RECIPROCAL HOMEOSTATIC REACTIONS TO CHRONIC GLUTAMATE RECEPTOR INACTIVATION IN DEVELOPING CEREBRAL CORTEX NETWORKS

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Chronic blockade of excitatory glutamatergic synaptic receptors in co-cultured organotypic rodent neocortex explants leads to a compensatory up-regulation of otherwise inactive input channels so as to maintain almost normal levels of ongoing bursts of action potentials. We report here that this homeostatic return of spontaneous, now kainate receptor driven, firing is accompanied by a reciprocal down-regulation of the blocked AMPA and NMDA receptors, such that the developing cortical network is protected from becoming hyperactive when these synaptic inputs again become able to transmit normally.

Intrinsically generated bioelectric currents, periodically triggering polyneuronal bursts and isolated spikes, is a well-nigh universal feature of neural networks, especially during early ontogeny *[1]*. Spontaneous burst activity (SBA) is characteristically organized as nested clusters of action potentials on increasingly higher order time-scales, which show a strong capacity for reacting homeostatically to experimental interference *[2]*. Thus, NMDA receptor blockade in developing organotypic rodent visual cortex cultures begins within hours to be compensated for by steadily rising firing rates, which regain stable control levels within 24h *[1,2]*. Although action potential discharges are then purely AMPA receptor driven, 2-week-old chronically treated cultures show no augmentation of spontaneous activity, and continue to burst normally, upon release of their NMDA receptors from the pharmacological blockade.

Combined blockade of NMDA and AMPA receptors eliminates SBA throughout the treatment period in isolated cortex cultures *[1]* but fails to do so in co-cultured explants which have been enabled to cross-innervate one another *[3]*. In such preparations, kainate receptor-mediated SBA takes over for the blocked excitatory receptors *[2]*, as could be shown by acute application of the highly selective kainate blocker *LY382284* (courtesy of Eli Lilly & Co). Spontaneous firing levels and burst intensities failed to be fully restored, however, primarily owing to a dearth of exceptionally active explants in the experimental group (see table 1: 'growth medium'). When such cultures were subsequently assayed in control medium the treated explants became more active (table 1) but, here too, did not exceed the values recorded in the control group. Although spontaneous bursts tended to be somewhat longer than normal, they were correspondingly less frequent and intense *[4]*. Three-week-old cultures gave comparable results, on the whole, but were much more individually variable.

One might have expected that even if, by virtue of the homeostatic restoration of SBA, blocked glutamate receptors had been prevented from becoming sensitized (and inhibitory interneurons from declining in number and/or efficacy) during the treatment period, they still ought to have contributed to intensified spontaneous firing when acting in synergy with the up-regulated kainate receptors. Their failure to do so suggests that, in accordance with a 'Hebbian' weakening of inactive or ineffective synapses *[5,6]*, the blocked excitatory receptors were in fact effectively down-regulated in 'forward reference', as it were, to an eventual restoration of normal physiological conditions. This deduction has now been confirmed experimentally by separately assaying the AMPA and NMDA contributions to glutamatergic driven spike-trains (see table 1: the two '+LY' groups),

For the following experiment, organotypic visual cortex explants were co-cultured, maintained and electrophysiologically monitored as described in our earlier reports *[2-4]*. After being grown for 2 weeks in medium containing the selective AMPA and NMDA receptor blockers, *DNQX* and *APV*, these cultures were recorded with *LY382284* added to the growth medium in the place of either DNQX or APV. In this way NMDA and AMPA receptor-driven firing, respectively, could be separately measured and compared with control cultures assayed in the same manner. After a 10 minute period of acclimatization, during which a spontaneously active site within the small pyramidal cell zone corresponding to layers II-III *[3]* was looked for, the ongoing spike train was recorded for 10 min. and subsequently analyzed off-line. Under control conditions, given the fact that their separate contributions add up to only about half the normal rate (table 1), AMPA and NMDA receptors appear to act synergistically rather than additively in generating a given firing level. Their contribution is more or less equal, however, which is to be expected in the middle of the developmental transition period from predominantly NMDA- to AMPA-driven synaptic activity *[1]*. Following chronic blockade, in contrast, neither receptor type is able to sustain more than a bare minimum rate of spontaneous firing (table 1), thus accounting for the absence of measurable hyperactivity in the ADN group upon return to normal growth medium. Bursting patterns were also similar to those seen in control cultures (data not shown).

This first-time ever demonstration of SBA-dependent kainate receptor plasticity in developing cortex tissue was made possible through the use of co-cultured explants, possibly owing in part to their greatly enhanced dendritic arborisation in comparison with isolated explants *[3]*. Kainate receptors are densely distributed along cortical dendrites *[7]*, namely, and might therefore require a critical amount of morphological substrate in order to display clearcut signs of plasticity. Furthermore, organotypic co-cultures - especially of the recently developed ‘mega’ variety - exhibit firing patterns which much more closely approximate the *in vivo* picture than do simpler preparations *[9]*. Kainate receptors in thalamocortical co-cultures have also been shown to react homeostatically, under stimulation protocols leading to either longterm potentiation or depression *[8]*. The conventionally used isolated neocortex preparation thus appears to lack some essential feature needed for full physiological maturation, a fact which needs to be considered when extrapolating experimental results to the intact organism.

Restoration of spontaneous activity in co-cultured cortical slices occurs even when the kainate receptors, too, are chronically blocked during cortical network formation *[4]*. This multiplicity of linked mechanisms for maintaining firing levels *[2]* further underscores the importance of intrinsically generated bioelectric activity for adaptively modulating cortical development *[1]*. Without such return of function, the secondary decline in efficacy of the AMPA and NMDA receptors which is reported here for the first time obviously could not have taken place. That this down-regulation was indeed induced by the compensatory kainate-driven action potentials is implied by the fact that paroxysmal hyperactivity does result when spontaneous spiking is successfully suppressed, e.g., by tetrodotoxin *[4]*. The present experiment suggests that not only ongoing activity levels but also the net sum of excitatory synaptic receptor sensitivities are homeostatically regulated. That ‘secondary’ homeostatic plasticity (i.e., anticipatory desensitization of AMPA and NMDA receptors) is not restricted to a narrow developmental window is suggested by the fact that 3-week-old cultures gave fully comparable SBA values *[4]*. The close morpho-physiological approximation of organotypic co-cultures to the intact neocortex *[3,9]* suggests that the intact brain, too, will be found to possess a hitherto unexpected breadth of ‘neuroplastic’ potencies.

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Table 1. AMPA versus NMDA receptor driven spontaneous firing following chronic blockade of AMPA together with NMDA receptors for two weeks *in vitro* ('ADN growth medium' = combined APV + DNQX treatment). The respective glutamatergic receptor evaluations were carried out in control medium to which either APV (i.e., AMPA receptor assay) or DNQX (NMDA receptor assay) had been added, plus the selective kainate receptor blocker ('LY 382284': ref. 10).

rec in:	growth medium~	control medium^	(" +LY) +APV	("+LY) +DNQX
CON	1.09 - **2.17** - 7.20 (n=24)	1.19 - **2.27** - 4.93 (n=19)	0.50 - **0.56** - 0.85 (n=6)	0.14 - **0.51** - 0.70 (n=11)
ADN	0.74 - **1.12** - 2.03 (n=23)*	0.99 - **1.93** - 7.40 (n=12)	0.10 - **0.26** - 0.34 (n=5)*	0.06 - **0.10** - 0.35 (n=17)*

median firing rates (bold-face) +- 50-percentile ranges (spikes per second)
* p<.05 vis á vis the CON (control) group: Mann-Whitney 'U' test.
~ data taken from ref. #2; ^ data taken from ref. #4.

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