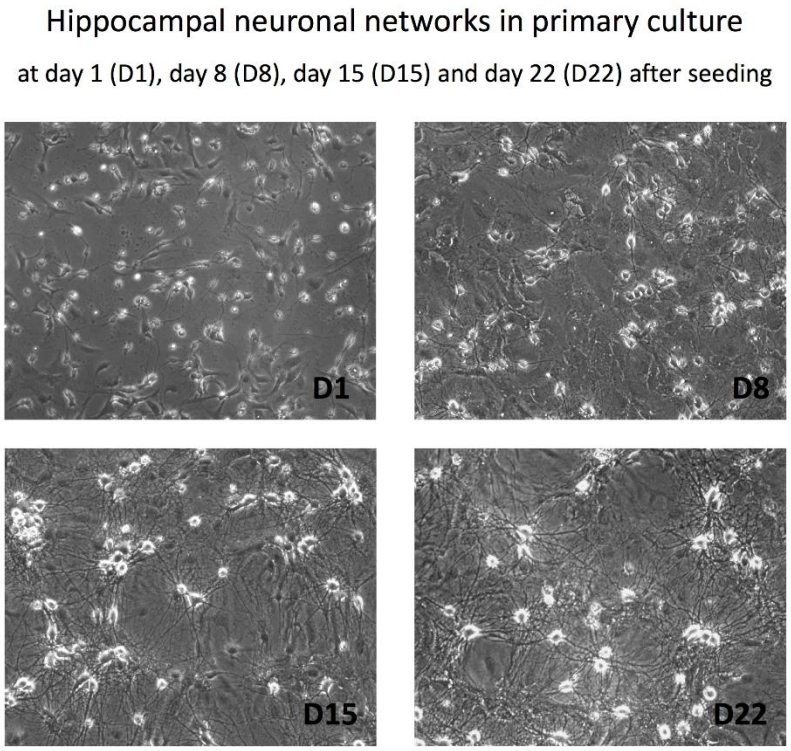
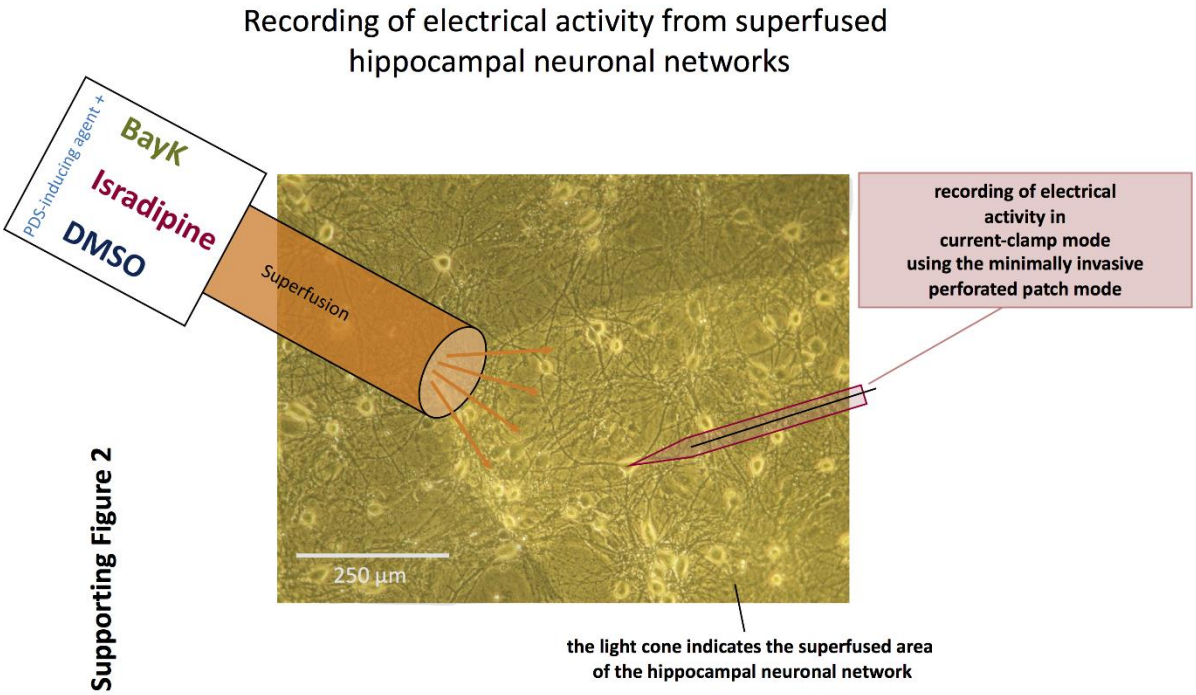
Supporting Information

Supporting Figure 1

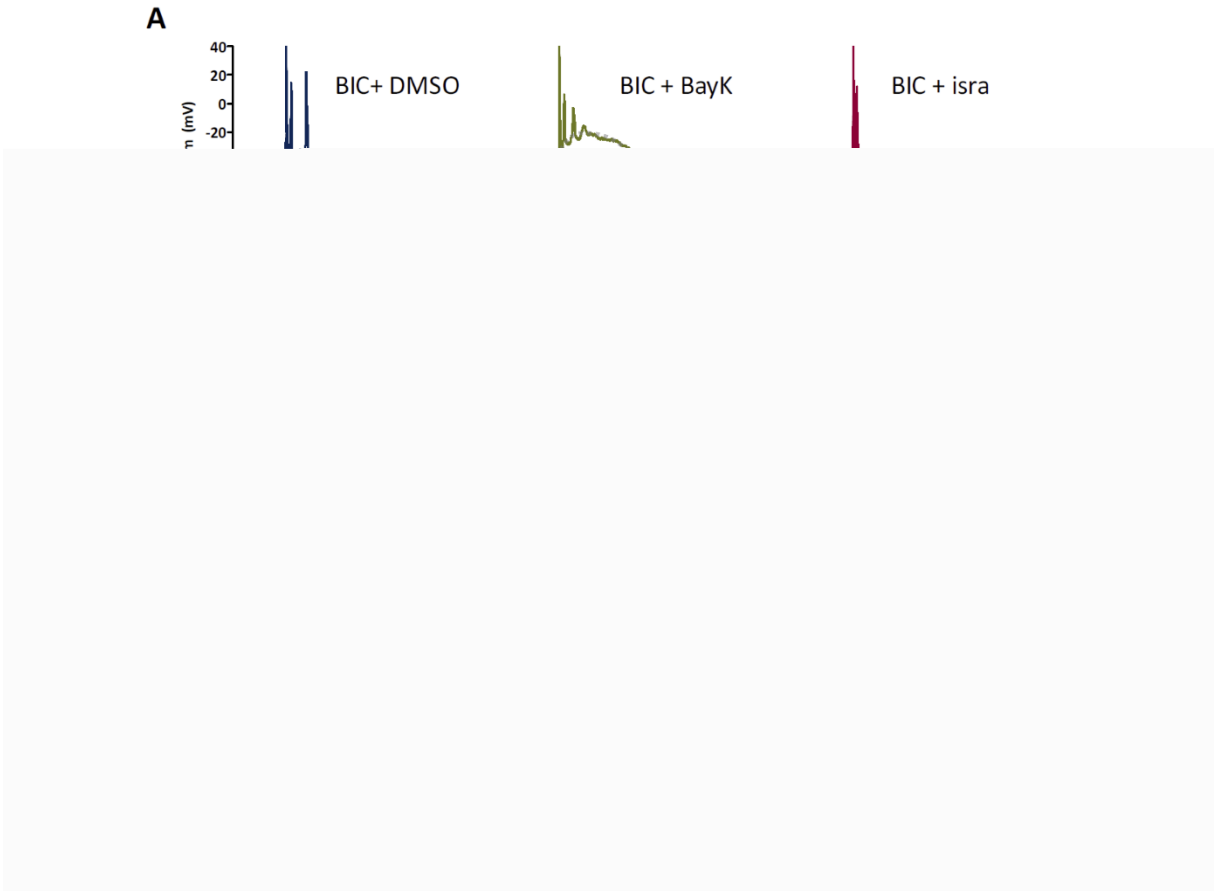


***Supporting Figure 1*, Formation of hippocampal neuronal networks in primary culture:** cells dissociated from rat or mouse hippocampal tissue were seeded in the centre of a 35 mm poly-D-lysine-coated culture dish. Non-attached cells were removed by medium exchange after 18 hours (attached cells were photographed, D1). Growth of non-neuronal cells was blocked by addition of the anti-mitotic agent cytosine arabinoside after 3 to 4 days. Neurons were then allowed to differentiate on the glial cell layer and pictures were taken 8, 15 and 22 days after seeding (D8-D22). Experiments were performed on these neuronal networks between days 12 and 20. Neuronal density amounted to about 100 to 150 cells/mm^2.



Supporting Figure 2

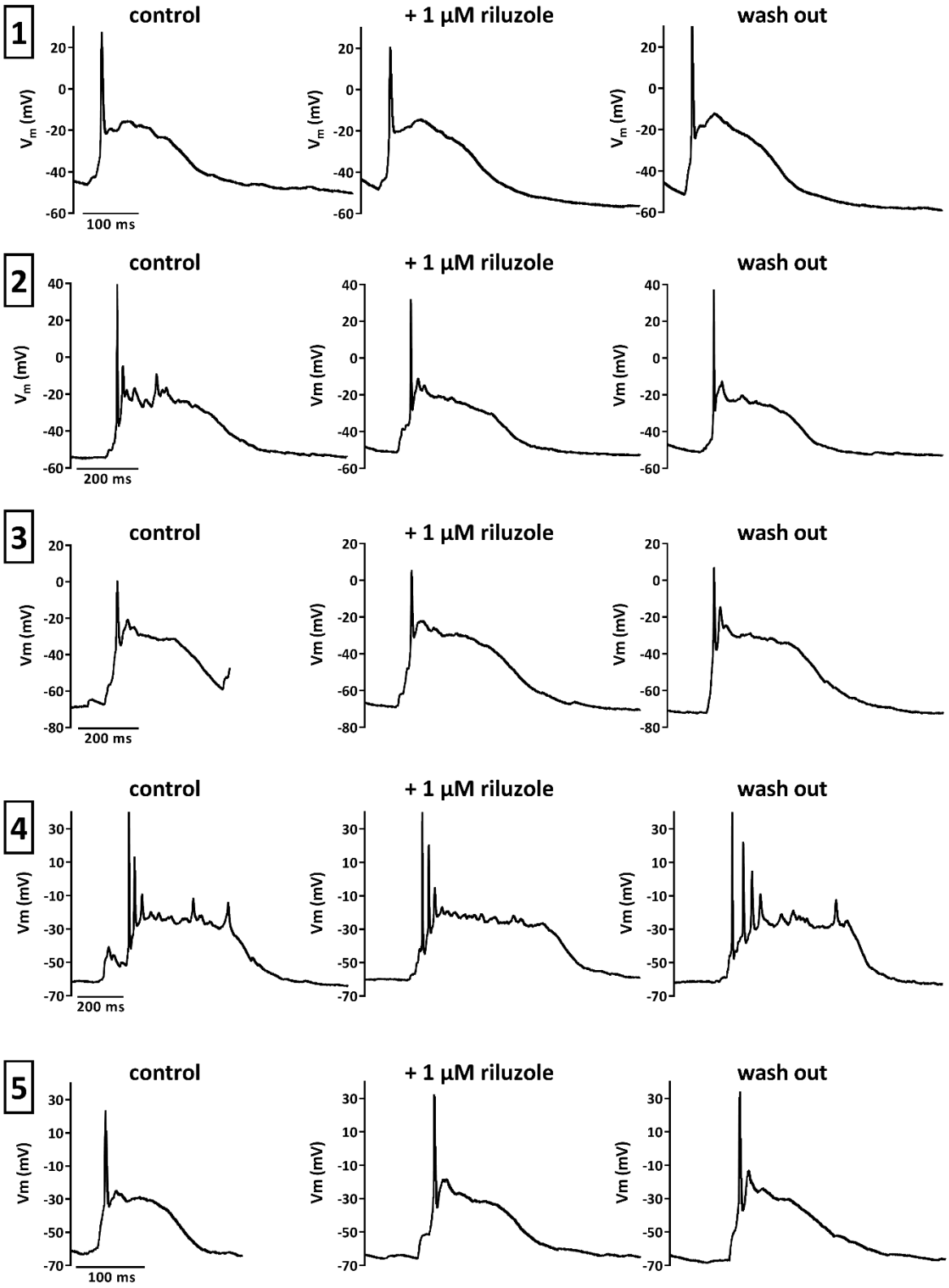
***Supporting Figure 2*, Perforated patch-clamp recording of continuously superfused primary hippocampal neuronal networks:** During the experiments, neurons were superfused with the same external solution as present in the bath, and the latter was supplemented with test compounds or vehicle as indicated. Recordings were made from individual neurons in perforated-patch mode using a glass micropipette (indicated on the right) filled with internal solution containing amphotericin B (500 µg/ml) as the pore forming agent. The perforated patch technique was chosen to minimize experimental alterations of the cell interior.



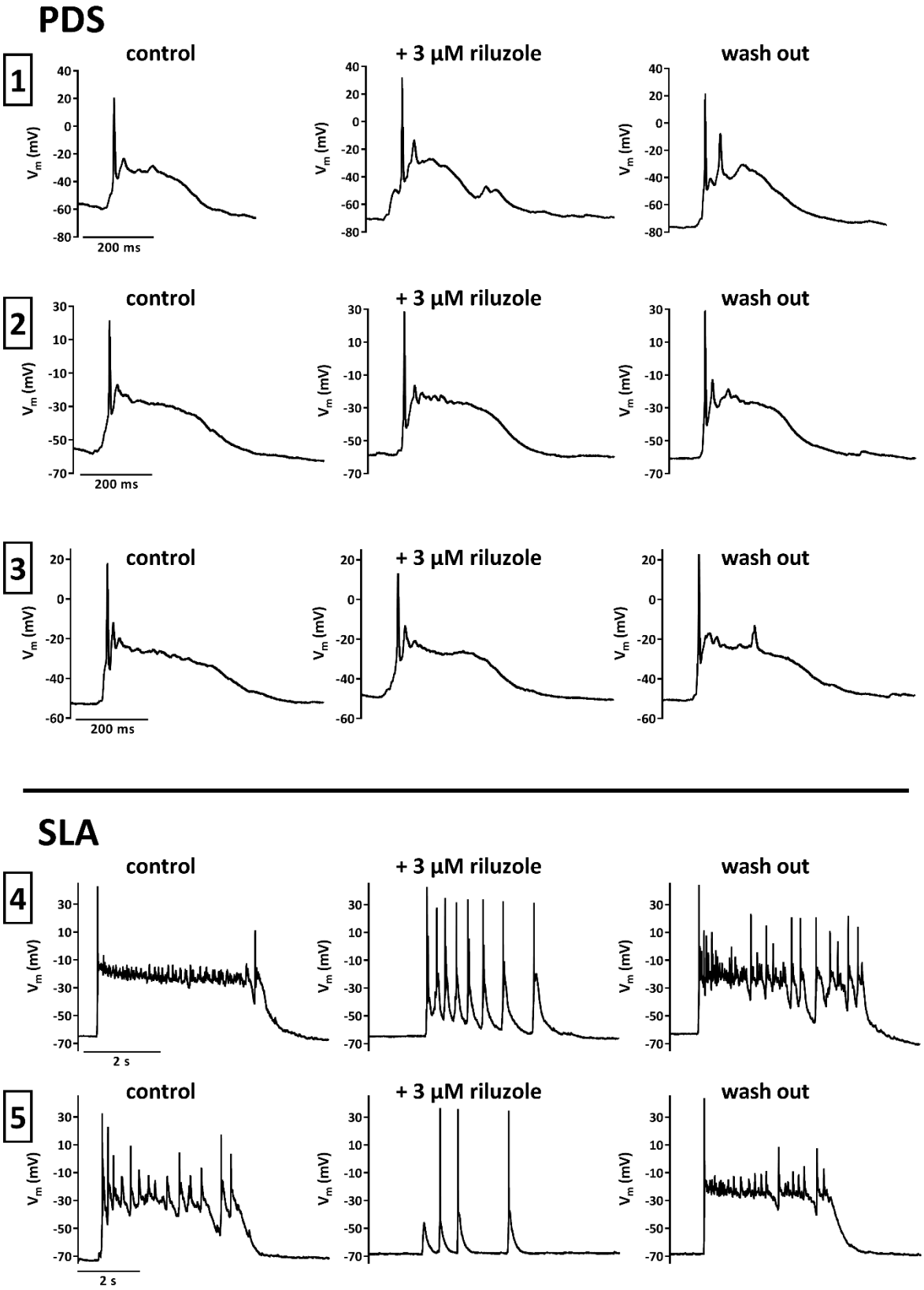
***Supporting Figure 3*, PDS involve an interplay of synaptic activity and activation of L-type voltage-gated calcium channels (LTCCs): A**) Depolarizing events recorded upon application of 10 µM bicuculline in the presence of either DMSO (vehicle), 3 µM Bay K8644 (BayK) or 3 µM isradipine (isra). Note the distinct depolarizing waves (dotted grey line) present in the recordings made under control conditions (DMSO) and in the presence of BayK, but not in the presence of isradipine. **B**) Overlay of the depolarizing events displayed in A. **C**) Bar graphs illustrating area-under-the curve analysis of depolarizing events as shown in A and B, recorded under the three conditions of LTCC availability. Note that PDS require LTCC activity, or - in other words - PDS are promoted by a dihydropyridine type LTCC agonist (BayK), but do not shape up as long as LTCCs are inhibited by the dihydropyridine type antagonist. **D**) PDS evoked in the presence of 10 µM bicuculline (BIC) and 3 µM BayK are eliminated reversibly by co-application of the AMPA/kainate-receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 µM). Left trace: control, middle trace: + CNQX (note different time scale), right trace: after wash-out of CNQX, but in the continued presence of BIC + BayK. The elimination of PDS by CNQX application was confirmed in three further neurons.

Supporting Figure 5. **Potentiation of either LTCC isoform enhances 1050 ms-long current-induced depolarizations, but only in $Ca_v1.3^{-/-}$ neurons afterdepolarizing potentials are elicited.**

Same as above, but here voltage responses to 1050 ms-long current injections are compared. As for panels **A1**-**A3** and **B1**-**B3**, please see the legend to Supporting Figure 4 for details. **C** shows an evaluation of the afterdepolarizations according to the area below the curve, as described for data presented in Fig.4 in the Methods section (chapter "Data analysis and presentation") of the main text. Statistical analysis was performed using Mann-Whitney test for data from 7 ($Ca_v1.2DHP^{-/-}$ neurons) and 6 ($Ca_v1.3^{-/-}$ neurons) experiments.



Supporting Figure 6, panel A



Supporting Figure 6, panel B

***Supporting Figure 6*. PDS of primary hippocampal neurons do not require persistent sodium current ($I_{Na,p}$).** Low concentrations of riluzole (1-3 µM), corresponding to the IC_{50} of $I_{Na,p}$ inhibition (Urbani and Belluzzi, 2000, Eur J Neurosci. 12:3567-3574; Wu et al.,2005, J Neurophysiol.93:2710-2722), do not alter the PDS elicited in $Ca_v1.2DHP^{-/-}$ neurons upon superfusion of the neuron with 10 µM bicuculline + 3 µM BayK. This is illustrated in **panel A** by direct comparison of representative PDS recorded under control conditions (DMSO), in the presence of 1 µM riluzole, and after washout of this compound for five cells (1-5), and in **panel B** for 3 different cells (1-3) treated with 3 µM riluzole. The recordings labelled with 4 and 5 in panel B depict two examples where 3 µM riluzole showed a distinct inhibition of bicuculline + BayK-induced seizure-like discharge activity (SLA). Hence, in primary hippocampal neurons, $I_{Na,p}$ is rather involved in long-lasting epileptiform activity than in PDS.