Characterisation of gamma oscillations in the medial entorhinal cortex *in vitro*

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Networks of neurones in mammalian cortex have the propensity to adopt synchronous oscillations in the gamma frequency range (~20–80 Hz) due to definite stimuli. The appearance of these oscillations during sensory stimulation has led to the suggestion that they are involved in cognitive functions such as memory formation. The medial temporal lobe appears to be crucial in the formation of long-term memories. Indeed, consolidation of information in associational cortical areas is believed to be important. One such associative area is the medial entorhinal cortex (mEC). Previously, *in vivo* experiments have demonstrated that the entorhinal cortex can generate gamma frequency oscillations in response to various stimuli (Chrobak *et al.* 2000). Using the combined entorhinal-hippocampal slice we can report that application of the specific AMPA/kainate receptor agonist kainic acid (400 nM; $n = 16$) induces gamma activity in the mEC.

Combined entorhinal-hippocampal slices (~450 μm), were taken from adult (200–250 g) Wistar rats after terminal anaesthesia using ketamine-xylazine (administered intramuscularly), and intracardial perfusion with artificial cerebrospinal fluid (ACSF) in which NaCl was replaced with sucrose. Slices were maintained at an interface of oxygenated ACSF and humidified (95 % O_2–5 % CO_2) gas at 36 °C. Extracellular field recordings were made in the mEC, using glass microelectrodes containing ACSF (resistance < 3 MΩ). Intracellular recordings were made in neurones, using glass microelectrodes (resistance 100–120 MΩ) containing 1.5 M KCH_3SO_4.

Once initiated, oscillations were stable for several hours. Whilst activity within lamina (< 1 mm) was highly synchronous and with no apparent phase lag, activity recorded across deep and superficial layers showed a 180 deg phase angle shift. A laminar profile of the mEC illustrated a distinct phase shift between layer II and layer III. In order to clarify this phase reversal issue we carried out intracellular recordings simultaneously with field recordings. These recordings revealed IPSPs at depolarised holding potentials (⩾ −40 mV), occurring at frequencies within the gamma range. Bath application of bicuculline (2 μM) abolished both IPSPs and field gamma. IPSPs in layer III were phase locked, and in antiphase with field activity in layer III. Conversely, intracellular events in layer III were phase locked and in phase with field activity in layer II. Finally, IPSPs in layer II were phase locked but antiphase with field activity in deep layers.

GABAergic activity was crucial to network activity. IPSP trains were observed in both deep and superficial layers with no phase reversal. Thus the phase shift observed in the mEC field recordings was probably due to a current sink/source phenomenon.

Chrobak, J.J. *et al.* (2000). *Hippocampus* **10**, 457–465.

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All procedures accord with current UK legislation.

The role of gap junctions in the generation of hippocampal gamma frequency oscillations

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Gamma frequency oscillations (30–80 Hz) can be evoked in the hippocampal slice by a number of methods, including bath application of either carbachol or kainate. This gamma frequency network activity is dependent on both electrical and chemical interactions and is abolished by the gap junction blockers octanol and carbenoxolone – indicating dependence upon gap junction conductances. However, computer modelling has predicted that as long as there is a high level of ectopic action potential activity in principal cell axons, gap junctions are not essential for this pharmacological gamma frequency activity (Traub *et al.* 2000). Gap junctional dependence of kainate induced gamma frequency oscillations was investigated using mouse hippocampal slices. All animals were humanely killed. Male adult mice were anaesthetised with inhaled isoflurane prior to intramuscular injection of ketamine (⩾ 100 mg kg^{-1}) and xylazine (⩾ 10 mg kg^{-1}). When all response to noxious stimuli, such as pedal withdrawal reflex, had terminated, the animals were intracardially perfused with ~25 ml of modified artificial cerebrospinal fluid (ACSF). Slices were maintained at 34°C at the interface between oxygenated ACSF and a mixture of 95 % O_2 and 5 % CO_2. Gamma oscillations were induced using kainate (200–300 nM) and the potassium channel blocker, 4-aminopyridine (4-AP), was applied to increase neuronal and axonal excitability. When carbenoxolone (100 μM) was bath applied to established kainate-induced oscillations there was a 90 ± 3.42 % block of gamma frequency activity within 60 min ($n = 8$). However, in contrast, carbenoxolone failed to cause a complete block of the oscillatory activity when in the presence of 40 μM 4-AP ($n = 6$). In fact 15–30 min application of carbenoxolone caused an increase in oscillatory power of 130 ± 82.2 % ($n = 6$). At 60 min carbenoxolone had still not fully abolished the oscillation, which was at 35 ± 45.1 % compared with kainate and 40 μM 4-AP. This suggests that partial block of gap junctions was able to produce a recovery of the gamma oscillation. With a longer application time carbenoxolone did decrease the activity but it was never completely abolished. This indicates that 4-AP was able to sufficiently increase axonal excitability such that gap junctions were no longer essential for the maintenance of the gamma frequency oscillation.

Traub, R.D. *et al.* (2000). *Eur. J. Neurosci.* **12**, 4093–4106.

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Asynchronous firing in single rat subthalamic neurones in brain slices provides no evidence for intrinsic interconnectivity

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In Parkinson's disease the neurones of the subthalamic nucleus (STN) show increased synchrony and oscillatory burst discharge,

probably reflecting a breakdown of parallel processing in basal ganglia (BG) circuitry. To understand better the mechanisms underlying this transition, we sought to mimic this change in firing pattern within sagittal slices of midbrain taken from humanely killed rats, at 32 °C. The firing patterns of up to four simultaneously extracellularly recorded STN neurones were analysed using burst and oscillation detection programs (Kaneoke & Vitek, 1996), and correlated activity between pairs of neurones also assessed. Data are shown as means ± S.E.M. Statistical comparisons used Student's paired *t* tests, or one-way ANOVA followed by Dunnet's test, with $P < 0.01$ considered significant.

In control conditions 345/354 STN neurones fired tonically, with an oscillation at 8.3 ± 0.24 Hz. There was no significant cross-correlated activity in 299/300 pairs of neurones ($P > 0.01$). The excitation caused by 30 μM muscarine reversibly induced burst firing in 1/19 cells, but no correlation was seen in any of 17 neuronal pairs. Similarly, the excitation by 30 μM (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD) reversibly induced bursting in 2/24 cells, but activity in 26 neuronal pairs remained uncorrelated. 6-Cyano-7-nitroquinoxaline-2, 3-dione (CNQX; 10 μM) and DL-2-amino-5-phosphonovaleric acid (APV; 100 μM) did not change firing rate or pattern ($n = 22$), providing no evidence for a role of glutamatergic collaterals within the STN under these conditions.

Tetraethylammonium (TEA; 10 mM) caused bursting in 77/105 neurones, with an oscillation at 0.79 ± 0.1 Hz and 14.4 ± 2.0 spikes at mean interval of 52.4 ± 8.0 ms per burst. These parameters were unchanged by CNQX and APV ($n = 13$). Muscarine (30 μM) caused a reversible increase in oscillation frequency in 12/15 neurones in TEA from 0.74 ± 0.15 to 1.10 ± 0.16 Hz (without change in spikes/burst or interval). Bursting was unchanged by 30 μM ACPD in 11/12 neurones. Correlated activity was seen in 5/54 neuronal pairs in TEA alone, 2/11 pairs in TEA and muscarine, and 1/12 pairs in TEA and ACPD. Of all correlated pairs, only one (in TEA alone) showed significant correlation over a lag of < 100 ms, rendering a contribution from a local synaptic network unlikely.

The bursting seen here is generally unaccompanied by the synchronous activity that appears (pathologically) *in vivo*, and probably reflects intrinsic STN neuronal properties, rather than network activity. The circuitry needed to produce synchrony in the STN is probably not intrinsic to the STN itself, but probably requires connections with other BG nuclei, and/or the cortex, which are absent in this preparation.

Kaneoke, Y. & Vitek, J.L. (1996). *J. Neurosci. Meths.* **68**, 211-223.

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Pharmacologically induced γ to β shift in rat hippocampus *in vitro*

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In vivo, presentation of a novel sensory stimulus is associated with a characteristic biphasic response in human electro-encephalograph recordings, consisting of an initial γ frequency (30–80 Hz) component and subsequent β frequency (15–30 Hz) component (Hanschel *et al.* 2000). The β component is thought to be associated with mnemonic processes related to the stimulus because the β component of the response habituates with continued presentation of the 'novel' stimulus. An analogous phenomenon has been observed *in vitro*. Applying 2-site strong tetanic stimulation to CA1 of rat hippocampus causes an initial gamma frequency response, followed by a later β frequency response (Whittington *et al.* 1997). Computer modelling suggests that enhanced recurrent excitation between pyramidal cells combined with recovery of the pyramidal cell spike afterhyperpolarisation (AHP) could promote synchronous 'beat skipping' by pyramidal cells, despite ongoing gamma frequency interneuronal activity, leading to a γ/β spectral shift (Bibbig *et al.* 2001).

We present here pharmacological data that may help to elucidate the mechanism underlying the γ/β shift *in vitro*. Hippocampal slices (450 μm) were obtained from rats, which had been anaesthetised humanely with isoflurane, prior to injection with xylazine (> 100 mg kg^{-1}) and ketamine (> 10 mg kg^{-1}) intra-muscularly. Brains were excised quickly and placed in ice-cold sucrose/ACSF. γ oscillations were elicited by application of the metabotropic glutamate receptor agonist (s)-3,5 dihydro-phenylglycine (DHPG). Subsequent application of 5-hydroxy-tryptamine (5-HT) caused a reversible spectral shift in extracellularly recorded field potentials from γ to β frequency (mean gamma frequency = 33.2 ± 0.52 Hz, mean beta frequency = 20.9 ± 1.58 Hz, shift highly significant; $P < 0.001$, $n = 16$, RMS test). Intracellular recordings from pyramidal cells demonstrated a 2-fold increase in excitatory postsynaptic potential (EPSP) amplitude (increase highly significant; $P < 0.001$, $n = 2900$ events from 5 cells, RMS test), and halving of EPSP frequency. Inhibitory postsynaptic potential (IPSP) frequency remained within the γ band during β activity. Additionally, an decrease in pyramidal cell slow AHP amplitude was observed during β activity, which was associated with spike 'beat skipping'. Application of 5-HT to the quiescent slice did not evoke synaptic activity and tended to cause long-term depression of stimulus-evoked EPSP amplitude, suggesting a role for intrinsic network mechanisms in promoting γ to β shifts.

The mechanism by which 5-HT causes an increase in EPSP amplitude may represent a common mode of action of SSRIs and AMPAkines used to treat depression.

Bibbig, A. *et al.* (2001). *J. Neurosci.* **21**, 9053–9067.

Hanschel, C. *et al.* (2000). *PNAS* **97**, 7645–7650.

"Whittington, M. *et al.* (1997). *PNAS* **94**, 12198–12203.

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Differential involvement of proximal and distal dendrite-targeting interneurons during hippocampal γ oscillations

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Synchronous γ frequency oscillations (20–80 Hz) represent a temporally coherent activity and are thought to be important in cortical information processing. Hippocampal interneurons have a pivotal role in driving inhibition-based rhythms, including gamma frequency network oscillations. It is, however, unknown

whether different types of interneurons show a different degree of involvement during γ activity. Therefore, using brief pressure ejection of kainate, an approach that allowed us to generate network oscillatory activity in submerged slices, we investigated how the anatomical heterogeneity of hippocampal interneurons is reflected in their behaviour during γ frequency oscillations.

Hippocampal slices (450 μm thickness) were prepared from C57 mice (P 18–25). All animals were humanely killed. Whilst measuring extracellular field potentials whole-cell patch-clamp recordings in current and voltage clamp mode were obtained from stratum oriens/alveus interneurons in area CA3. Patch pipettes contained the marker biocytin and all labelled neurons were processed for *post-hoc* anatomical identification. Student's *t* test was used for statistical comparisons. Average values are expressed as means ± S.E.M.

Our data demonstrate a differential degree of involvement of two types of stratum oriens interneurons in network activity, distal dendrite targeting O-LM and proximal dendrite targeting trilaminar cells. The kinetics of the EPSP/Cs and the amplitude of synaptic events of these cells during oscillations were significantly different. The mean amplitude of excitatory synaptic currents in O-LM interneurons was 54.3 ± 1.6 pA, being significantly smaller than those in trilaminar cells (150.1 ± 8.8 pA; $P < 0.05$). Likewise, the 10–90 % rise time of averaged EPSCs in O-LM interneurons was slower (0.84 ± 0.09 ms) than those in trilaminar cells (0.53 ± 0.05 ms). The mean decay time constant of EPSCs was 2.52 ± 0.20 ms in O-LM cells and 1.40 ± 0.23 ms in trilaminar cells ($P < 0.05$). Moreover, although both classes of cells received a similar sustained barrage of high-frequency (~300 Hz) rhythmic input, modulated at gamma frequency, their discharge patterns were significantly different. O-LM interneurons consistently fired at lower frequencies in the theta frequency range (mean 9.0 ± 2.7 Hz, $n = 7$), whereas trilaminar interneurons generated at least one action potential per gamma cycle, frequently firing spike doublets ($n = 4$).

These observations demonstrate that distally targeting O-LM interneurons generate theta frequency-modulated output during field γ, whereas trilaminar interneurons phase postsynaptic cells with γ frequency IPSPs and presumably play a critical role in the generation and maintenance of rhythmic oscillatory activity in this frequency band.

All procedures accord with current UK legislation.

Amphetamine and cocaine modulate kainate-evoked γ frequency oscillations in the rat hippocampus *in vitro*

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Psychostimulants such as amphetamine and cocaine mediate their effects, at least in part, via the dopamine neurotransmitter system. The hippocampus contains all five (D1–D5) subtypes of dopamine receptors and modulation of dopamine function has been reported to have a wide variety of effects, including alterations in excitatory and inhibitory synaptic transmission, and electrical coupling via gap junctions. Several different types of synchronized network activity have been described in the hippocampus that depend upon both synaptic interactions and gap junction-dependent electrical transmission. We therefore wished to determine what effect amphetamine and cocaine might have on the generation of rhythmic activity in the hippocampus.

Hippocampal slices (450 μm thick) were prepared from adult, male Wistar rats terminally anaesthetised with inhaled isoflurane and injection (I.M.) of ketamine ($\geqslant 100$ mg kg^{-1}) and xylazine ($\geqslant 10$ mg kg^{-1}). Animals were perfused transcardially with artificial cerebrospinal fluid (ACSF) in which NaCl was replaced with equimolar sucrose. Slices were maintained in an interface recording chamber perfused with ACSF and oxygenated with 95 % O_2 and 5 % CO_2 at 34 °C. Extracellular field recordings were made in stratum radiatum or stratum pyramidale of the CA3 and CA1 regions. Changes in oscillatory activity were assessed by measuring the area between 20 and 80 Hz from the power spectra. Data are expressed as means ± S.E.M. Bath application of kainate (300 nM) elicits a persistent γ frequency oscillation that depends upon both excitatory and inhibitory synaptic interactions. Amphetamine sulphate (10–30 μM) caused a mean decrease of 53 ± 32 % in the power of the gamma frequency activity ($n = 8$). This reduction was reversible on washout of amphetamine. Application of cocaine also elicited a reduction in the kainate-evoked γ frequency activity with a mean decrease of 70 ± 11.8 % ($n = 6$). We also assessed the effect of amphetamine on ultrafast oscillations (80–200 Hz) that occur in the absence of synaptic transmission and depend upon electrical transmission via gap junctions. However, fast oscillatory activity (evoked by pressure ejection of potassium in the presence of blockers of fast glutamaterigc and $GABA_A$ergic transmission) persisted in the presence of amphetamine. The same duration application of amphetamine failed to reduce the power of the fast oscillation (11.3 ± 1.4 %; $n = 3$). This result suggests that amphetamine selectively reduces kainate-evoked oscillations but does not affect the gap junction-dependent fast oscillatory activity. In conclusion, some of the well-known effects of psychostimulants, such as those on attentional mechanisms, may be due to effects on cortical network activity.

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All procedures accord with current UK legislation.

Effects of nitric oxide on electrotonically coupled rat sympathetic preganglionic neurones *in vitro*

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A population of sympathetic preganglionic neurones (SPNs) are electrotonically coupled (Logan *et al.* 1996). Oscillations in membrane potential, manifest as low-pass filtered remnants of presynaptic action potentials in coupled SPN, are a characteristic feature of these neurones. Nitric oxide (NO) induces membrane hyperpolarisation and inhibits oscillatory activity in coupled SPN (Spanswick *et al.* 1996). One explanation for these observations is that NO modulates electrical synapses in SPN. We therefore used whole-cell recording techniques in an isolated spinal cord preparation to investigate the effects of NO-dependent signalling on electrical synapses in SPNs.

Neonatal Wistar rats (6–14 days) were terminally anaesthetised with halothane (4 % in O_2), decapitated, the spinal cord removed and 400 μm transverse thoracolumbar spinal cord slices prepared as described previously (Logan *et al.* 1996).

Simultaneous whole-cell recordings were made from 12 pairs of SPNs. In 3 pairs, electrotonic coupling was directly demonstrated by injection of current pulses into one SPN, which produced membrane responses in both SPNs. The mean coupling

coefficients (CC) and junctional conductances (G_j) were 0.22 ± 0.02 (mean ± S.E.M.) and 0.91 ± 0.06 nS, respectively.

Bath application of the NO donor, *S*-nitrosoglutathione (SNOG, 100–200 μM) induced membrane hyperpolarisation, cessation of spontaneous activity and a decrease in neuronal input resistance in all neurones tested ($n = 13$). In the presence of SNOG ($n = 3$), mean CC and G_j were 0.23 ± 0.03 and 0.96 ± 0.15 nS, respectively. Consequently, NO had no significant effect on either CC or G_j ($P = 0.23$ and 0.60, respectively, Student's paired *t* test). A principle mode of action of NO is to raise intracellular cGMP levels via the activation of soluble guanylyl cyclase. Therefore the effects of 8-bromo-cGMP were compared with those of the NO donor. 8-Bromo-cGMP (50 μM) mimicked the effects of SNOG, inducing membrane hyperpolarisation, and a reduction in neuronal input resistance in all neurones tested ($n = 2$). CC and G_j ranged from 0.19 to 0.26 and 0.76 to 1.01 nS, respectively, in control conditions. Corresponding values in the presence of 8-Bromo-cGMP were 0.17 to 0.24 and 0.87 to 0.95 nS, respectively. Thus, 8-Bromo-cGMP was also without effect on CC and G_j.

These data suggest that NO, via cGMP-dependent and -independent pathways, acts to inhibit electrotonically coupled SPN, but is without direct effect on electrical synapses.

Logan, S.D. *et al.* (1996). *J. Physiol.* **495**, 491–502.

Spanswick, D. *et al.* (1996). *J. Physiol.* **494.P**, 78–79*P*.

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Anandamide transport inhibition, but not inhibition of fatty acid amide hydrolase (FAAH) blocks the induction of long-term potentiation (LTP) in the CA1 neurons of the rat hippocampal slice

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The sleep-inducing properties of cis-oleamide (cOA) may reflect quenching of the FAAH enzyme, which degrades the endocannabinoid anandamide (Mechoulam *et al* 1997). cOA is itself an inhibitor of FAAH and we have previously reported that at a dose of 32 μM it fails to inhibit LTP. Here we investigate the effects of FAAH inhibition (1,1,1-trifluoro10(Z)-nonadecen-2-one (TFNO) ± cOA) and of uptake inhibition (AM404) on LTP induction by high-frequency stimulation (HFS) in the hippocampal slice.

Male Wistar rats (*ca* 150 g) were humanely killed and transverse hippocampal slices (400 μm) were isolated. After $\geqslant$ 1 h, slices were perfused (2–3 ml min^{-1}) with ACSF (composition (mM): NaCl 124, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 26, NaH_2PO_4 1.25, D-glucose 10, equilibrated with carbogen) at 34.6 ± 0.2°C ($n = 10$). ACSF was supplemented with 0.1 % of DMSO and BSA to aid the dissolution of cOA. Extracellular fEPSPs were obtained from the stratum radiatum in response to stimulation of the Schaffer collaterals (0.1 ms pulses, 0.05 Hz). We quantified changes in the initial slope (20–80 % of maximum amplitude) 30 min after the conditioning protocols (all ligands were applied at least 60 min before the conditioning train was given at 100 Hz for 1 s). Values are expressed as % of mean ± S.E.M. (normalised to pretreatment). A *P* value of less than 0.05 was significant.

The HFS protocol caused a significant and sustained increase in the slope of the fEPSP compared with untreated controls. (159.8 ± 6.4 %, $n = 4$). TFNO (1 μM) did not block the induction of LTP when compared with the controls (171.3 ± 12.85 %, $n = 4$, $P > 0.05$, unpaired *t* tests). cOA (32 μM), in the presence of TFNO (1 μM), did not inhibit HFS-LTP(166.0 ± 12.9 %, $P > 0.05$, $n = 4$, unpaired *t* tests). On the contrary, AM404 (20 μM) significantly attenuated LTP after 30 min (137.0 ± 2.1 %, $n = 4$, $P < 0.001$, unpaired *t* test).

Selective inhibition of the metabolism of anandamide by TFNO does not block CA1 LTP, which is sensitive to exogenous anandamide (Terranova *et al.* 1995). Combination of TFNO and cOA did not block LTP either, which emphasises oleamide's lack of CB1 agonist properties in our hands. The transport inhibitor AM404 does attenuate LTP. This has some parallels *in vivo* where systemically administered FAAH inhibitors or anandamide transport inhibitors potentiate the actions of the endocannabinoids, but only the latter increases anandamide titres in plasma (Giuffrida *et al.* 2000).

Giuffrida, A. *et al.* (2000). *Eur. J. Pharmacol.* **408**, 161–168.

Mechoulam, R. *et al.* (1997). *Nature* **389**, 25–26.

Terranova, J.P. *et al.* (1995). *Naunyn-Schmied. Arch. Pharmacol.* **352**, 576–579.

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Developmental changes in pairing-induced synaptic plasticity in CA1 hippocampus

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Correlated pairings of single pre- and postsynaptic action potentials are sufficient to induce long-term plastic changes in developing hippocampal synapses (Bi & Poo, 2001). In contrast, previous work from our group has suggested that postsynaptic burst-spiking is necessary to induce long-term potentiation (LTP) during a pairing protocol in adult acute hippocampal slices (Pike *et al.* 1999). The aim of the present experiments was to investigate the developmental profile of the effectiveness of single postsynaptic spikes *vs.* postsynaptic bursting activity for induction of LTP. Hippocampal slices (300 μm) were made from Wistar rats (P12–P29), decapitated under isoflurane anaesthesia in accordance with UK Animals (Scientific Procedures) Act, 1986. Patch-clamp recordings were made from CA1 pyramidal neurones in submerged slices (25–28 °C). Two afferent Schaffer collateral inputs were alternately stimulated at 0.2 Hz and EPSP amplitude measured throughout the experiment.

Following a stable EPSP baseline of at least 12 min, one afferent input was paired firstly with a postsynaptic single spike, repeated 30 times, then 20 min later with a postsynaptic burst, again repeated 30 times.

Single spike pairing induced a significant potentiation of the test input 20 min after pairing in young animals (P12–15: 186 ± 21 % (Student's *t* test, mean ± S.E.M.), $n = 11$, $P < 0.05$). The effectiveness of single-spike pairing to potentiate the test input was negatively correlated with developmental age (P12–29: $n = 34$, $r = -0.417$, $P < 0.05$). In contrast, burst-spike pairing significantly increased EPSP amplitude in older animals (P25–29: 168 ± 24 %, $n = 8$, $P < 0.05$) but caused no further increase in

EPSP amplitude in younger animals (P12–15: 100 ± 5 %, $n = 9$). No significant correlations between developmental age and synaptic efficacy were observed in the control input.

These data suggest that there is a gradual developmental shift in the induction rules for synaptic plasticity in the hippocampus from young animals to adults.

Bi, G.-Q. & Poo, M.-M. (2001). *Ann. Rev. Neurosci.* **24**, 139–166.
Pike, F.G. *et al.* (1999). *J. Physiol.* **518**, 571–576.

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Laminar differences in plasticity in striate cortex of cats

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We have previously reported to the Society that in cats in which monocular retinal lesions were made in adulthood or adolescence cells in the lesion projection zone (LPZ) of area 17 recovered sensitivity to photic stimuli but the receptive fields (RFs) were now ectopic, i.e. outside the lesion (Burke *et al.* 2000). When the lesions were made in adult cats (AL), stimuli presented via the lesioned eye gave lower peak discharge rates and lower cut-off velocities than those presented via the non-lesioned eye. By contrast, in kitten-lesioned cats (KL) the cut-off velocities and the peak discharge rates were similar for stimuli presented via the lesioned and the non-lesioned eye. There was, however, a difference in the time from lesion to experiment between the AL and KL groups, 2–24 weeks *vs.* 28–68 weeks. It was, therefore, important to see if this factor could be responsible for the neural effects. Ectopic RFs are believed to be mediated via axon collaterals of pyramidal cells interconnecting mainly in laminae 2 and 3. We, therefore, also investigated the location of the LPZ neurones (supragranular (SG) – laminae 1–3: granular/infragranular (G/I) – laminae 4–6).

In addition to the two groups already described (AL 4 cats; KL 5 cats) we prepared a third group in which the lesion was made in the adult cat but the cat then survived 3.5–4.5 years (AL/L 3 cats). Retinal lesions 8–12 deg in diameter were made in cats anaesthetized with xylazine (3 mg kg^{-1}) and ketamine (30 mg kg^{-1}). Single neurones in area 17 were studied in cats anaesthetized with 0.5–0.7 % halothane in 67/33 N_2O/O_2, given gallamine triethiodide 7.5 mg kg^{-1} h^{-1} I.V. and artificially respired. EEG, ECG, end-tidal CO_2, lung pressure and deep body temperature were monitored and kept within normal limits. Animals were humanely killed at the end of the experiments.

In the G/I laminae we found no difference between AL and AL/L cats but a significant difference between AL/L and KL cats and between AL and KL cats with regard to peak discharge rates and cut-off velocities (Wilcoxon, $P < 0.05$). Thus it seems that the interval between lesion and experiment is not a critical factor.

By contrast, in the SG laminae there was no difference between AL and KL cats with respect to peak discharge rates and cut-off velocities. Thus following monocular retinal lesions there appears to be a critical period for the LPZ cells recorded from the G/I layers of area 17, after which the presumed cortical mechanisms underlying establishment of ectopic RFs are not capable of good compensation for the loss of the retinal input.

Burke, W. *et al.* (2000). *J. Physiol.* **528.P**, 75–76*P*.

All procedures accord with current National and local guidelines.

Age-dependent long-term depression at CA3–CA1 synapses in mouse hippocampal slices

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Low-frequency stimulation (LFS) is widely used to induce long-term depression (LTD) at rodent CA3–CA1 hippocampal synapses. The relationship between the efficacy of LFS induction and postnatal age remains controversial in the rat, and has yet to be studied in the mouse. The data presented here strongly suggest that LFS-induced LTD is highly age dependent at mouse hippocampal synapses.

Briefly, transverse hippocampal slices (400 μm) were prepared from humanely killed B6xCBA mice aged between postnatal days P6–P116. Slices were maintained at 28°C in an interface chamber and perfused with oxygenated artificial cerebrospinal fluid. Field excitatory postsynaptic potentials (fEPSPs) were recorded using microelectrodes filled with 1 M sodium acetate placed in the stratum radiatum of the CA1 field. Stimulation of CA3 Shaffer collaterals was performed with monopolar stainless-steel electrodes. Test shocks were applied at 30 s intervals, set to evoke either 50 % of the maximum response or a maximal sub-threshold population spike response. LFS consisted of 900 shocks at 1 Hz and LTD defined as a stable reduction (> 10 %) of the fEPSP slope 1 h post-conditioning. Data are expressed as the per cent change in fEPSP slope ± S.E.M. Statistical analysis was carried out using Dunnett's multiple comparison test for significance with respect to adult slices (P100+).

LFS conditioning induced a highly significant, age-dependent LTD, apparent from postnatal day P6 but no longer significant by P18. Slices prepared from animals aged P6–9 exhibited LTD (−28.97 ± 5.99 %; $n = 8$, $P < 0.01$) in all experiments. By age P10–13, LTD (−19.8 ± 2.27 %; $n = 39$, $P < 0.01$) was induced in 77 % of experiments. Similarly, in slices from P14–17 LTD (−20.03 ± 4.85 %; $n = 15$ $P < 0.01$) was induced in 80 % of experiments. By P18–21 (−4.585 ± 4.22 %; $n = 14$) LTD is induced in 29 % of experiments but the group mean is no longer significant ($P > 0.05$). P22–28 (−1.812 ± 3.48 %; $n = 15$) and P29–49 (4.365 ± 5.99 %; $n = 6$) exhibited LTD in 13 and 17 % of experiments, respectively. P50–100 (9.39 ± 4.71 %; $n = 7$) and P100+ (10.942 ± 4.69 %; $n = 9$) failed to exhibit LTD. LFS-induced LTD was also homosynaptic (P12; $n = 4$) and reversibly blocked by the NMDA receptor antagonist D-(−)-2-amino-5-phosphonopentanoic acid (25 μM AP5) (−5.49 ± 4.08 %); following removal of AP5 a second period of LFS conditioning successfully induced LTD (−23.47 ± 5.56 %; $n = 6$; $P < 0.05$, Student's unpaired t test; P12–15).

Our data demonstrate that, in mouse, LFS (900 shocks at 1 Hz) -induced LTD at CA3-CA1 synapses requires the activation of NMDA receptors and is highly age dependent.

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All procedures accord with current UK legislation.

Effect of bicuculline on EPSP amplitude in Purkinje cells of *mdx* mice

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The absence of the protein dystrophin causes a muscular dystrophy in *mdx* mice (the most commonly used murine model of Duchenne muscular dystrophy in humans). Dystrophin is normally expressed in a range of tissues including areas of the CNS (Anderson *et al.* 2002). In Purkinje cells from normal mice the P-dystrophin isoform is organised in punctate clusters at the postsynaptic densities co-localised with the $GABA_A$ receptor subunit clusters. Kneusel *et al.* (1999) showed a marked decrease in $GABA_A$ receptor clusters immunoreactive for $\alpha 1$ and $\alpha 2$ subunits in cerebellar Purkinje cells of *mdx* in which the P-dystrophin isoform is absent.

Experiments were performed on cerebellar slices taken from *mdx* mice (C57Bl/10 *mdx*) and age-matched controls (C57Bl/10 ScSn). Mice were anaesthetised with halothane and then killed by cervical dislocation. The cerebellum was rapidly removed, bissected and transferred to ice-cold, carbogenated artificial cerebrospinal fluid (ACSF). Parasagittal slices (250 μm) were cut and transferred to carbogenated ACSF at room temperature. Cerebellar Purkinje cells were identified using IR-DIC optics and ×40 immersion lens. Intracellular recordings were obtained from Purkinje cells using glass micropipettes (~120 MΩ) filled with 2 M potassium acetate. EPSPs were evoked by electrical stimulation using a concentric stimulating electrode positioned in the molecular layer of the cerebellar slice close to the Purkinje cell under study. Where the SD in each group was equal, a Student's *t* test was used to statistically compare control and *mdx* groups, and where there was a significant difference between the S.D. from each group a Mann-Whitney test was used. Data are presented as means ± S.E.M.

The resting membrane potential for our sample of control Purkinje cells had a mean of −64 mV (± 2.7; $n = 10$) and for *mdx* cells a mean of −69 mV (± 3.2; $n = 11$), which did not differ significantly ($P = 0.34$, $n = 21$, t test). The mean of the input resistance for control Purkinje cells of 13 MΩ (± 1.7, $n = 8$) did not differ significantly from the mean for *mdx* cells of 15 MΩ (± 3.8, $n = 7$) ($P = 0.867$, $n = 15$, Mann-Whitney). The average of the increase in EPSP amplitude in control Purkinje cells on application of bicuculline was 75.8 % (± 23; $n = 10$) and for *mdx* cells was 34.8 % (± 9.2; $n = 11$). Although the difference in the means is not quite statistically significant at the 5 % level ($P = 0.057$, Mann-Whitney), the data clearly indicate a difference in the extent to which the amplitude of the net evoked EPSP of control cells and *mdx* cells is affected by the $GABA_A$ receptor antagonist.

Our results provide functional data that are consistent with the histological findings of a reduction in $GABA_A$ channel clusters in Purkinje cells of dystrophic mice.

Anderson, J.L *et al.* (2002). *Brain* **125**, 4–13.

Knuesel *et al.* (1999). *Eur. J. Neurosci.* **11**, 4457–4462.

All procedures accord with current National and local guidelines.

cis-Oleamide does not modulate serotonergic transmission in CA1 pyramidal cells of the rat hippocampal slice

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Oleamide (cOA) is a sleep-inducing brain lipid, which has been reported to be a potent modulator (1–100 nM) of serotonergic receptors in oocytes and cell lines ($_{2A/C}$ and $_{1A}$ subtypes) (e.g. Boger *et al.* 1998). *In vivo* studies provide evidence for, and against, a serotonergic mechanism (Cheer *et al.* 1999; Federova *et al.* 2001). The CA1 region of the hippocampus bears somatic $5HT_{1A}$ receptors, which mediate hyperpolarisation and a concentration-dependent depression of the population spike (Pugliese *et al.* 1998). We have assessed the ability of cOA to modulate serotonergic transmission in the *in vitro* hippocampal slice exposed briefly to exogenous 5HT.

Male Wistar rats (*ca* 150 g) were humanely killed and sagittal brain slices (400 μm) were prepared for superfusion at 35 °C with ACSF (mM): NaCl 119, KCl 3, $MgCl_2$ 1, $CaCl_2$ 2, $NaHCO_3$ 26.2, NaH_2PO_4 1.25, D-glucose 10, equilibrated with 95 % O_2 and 5 % CO_2). Field population spikes (fPS) from CA1 stratum pyramidale were recorded following stimulation of the Schaffer collaterals using a bipolar stimulus (0.1 ms pulses at 0.05 Hz) at a stimulus intensity giving *ca* 40 % of the maximal fPS. ACSF was supplemented with 0.1 % DMSO plus 0.1 % BSA to aid the dissolution of cOA. Results are normalised as % of pretreatment fPS amplitude ± S.E.M. Paired t tests were used to compare averaged responses before and after oleamide.

5HT (0.1–100 μM) caused a concentration-dependent depression of the population spike (EC_{50}: *ca* 10 μM), which was maximal between 64 and 100 μM ($n = 5$). We selected a concentration of 3.2 μM (*ca* EC_{25}) to measure the effects of repeated 5HT application. Three 10 min applications of 5HT alone (with 15 min wash-out to avoid desensitisation) were applied followed by a further three applications in the presence of 1 μM cOA (30 min pretreatment). cOA did not attenuate the amplitude of population spikes when applied alone at this concentration, nor did it potentiate the depressant effects of 5HT after 30–60 min of superfusion. The responses obtained with repeated application of 5HT in control saline were not significantly different from those obtained with cOA (5HT against 5HT and cOA, 30.7 ± 2.7 % against 28.5 ± 3.7 %, $P > 0.05$, $n = 5$)

We conclude that cOA, at a concentration that would saturate recombinant receptors in expression systems, does not modulate serotonergic transmission in the CA1 neurons. $5HT_{2A/C}$ receptors also mediate inhibitory responses on the CA1 soma. We are currently examining the possibility that such low-affinity metabotropic receptors are differentially sensitive to oleamide in these functional CNS circuits.

Boger, D.L. *et al.* (1998). *Curr. Pharm. Des.* **4**, 303–314.

Cheer, J.F. *et al.* (1999). *Neuropharmacology* **38**, 533–541.

Federova, I. *et al.* (2001). *JPET* **299**, 332–342.

Pugliese, A.M. *et al.* (1998). *Br. J. Pharmacol.* **124**, 93–100.

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All procedures accord with current UK legislation.

Cell death following inhibition of amyloid β protein production in primary cultures of rat central neurones

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The amyloid β protein (Aβ) is derived by sequential β- and γ-secretase cleavage of amyloid precursor protein and is widely regarded as the principal toxic factor of Alzheimer's disease (AD). Newly developed inhibitors of β- and γ-secretase prevent Aβ production and represent a potential therapeutic strategy in AD (Sinha *et al.* 1999; Selkoe, 2001). We have recently proposed a physiological role for Aβ (Ramsden *et al.* 2001), and so chose to investigate the impact of secretase inhibitors on the viability of cultured cells.

Primary cultures of rat cortical or cerebellar granule neurones (CGN) were prepared from humanely killed animals as previously described (Ramsden *et al.* 2001). Immortalised cell lines (SH-SY5Y, DDT1FM2, HEK293) and primary cultures of a teratorhabdoid tumour were grown using the methods of Shukla *et al.* (2001). Cell viability was assessed using the MTT assay (Shukla *et al.* 2001). All values are given as means ± S.E.M.

Following 24 h treatment with 2-naphthoyl-VF-CHO (γ-IV; 10 μM), an inhibitor of γ-secretase activity, the viability of cortical neurones was reduced by 57 ± 5 % ($n = 9$, $P < 0.01$, Student's unpaired t test). This neurotoxic effect was also seen with the β secretase inhibitor H-KTEEISEVN-stat-VAEF-OH (βSI; 100 nM, 54 ± 4 % cell death, $n = 9$) and could be reproduced using cerebellar granule neurones and the human neuroblastoma cell line SH-SY5Y (62 ± 6 and 43 ± 10 % cell death, respectively). In contrast, cells of a non-neuronal origin were unaffected by inhibition of amyloidogenic pathways. In HEK293 cells 24 h incubation with γ-IV resulted in a small, non-significant increase in cell viability of 6.4 ± 1.9 % ($n = 8$) and in DDT1FM2 cells and a teratorhabdoid carcinoma similar increases (2.1 ± 3.0 and 2.0 ± 2.5 %, $n = 8$ in each case) were seen, respectively. Crucially, the cell death observed in neurones could be prevented by co-application of Aβ1–40 at physiologically relevant concentrations. At a concentration of 1 nM, Aβ reduced cell death in cortical neurones caused by γ-IV to 4 ± 7 % ($n = 9$). This protective effect was not seen with the pathological 1–42 or synthetic 25–35 size forms.

These data suggest that the production of Aβ is a requirement for neuronal viability and support a physiological role for this peptide. Importantly, these data suggest that interfering with amyloidogenesis as a therapeutic approach in AD may result in unforeseen neurotoxic effects.

Ramsden, M. *et al.* (2001). *J. Neurochem.* **79**, 699–712.

Selkoe, D.J. (2001). *Neuron* **32**, 177–180.

Shukla, C. *et al.* (2001). *J. Cell Path.* **5**, 241–249.

Sinha, S. *et al.* (1999). *Proc. Natl Acad. Sci. USA* **96**, 11049–11053.

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Feedback potentiation of presenilin expression in human neuroblastoma (SH-SY5Y) cells by amyloid β peptide$_{1–40}$, but not amyloid β peptide$_{1–42}$

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Amyloid β peptides (Aβs) of Alzheimer's disease (AD) are formed from the cleavage of amyloid precursor protein (APP) by β- and γ-secretase. γ-Secretase is known to be a complex including members of the presenilin protein family which also have direct effects on IP_3-sensitive intracellular Ca^{2+} stores (Leissring *et al.* 2000; Smith *et al.* 2002). Here, we report a novel effect of Aβs on presenilin expression.

SH-SY5Y cells were cultured as previously described and, either plated onto coverslips and loaded with fura-2 AM for the measurement of muscarine-evoked increases in $[Ca^{2+}]_i$ or lysed into protein extraction reagent for standard Western immunoblotting (Smith *et al.* 2001). Blots were probed with antibodies raised against c or n terminal fragments (CTF and NTF) of PS-1 and the NTF of PS-2. All values are means ± S.E.M. and $n > 3$. Statistical significance ($P < 0.01$) was tested by one-way ANOVA with a Bonferroni multiple comparison post test.

Treatment with A$\beta_{1–40}$ (1 μM, 24 h) caused a 1.8 ± 0.3 (NTF)- and 4.0 ± 0.8 (CTF)-fold increase in expression of full length and a 2.2 ± 0.06 (NTF)- and 2.6 ± 0.6 (CTF)-fold increase in cleaved PS-1 relative to control values. The A$\beta_{25–35}$ fragment mimicked this effect of A$\beta_{1–40}$ with a 3.3 ± 1.8 (NTF)- and 3.3 ± 0.8 (CTF)-fold increase in full length and a 2.4 ± 0.06 (NTF)- and 1.4 ± 0.1 (CTF)-fold increase in cleaved PS-1. The reverse sequence peptide A$\beta_{40–1}$ and A$\beta_{1–42}$ were without effect. This effect of A$\beta_{1–40}$ could be due to the production of reactive oxygen species (ROS) and so cells were treated with H_2O_2 (40 or 150 μM, 24 h) which had no effect on PS-1 expression. Co-incubation with both A$\beta_{1–40}$ (1 μM) and one of the anti-oxidants melatonin (150 μM), ascorbic acid (200 μM) or ebselen (10 μM), for 24 h did not prevent the increase in PS-1 levels. Similar results were obtained for PS-2 expression.

A$\beta_{1–40}$ and A$\beta_{25–35}$ (1 μM, 24 h) caused a significant increase in the size of muscarine-sensitive Ca^{2+} stores in these cells (control, 23.6 ± 5.7; 1–40, 61.7 ± 5.2; 25–35, 65.4 ± 5.6), an effect that was not seen after treatment with the 40–1 or 1–42 peptides. The effect of A$\beta_{1–40}$ on Ca^{2+} stores was not prevented by melatonin or ebselen.

The present study shows for the first time that A$\beta_{1–40}$ positively regulates the expression of the enzyme responsible for the production of Aβ peptides. The mechanism is unknown but does not involve the production of reactive oxygen species. This positive feedback loop may contribute to the deposition of amyloid peptides and so the progressive neurodegeneration seen in AD.

Leissring, M.A. *et al.* (2000). *J. Cell Biol.* **149**, 793–797.

Smith, I.F. *et al.* (2001). *J. Neurochem.* **79**, 877–884.

Smith, I.F. *et al.* (2002). *FASEB J.* **16**, A505.

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Cell volume regulation in CAD cells using a video-imaging technique

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When a cell is exposed to hypotonic conditions it increases in size through osmotic swelling, activating a mechanism known as regulatory volume decrease (RVD) which returns the cell back to its original volume mainly through KCl efflux. This adaptive response is particularly important in the brain, as swelling can lead to ischaemic and anoxic brain damage.

This study investigates the conditions necessary for cell volume regulation in a neuronal cell line CAD (Qi *et al.* 1997), and suggests a role for chloride (Cl^-) channels in RVD. Cells were perfused with a Hepes-buffered isotonic solution (300 mosmol kgH_2O^{-1}) for 3 min followed by a 30 % hypotonic solution (210 mosmol kgH_2O^{-1}) for up to 47 min at 37 °C unless otherwise stated. Digital photographs were taken every minute and cell volume measurements were calculated using the Scion Image computer software package. All cell volume measurements are expressed as relative cell volumes with respect to control values. Changes in cell volumes during hypotonic exposure (mean volume change ± S.E.M.) were compared using a Student's unpaired *t* test or ANOVA followed by the Tukey HSD test.

On exposure to a 30 % hypotonic solution cells swelled to 1.32 ± 0.013 in the first 4 min and recovered to 1.23 ± 0.017 ($n = 9$) within 15 min. Cells exposed to a 23 % hypotonic solution (231 mosmol kgH_2O^{-1}) swelled to 1.24 ± 0.007 and recovered to 1.17 ± 0.003 ($n = 3$) within 15 min. Although cell swelling is affected by the degree of hypotonicity ($P < 0.001$) there was no difference in the mean cell volume changes after 15 min hypotonic exposure ($P > 0.10$). However, RVD is temperature dependent as a significant difference in mean cell volume change was observed when cells were exposed to a 23 % hypotonic solution for 15 min at 21 or 37 °C ($n = 3$, $P < 0.005$).

The role of Cl^- channels in RVD was investigated using the volume-regulated anion channel (VRAC) antagonists tamoxifen and 4,4′-diisothiocyanatostilbene-2,2′-disulphonic acid (DIDS). On exposure to a 30 % hypotonic solution containing 10 μM tamoxifen, cells swelled to 1.40 ± 0.012 and recovered to 1.38 ± 0.023 ($n = 5$) within 15 min, and 1.29 ± 0.015 ($n = 3$) within 47 min. On exposure to a 30 % hypotonic solution containing 100 μM DIDS, cells swelled to 1.35 ± 0.012 and recovered to 1.32 ± 0.012 ($n = 5$) within 15 min, and 1.29 ± 0.025 ($n = 4$) within 47 min. In both cases changes in mean cell volume at 15 and 47 min were significantly different from control values ($P < 0.01$).

In conclusion CAD cells act as near perfect osmometers and are capable of regulating their volume on exposure to a hypotonic medium. Furthermore, these results suggest that a volume-regulated Cl^- channel is important for RVD in CAD cells.

Qi, Y. *et al.* (1997). *J. Neurosci.* **17**, 1217–1225.

Chronic hypoxia inhibits Na^+–Ca^{2+} exchange and elevates mitochondrial $[Ca^{2+}]$ in rat type 1 cortical astrocytes

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Periods of chronic hypoxia (CH) are well known to alter Ca^{2+}-dependent mechanisms in a variety of cell types and such effects may contribute to disturbances of Ca^{2+} homeostasis associated with Alzheimer's disease (e.g. Taylor *et al.* 1999; Smith *et al.* 2001). Here, we have investigated bradykinin-evoked changes of $[Ca^{2+}]_i$ in rat type 1 cortical astrocytes, and how these changes were modified by culturing cells in CH (24 h, 2.5 % O_2). $[Ca^{2+}]_i$ was monitored in fura-2-loaded cells as previously described (Smith *et al.* 2001). Transient rises of $[Ca^{2+}]_i$ above baseline were integrated and results are presented as means (± S.E.M.) ratio unit seconds (r.u.s.). Statistical significance was determined using Student's unpaired *t* tests.

In Ca^{2+}-free solution (replaced with 1 mM EGTA), 100 nM bradykinin evoked rises of $[Ca^{2+}]_i$ in control cells of 8.04 ± 0.92 r.u.s. ($n = 14$), which declined to basal levels with a $t_{½}$ of 43.6 ± 3.7s. Ca^{2+} transients in CH cells were significantly greater than in controls at 17.7 ± 1.5 r.u.s. ($n = 13$, $P < 0.01$) and declined with a $t_{½}$ of 61.7 ± 8.6 s ($P < 0.04$). Upon removal of external Na^+ from the perfusate (replaced with *N*-methyl-D-glucamine), transient rises of Ca^{2+} in control cells (11.47 ± 0.93 r.u.s., $n = 8$) were significantly greater than in the presence of Na^+ ($P < 0.03$). Under CH conditions, transient responses to bradykinin were not significantly altered by Na^+ removal (23.56 ± 4.4 r.u.s., $n = 8$). Bath application of 10 μM FCCP/ 2.5 mg ml^{-1} oligomycin evoked rises in $[Ca^{2+}]_i$ due to release from mitochondria which were significantly greater ($P < 0.05$) in CH-treated cells at 4.98 ± 0.82 r.u.s. ($n = 8$) than were observed in control cells (1.93 ± 0.62 r.u.s., $n = 8$). Subsequent application of 100 nM bradykinin in the continued presence of FCCP and oligomycin caused rises of $[Ca^{2+}]_i$ in control cells (19.6 ± 2.0 r.u.s., $n = 13$), which were not significantly different from those observed in CH cells (20.5 ± 2.4 r.u.s., $n = 9$).

Our results indicate that CH causes an increase in the Ca^{2+} content of mitochondria which in turn results in an apparent increase in responses to bradykinin due to a decreased ability of mitochondria to buffer Ca^{2+} released from the endoplasmic reticulum. In addition, Na^+–Ca^{2+} exchange appears inhibited in CH cells, which may also be due to elevated mitochondrial Ca^{2+} levels (Opuni & Reeves, 2000).

Opuni, K. & Reeves, J.P. (2000). *J. Biol. Chem.* **275**, 21549–21554.

Smith, I.F. *et al.* (2001). *J. Neurochem.* **79**, 887–884.

Taylor, S.C. *et al.* (1999). *J. Biol. Chem.* **274**, 31217–31223.

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Properties of $P2X_7$ receptors at mammalian motor nerve terminals

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We have previously identified adenosine triphosphate (ATP)-gated $P2X_7$ receptor ($P2X_7R$) subunits on mammalian motor nerve terminals (Deuchars *et al.* 2001). Here we report further on some properties of the $P2X_7R$ at the neuromuscular junction.

First, we tested if $P2X_7R$-mediated responses are influenced by a receptor protein tyrosine phosphatase-β (RPTPβ), which is associated with the $P2X_7R$ complex (but not the $P2X_2R$) and dephosphorylates the receptor (Kim *et al.* 2001). Transversus abdominis muscle and intercostal nerves were dissected from humanely killed young adult mice and placed into oxygenated physiological saline, and nerve terminals loaded with RH414 via stimulation of intercostal nerve stumps. Application of the $P2X_7R$ agonist benzoyl ATP (BzATP; 30 μM) stimulated vesicle release as demonstrated by nerve terminal destaining. In the presence of RPTPβ inhibitor, bisperoxo-(1,10-phenanthrolone)-oxovanadate (BvP: 100 μM), nerve terminal destaining was potentiated (BzATP, 23 ± 4 %, $n = 6$; BzATP + BvP, 40 ± 9 %, $n = 6$; control, 9 ± 1 %, $n = 5$; mean relative destain ± S.E.M.). The native agonist of the $P2X_7R$, ATP, was also able to produce destaining/vesicle release. 10 mM ATP applied in an essentially calcium/magnesium-free saline solution evoked a degree of destaining greater than that by 30 μM BzATP (42 ± 7 %, $n = 5$).

Since many (approximately 25 %) motor nerve terminals did not destain with agonist application we conducted immuno-electron microscopy to determine if lack of response was related to absence of $P2X_7R$ immunoreactivity. Mouse flexor digitorum brevis muscle was fixed in 4 % paraformaldehyde and 0.2 % glutaraldehyde solution. Subsequently, 50 μm sections were labelled with anti-$P2X_7R$ antibodies (Alomone, 1:1000), visualized with diaminobenzidine (DAB). Only a proportion of motor nerve terminals possessed the $P2X_7R$ – even in ultra-thin section heterogeneity was apparent within a single field of view. Further examination of DAB-labelled motor nerve terminals subsequent to BzATP stimulation revealed that many appeared depleted of synaptic vesicles, compared with unlabelled terminals. In addition, vesicle depletion was blocked by oxidised ATP (100 μM), Brilliant Blue G (1 μM) and calmidazolium (100 μM).

Taken together, these data suggest that $P2X_7R$ are located on only a proportion of mammalian motor nerve terminals, and that these can be activated by pharmacological and native agonists. Activation results in release of vesicles as demonstrated by vital imaging and electron microscopy.

Deuchars, S. *et al.* (2001). *J. Neurosci.* **15**, 7143–7152.

Kim, M. *et al.* (2001). *EMBO J.* **20**, 6347–6358.

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All procedures accord with current UK legislation.

Effects of subunit-selective dendrotoxins on spontaneous inhibitory postsynaptic currents in cerebellar Purkinje cells of Kv1.1 null mutant mice

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We are interested in determining the role of specific voltage-gated (Kv) potassium channels (and the different subunit proteins that comprise them) in neuronal excitability. Kv1.1 channel subunits are densely localised in the axon terminals of cerebellar basket cells, which impinge on cerebellar Purkinje cells. In this study we investigated the role of Kv1.1 subunits electrophysiologically using Kv1.1 null mutant mice (Smart *et al.* 1998), and selective potassium channel toxins. Whole-cell patch-clamp recordings of spontaneous inhibitory postsynaptic currents (sIPSCs) were obtained from Purkinje cells in parasagittal cerebellar slices from Kv1.1 null mice (postnatal day 25). Wild-type littermates of the same age were used as controls, and experiments were performed blind. Mice were bred and humanely killed under UK Home Office licence, in accordance with UK legislation.

Both amplitude and frequency of sIPSCs were significantly greater in Kv1.1 null mutant mice ($n = 16$), compared with wild-type littermates ($n = 12$, $P < 0.05$, Student's unpaired t test). In the presence of δ-dendrotoxin (δ-DTX, 100 nM), a selective Kv1.1 subunit blocker, the amplitude and frequency of sIPSCs was increased significantly in wild-type mice ($P < 0.05$). The changes in sIPSC amplitude and frequency were quantified as an activity index (the activity index is the product of the toxin/control amplitude ratio and the toxin/control frequency ratio; see Southan & Robertson, 1998). The activity index for δ-DTX in wild-type mice was 2.1 ± 0.2 ($n = 12$, mean ± S.E.M.). In Kv1.1 null mutant mice, the application of δ-DTX (100 nM) had no effect on sIPSC amplitude and frequency (activity index 1.0 ± 0.1, $n = 6$, $P < 0.01$ compared with wild-type). α-DTX, a Kv1.1 and 1.2 subunit blocker (100 nM), was applied following the addition of δ-DTX in all experiments. In wild-type mice, α-DTX further increased the amplitude and frequency of sIPSCs above that observed in δ-DTX ($n = 10$, activity index 1.9 ± 0.3). In Kv1.1 null mutants, α-DTX caused an increase in the amplitude and frequency of sIPSCs, similar to that observed in wild-type mice ($n = 4$, activity index 2.1 ± 0.3).

The Kv1.1 subunit is therefore not essential for synaptic transmission at this key cerebellar synapse, but its absence in the null mutant appears to increase the overall inhibitory tone. Future studies are planned with the other components of Kv1 subunit channels present at this synapse.

Smart, S.L. *et al.* (1998). *Neuron* **20**, 809–819.

Southan, A.P. & Robertson, B. (1998). *Br. J. Pharm.* **125**, 135–138.

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Up-regulation of Na^+ current attributed to $Na_V1.9$ (NaN) changes firing properties of small diameter sensory neurones

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Tetrodotoxin-resistant (TTX-r) Na^+ currents are involved in signal transduction and transmission in small diameter axons (e.g. reviewed by Baker & Wood, 2001). Small diameter dorsal root ganglion (DRG) neurones generate TTX-r Na^+ currents that can be discriminated by their biophysical characteristics (e.g. Rush *et al.* 1998; Cummins *et al.* 1999). The $Na_V1.8$ channel underlies high-threshold currents, and another TTX-r current (attributed to $Na_V1.9$) can be functionally isolated in neurones from $Na_V1.8$ null-mutant animals, although it can also be recorded in wild-type. This kinetically slow current has a voltage threshold close to −65 mV (Cummins *et al.* 1999), and generates a large persistent component.

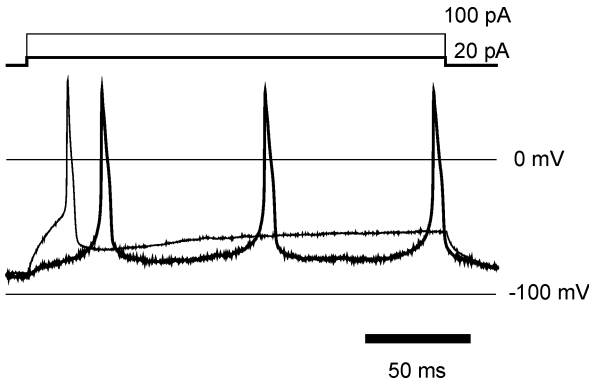

Figure 1. Persistent current up-regulation causes breakdown in accommodation to just suprathreshold depolarization. Recordings made before (fine trace) and after (heavy trace) current up-regulation. Change in holding current is indicated next to current waveform.

DRG cultures were prepared from wild-type and $Na_V1.8$ null-mutant mice, and from 3-week-old rats. The animals were killed humanely and the neurones prepared by enzymatic dissociation of the isolated ganglia. Voltage-clamp and current-clamp recordings were made within 2 days using the whole-cell patch-clamp technique, from neurones less than 25 μm in apparent diameter. TTX-r persistent Na^+ current amplitude was up-regulated over 5 min by the inclusion of 500 μM GTP or GTP-γ-S in the recording pipette, up to one order of magnitude, but not by GDP. Following up-regulation, the voltage threshold fell by 15.74 ± 2.24 mV ($n = 6$) when measured in current-clamp from a holding potential of −90 mV. In neurones not generating the current, threshold increased by 3.72 ± 1.29 mV ($n = 27$; $P < 0.0001$, Student's two-tailed, unpaired t test) over a similar period and threshold fell by 1.05 ± 0.46 mV ($n = 15$; $P < 0.001$, Student's two-tailed, unpaired t test) with GDP internal. All values are given as means ± S.E.M., and data from $Na_V1.8$ null-mutant and wild-type neurons are pooled. The fall in voltage threshold led to a breakdown of accommodation with just supra-threshold depolarization (Fig. 1). We suggest that up-regulation of the persistent current could induce sustained firing in pain fibres and may represent a way in which the excitability of C-fibres can be changed by inflammatory mediators.

Baker, M.D. & Wood J.N. (2001). *Trends Neurosci.* **22**, 27–31.

Cummins, T. *et al.* (1999). *J. Neurosci.* **RC43**, 1–6.

Rush, A.M. *et al.* (1998). *J. Physiol.* **511**, 771–789.

We thank the MRC and The Wellcome Trust for support.

All procedures accord with current UK legislation.

Contrasting localisation of the $P2X_7$ receptor in different regions of the CNS in excitatory terminals identified by the presence of the vesicular glutamate transporters VGluT1 and VGluT2

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We recently demonstrated the $P2X_7$ receptor subunit is present in presynaptic terminals in the CNS which have the morphology and localisation of excitatory terminals (Deuchars *et al.* 2001; Sperlágh *et al.* 2002). Here we first aimed to confirm this localisation using a new antibody to the $P2X_7$ receptor. Secondly we examined if the receptor subunit is present in glutamatergic excitatory terminals identified using antisera to the vesicular glutamate transporters VGluT1 and VGluT2.

Male Wistar rats (150–200 g) were humanely killed by anaesthesia with sodium pentobarbital (Sagatal, 60 mg kg^{-1} I.P.) and transcardial perfusion with 500 ml of fixative (4 % paraformaldehyde/0–0.1 % glutaraldehyde). To confirm presynaptic localisation of the $P2X_7$ receptor, 50 μm sections of the medulla and forebrain were prepared for light and electron microscopy (Deuchars *et al.* 2001), using an antibody raised against amino acids 60–323 of the $P2X_7$ receptor (Kim *et al.* 2001). To detect glutamatergic terminals, sections were incubated for 12–24 h with rabbit anti-$P2X_7$ (Alomone Labs, 1:1000, aa 576–595) and guinea-pig anti-VGluT1 or anti-VGluT2 (1:3000; Varoqui *et al.* 2002) and primary antibodies detected with donkey anti-rabbit Cy3 (1:1000, Jackson Immunochemicals) or biotinylated anti-guinea-pig (1:200, Jackson) followed by Streptavidin $ALEXA^{488}$.

At light microscope level both primary antibodies for the $P2X_7$ receptor subunit revealed prominent staining of glial cells, well known to express $P2X_7$ receptors. In addition, both resulted in fine punctate staining of the neuropil which, at the electron microscopic level, was revealed to be due to immunoproduct within presynaptic terminals, confirming our previous findings with the anti-rabbit antisera. In the medulla oblongata the $P2X_7$ receptor was co-localised with VGluT2 but not VGluT1. In a surprising contrast, $P2X_7$ was co-localised with VGluT1 but not VGluT2 in the hippocampus.

These data are consistent with the $P2X_7$ receptor having a role in modulating glutamate release from presynaptic terminals in the CNS. However, it is clear that not all glutamate-releasing terminals bear the $P2X_7$ receptor. Furthermore, the expression of the $P2X_7$ receptor may be correlated with that of either VGluT1 or VGluT2 depending on the CNS region examined.

Deuchars, S.A. *et al.* (2001). *J. Neurosci.* **21**, 7143–7153.

Kim, M. *et al.* (2001). *J. Biol. Chem.* **276**, 23262–23267.

Sperlágh, B. *et al.* (2002). *J. Neurochem.* **81**, 1–16.

Varoqui, H. *et al.* (2002). *J. Neurosci.* **22**, 142–155.

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Mechanism of spontaneous calcium oscillations in ventrobasal thalamus astrocytes

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Astrocytes in the neonate rat ventrobasal (VB) thalamus (obtained from humanely killed animals) display spontaneous $[Ca^{2+}]_i$ oscillations that are dependent on Ca^{2+} release from intracellular stores and are independent of neuronal activity since they are not blocked by tetrodotoxin. To further clarify the mechanism of these spontaneous oscillations, slices of neonate rat VB were prepared as previously described (Parri *et al.* 2001), loaded with the calcium indicator fluo-4 and imaged from an area of 206 × 158 μm using a Noran Odyssey confocal microscope. Whole-cell patch-clamp recording was performed using an Axopatch 200B amplifier.

To confirm the intrinsic nature of these oscillations slices were incubated with 4 μM Bafilomycin A1 for 1 h to block vesicular neurotransmitter release. In control conditions synaptic stimulation (5 × 2 ms stimuli of 300 μA at 50 Hz) elicited inward currents of 243 ± 70 pA ($n = 4$ of 4 cells) in thalamocortical neurones. Currents were abolished in neurons from slices treated with Bafilomycin A1 ($n = 4/4$), indicating that neurotransmitter release was blocked. In control conditions the number of astrocytes exhibiting spontaneous $[Ca^{2+}]_i$ oscillations was 7.25 ± 0.5 (in 600 s) ($n = 4$ slices), and 7.75 ± 1.9 ($n = 4$ slices) in Bafilomycin A1-treated slices, showing that oscillations were not dependent on neurotransmitter release. In slices incubated with 20 μM of the phospholipase C inhibitor U73122, the number of cells responding to 50 μM trans-ACPD was reduced from 17.28 ± 2.7 ($n = 8$) in control conditions to 5.12 ± 0.54 ($n = 8$), but the number of spontaneously oscillating astrocytes was unaffected (3.37 ± 1.2 in control and 3.87 ± 0.89 (in 300 s) ($n = 8$) in U73122-treated slices).

The number of cells exhibiting spontaneous oscillations in a 300 s period increased from 2.8 ± 0.8 in 1 mM $[Ca^{2+}]_o$ to 4.6 ± 0.55 in 2.5 mM $[Ca^{2+}]_o$ and 9.33 ± 0.67 in 5 mM $[Ca^{2+}]_o$ ($P < 0.005$, ANOVA, compared with 1 mM $[Ca^{2+}]_o$) ($n = 4$ slices). The number of transients exhibited by each spontaneously active astrocyte was 1.64 ± 0.32 in 1 mM, 1.5 ± 0.12 in 2.5 mM and 2.46 ± 0.34 in 5 mM $[Ca^{2+}]_o$ ($P < 0.05$ compared with 1 mM $[Ca^{2+}]_o$). Application of 10 μM of the SERCA blocker cyclopiazonic acid (CPA) resulted in a slow increase in $[Ca^{2+}]_i$ levels in 56 ± 23 % ($n = 3$ slices) of astrocytes, and also induced $[Ca^{2+}]_i$ transients in 68 ± 22 % of astrocytes ($n = 3$ slices), suggesting that an accumulation of cytoplasmic Ca^{2+} can elicit $[Ca^{2+}]_i$ release in thalamic astrocytes.

These data show that spontaneous thalamic astrocytic $[Ca^{2+}]_i$ oscillations are intrinsic, not dependent on neurotransmitter release, or on receptor activation of PLC pathways. The effects of modifying $[Ca^{2+}]_o$, and the effect of CPA in inducing transients, suggest a role for Ca^{2+} in inducing $[Ca^{2+}]_i$ release.

Data are espressed as means ± S.E.M. Statistical significance was measured with Student's unpaired *t* test.

Parri, H.R. *et al.* (2001). *Nat. Neurosci.* **4**, 803–812.

All procedures accord with current UK legislation.

Oscillatory behaviour of intracellular Ca^{2+} in supporting cells of the organ of Corti

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The level of ATP in cochlear fluid has been shown to increase in response to sound over-stimulation. The two most prominent types of supporting cells in the organ of Corti, Deiters' and Hensen's cells, have been shown to respond to focal application of ATP with transient elevation of intracellular Ca^{2+} levels (Lagostena *et al.* 2001; Lagostena & Mammano, 2001). Here we used an organotypic preparation from humanely killed rats (Sobkowicz *et al.* 1975) of the immature cochlea (prepared on postnatal day 1 and utilised up to 2 days in culture) to follow the intercellular spread of ATP-induced Ca^{2+} signals. Bolus application of ATP (final concentration 50–100 μM) in Ca^{2+}-free HBSS (containing (mM): KCl 5.33; KH_2PO_4 0.44; $NaHCO_3$ 4; NaCl 138; Na_2HPO_4 0.3; glucose 5, 6; pH 7.4) produced an initial generalised increase of intracellular Ca^{2+} concentration (Fig. 1*A*), followed by generation of oscillatory Ca^{2+} responses in selected supporting cells (Fig. 1*B*).

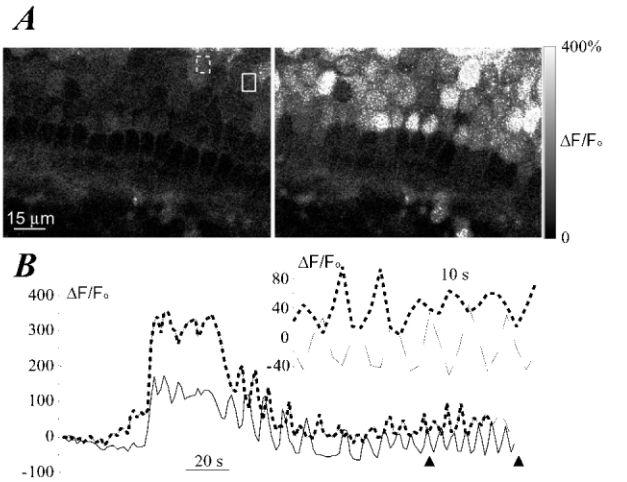

Figure 1. Response to ATP in the organ of Corti. *A*, confocal images of an organ of Corti culture loaded with fluo-3 AM-ester (5 μM) for 45 min at 37 °C (left, control), and at the peak of the response to ATP (right). *B*, Ca^{2+}-induced fluorescence change averaged within the two rectangular regions of interest shown in *A;* inset: expanded waveforms from the time window within arrowheads.

In a given cell, oscillations occurred with a frequency of 0.163 ± 0.012 Hz ($n = 4$, mean ± S.E.M.). A clear pattern emerged during the oscillatory phase of the responses, characterised by intercellular propagation of Ca^{2+} waves. Propagation velocity was 9.6 ± 1.4 μm s^{-1} ($n = 18$). Oscillatory responses were either absent or greatly attenuated if Ca^{2+} (2 mM) was included in the extracellular medium. Facilitation of ATP responses in Ca^{2+}-free medium is consistent with waves being carried by a mechanism that involves ATP-induced ATP release through open connexin hemichannels (Cotrina *et al.* 1998).

Cotrina, M.L. *et al.* (1998). *Proc. Natl Acad. Sci. USA* **95**, 15735–15740.

Lagostena, L. *et al.* (2001). *J. Physiol.* **531**, 693–706.

Lagostena, L. & Mammano, F. (2001). *Cell Calcium* **29**, 191–198.

Sobckowicz, H.M. *et al.* (1975). *J. Neurocytol.* **4**, 543–572.

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Large-scale neuronal network models based on a probabilistic connectivity principle

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The use of models of large-scale neuronal networks has become indispensable in studying information processing in various brain areas, such as the hippocampus (Traub *et al.* 1994), the cortex (Traub *et al.* 1996), or the thalamocortical network (Destexhe & Sejnowski, 2001). At the same time, constructing and implementing such models is fraught with difficulties of both conceptual and technical nature because the exact connectivity of the network is usually not known, and because of the problems that arise when large systems of ordinary differential equations are to be solved.

To avoid some of these problems, we used a novel approach based on a principle of probabilistic connectivity of the network. We thus obtained the mathematically expected (average) behaviour of neurones representing whole neurone pools. This method was tested on three network models of general nature, but with detailed biophysical neurone and synaptic models.

The first simple example consists of one pool of 300 identical neurones that is connected with NMDA-type synapses to neurones of a second pool (of 300 neurones), and both pools receive independent afferent inputs. This model shows that the effect of the activities of all neurones of a pool are taken into account when computing the overall output of the pool. The second example demonstrates that computationally difficult cases, such as the behaviour of networks with mutually strong excitatory connections between two neurone pools can reliably be simulated. Finally, our method also works with a heterogeneous network of three neurone pools with different neurone types and excitatory (AMPA, NMDA) and inhibitory ($GABA_A$) synaptic connections.

These results suggest that models based on the probabilistic connectivity principle are a viable alternative to detailed deterministic network models.

Destexhe, A. & Sejnowski, T.J. (2001). *Thalamocortical Assemblies.* Oxford University Press, Oxford.

Traub, R.D. *et al.* (1994). *J. Physiol.* **478**, 379–393.

Traub, R.D. *et al.* (1996). *Nature* **383**, 621–624.

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Compound spikelets correlate with high-threshold bursting in thalamocortical (TC) neurones *in vitro*

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In the presence of the metabotropic glutamate receptor (mGluR) agonists, *trans*-ACPD (50–200 μM) or DHPG (50–100 μM), thalamocortical neurones of the cat dorsal lateral geniculate nucleus (obtained from humanely killed animals) maintained *in vitro* exhibit stereotypical, small depolarising potentials or spikelets. In the majority of cases (80%), these spikelets are isolated rhythmic (~10 Hz) events that represent the electrotonic transmission of single action potentials via gap junctions (Hughes *et al.* 2002).

In cells exhibiting spikelets, we now report that in a small number of cases (20%) they appear in rhythmic bursts which ride on a small depolarisation, and which we term compound spikelets. As with isolated spikelets, compound spikelets are blocked by carbenoxolone (100 μM) and usually accompanied by dye coupling (75%). The interburst frequency of compound spikelets is 4.6 ± 0.8 Hz whilst their intraburst frequency is 66 ± 4 Hz ($n = 4$). These values correspond closely to those exhibited by a novel form of burst firing (interburst frequency range 3–12 Hz; intraburst frequency = 74 ± 5 Hz; $n = 52$) that occurs at membrane potentials above −50 mV (high-threshold bursting, HTB) in a small percentage (25%) of TC neurones in the presence of *trans*-ACPD or DHPG. Therefore we suggest that compound spikelets are generated by electrotonically transmitted high-threshold bursts. Isolated spikelets and compound spikelets differ in their ability to entrain firing: isolated spikelets entrain single spike activity at >15 Hz, whereas compound spikelets entrain HTB at 3–12 Hz. The latter finding suggests that TC neurones should generate synchronized oscillations at 3–12 Hz, a suggestion confirmed by extracellular multi-unit recordings. Furthermore, dual intra-and extracellular recordings performed in the presence of 6-cyano-7-nitro-quinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphono-valeric acid (APV) and bicuculline methiodide (BMI) directly demonstrate that (i) these oscillations are not dependent on fast chemical synaptic transmission, (ii) they are driven by HTB and (iii) that their frequency is largely determined by how depolarised the TC neurone population is. In conclusion, our results suggest that in the presence of mGluR agonists, TC neurones generate one of two distinct types of firing pattern: single spike activity or HTB, with single spikes being reflected electrotonically as isolated spikelets and HTB as compound spikelets. The combination of HTB and compound spikelets can generate gap junction driven synchronized oscillations at α (8–12 Hz) and θ (3–7 Hz) frequencies that may have relevance to the EEG rhythms that characterize relaxed wakefulness and early sleep stages, respectively (data are quoted as means ± S.E.M.).

Hughes, S.W. *et al.* (2002). *Neuroscience* **110**, 395–401.

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Tetanus-induced γ to β oscillations in the rat hippocampus *in vivo*

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Tetanic stimulation of area CA1 of hippocampal slices induces a slow depolarisation and sustained rhythmic firing. The relative roles of $GABA_A$ergic and metabotropic glutamatergic mechanisms in the depolarisation and of ephaptic interactions and IPSPs in the synchronisation of firing have been controversial (Bracci *et al.* 1999 *vs.* Whittington *et al.* 2001). In order to judge which mechanisms contribute under most physiological conditions we studied tetanus-induced oscillations *in vivo*.

Male Sprague-Dawley rats (280–350 g) were anaesthetised with 2.0 mg kg^{-1} I.P. urethane and fixed in a stereotaxic frame. Bipolar stimulation electrodes were placed in the alveus and in stratum radiatum of hippocampus area CA1, 0.15 mm posterior to a 16-channel field potential recording probe. A 10 μl Hamilton syringe needle was placed in the ipsilateral ventricle, to apply drugs I.C.V. Rats were killed humanely at the end of the experiment.

The threshold stimulus intensity for evoking a fast γ oscillation (90 ± 4 Hz, mean ± S.E.M., $n = 8$) with a 10-pulse 100 Hz tetanus was 8 ± 1 V. Large-amplitude population spikes were induced with a small increase of stimulus intensity. At 1.5 × threshold intensity the tetanus evoked population spikes (maximum amplitude: 13 ± 1 mV, maximum field strength: 54 ± 6 V m^{-1}) starting at γ (71 ± 3 Hz), slowing down to β (11 ± 2 Hz) frequencies after 0.5 s, with small population spikes continuing at γ frequency (75 ± 2 Hz) between the large population spikes. Current source density analysis revealed that the discharge-related sink was confined to strata pyramidale/oriens and that the spontaneous discharge often started in stratum oriens. I.C.V. application of the metabotropic glutamate receptor antagonist MCPG (10 μl, 50 mM) reversibly reduced the amplitude and duration of the oscillation. The effects of MCPG were reversed by increasing stimulus intensity, or by co-application of the $GABA_B$ receptor antagonist CGP 55845A (1 μl of 0.2 mM I.C.V.). In contrast the $GABA_A$ receptor antagonist bicuculline (1 μl of 5 mM I.C.V.) completely blocked the oscillation at all intensities tested and in the presence of CGP 55845A.

These observations closely resemble our observations in interface recording chambers. The activity profile over the cell axis suggests strong ephaptic interactions due to ongoing high-frequency discharges in the non-tetanised dendrites. The pharmacological profile suggests that mGluR activation potentiates GABA release, necessary for a depolarising GABA response. These observations are consistent with those *in vitro* and indicate that depolarising GABA and strong ephaptic interactions play an important role in oscillations *in vivo*.

Bracci, E. *et al.* (1999). *J. Neurosci.* **19**, 8102–8113.

Whittington, M.A. *et al.* (2001). *J. Neurosci.* **21**, 1727–1738.

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Effects of ketamine on gamma oscillations in the entorhinal cortex

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Previously we have demonstrated that gamma band (20–80 Hz) oscillations can be elicited in the medial entorhinal cortex (mEC) by the application of nanomolar concentration of kainate (Cunningham *et al.* 2002). The mEC is an association centre located in a strategic position in the cortical-hippocampal axis of the medial temporal lobe. It is known that abnormalities in the mEC have been implicated in the pathology of schizophrenia (Harrison, 1999). Ketamine is a dissociative anaesthetic agent, known to induce severe psychosis in humans. The aim of this work was to investigate the action of ketamine on gamma oscillations in the mEC.

Combined entorhinal-hippocampal slices (~450 μm) were taken from adult (200–250 g) Wistar rats after terminal anaesthesia using ketamine-xylazine (administered intramuscularly), and intracardial perfusion with artificial cerebrospinal fluid (ACSF) in which NaCl was replaced with sucrose. Slices were maintained at an interface of oxygenated ACSF and humidified (95 % O_2 and 5 % CO_2) gas at 36 °C. Extracellular field recordings were made in the superficial and deep layers of the mEC, using glass microelectrodes containing ACSF (resistance < 3 MΩ). Student's *t* test was used to compare the data.

Gamma activity was evoked by bath perfusion of the AMPA/kainate receptor agonist kainic acid (400 nM) alone. Ketamine was bath applied at concentrations of 10, 20 and 50 μM. At concentrations of 20 μM ketamine, superficial gamma peak power and area was significantly reduced ($P < 0.05$; $n = 8$), although in the deep layers no such effect was observed. In both layers, the peak frequency of the gamma activity was reduced significantly at 20 μM ketamine ($P < 0.05$). In terms of rhythmicity, the superficial layers were also selectively more sensitive than deep layers. Auto-correlation analysis of activity in both layers revealed significant ($P < 0.05$) reductions in this behaviour at 10 μM in the superficial layers, whereas a significant effect was not seen until a concentration of 20 μM in the deep layer. Ketamine is known to act at NMDA receptors. Indeed, gamma activity in the mEC was shown to be inhibited by the application of the non-competitive NMDA antagonist D-APV. However, the pre-application of D-APV was unsuccessful in occluding the action of ketamine on residual gamma activity in the mEC.

These results demonstrate that gamma activity in the mEC is disrupted by ketamine. Superficial gamma activity was particularly sensitive and since the superficial layers are the main output of the mEC to the hippocampus and higher cortical areas, this may have important implications in the study of psychosis. In addition, these data suggest that the effects of ketamine on gamma activity involve mechanisms in addition to non-competitive NMDA blockade.

Cunningham, M.O. *et al.* (2002). *FENS Abstr.* **1**, A146.1.

Harrison, P.J. (1999). *Brain* **122**, 593–624.

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All procedures accord with current UK legislation.

The role of $GABA_A$ and glycine receptor-mediated inhibition on theta frequency oscillations in substantia gelatinosa neurones of the rat spinal cord *in vitro*

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Extracellular recordings in rat spinal nociceptive neurones *in vivo* by Sandkuhler & Eblen-Zajjur (1994) have shown oscillatory activity in the theta frequency range (6–12 Hz). We have previously demonstrated that theta frequency rhythms can be evoked in the substantia gelatinosa (a dorsal horn lamina important for nociceptive processing) by transient elevation of extracellular potassium (Asghar *et al.* 2002). GABA and glycine play an important inhibitory role in the spinal dorsal horn and several studies in hippocampal neurones have shown that inhibitory interneurons are important in generating network oscillations. Therefore, the present investigation aimed to determine whether antagonism of $GABA_A$ or glycine inhibitory receptors could influence potassium-induced theta oscillations in the substantia gelatinosa.

Spinal cords were removed from Wistar rats (age 12–14 days) terminally anaesthetised with urethane (2 g kg^{-1} I.P.). Transverse slices of 300 μm thickness were cut from lumbar segments and placed in an interface recording chamber perfused with artificial cerebrospinal fluid (ACSF) at 32 °C. Extracellular field recordings were made from the substantia gelatinosa lamina of the spinal cord using ACSF-filled glass microelectrodes (1–2 MΩ). Potassium (KCH_3SO_4, 1.5 M) was pressure ejected (10–20 ms duration) from a microelectrode (1–2 MΩ) placed close to the field recording electrode. Power spectra were analysed to reveal the mean peak frequency and amplitude in the 6–12 Hz band. The area under the peak was calculated between 6 and 12 Hz. Data are expressed as means ± S.E.M.

Brief pressure ejection of potassium evoked a field oscillation of 5–15 s duration which had a mean peak frequency of 7.3 ± 0.3 Hz ($n = 12$). Bath application of the $GABA_A$ receptor antagonist, bicuculline (30 μM), significantly reduced the peak amplitude (% reduction of 81 ± 4, $P < 0.005$, paired t test, $n = 6$) and the area (% reduction of 76 ± 5, $P < 0.01$, paired t test, $n = 6$). The glycine receptor antagonist, strychnine (10 μM), also significantly reduced the peak amplitude (% reduction of 69 ± 9, $P < 0.05$, paired t test, $n = 6$) and the area (% reduction of 67 ± 8, $P < 0.05$, paired t test, $n = 6$). There was no apparent effect of either bicuculline or strychnine on the peak frequency.

These data demonstrate that theta frequency oscillations evoked in the substantia gelatinosa are partly dependent upon $GABA_A$ and glycine inhibitory neurotransmission. This inhibitory neurotransmitter-dependent rhythmic activity could play a role in the somatosensory processing of nociceptive signals.

Asghar, A.U.R. *et al.* (2002). *J. Physiol.* **539.P**, 153*P*.

Sandkuhler, J. & Eblen-Zajjur, A.A. (1994). *Neuroscience* **61**, 991–1006.

All procedures accord with current UK legislation.

The use of organotypic slice cultures for the biolistic transfection of cells in the dorsal horn

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An ability to transfect neurons in the spinal cord with an efficient high throughput system would hold many advantages for studying gene function. This could be achieved by using an appropriate culture system and a biolistic method for the introduction of DNA constructs (Arnold & Heintz, 1997). To this end, organotypic spinal slice cultures were assessed electrophysiologically and morphologically for their suitability as a model system for the study of nociceptive processing. In addition, cultures were subjected to biolistic transfection with an enhanced green fluorescent protein DNA construct with a cytomegalovirus promoter (CMV-EGFP) and the level of transfection assessed.

Wistar rats aged 8 days (15–22 g) were terminally anaesthetised with urethane (2 g kg^{-1} I.P.). The spinal cord was removed and 350 μm transverse slices were cultured by a modified interface method adapted from Stoppini *et al.* (1991). Whole-cell patch-clamp recordings were made from the superficial dorsal horn in the presence of bicuculline (30 μM) and strychnine (10 μM). Biocytin (0.1 %) was included in the electrode for recovery and visualisation of the neuron. Graded focal stimulation with a bipolar electrode evoked EPSCs of increasing amplitude at a constant latency indicative of monosynaptic events. The selective AMPA antagonist CNQX (20 μM) completely inhibited EPSCs (cells held at −60 mV) and the μ-opioid agonist DAMGO (1 μM) partially inhibited EPSCs. Recovery of biocytin-filled neurons revealed preservation of an extensive dendritic network in the majority of cells. Immunostaining for neuron-specific nuclear protein (NeuN) confirmed a dense cell population throughout the dorsal horn. In CMV-EGFP transfection studies, slice cultures were shot with a PDS-1000/He particle delivery system (Biorad) with a vacuum evacuated to 91 kPa (27″ Hg) and under a pressure of 7584 kPa (1100 p.s.i.). To facilitate neuronal survival the slices were placed on an ice-cold sterile agar plate and covered with a nylon baffle. Cultures were assessed at > 40 h post-shooting and a number of transfected cells in superficial and deep laminae were evident. Double-labelling for glial fibrillary acidic protein compared with NeuN was used to evaluate relative levels of transfection in different cell populations. However, as no co-localisation with the cellular markers GFAP or NeuN was evident, the identity of the transfected population remains to be established.

These findings show that organotypic culture combined with an optimised biolistic protocol could provide a useful model system for the study of genes relevant to nociceptive processing in the spinal cord.

Arnold, D.B. & Heintz, N. (1997). *Proc. Natl Acad. Sci. USA* **94**, 8842–8847.

Stoppini, L. *et al.* (1991). *J. Neurosci. Meth.* **37**, 173–182.

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All procedures accord with current UK legislation.

Regulation of thalamic output by the metabotropic glutamate receptor-mediated component of the cortico-thalamic EPSP; a simulation study

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The precise role of the metabotropic glutamate receptor-mediated (mGluR) component of the corticothalamic EPSP in the regulation of thalamic output is still not fully understood. Using a multi-compartment model of a thalamocortical neurone (Antal *et al.* 2001), we have now investigated the ability of cortical EPSPs with different sizes of mGluR component to perturb the δ oscillation and to modulate the efficacy of the retinal input. Four spatial distributions of cortical synapses were used: symmetric distal, with synapses distributed among all distal dendrites; symmetric proximal, with synapses on all secondary dendrites; single-dendrite, with synapses on all distal segments of one dendrite; single-segment, with a single distal synapse. Retinal synapses had symmetric proximal location.

The presence of an mGluR component in the cortical EPSP increased its ability to perturb the δ oscillation. Without an mGluR component the cortical EPSP could only change the oscillation cycle when it arrived on the decay phase of the low threshold Ca^{2+} potential. With an mGluR component ($\geqslant 1$ mV amplitude), however, it was able to completely reset the oscillation, independently from the time of arrival and synapse location.

The effect of subthreshold cortical EPSPs on retinal EPSPs arriving 10–500 ms after the cortical input was investigated. Cortical EPSPs facilitate retinal transmission when the delay was < 160 ms. The time-window for facilitation increased with the amplitude of the mGluR component, and depended on the location of cortical synapses, with the longer facilitation interval occurring with the single segment distribution. For delays ranging from 160 to 360 ms, the ability of retinal EPSPs to evoke low threshold Ca^{2+} potentials and action potentials decreased, as a retinal EPSP that alone induced a burst of action potentials did not evoke either potentials after a cortical EPSP. With increasing mGluR components, this suppressive effect of cortical ESPs became larger and less dependent on synaptic location.

Antal, K. *et al.* (2001). *Thal. Rel. Syst.* **1**, 105–116.

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