available at www.sciencedirect.com

SCIENCE DIRECT®

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Short Communication

Homeostatically regulated spontaneous neuronal discharges protect developing cerebral cortex networks from becoming hyperactive following prolonged blockade of excitatory synaptic receptors

Michael A. Corner, Robert E. Baker, Jaap van Pelt*

Netherlands Institute for Neuroscience, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands

ARTICLE INFO

Article history:
Accepted 5 May 2006
Available online 11 July 2006

Keywords:
Rat neocortex
Organotypic tissue culture
Activity-dependent development
Ionotropic glutamate receptor
Cholinergic synaptic drive
Tetrodotoxin

Abbreviations:
APV, DL-2-amino-5-phosphonopentanoic acid (50 μM, Sigma)
APN, atropine (5 μM, Sigma)
BIC, bicucculine (200 μM, Sigma)
DBE, dihydro-beta-erythroidine hydrobromide (20 μM, Sigma)
DNQX, 6,7-dinitroquinoxaline-2-3-dione (100 μM, Sigma)
LY, 'LY382284' (50 μM, courtesy of Eli Lilly and Co, Indianapolis)
TTX, tetrodotoxin (1 μM, Sigma)

ABSTRACT

In order to further examine the role of spontaneous action potential (SAP) discharges in neocortical development, amino-acid-mediated synaptic transmission was selectively blocked in an improved organotypic neocortex culture preparation. Contralateral occipital cortex slices from neonatal rats were co-cultured for several weeks in a ventricle-to-ventricle orientation known to greatly enhance cyto-morphological and electrophysiological maturation. Such preparations are highly resistant to attempts to suppress neuronal firing by blocking ionotropic glutamate receptors: not only can kainate receptors partly substitute for NMDA- and AMPA-mediated neurotransmission when these receptors are pharmacologically blocked, but (muscarinic) cholinergic receptors also begin to drive SAP activity when the kainate receptors, too, are chronically blocked. Only tetrodotoxin proved able to eliminate SAPs altogether in these co-cultures, while GABAergic receptor blockade (using bicucculine) led to persistent epileptiform discharges. Treatment effects were assayed upon transfer to control medium by means of a quantitative analysis of spontaneously occurring polyneuronal spike trains. Total suppression of action potentials for several weeks (by tetrodotoxin treatment) led, as in earlier experiments, to strongly intensified burst firing upon transfer to control medium. Chronic glutamate receptor blocked cultures, on the other hand, showed only minor deviations from control firing levels and patterns when assayed in normal medium. Protection against the development of hyperactivity despite partial blockade of synaptic transmission was roughly proportional to the degree to which spontaneous firing during the treatment period approximated normal SAP levels. This homeostatic response to chronically reduced excitatory drive thus differs from earlier results using isolated organotypic cortex cultures, in which the restoration of SAP activity failed to prevent the development of network hyperactivity. Chronic bicucculine treatment, in contrast, had little or no homeostatic effect on SAP firing patterns; on the contrary, opposite to earlier findings using isolated occipital cortex explants, paroxysmal discharges persisted even after transfer to control medium.

* *Corresponding author*. Fax: +3120 696 1006.
E-mail address: m.corner@nin.knaw.nl (M.A. Corner).

0006-8993/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.brainres.2006.05.026

Intrinsically generated 'spontaneous' action potentials (SAPs) have in recent years been established as a crucial factor in the refinement of neural networks throughout the developing central nervous system (CNS), from the selective innervation of target cells to the balance between excitatory and inhibitory synaptic drive and trans-membrane ion flow (Corner, 1994; Corner et al., 2005; Ramakers et al., 1990, 1991). Activity dependence at early stages of physiological development typically takes the form of a homeostatic response whereby the network gradually compensates for abnormal increases or decreases in spontaneous firing (Burrone and Murthy, 2003; Corner et al., 2002, 2005; Davis and Bezprozvanny, 2001; Hasselmo, 1995; Houweling et al., 2005; Kubitzer and Kahn, 2003; Ramakers et al., 1990, 1991; Turrigiano and Nelson, 2000, 2004). In this way, SAPs appear to serve in the developing brain as a monitor of overall network excitability, with levels above or below some preset 'optimal' range – the ontogenetic origins of which are unknown – leading to down- or up-regulation, respectively, of mechanisms responsible for maintaining optimal levels of neuronal activity (Corner and Ramakers, 1992).

Our earlier study of activity-dependent maturation in isolated organotypic occipital cortex slices demonstrated that, although SAP activity quickly recovers to control levels under conditions of chronic NMDA receptor blockade, such (APV-treated) cultures nevertheless exhibit severe hyperactivity when assayed under control conditions following several weeks of such treatment (Corner et al., 2002). Control firing levels in these isolated neocortex explants are considerably less than in the intact brain, however, so it is conceivable that, if SAP levels are high enough, intrinsic activity would be able to promote normal development even with the NMDA receptors blocked. With the discovery, then, that dendritic arborization (Baker and van Pelt, 1997) and spontaneous firing levels (Corner et al., 2005) become greatly enhanced when occipital cortex slices are co-cultured in close apposition to one another, thus enabling extensive cross-innervation to take place, further investigation of the effects of chronic glutamate receptor blockade on the development of spontaneous activity patterns was called for.

To our surprise and initial consternation, it proved impossible in this improved 'model system' to permanently suppress SAP discharges by blocking glutamatergic synaptic transmission. Even a combined blockade of NMDA and AMPA receptors (chronic APV + DNQX treatment) failed to prevent the early appearance of SAPs, with levels of firing in treated co-cultures attaining control values already by 1 week in vitro (Corner et al., 2005). Using a selective kainate receptor blocker (LY: Bleakman and Lodge, 1998), it could be established that – in contrast to its negligible role under normal conditions [see Corner et al., 2005] – this receptor subtype was now providing the major excitatory synaptic drive. Even when all glutamatergic interactions were then suppressed by means of a cocktail consisting of three selective ionotropic receptor blockers (chronic 'LAD' treatment: LY + APV + DNQX), SAPs were already present in 1-week-old explants and persisted throughout the rest of the culture period (Corner et al., 2005). It could be shown by acute APN treatment that most of the excitatory drive had now become cholinergic (muscarinic) in nature, with a small additional contribution from an unknown (non-cholinergic) source. The latter became more prominent in subsequent experiments where, together with the abovementioned glutamate receptors, muscarinic receptors were chronically blocked using APN ('LADA' treatment: see Table 4).

The present report examines the effects of these varied treatments on functional development by comparing SAP patterns in the experimental groups following transfer to control recording medium after 1–3 weeks of culture in vitro. A parallel experiment was carried out using the sodium channel blocker tetrodotoxin (TTX), which eliminates SAPs altogether (Corner and Ramakers, 1992; Corner et al., 2002; Ramakers et al., 1990, 1991). This experiment served to confirm that the neocortex co-culture system is not immune to activity-dependent disturbances during development. In addition, the GABAa receptor blocker bicuculline (BIC) – which induces a persistent paroxysmal discharge pattern – was studied in order to see if, as in dissociated cell cultures (Corner and Ramakers, 1992; Ramakers et al., 1990, 1991), homeostatic adjustments to abnormal electrophysiological activity operate in both directions.

Spontaneous firing was chosen as the dependent variable in this study because it is one of the most sensitive indicators of physiological health in developing neuronal networks [e.g., Corner, 1994] as well as being a direct expression of the myriad processes which determine their excitability [for a review, see Corner and Ramakers, 1992; Corner et al., 2002].

Experimental procedures. Explants of neonatal (P5) rat occipital cortex were prepared as described previously (Baker and van Pelt, 1997) and co-cultured pairwise (left and right cerebral hemispheres) for 1 to 3 weeks in a specially developed serum-free medium (R16: Romijn et al., 1988). All explants in a given run came from a single pup, with each age/treatment group comprising at least three runs, and usually quite a few more. Each run included cultures from the different treatment groups used in a given statistical comparison. After allowing several minutes in the recording setup for accommodation and to locate an active extracellular site – and again following medium

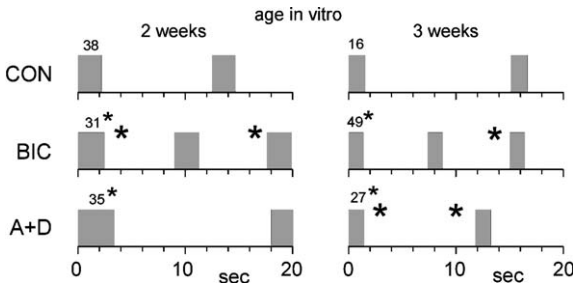

Fig. 1 – Visual representation of the characteristic burst pattern in six age/treatment groups when assayed in control recording medium (DMEM), based on the *median* values in Table 1. The mean duration and period are given on the horizontal axis, and the number of spikes constituting a burst indicated above the first one in a train. An asterisk immediately following a burst signifies a significant difference from control values for the *duration*; an asterisk immediately preceding a burst signifies a difference in the *period*; and an asterisk next to the number of spikes in a burst signifies a difference in intra-burst firing *intensity* (spikes/s: see Table 1).

Table 1 – Spontaneous firing patterns as assayed in control recording medium (DMEM) following 2 or 3 weeks of culture in the presence of selective amino acid receptor blockers

Group	Two weeks in vitro			Three weeks in vitro		
	Control (19)	BICUC (15)	APV + DNQX (12)	CONTROL (13)	BICUC (9)	APV + DNQX (23)
MFR [a,b]	2.27 (1.19–4.93)	2.28 (1.05–3.90)	1.93 (0.99–7.40)	1.47 (1.15–2.52)	3.77 (2.57–4.56) *	1.16 (0.44–3.56) *,#
Mode [a,b]	6 (5–7)	35 (10–100) **	23 (8–70) *	8 (5–27)	5 (3–9) #	20 (6–25) **
CV60 [a,b]	67 (35–85)	99 (91–120) *,#	120 (92–178) **	109 (103–138)	67 (53–107) *	69 (47–99) **
1000 ms criterion						
Duration [a]	2.23 (0.76–4.61)	2.40 (1.33–3.02) **	3.44 (1.84–5.79) #	1.56 (1.27–3.19)	1.41 (0.48–1.90)	1.59 (0.33–4.70) *
Intensity [a,b]	17.1 (9.76–34.9)	13.1 (7.98–32.7) *	10.3 (8.08–17.4) *	10.5 (7.10–17.5)	34.6 (23.9–52.7) **	16.9 (6.08–25.3) *,#
Period [a,b]	12.5 (7.96–27.5)	8.90 (6.43–12.9) **	18.0 (9.20–21.4) #	15.2 (14.5–19.5)	7.50 (6.60–14.1) *	11.9 (8.64–16.0) *,#

MFR: mean firing rate in spikes per second; *Mode* gives the modal interspike interval in milliseconds (ms); *CV60* is the coefficient of variation (in percent) over all 60 s bins in the activity/time plot. Mean burst durations (*Duration*) are given in seconds; *Intensity* is the mean number of spikes per second during bursts; *Period* is the mean time (in seconds) elapsing between the onset of successive bursts. All distributions are represented by the median value for the indicated group and the 50 percentile range, with the number of preparations being given above in parentheses.

[a] Overall *treatment* effect at 2 weeks in vitro.

[b] Overall *treatment* effect at 3 weeks in vitro ($P < 0.05$).

* $P < 0.05$.

** $P < 0.01$ vis a vis the control group.

\# $P < 0.05$ between the corresponding BICUC and APV + DNQX groups.

changes – polyneuronal action potentials [1–3 units: see Corner et al., 2005] were assayed in control medium for 10–15 min. The spike trains (from a single recording site per explant) were computer-analyzed off-line as described previously (Corner et al., 2002, 2005). Only preparations in which an active site was able to be located during the initial screening (up to 5 sites, ~2 min per site) were selected to be included in the assay (90–95% of the explants in any of the age or treatment groups met this criterion).

For burst detection, we employed the *<1000 ms* interspike interval criterion used previously for defining (network) bursts (because of their typically widespread occurrence throughout the network: e.g., Corner et al., 2005; van Pelt et al., 2004a,b). As in earlier electrophysiological studies (Corner and Ramakers, 1992; Corner et al., 2002, 2005; Ramakers et al., 1990, 1991), the coefficient of variation (CV) was calculated over the number of spikes in successive 60 s bins in order to assess the degree to which mean SAP levels fluctuate from minute to minute. *Mean firing rates* over the entire spike train were also used for statistical comparisons among age and treatment groups, as were the *modal interspike intervals* estimated from smoothed semi-log histogram plots (Corner et al., 2002, 2005; Corner and Ramakers, 1992).

The following parameters were calculated per preparation: (i) mean burst *duration*, (ii) mean spike rate (*intensity*) during bursts, and (iii) the mean time elapsing between the onset of successive bursts (*period*). Medians and 50-percentile ranges for each group are presented in the form of tables, which also include the results of non-parametric statistical comparisons. A preliminary analysis of variance (Bonferroni or Chi-square) was made whenever multiple groups needed to be compared followed by pairwise comparisons using the Fischer, Chi-square or Mann–Whitney *U* tests (depending on the sample sizes and the distribution of ranks: Siegel, 1956). Treatment effects in a given group of cultures were tested for statistical significance using the Sign or the Wilcoxon matched-pairs, signed-ranks test. Two-tailed comparisons were made

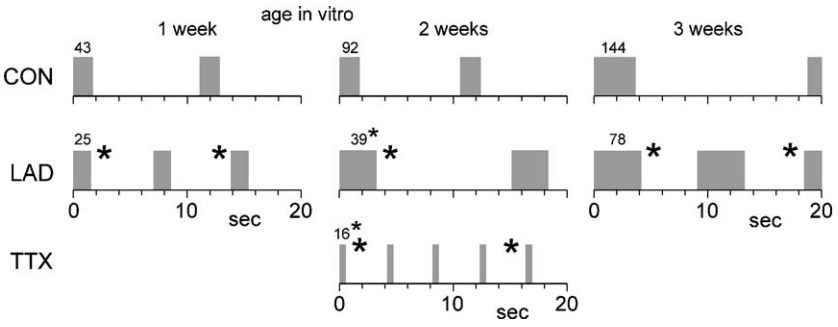

Fig. 2 – Visual representation of the characteristic burst pattern in seven age/treatment groups when assayed in control growth medium (R16), based on the *median* values in Tables 2 and 3. The mean duration and period are given on the horizontal axis, and the number of spikes constituting a burst indicated above the first one in a train. An asterisk immediately following a burst signifies a significant difference from control values for the *duration*; an asterisk immediately preceding a burst signifies a difference in the *period*; and an asterisk next to the number of spikes in a burst signifies a difference in intra-burst firing *intensity* (spikes/s: see Table 2, 3).

Table 2 – Effects of simultaneous chronic blockade of kainate, NMDA and AMPA receptors ('LAD' group) in 1-, 2- and 3-week-old cultures, as assayed in control growth medium (R16)

	One week		Two weeks		Three weeks	
	Control (20)	LAD (31)	Control (25)	LAD (20)	Control (16)	LAD (16)
MFR [a,b]	2.52 (1.75–3.41)	2.87 (0.97–6.03) **	5.87 (3.08–7.21)	3.92 (1.28–6.98)*	4.98 (2.50–8.48)	8.12 (4.82–9.18) *
Mode [a,b]	10 (9–25)	25 (7–78) **	3 (2–6) [c]	25 (8–200) **	4 (2–7)	7 (5–9)
CV60 [a,b]	102 (91–116)	53 (31–75) **	69 (50–80) [c]	52 (35–82) [c]	90 (52–131)	82 (69–90) *
1000 ms criterion						
Duration [a,b]	1.76 (1.19–2.61)	1.58 (0.63–2.70)	1.78 (0.24–4.45) [c]	3.50 (1.57–7.07) **,[c]	3.94 (1.43–6.14)	3.98 (2.13–5.16)
Intensity [b]	24.6 (16.2–30.7)	15.9 (6.46–33.3) **	51.8 (35.1–71.3) [c]	11.0 (7.30–23.3) **,[c]	36.6 (29.9–44.9)	19.5 (12.3–37.9) **
Period [a,b]	10.9 (9.81–20.4)	6.91 (4.71–12.9) **	10.6 (5.72–26.1) [c]	15.0 (8.92–25.7) [c]	18.7 (7.64–33.3)	8.80 (7.55–16.3) *

Abbreviations as in Table 1.
[a] $P < 0.05$ for age differences among the control groups.
[b] $P < 0.05$ for age differences among LAD groups.
[c] $P < 0.01$ vis a vis the 2-week-old TTX group (cf. Table 3).
* $P < 0.05$.
** $P < 0.01$ vis a vis the corresponding control group.

between groups of cultures run in parallel, using slices taken either from the same animal or from littermates, with three or more runs being pooled for each age and treatment group.

Empirical findings. Combined treatment with APV and DNQX led initially to longer modal interspike intervals, lower firing rates ('intensity') during bursts, and larger minute-to-minute fluctuations in the mean firing level (*CV60*) than were observed in untreated cultures upon assay in control recording medium (Fig. 1; Table 1: 2 weeks). At 3 weeks in vitro, in contrast, APV + DNQX-treated cultures were characterized by attenuated minute-order fluctuations (*CV60*), but with a much shorter cycle length (see Fig. 1), along with exceptionally large inter-individual variances in most of the burst parameters and in the mean firing rates (see Table 1: 50% ranges).

Already after 1 week in vitro, total glutamate receptor blockade ('LAD' group) led to a broad spectrum of deviations from control firing patterns when assayed in control growth medium (see Fig. 2; Table 2). It is especially striking that the experimental group was characterized by exceptionally large inter-individual variations in all parameters: some explants were much less active than any of the controls while others were strongly hyperactive (Table 2: *MFR*); some displayed relatively short and intense bursts of activity while others were characterized by abnormally long and sluggish bursts, causing the interspike interval histogram to shift strongly to the right (Table 2: *Mode*).

Large inter-individual differences (Table 2; note the 50% ranges) also distinguished 2-week-old LAD-treated cultures from the corresponding controls, though to a lesser extent than at 1 week in vitro. Nor had the LAD-treated group as yet developed any signs of hyperactivity in comparison with untreated explants (Fig. 2; Table 2). After 3 weeks of LAD treatment in vitro, in contrast, the extreme inter-individual differences seen in the younger LAD-treated cultures had for the most part disappeared to be replaced by a consistently much higher mean SAP level than in the controls (Table 2: *MFR*). In addition, the incidence of 'network' bursts (Fig. 2; Table 2: *1000 ms*) was now considerably higher in the experimental group.

In contrast to the LAD-treated group, 2-week-old TTX-treated explants consistently displayed paroxysmal spike bursts when assayed in control medium (see Fig. 2; Table 3). Thus, a rapid succession of extremely short bursts of high initial intensity appeared, while minute-to-minute fluctuations in the mean firing rate were minimal. This pattern differed markedly from that induced by acute GABAergic disinhibition (ACU-BIC group), where abnormally long bursts and high firing rates were observed (Table 3).

Chronic disinhibition produced an opposite homeostatic developmental effect to that of TTX: upon transfer to control medium, the paroxysmal spike bursts induced by BIC throughout 2 weeks of culture [see Corner et al., 2005] became transformed into spike trains in which modal interspike intervals were dramatically longer than in the controls (Fig. 1; Table 1). The intensity of intra-burst firing was significantly less, moreover, and the minute-order fluctuations (*CV60*) more extreme than in control cultures—again the opposite of the

Table 3 – Effects of suppression of action potentials (TTX group), as assayed in control growth medium (R16) following 2 weeks of culture in vitro, compared with the effects of acute disinhibition of 2-week-old untreated cultures (ACU-BIC group)

	TTX (15)	ACU-BIC (12)
MFR [a]	5.08 (3.58–5.70)	21.8 (20.7–23.8) **
Mode [a]	2 (1–2) **	6 (5–7) *
CV60	54 (47–65) *	53 (43–62)
1000 ms criterion		
Duration [a]	0.39 (0.29–0.75) **	4.64 (3.14–7.54) **
Intensity	40.0 (33.1–47.4) **	49.9 (43.7–55.9)
Period	4.11 (2.81–5.87) **	9.15 (7.44–15.9)

Abbreviations as in Table 1.
[a] $P < 0.01$ for the difference between the TTX and the ACU-BIC group.
* $P < 0.05$.
** $P < 0.01$ vis a vis the 2-week-old control group (cf. Table 2).

Table 4 – Spontaneous firing patterns in co-cultured neocortex explants at 2 weeks in vitro, recorded in a growth medium containing a tripartite 'cocktail' of glutamate receptor blockers supplemented by atropine ('LADA' group), followed by assay in control medium (R16)

	Recorded in: growth medium		(idem) With added 'DBE'		Control medium	
	Control (20)	LADA (26)	Control (12)	LADA (12)	Control (20)	LADA (14)
MFR	4.54 (2.67–5.62)	2.10 (0.98–3.89) **	1.83 (1.64–6.33)	3.32 (1.56–6.53)	5.44 (2.67–11.1)	3.01 (1.61–3.39) **
Mode	5 (4–7)	300 (8–850) **	4 (4–5)	200 (30–300) **	5 (3–6)	5 (4–13)
CV60	96 (79–159)	26 (41–92) **	87 (59–116) [a]	53 (46–64) *	103 (57–159)	48 (39–59) **
1000 ms criterion						
Duration	2.12 (1.71–2.40)	1.70 (1.04–3.82)	2.58 (1.93–2.82)	6.02 (1.91–10.3) *, [a]	2.31 (1.71–2.96)	1.28 (0.83–1.77) *
Intensity	29.4 (22.4–48.0)	5.11 (3.09–11.8) **	16.3 (9.55–39.5) [a]	6.34 (3.03–6.77) **	36.4 (19.9–48.3)	12.5 (12.0–18.1) **
Period	14.2 (12.5–24.2)	9.07 (7.34–17.0) **	18.8 (16.0–22.6)	8.30 (7.17–20.4) *	17.9 (10.1–24.2)	6.74 (5.00–9.21) **

In both groups, the acute effect of a selective nicotinic receptor blocker (DBE) was also studied when added to their respective growth media
Abbreviations as in Table 1.
[a] $P < 0.05$ for the acute effect of adding DBE to the medium.
* $P < 0.05$.
** $P < 0.01$ for the LADA vs. the corresponding control group.

TTX-induced effects. After 3 weeks of intense paroxysmal activity in BIC-containing medium, however, the intensity and incidence of network bursts have become abnormally high while minute-order fluctuations are now smaller than in the control group (Fig. 1; Table 1).

In a final attempt to eliminate SAP activity during early network formation by blocking excitatory neurotransmission, the muscarinic receptor blocker atropine was added to the 'LAD cocktail' in a dosage which had proven to be effective in eliminating SAPs in chronically LAD-treated explants. Even in this 'LADA' growth medium, however, considerable spontaneous spiking was present at 2 weeks in vitro, although it differed considerably from the control patterns (Table 4). Not only were mean firing rates (*MFR*) and minute-to-minute fluctuations (*CV60*) only about half of those measured in control cultures, a greatly reduced tendency existed for the action potentials to occur in intense bursts (Table 4: *Mode*). Doubling the dose of atropine had no noticeable effect on SAP activity, which also appeared to be largely unaffected by acute

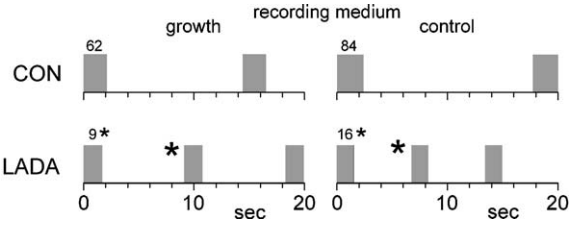

Fig. 3 – Visual representation of the characteristic burst pattern in four age/treatment groups when assayed in control growth medium (R16), based on the *median* values in Table 4. The mean duration and period are given on the horizontal axis, and the number of spikes constituting a burst indicated above the first one in a train. An asterisk immediately following a burst signifies a significant difference from control values for the *duration*; an asterisk immediately preceding a burst signifies a difference in the *period*; and an asterisk next to the number of spikes in a burst signifies a difference in intra-burst firing *intensity* (spikes/s: see Table 4).

pharmacological blockade of nicotinic cholinergic receptors (Table 4: 'added DBE'). Control explants, in contrast, showed a slight but significant reduction in spiking intensities when their nicotinic receptors were blocked using DBE.

When transferred to control medium for assay after 2 weeks in vitro, LADA-treated explants showed none of the hyperactivity and stereotyped bursting that were such dramatic sequelae of prolonged TTX exposure (see above). On the contrary, overall activity levels were even lower and burst firing less intense than in the corresponding control cultures (Fig. 3; Table 4). They resemble TTX-treated cultures (cf. Fig. 2; Table 3) to some extent, however, with respect to (i) a 3- to 4-fold higher incidence of network bursts which, in addition, are relatively short (see Fig. 3), and (ii) a greater regularity of minute-to-minute mean firing levels in comparison with the controls (Table 3: *CV60*). In all these respects, the 'LADA' group deviated more strongly from control values than did the LAD-treated explants (cf. Fig. 2; Table 2), suggesting that the presence of effective cholinergic synaptic drive in the latter group contributed to the partial normalization of physiological maturation in the absence of glutamatergic excitatory interactions. That homeostatic protection may be only temporary, however, is shown by the appearance of hyperactive neurons in LAD-treated cultures between 2 and 3 weeks in vitro, albeit in a much less severe form than following 2 weeks of chronic exposure to TTX.

Final remarks. It can be concluded from the present experiments that spontaneous neuronal firing is essential for normal physiological development, not only in the reduced culture systems previously reported (Corner, 1994; Corner et al., 2002, 2005; Corner and Ramakers, 1992; Ramakers et al., 1990, 1991) but also in the present, more realistic, neocortical co-culture 'model' system. Even abnormally low levels or distorted patterns of SAP activity afforded at least partial protection against the paroxysmal hyper-excitability that typically appears in chronically silenced cultures when subsequently assayed under control conditions. It is perhaps not surprising, therefore, to find that there exists a wide variety of homeostatic mechanisms which act on short notice to oppose any tendency for spontaneous firing to become

seriously reduced (Corner, 1994; Corner et al., 2002, 2005). That this novel form of neuro-plasticity is much less evident in isolated (Corner et al., 2002) than in co-cultured cortical explants (the present report) could in part be a consequence of the poor dendrite outgrowth observed in such preparations (Baker and van Pelt, 1997). The greatly enhanced dendritic arborizations observed in co-cultured explants (Baker and van Pelt, 1997) presumably contain most of the kainate, muscarinic and unknown other receptors, the up-regulation of which enables SAP patterns to become re-established when glutamatergic synaptic transmission is eliminated.

Given the compensatory up-regulation of kainate and muscarinic receptors in, respectively, chronically A + D- and LAD-treated co-cultures (Corner et al., 2005), it was surprising to find that spontaneous firing rates seldom exceeded control levels when assayed in normal medium (the present report). SAP homeostasis under these experimental growth conditions apparently not only prevented the inactive synaptic receptors from becoming hypersensitive (which would have resulted in exaggerated bioelectric activity upon transfer to control medium), it actually seems to have caused them to become proportionally *less* reactive than in the control cultures. This form of plasticity would represent yet another level of protection against the development of hyper-excitability in immature cortical networks, this time operating with 'forward reference' to an eventual restoration of normal environmental conditions [e.g., Houweling et al., 2005]. The underlying mechanism here could be based simply on the classical Hebbian principle whereby soma-dendritic spiking can depress the sensitivity of concomitantly non-active post-synaptic receptors [e.g., Turrigiano and Nelson, 2000].

Since the organotypic *co-culture* technique, as used in the present experiments, gives a much closer approximation to development in situ than do isolated explants, in which SAPs can be fully suppressed by glutamate receptor blockers for prolonged periods with little or no sign of adaptation [see Corner et al., 2002, 2005], we may conjecture that the intact developing brain is similarly buffered against abnormal reductions in excitatory synaptic drive. In view of the variety and complexity of functional adaptations to prolonged activity deprivation, as exemplified by the present results, computer simulations of neural network dynamics [e.g., Hasselmo, 1995, Houweling et al., 2005] may prove to be a *sine qua non* for analyzing the respective contributions of the myriad cellular processes that regulate neuronal excitability and functional connectivity during early ontogeny [for recent reviews, see Corner et al., 2002; Kubitzer and Kahn, 2003; Moody and Bosma, 2005; Turrigiano and Nelson, 2004].

REFERENCES

Baker, R.E., van Pelt, J., 1997. Co-cultured but not isolated cortical explants display normal dendritic development: a long-term quantitative study. Dev. Brain Res. 98, 21–27.

Bleakman, D., Lodge, D., 1998. Neuropharmacology of AMPA and kainate receptors. Neuropharmacology 37, 1187–1204.

Burrone, J., Murthy, V.N., 2003. Synaptic gain control and homeostasis. Curr. Opin. Neurobiol. 13, 560–567.

Corner, M.A., 1994. Reciprocity of structure–function relations in developing neural networks. Prog. Brain Res. 102, 3–31.

Corner, M.A., Ramakers, G.J.A., 1992. Spontaneous firing as an epigenetic factor in brain development—Physiological consequences of chronic tetrodotoxin and picrotoxin exposure in cultured rat neocortex neurons. Dev. Brain Res. 65, 57–64.

Corner, M.A., van Pelt, J., Wolters, P.S., Baker, R.E., Nuytinck, R.H., 2002. Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks—An inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny. Neurosci. Biobehav. Rev. 26, 127–185.

Corner, M.A., Baker, R.E., van Pelt, J., Wolters, P.S., 2005. Compensatory physiological responses to chronic blockade of amino acid receptors during early development in spontaneously active organotypic cerebral cortex explants cultured in vitro. Prog. Brain Res. 147, 231–248.

Davis, G.W., Bezprozvanny, I., 2001. Maintaining the stability of neural function: a homeostatic hypothesis. Annu. Rev. Physiol. 63, 847–869.

Hasselmo, M.E., 1995. Neuromodulation and cortical function: modeling the physiological basis of behavior. Behav. Brain Res. 67, 1–27.

Houweling, A.R., Bazhenov, M., Timofeev, I., Steriade, M., Sejnowski, T.J., 2005. Homeostatic synaptic plasticity can explain post-traumatic epileptogenesis in chronically isolated neocortex. Cereb. Cortex 15, 834–845.

Kubitzer, L., Kahn, D., 2003. Nature versus nurture revisited: an old idea with a new twist. Prog. Neurobiol. 70, 33–52.

Moody, W.J., Bosma, M.M., 2005. Ion channel development, spontaneous activity, and activity-dependent development in nerve and muscle cells. Physiol. Rev. 85, 883–941.

Ramakers, G.J.A., Corner, M.A., Habets, A.M., 1990. Development in the absence of spontaneous bioelectric activity results in increased stereotyped burst firing in cultures of dissociated cerebral cortex. Exp. Brain Res. 79, 157–166.

Ramakers, G.J.A., Habets, A.M., Corner, M.A., 1991. Abnormalities in the spontaneous firing patterns of cultured rat neocortical neurons after chronic exposure to picrotoxin during development in vitro. Brain Res. Bull. 25, 429–432.

Romijn, H.J., de Jong, B.M., Ruijter, J.M., 1988. A procedure for culturing rat neocortex explants in a serum-free nutrient medium. J. Neurosci. Methods 23, 75–83.

Siegel, S., 1956. Non-Parametric Statistics for the Behavioral Sciences. McGraw-Hill Kogakusha, Tokyo.

Turrigiano, G.G., Nelson, S.B., 2000. Hebb and homeostasis in neuronal plasticity. Curr. Opin. Neurobiol. 10, 358–364.

Turrigiano, G.G., Nelson, S.B., 2004. Homeostatic plasticity in the developing nervous system. Nat. Rev., Neurosci. 5, 97–107.

van Pelt, J., Corner, M.A., Wolters, P.S., Rutten, W.L., Ramakers, G.J.A., 2004a. Longterm stability and developmental changes in spontaneous network burst firing patterns in dissociated rat cerebral cortex cell cultures on multi-electrode arrays. Neurosci. Lett. 361, 86–89.

van Pelt, J., Wolters, P.S., Corner, M.A., Rutten, W.L., Ramakers, G.J., 2004b. Long-term characterization of firing dynamics of spontaneous bursts in cultured neural networks. IEEE Trans. Biomed. Eng. 51, 2051–2061.