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MCN Molecular and Cellular Neuroscience

www.elsevier.com/locate/ymcne
Mol. Cell. Neurosci. 31 (2006) 805 – 816

Network stability through homeostatic scaling of excitatory and inhibitory synapses following inactivity in CA3 of rat organotypic hippocampal slice cultures

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Received 4 October 2005; revised 6 December 2005; accepted 11 January 2006
Available online 24 February 2006

Homeostatic plasticity is a phenomenon whereby synaptic strength is scaled in the context of the activity that the network receives. Here, we have analysed excitatory and inhibitory synapses in a model of homeostatic plasticity where rat organotypic hippocampal slice cultures were deprived of excitatory synaptic input by the NMDA and AMPA/KA glutamate receptor antagonists, AP5 and CNQX. We show that chronic excitatory synapse deprivation generates an excitable CA3 network where enhanced amplitude and frequency of spontaneous excitatory post-synaptic potentials were associated with increased glutamate receptor subunit expression and increased number and size of synapsin 1 and VGLUT1 positive puncta. Intact spontaneous inhibitory post-synaptic potentials coincided with persistent expression of the GABA-A receptor alpha subunit and GAD65 and an enhancement of parvalbumin-positive puncta. In this model of homeostatic plasticity, scaling up of synaptic excitation and maintenance of fast synaptic inhibition promote an excitable, but stable, CA3 network.

Keywords: Hippocampus; Synapsin 1; Glutamate; VGLUT1; Synapsin 1; GABA; GABA-A receptor; NR1; GluR1; Homeostatic plasticity; Homeostatic scaling; EPSP; IPSP

Introduction

Over the past decade, there has been a transformation in our understanding of activity-dependent synaptic plasticity especially regarding the development and refinement of synaptic connections. Throughout life, axonal arbours and synaptic connections are continuously reshaped, refined and reconfigured by activity-dependent mechanisms (Goodman and Shatz, 1993; Munno and Syed, 2003). These activity-dependent mechanisms can be categorised into Hebbian plasticity including long-term potentiation and depression (LTP and LTD) and homeostatic plasticity (Turrigiano et al., 1998; Davis and Bezprozvanny, 2001; Burrone and Murthy, 2003). Hebbian and homeostatic plasticity are complementary yet contrasting. Whilst Hebbian plasticity can act to destabilise neuronal networks in either a positive or negative direction, homeostatic plasticity promotes network stabilisation by returning synaptic strength and neuronal firing to within a set range (Turrigiano, 1999; Turrigiano and Nelson, 2004; Burrone and Murthy, 2003).

The concept of physiological homeostasis was originally championed by Cannon in 1932 (Cannon, 1939). However, the idea that neuronal networks could undergo the same form of adaptation was only recently suggested (Davis and Goodman, 1998; Turrigiano et al., 1998). This may be because up until recently, the idea seemed contradictory, how can homeostatic regulation of neuronal activity also permit the changes in synaptic strength required by Hebbian plasticity and learning ? Rather, the view is that instead of returning neuronal activity to a fixed level, homeostatic plasticity acts like a 'net' ensuring that neuronal networks do not reach the extremes of their activity. This is particularly important for highly epileptogenic structures such as the hippocampus and cortex.

The classical way to model homeostatic plasticity is to subject neurones to a period of long-term inactivity or overactivity. Previous studies examining homeostatic plasticity in a variety of neuronal preparations have reported increases in neuronal excitation entirely consistent with the network acting to correct the inactivity by upregulating synaptic excitation. This has been measured as an increase in the amplitude and frequency of spontaneous miniature excitatory post-synaptic currents (mEPSCs) indicating direct changes in the properties of the synapse, increases in glutamate receptor expression (O'Brien et al., 1998; Turrigiano et al., 1998; Watt et al., 2000; Bacci et al., 2001; Burrone et al., 2002; Kilman et al., 2002; Galvan et al., 2003) and increases in synapse size, including the pre-synaptic active zone and number of vesicles (Murthy et al., 2001; De Gois et al., 2005).

Although homeostatic plasticity has been examined in dispersed primary cortical and hippocampal cultures, there are fewer examples

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.mcn.2006.01.009

where the consequences of changes in synaptic excitation within intact neuronal networks have been studied (Aptowicz et al., 2004; Galvan et al., 2003; Royer and Pare, 2003). There are fewer studies where the impact of changes in synaptic inhibition has been examined. Intuitively, homeostatic plasticity in a neuronal network deprived of excitatory synapse input should down-scale synaptic inhibition, as it can be argued that it is not required. This is the case in dispersed neocortical cultures where homeostatic plasticity reduces the frequency of miniature inhibitory post-synaptic currents (mIPSCs) (Kilman et al., 2002); however, this could have grave consequences to additionally destabilise the network in the presence of enhanced excitation.

In the present study, we therefore chose to examine the phenomenon of homeostatic plasticity using organotypic hippocampal slice cultures where both synaptic excitation and inhibition exist together to balance the output properties of the hippocampus (Stoppini et al., 1991). To do this, we subjected developing hippocampal slice cultures to a period of synapse inactivity by the chronic application of NMDA and AMPA/Kainate glutamate receptor antagonists, AP5 and CNQX. The result, on release from the inactivity, was enhanced synaptic excitation via pre- and post-synaptic mechanisms, but where sufficient fast synaptic inhibition ensured the stability of this highly epileptogenic region.

Results

Evoked fEPSP excitability in CA3 is increased in AP5/CNQX-treated organotypic hippocampal slice cultures but there is no alteration in cell number or viability compared with controls

Evoked fEPSPs, arising from the stimulation of mossy fibres, in control organotypic hippocampal slice cultures and AP5/CNQX-treated sister cultures revealed an increase in excitability in treated slice cultures, see Fig. 1A. In response to a range of stimuli (0–40 V)

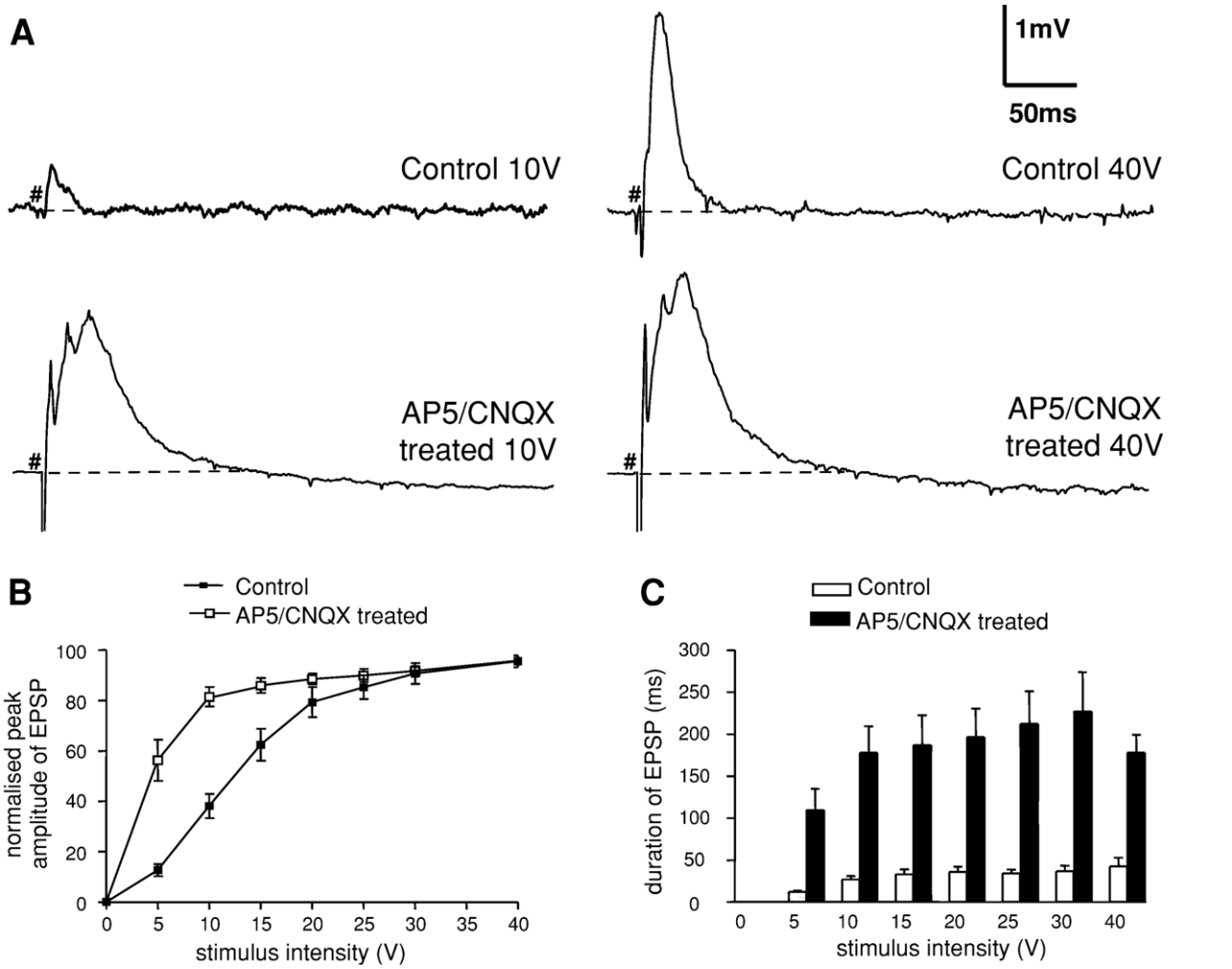

Fig. 1. Network excitability is increased in AP5/CNQX-treated organotypic hippocampal slice cultures compared with control. (A) Representative extracellular recordings evoked from the CA3 region of control and AP5/CNQX-treated organotypic hippocampal slice cultures using 10 V or 40 V stimulation (as stated). Recordings are the average of a minimum of three successive events. (B) fEPSP peak amplitude stimulus response curves evoked from control (n = 10) and AP5/CNQX-treated slice cultures (n = 12), values are means ± SEM. The curve is shifted to the left in AP5/CNQX-treated slice cultures indicating increased excitation, for a given stimulus input. (C) fEPSP duration stimulus response curves evoked from control (n = 10) and AP5/CNQX-treated slice cultures (n = 12), values are means ± SEM. The duration of the fEPSP is increased at all stimulus strengths in AP5/CNQX-treated slice cultures compared to control. #Denotes the stimulation artefact.