Rapid regionally restricted pH shifts in neurones induced by the UV photolysis of 2-nitrobenzaldehyde

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Hitherto three types of techniques have been used to change intracellular pH (pHi); superfusion of weak acids or bases (Jacobs, 1920), cytosolic injection of acid or alkali, or the activation of channels or transporters (e.g. the Ca\(^{2+}\)-H\(^+\) pump, Schwiening et al. 1993) that allow transmembrane H\(^+\) or HCO\(_3\)\(^-\) fluxes. Here I describe a fourth technique, the regional UV-induced release of protons (Ciamician & Silber, 1901; George & Scaiano, 1980) that allows for extremely rapid and localized acidic pH shifts. This technique should provide a simple, cheap and highly controllable way of generating local pH signals independently of changes in other ion concentrations (e.g. Ca\(^{2+}\)).

Helix aspersa neurones were isolated from the ganglia following enzymatic digestion and mechanical agitation (Schwiening & Willoughby, 2002). Snail Ringer solution containing the isolated neurones was placed in a superfusion chamber on a Zeiss LSM 510 confocal microscope and the cells were allowed to settle for ~1 h. A cell with an axonal projection was selected and patch clamped in the whole-cell configuration (pipettes ~1.5 MW tip). ratiometric pH-sensitive fluorescence was imaged (488 nm excitation, 505 nm emission) using a 63x water-immersion c-apochromat objective (1.2 NA). In some experiments ratiometric pH recordings were made using alternating 488 nm and 357 nm excitation of the dye HPTS. HPTS-sensitive fluorescence was imaged (488 nm excitation, 505 nm emission) using a 63x water-immersion c-apochromat objective (1.2 NA).

Spatially restricted acidic pH shifts, induced by the flash release of acid, are shown at low magnification in Fig. 1, and at higher magnification in Fig. 2. In both cases NBA was applied several min before the start of the trace. NBA appears to be highly membrane permeant since prolonged illumination with UV light yields a larger change in pH (−0.8 pH unit) than expected from the complete release of 2 mM H\(^+\) with a buffering power of 10 mM H\(^+\) (1 cell volume)\(^{-1}\). Also, when bath applied, NBA diffuses rapidly into the cell as judged by the appearance of UV-induced pH shifts. It is unlikely that HPTS photobleaching underlies the F/F\(_0\) changes presented here since UV illumination in the absence of NBA did not produce any change in HPTS fluorescence, furthermore ratiometric recording of pH using HPTS produced similar results to the single wavelength records shown here.

This demonstration is, to the best of my knowledge, the first time acid has been both locally flashed released and recorded inside a cell. Such release of intracellular acid is by far the most rapid and controllable method for altering regional pHi. Photolysis of NBA, or its derivatives (Abbruzzetti et al. 2003), should facilitate the investigation pH, microdomain signalling targets in normal neuronal function.


I thank the MRC for providing the LSM510 and their high rating of my recent grant applications. However, I deplore their failure to support any of my current work, including that presented here, which has been supported by the University.

Figure 1. A, pH-sensitive fluorescence recorded in the axon foot region of the cell shown in Fig. 1B. Two single UV illuminations (dwell time 39 µs m\(^{-1}\)) were followed by a series of 7 illuminations over 1s. B, confocal image of axon foot (128 x 67 pixels). C, confocal F/F\(_0\) image before acidifications showing the four ROI plotted in A, D, confocal F/F\(_0\) at the peak (*) of the acidification (average of 18 images).
Inputs to the suprachiasmatic nucleus from the arcuate nucleus are modulated by leptin

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The hypothalamic suprachiasmatic nucleus (SCN, the clock nucleus) is part of a network regulating feeding and metabolism. The hypothalamic arcuate nucleus (ARCN) may be involved in food intake regulation while the SCN modulates the timing of feeding. The input to the SCN from the retrochiasmatic area of the ARCN provides a route by which the SCN may be influenced by neuroendocrine cues. The SCN has been reported to contain receptors to leptin (Hakansson et al., 1998), the peptide secreted by adipose tissue and involved in the modulation of the neural control of feeding. The aim of the present study was to determine if leptin influenced the activity of cells in the SCN or their responses to stimulation of the ARCN.

Conventional extracellular recordings were made from single units in the SCN in 500 μm sagittal hypothalamic slices of brains removed from anaesthetised (urethane 1.2 g/kg I.P.) male Wistar rats and their responses to ARCN stimulation were assessed by peristimulus time histograms.

A total of 24 of the 35 SCN cells responded to stimulation of the ARCN. Excitatory (n = 12), inhibitory (n = 7), and complex responses (excitation followed by inhibition or vice versa; n = 5) were seen. The responses had a short latency (< 20 ms) after the stimulus pulse. Typically excitatory responses had a short duration (< 25 ms) and inhibitory responses were longer (> 40 ms). After the administration of leptin (20 nm) the mean spike frequency of SCN cells was reduced (P < 0.001, Wilcoxon signed rank test) while mean log interspike interval (ISI) was increased in duration (P = 0.033, Wilcoxon signed rank test).

The entropy of the log ISI probability distribution was also increased by 0.16 ± 0.07 (mean difference ± s.e.m., P = 0.033, paired t test) a change in the regularity of the recorded spike train. The input to the SCN from the ARCN was significantly influenced by leptin in 12 of 35 experiments. Excitatory responses were reduced in 7 cases and increased in 2 cases. In one case an excitatory response became evident only in the presence of leptin. Leptin did not appear to alter inhibitory responses that were seen before its administration but in two experiments inhibitory responses became evident only in the presence of leptin. The effects of leptin developed relatively slowly (> 10 min) and in most cases were completely or partially reversible following washout with control solution for 20–30 min.

The present results show that leptin can influence the activity of SCN units and modulate the input to the SCN from the ARCN. The effects of leptin involved both a change in the regularity of the ISI of the spike trains recorded from SCN neurons and a reduction in their firing rate. The heterogeneity of the responses probably, reflects the diversity of cell types in the SCN.


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All procedures accord with current UK legislation

Daily rhythms of spike coding in the suprachiasmatic nucleus

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Although the suprachiasmatic nucleus (SCN) is widely regarded as the main circadian pacemaker in the mammalian brain, evidence for a rhythm of firing activity based on extensive single unit recording in intact animals has been reported only recently (Saeb-Parsy & Dyball, 2003). There were significant differences in mean spike frequency at different times of the day but the differences were not clearly evident unless activity was averaged over arbitrary time epochs. The use of such averaging techniques represents a substantial loss of information. We have found that parameters that measure the irregularity of spike activity of cells in the SCN show more obvious changes over the day/night cycle than mean spike frequency.

Using a ventral surgical approach, extracellular single unit activity was recorded from SCN cells in urethane (1.2 g/kg I.P.) anaesthetised rats in vivo. All rats were killed humanely at the end of the experiments. Conventional variance measures were not used to quantify the variability of inter-spike intervals because of the non-Gaussian distribution of the intervals. To measure the irregularity of spike activity, we used the entropy (a widely used measure of disorder) of the log interval distribution. Such a measure quantifies the variability of intervals and thus the coding capacity of each spike. The entropy was plotted against the respective zeitgeber and showed an oscillation that could be described using a second order Fourier expansion with a peak in the mid-dark period.

Neurones within the SCN showed a significant rhythm in entropy (n = 166; P < 0.001) and there was a similar rhythm in the activity of cells recorded from perinuclear zone (PSCN) of the suprachiasmatic nucleus (n = 209; P = 0.0370). SCN and PSCN cells with caudal projections showed a significant rhythm (n = 78; P = 0.006) with a peak in the mid-light period whereas those without a caudal projection showed a rhythm with a peak in the mid-dark period (n = 297; P = 0.001). There was no evidence for a rhythm of activity for hypothalamic more than 1 mm distant from the SCN.

Thus measures of SCN cell activity in addition to mean spike frequency show a daily rhythm that is more obvious. Cells within the SCN show a clearer rhythm than cells in the PSCN and different populations of cells within the SCN show rhythms that appear to be different from each other.


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C39

Pinealectomy appears to eliminate the daily rhythm of firing activity in the suprachiasmatic nucleus that is seen in intact animals

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The suprachiasmatic nuclei (SCN) of the hypothalamus are widely regarded as the part of the brain that generates circadian rhythms in mammals. Secretion of the pineal hormone melatonin is one of a number of neuroendocrine events that show a daily rhythm (Perreau-Lenz et al. 2003), and melatonin in turn affects the circadian clock by mechanisms that are unclear (Pevet et al. 2002). It has recently been demonstrated using single-unit recordings from the SCN in intact animals that SCN cells show a daily rhythm of mean spike frequency (Saeb-Parsy & Dyball, 2003). The present study was undertaken to extend the earlier study and to explore the role played by melatonin in the generation of rhythms in the SCN by making recordings from pinealectomised rats.

Using the ventral surgical approach, single-cell recordings were made in anaesthetised (urethane, 1.2 g/kg I.P.) rats in vivo between 3 and 10 weeks after pinealectomy under isofluorane. The rats were killed humanely at the end of the experiments.

In pinealectomised rats, recordings were made from 192 SCN cells at different times of the day night cycle and the activity of each of them, recorded for a minimum of 10 min, was recorded. Activity was expressed both in terms of mean spike frequency and in terms of the entropy of the log interspike interval distribution. The entropy of the log interspike interval distribution quantifies the variability in the interval distribution and thus gives a measure of the coding capacity of each spike. Entropy was plotted against the respective Zeitgeber time. In intact animals such a plot showed an oscillation that could be described using a second order Fourier expansion with a peak in the mid-dark period. Monte-Carlo significance testing showed a significant rhythm in intact animals (Blumbera et al. 2003). In pinealectomised animals, however, the maximum amplitude (0.4092 bits) of the curve describing the SCN cell activity was below the amplitude found after fitting the corresponding curves to randomised data (maximum amplitude after 10,000 randomisations, 0.8139 bits), and thus the oscillation seen in real data was not statistically different from a flat line (P < 0.001, Monte-Carlo significance testing). Therefore, after pinealectomy no rhythm in the activity of the SCN cells with the dark/light period was detected. Mean spike frequency showed a similar lack of change in pinealectomised animals. It thus appears that at least some aspects of the rhythmic activity of the SCN do not persist after pinealectomy.

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C40

Microamperometric recording of noradrenaline release from visualised axonal varicosities of A1 noradrenergic neurones

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The characteristics of noradrenaline (NA) release from central neurones have largely been inferred from experiments in model systems such as bovine adrenal chromaffin cells or the PC12 cell line (Ninomiya et al. 1997;Teschemacher & Seward, 2000). In these models it is possible to place microamperometric electrodes on individual cells and monitor exocytosis of NA. Similar experiments in native brain noradrenergic (NAergic) neurones have not been possible because the release sites are difficult to visualise and access. We have developed a method which allows direct recording of NA release from NAergic neurones from specific brainstem nuclei.

To this end organotypic brainstem slice cultures are prepared from humanely killed 9-day-old rats. Cultures are subsequently transfected with an adenoviral vector which causes expression of EGFP selectively in NAergic neurones under the control of an artificial promoter (PRSx8; Hwang et al. 2001). After >3 days, clusters of fluorescent neurones can be visualised using conventional epifluorescence and confocal microscopy in living slices in the areas consistent with the location of the known brainstem NAergic and adrenergic cell groups (A1, A2, A6, C1). In addition to cell bodies, multiple processes including characteristic beaded axons can be traced for hundreds of micrometers. These axons were approached by microamperometric probes (5 μm tip diameter). Oxidative current spikes were detected as described for vesicular catecholamine release in isolated cell models. This method will be used to study modulation of NA release from the different cellular compartments of identified NAergic neurones and can be combined with further genetic manipulation of the same cells.


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C42

Clathrin-mediated retrieval of vesicles at hippocampal synapses

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Exocytosis of neurotransmitter from a synaptic vesicle is followed by efficient retrieval of its constituent membrane and proteins. Several different mechanisms of endocytosis occur at synapses, but their relative importance has been unclear (Royle & Lagnado, 2003). We have investigated the role of clathrin-mediated endocytosis (CME) by using RNA interference (RNAi) to selectively knock-down the expression of clathrin heavy chain (CHC) in hippocampal neurons.

Primary cultures of rat hippocampal neurones were prepared
A role for peroxisome proliferator activated receptors in the β-amyloid (Aβ1–40) mediated impairment of hippocampal long-term potentiation in vitro

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Alzheimer’s disease (AD) is a neurodegenerative disorder associated with accumulation of the β-amyloid peptide (Aβ). Aβ has been shown to impair hippocampal long-term potentiation (LTP) in vivo and in vitro (Freir et al. 2003; Costello & Herron, 2003). LTP is an activity-dependent increase in synaptic efficacy, widely accepted as a cellular model of memory. Peroxisome proliferator activated receptors (PPARs) are a class of nuclear receptor transcription factors, regulated by mitogen activated protein kinases (MAPKs). PPARγ expression is elevated in AD (Kitamura et al. 1999), and PPAR agonists have been shown to prevent Aβ-stimulated activation of microglia and production of proinflammatory cytokines (Combs et al. 2000). We have identified a role for MAPKs in the depressant effects of Aβ on LTP in vitro (Costello & Herron, 2003). Here we investigate the effects of PPARγ agonists on synaptic transmission and on the Aβ1–40-mediated impairment of LTP.

Experiments were performed on hippocampal slices (350 μm) from humanely killed male Wistar rats (50–100g). The Schaffer collateral-aminissural pathway was stimulated at 0.033 Hz and field excitatory postsynaptic potentials (EPSPs) were recorded in area CA1. LTP was induced by applying high frequency stimuli (HFS; 10 trains of 10 pulses at 200 Hz, repeated 3 times at 20 s intervals). Control LTP was recorded 1 h post-HFS (167 ± 4 %, %EPSP slope ± S.E.M., n = 8).

Aβ1–40 (200 nM) significantly reduced LTP (137 ± 6 %, n = 5, P < 0.001) in vitro. The PPARγ agonist troglitazone (20 μM) significantly enhanced LTP (174 ± 10 %, n = 5, P < 0.05), when applied 30 min prior to HFS. Cigitazone (20 μM) however did not affect LTP significantly (161 ± 9 %, n = 4), but enhanced baseline transmission (108 ± 4 %, n = 4, P < 0.05) 30 min following application. The endogenous PPARγ agonist, 15-deoxy-D12,14-PGF2α (5 μM), reduced LTP significantly (143 ± 2 %, n = 5, P < 0.001), but also increased baseline transmission (109 ± 3 %, n = 5, P < 0.05) 30 min following application. Ciglitazone (20 μM) however did not significantly compared with control values or Aβ1–40 alone (152 ± 9 %, n = 5). This suggests that enhancing PPARγ activity may reduce the impairment of LTP mediated by Aβ1–40. We also suggest involvement of PPARγ agonist in the regulation of synaptic transmission and LTP in the CA1.

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All procedures accord with current national and local guidelines

Disrupted synaptic transmission in hippocampal slices taken from aged TAS10 mice

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Transgenic mice overexpressing amyloid precursor protein (APP) have previously been shown to have deficits in either hippocampal basal synaptic transmission (Fitzjohn et al. 2001) or synaptic plasticity (Chapman et al. 1999) but not both. We have studied synaptic transmission and plasticity in hippocampal slices taken from aged (12–14 months) TAS10 mice (Richardson et al. 2003) overexpressing APP harbouring the Swedish mutation (K670N and M671L), in an attempt to resolve these confounding reports. In addition, we have examined neuronal network behaviour induced by 4-aminopyridine (4-AP).

Wildtype (WT) and heterozygous transgenic mice (male and female) were killed humanely and 400 μm-thick horizontal hippocampal slices prepared in a standard manner. Data are presented as mean ± S.E.M. and statistical significance determined using Student’s unpaired t test, unless otherwise stated.

In TAS10 slices we observed a significant deficit in basal synaptic transmission in the CA3–CA1 Schaffer collateral pathway such that the mean ratio of the slope of the maximal fEPSP to fibre volley amplitude was approximately six-fold lower (0.63 ± 0.14, n = 14) than in WT slices (3.27 ± 0.55, n = 11; P < 0.001). Despite this deficit, relative levels of short-term synaptic

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C43

C44
plasticity in the CA1 region of TAS10 slices (paired pulse and multiple pulse facilitation, at frequencies of 1, 5 and 10 Hz) were normal. In addition, LTD induced by a theta burst stimulation paradigm, was normal in the CA1 of TAS10 slices (WT = 160 ± 12 % of baseline, n = 8, TAS10 = 186 ± 10 % of baseline, n = 8, P > 0.05 2-way ANOVA). The inter-event interval (IEI) of synchronous events induced by 100 µM 4-AP was significantly longer in TAS10 hippocampal slices (IEI, 6.9 ± 1.7 s; n = 8) compared with WT slices (IEI, 2.4 ± 0.6 s; n = 6, P < 0.05). Addition of NBQX (20 µM) and D-AP5 (50 µM) to the bath medium, isolated bicuculline-sensitive GABA-mediated synchronous events in both WT and TAS10 slices. These GABAergic events were similar in frequency in WT and TAS10 slices (IEI, 10.3 ± 1.2 s in WT slices cf. 10.8 ± 1.3 s in TAS10 slices), suggesting that GABAergic systems remain relatively unaffected by overproduction of β-amyloid.

This study suggests that the deficit in glutamatergic synaptic transmission, observed in several strains of APP overexpressing mice, may be coupled with alterations in synchronous network activity, which in turn may lead to deficient information processing.


All procedures accord with current UK legislation

C45

The latency to the anoxic depolarization of CA1 pyramidal cells in rat hippocampal slices exposed to different types of metabolic inhibition

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During brain anoxia or ischaemia a fall of ATP level leads to a sudden run-down of transmembrane ion gradients (the anoxic depolarization or AD). This releases glutamate by reversed uptake (Rossi et al. 2000), which triggers the death of neurons. By whole-cell clamping pyramidal cells (using a KCl-based, BAPTA and ATP containing internal solution) to sense the rise of [glutamate], accompanying the AD, we have investigated the energetic factors controlling the latency of the AD in area CA1 of hippocampal slices (from P12 rats killed humanely by cervical dislocation).

When oxygen and glucose (10 mM) were removed, glycolytic ATP production was blocked with iodoacetate (2 mM), and mitochondrial ATP production was blocked using rotenone (100 µM) and antimycin (100 µM), the AD occurred in ~7.5 mins (440 ± 17 (s.e.m.) s n = 29) at 33 °C. Allowing glycolysis fuelled by glycogen, when glucose and oxygen were removed and mitochondria were blocked, delayed the AD by a further ~5.5 mins (to 771 ± 37 s n = 8), and if external glucose was present the AD was prevented altogether (latency > 3922 ± 811 s n = 4). Allowing mitochondria to function when glycolysis was blocked increased the latency to the AD by over 6 mins (786 ± 78 s n = 13, compared with 409 ± 37 s n = 7), when mitochondria were blocked, both experiments done with oxygen present, glucose removed and iodoacetate added, suggesting that metabolites feeding mitochondria downstream of glycolysis (pyruvate and citric acid cycle intermediates) provide a significant energy store. Superfusing lactate (5 mM) in this situation did not prevent the AD occurring, and provided no extra increase in the latency to the AD (n = 7). Simply removing oxygen and glucose from the superfusion solution produced an AD after 17 mins (1021 ± 84 s n = 5). By comparison in vivo ischaemia produces an AD after ~2 mins, implying a ~3 mins latency at the 33 °C used here.

These data show that glycolysis alone can prevent the anoxic depolarization, and do not support the idea that most brain ATP production is powered by lactate supplied to neurons by glia. The long latency to the AD seen on simply removing oxygen and glucose may result from: (i) the presence of residual oxygen in superfusion solutions passing through the recording chamber, which has to be open to the air to allow electrode access; (ii) a lower metabolic rate for sliced tissue compared to tissue in vivo; (iii) the use of a non-physiologically high glucose concentration (10 mM) which gives the tissue an abnormally high reserve of glycogen.


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C46

Tonic release of glutamate in rat hippocampal slices by a DIDS-sensitive mechanism

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Tonic release of glutamate into the extracellular space of the hippocampus and striatum has been attributed to a cystine-glutamate exchanger which is blockable by the glutamate analogue CPG, and adaptation of this release in the striatum has been suggested to underlie relapse in the use of cocaine (Jabaudon et al. 1999; Baker et al. 2002, 2003). We monitored tonic glutamate release in area CA1 of hippocampal slices (from P12 rats killed humanely by cervical dislocation) using whole-cell patch-clamping to measure the glutamate receptor mediated current evoked in pyramidal cells when Na-dependent glutamate uptake was blocked with the non-transported glutamate analogue TBOA (200 µM). The superfusion solution contained TTX (0.1–1µM) to block action potentials. TBOA evoked a slowly developing inward current at ~30 mV which reached ~125 pA after 1.5 mins, and was abolished by the NMDA receptor blocker D-AP5 (50 µM). The TBOA-evoked current was not significantly affected by CPG (50µM), even though cystine (300 µM) increased the TBOA-evoked current 2.8 ± 1.9 fold (mean ± s.e.m., P = 0.04 by 2 tailed t test, n = 5) and sometimes evoked an inward current even in the absence of TBOA (as seen previously in cerebellum and cortex: Watt et al. 1999), demonstrating that cystine-glutamate exchange is present in area CA1. The TBOA-evoked current was reduced by 49 ± 11 % (P = 0.0004, n = 5) by DIDS (1 mM). DIDS has been claimed to block NMDA receptor mediated currents (Tauskela et al. 2003), but this was found to be an artefact (produced by the co-application of NMDA and DIDS) reflecting the superposition of a DIDS-evoked outward current on an NMDA-evoked inward current.

These data suggest that although cystine-glutamate exchange is present in the hippocampus it does not generate the tonic release of glutamate, and that the tonic release is mediated by a DIDS-sensitive mechanism.


Glutamate spillover and activation of mGluR1 in mouse cerebellar Purkinje cells

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At the cerebellar parallel fibre to Purkinje cell synapse, repetitive stimulation of a beam of parallel fibers evokes a fast AMPA receptor mediated synaptic current (EPSCfast), and a slow synaptic current (EPSCslow) mediated by metabotropic glutamate (mGluR1) receptors (Tempia et al. 1998). Here we examine how the EPSCslow magnitude depends on the number of activated synapses, which was determined from the size of the EPSCfast (in GABAzine, 100 μM) evoked by different stimulus strengths (Marcaggi et al. 2003).

We recorded from Purkinje cells in sagittal cerebellar slices from 2–3 week old mice killed humanely by cervical dislocation. Cells were clamped at −70 mV and the internal medium was Cs-glucuronate based with 0.1 mM CaCl2 buffered by 0.5 mM EGTA at pH 7.3. Stimulating in the molecular layer (10 pulses at 200 Hz), we observed a supralinear dependence of the amplitude of EPSCslow on the number of activated synapses, implying an interaction between different synapses. We previously showed that the AMPA receptor mediated EPSC was prolonged when more synapses were activated (again implying interaction between synapses), and that this prolongation was mimicked and amplified by inhibition of glial transporters, suggesting that it was due to glutamate spillover (Marcaggi et al. 2003). Knock-out of the main glial glutamate transporter GLAST approximately doubled the EPSCslow amplitude, and superimposed inhibition of GLT-1 by dihydrokainate further doubled the amplitude. In wild-type slices, superfused with solution containing the broad spectrum glutamate transporter blocker TBOA, when parallel fibres passing close to, but not contacting, the recorded cell were stimulated an EPSCslow could be detected, showing that spillover of glutamate could, in these conditions, elicit an EPSCslow. When stimulation was applied to the granular layer or white matter, rather than the molecular layer, so that activated synapses were more scattered in the Purkinje cell dendritic tree, the EPSCslow was approximately 1/3 of the size of the EPSCslow induced by molecular layer stimulation of the same number of synapses.

We conclude that the EPSCslow is strongly shaped by glial glutamate uptake and by the spatial arrangement of activated synapses on the Purkinje cell dendritic tree.


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A tonic glycine receptor-mediated current in lateral superior olive (LSO) neurones of the young rat

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50% for the EPSC amplitude. The AP wave form determines the calcium signal that controls vesicle fusion, by both controlling the opening of voltage-gated calcium channels and the driving force for calcium influx. Integrating our simulated AP wave forms into a model of a Calyx-of-Held type synapse (Borst & Sakmann, 1998) shows that both calcium peak current and total calcium ion influx have a CV of 9%, which translates (Augustine, 2001) into a change in vesicle release probability of over an order of magnitude. Synaptic reliability and variability has been in general attributed to mechanisms inside the cortical synapse, but our knowledge is based on paired soma recordings. Thus, it is difficult to dissociate synaptic and axonal stochastic effects. Although care has to be taken when relating data from large synapses to much smaller cortical synapses, our simulations suggest that axonal channel noise could have an impact on cortical synaptic transmission.

Faisal AA et al. (2002). IEEE WC'02 proceedings 1, 1661–1669.

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Cellular Neurophysiology 7P

C50

Morphology, intrinsic firing and synaptic properties of excitatory cells in layer 6 of rat and cat visual cortex

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Excitatory cells in layer 6 have diverse morphologies but fall into broad categories according to their long distance projections (Katz, 1987; Zhang & Deschenes, 1997). Corticocortical cells (CT) have apical dendritic tufts in layer 4 and local axon collaterals arborising in layer 4. Corticocortical cells (CC) are more varied, including short pyramids, inverted pyramids and bipolar cells, but lack apical tufts in layer 4 and have horizontally directed axon collaterals restricted to layers 5/6. These differences suggest distinct roles within the network.

We recorded and filled pairs of layer 6 cells in 450–500 μm slices from 5–6 week old male rats and adult male cats at 34°C (Anaesthesia: halothane + 60 mg kg⁻¹ pentobarbitone I.P. for rats; 70 mg kg⁻¹ α-chloralose + 6 mg kg⁻¹ pentobarbitone I.V. for cats. Animals were humanely killed. Further details in Thomson & West (2003).

Three firing types were observed in rat: tonic (8 cells), phasic (20), and phasitonic (phasic to small suprathreshold depolarisations but tonic to larger depolarisations, 10 cells). These cells have a more varied morphology; the majority were tonic (8) or phasitonic (6). In contrast, none of 21 CC-like cells was tonic and 17 were phasic. 1037 pairs of layer 6 cells in rat and 141 in cat were tested for synaptic connections, yielding 37 and 8 connections respectively. Average EPSP amplitude (at −72 mV) was 0.73 ± 0.43 mV (mean ± s.d., n = 22) and transmission failures ranged from 0–54% (12 ± 16%). Most filled cells had CC-like morphology and phasic firing properties. All connections from CC-like cells exhibited short-term synaptic depression: 2nd EPSP amplitudes were 15–60% of the 1st at intervals < 20 ms, recovering to ~80% at 60–100 ms. The one connection between CT-like cells recorded had qualitatively different short-term properties, exhibiting clear facilitation (> 150% at 15 ms interval) and post-tetanic potentiation (EPSPs following 6 spike trains at 3 s intervals were 2-3 those following single spikes).

These data support previous suggestions that CC cells make depressing synaptic connections whereas CT cells make facilitating connections (Beierlein & Connors, 2002; Tarczy-Hornoch et al. 1999). The phasic firing properties of CC cells and their depressing synaptic connections are best suited to signalling changes in incoming activity (novelty detection), whereas the ability of CT cells to fire tonically and their less phasic outputs...
may enable sustained input both to layer 4 and to the thalamus.


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C52
A selective Na⁺ conductance in dexamethasone-treated H441 airway epithelial cells

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Airway epithelia absorb Na⁺ from the airway surface liquid and this process is vital to lung function, develops in response to increased fetal levels of glucocorticoids, thyroid hormone and adrenaline (reviewed by Olver et al., 2004), and is dependent upon epithelial Na⁺ channels (Hummel et al. 1996). These channels are composed of three subunits (α, β and γ-ENaC) that form a selective Na⁺ conductance if co-expressed in Xenopus oocytes. However, several authors have failed to identify such selective Na⁺ channels in airway epithelia and Na⁺ influx in these cells has thus been attributed to non-selective cation channels, which may be formed by the ENaC subunits in an alternative, stoichiometric arrangement. This prompted us to explore the effects of dexamethasone upon Na⁺ conductance in H441 airway epithelial cells.

Figure 1. H441 cells were grown (~24 h) on glass coverslips under control conditions or in the presence of 0.2 µM dexamethasone before being studied using the perforated patch technique; all data are mean ± S.E.M. (n > 4). A, effects of amiloride (10 µM) upon V_m. B, the relationship between the amiloride-sensitive current (I_amil) and V_m in dexamethasone-treated cells under quasi-physiological ionic conditions (Na⁺) and after abolishing the transmembrane Na⁺ gradient by replacing Na⁺ with NMDG⁺ or Li⁺.

Measurements of V_m (Fig. 1A) showed that dexamethasone caused amiloride-sensitive depolarisation and voltage clamp studies (Fig. 1B) showed that the amiloride-sensitive currents in these cells normally reversed at 65.8 ± 1.8 mV, a potential close to ENa (67.9 mV). Lowering [Na⁺], to 10 mM, the concentration in the pipette solution, by NMDG⁺ substitution shifted V_m, to 5.3 ± 0.4 mV whilst replacing Na⁺ with Li⁺ caused a rightward shift in V_rev to 90.0 ± 31.8 mV (Fig. 1B). Dexamethasone thus evokes the expression of a selective Na⁺ conductance with a substantial permeability to Li⁺ and this conductance is thus similar to that seen in oocytes co-expressing α, β and γ-ENaC. These data (see also Voilley et al. 1994) thus suggest that selective, rather than non-selective, Na⁺ channels permit Na⁺ entry in airway epithelia.


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C53
Nociceptor-specific gene deletion using heterozygous NaV1.8-Cre recombinase mice

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Mouse null mutants provide useful insights into gene function, but perinatal lethality or developmental compensatory mechanisms may obscure behavioural phenotypes. It is desirable to be able to delete genes in specific tissues, preferably in an inducible manner. The Cre-loxP system allows tissue-specific Cre-recombinase mice to be crossed with mice containing floxed loxP-flanked genes to produce tissue-specific null mutants. NaV1.8 is a voltage-gated sodium channel expressed only in a subset of sensory neurons. More than 85% of these are nociceptors (e.g. Djouhri et al. 2003). We therefore inserted (knocked-in) the bacteriophage Cre-recombinase gene at the NaV1.8 locus in embryonic stem cells and generated transgenic mice expressing Cre recombinase under the control of the NaV1.8 promoter.

Figure 1. TTX-⁺ transient currents are normal in NaV1.8-Cre heterozygotes, but abolished in NaV1.8-Cre homozygotes. Mean NaV1.8 current density is unchanged with the functional loss of one NaV1.8 gene (n = 22, WT n = 22; means ± S.E.M.), whereas no transient TTX-⁺ Na⁺ currents were found in NaV1.8-Cre homozygotes (n = 26). Inset: left, centre and right panels, example currents recorded from WT, NaV1.8-Cre heterozygote and homozygote, respectively, in 250 nM TTX.
Analysis of the expression pattern of functional Cre recombinase using ROSA26 reporter mice demonstrated that Cre was expressed in an identical pattern to that of Na\(_{\text{v}1.8}\). Expression began at embryonic day 14 in small diameter neurons in dorsal root, trigeminal and nodose ganglia, but was absent in non-neuronal or CNS tissues. This pattern of expression was unaltered in adult animals. Studies of Na\(_{\text{v}1.8}\) heterozygous null mice have shown that these animals have normal electrophysiological and behavioural phenotypes (Akopian et al. 1999). Na\(_{\text{v}1.8}\)-Cre and wild-type (WT) mice were killed humanely and dorsal root ganglion cultures prepared. Tetrodotoxin-resistant (TTX-r) Na\(^+\) channel expression in humanely and dorsal root ganglion cultures prepared.

In naive animals, neither GW567675 (1–10 µM) nor GW567676 (1–10 µM) altered the frequency of spontaneous glutamatergic EPSCs or IPSCs. In contrast, in FCA-inflamed animals, the EP1 antagonists significantly increased the frequency of spontaneous EPSCs in 5/10 cells (by 48 ± 14%) and IPSCs in 6/13 cells (by 67 ± 9%), respectively. Similarly, analysis of miniature synaptic currents revealed that EP1 antagonism significantly increased mEPSC frequency in 12/17 cells (by 67 ± 9%) and glycinergic mIPSC frequency in 5/8 cells (by 73 ± 10%), respectively. These changes in miniature frequency occurred in the absence of changes to the amplitude or kinetics of either the EPSC or IPSC population. The modulation afforded by EP1 antagonism was eliminated in the presence of 10 µM prostaglandin E2.

These data suggest a presynaptic location for prostaglandin EP1 receptors on the terminals of glutamatergic and glycinergic neurones. Following peripheral inflammation, these prostaglandin EP1 receptors become tonically activated by an endogenous ligand(s) which results in decreased synaptic release of both glutamate and glycine in substantia gelatinosa. This effect provides a potential cellular basis for the antihyperalgesic effects of prostaglandin EP1 receptors in inflammatory pain states.

Akayama K et al. (2002). Anesthesiology 97, 1254–1262.

All procedures accord with current UK legislation
These results suggest a presynaptic location for TRPV1 receptors on the terminals of glutamatergic neurones. We hypothesise that during peripheral inflammation TRPV1 receptors in sensory terminals become spontaneously active, resulting in an increased vesicular release of glutamate. These results provide new functional evidence for the involvement of TRPV1 in chronic inflammatory pain and provide a cellular basis for the antihyperalgesic effects of TRPV1 receptor antagonists.


All procedures accord with current UK legislation

Second messengers of octopamine receptors in the pond snail Lymnaea stagnalis

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We recently showed a major role for octopamine as a transmitter in the buccal ganglia of the snail Lymnaea stagnalis (Vehovszky & Elliott, 2001). Most octopamine responses in insects are mediated by adenylyl cyclase-dependent mechanisms (see, Roeder 1999 for review). To determine if this is also the case in molluscs, we recorded simultaneously the responses in the B1, B2 and B3 motoneurons to bath-applied octopamine in the presence of pharmacological agents which interact with the cAMP pathway.

The CNS was isolated and pinned down in a bath, though which saline was pumped at 1ml/min. Penetration of the visually identified neurons B1, B2 and B3 using glass micropipettes was assisted by application of 0.1% protease (Sigma XIV). Signals were amplified and recorded to computer disk using DasyLab. Chemicals were dissolved in high Ca/high Mg saline or low Ca/high Mg saline, to reduce synaptically mediated background. Full details of the methods are given Vehovszky & Elliott (2001).

The B1 motoneuron is depolarised by octopamine, with a threshold of 5 μM. The amplitude of the response is enhanced by preincubation for 20 min with 10 μM IBMX (an inhibitor of phosphodiesterase). Application of the direct cyclase activator 50 μM forskolin also depolarises the B1 motoneuron, but the inactive analog 1, 9-dideoxyforskolin has no effect. The membrane-permeable cAMP analog 8-bromo-cAMP also depolarise the B1 motoneuron, at 2 μM. Effects of the cyclase blockers SQ 22536 and Rp-cAMPS were only seen at extremely high concentrations (1–3 μM) – this may indicate that the tissue sheath or glial cells prevent these drugs from reaching the neurons. The response was also blocked by the G-protein Antagonist GDP-β-S. These observations are consistent with the hypothesis that the B1 has an octopamine receptor that activates adenyly cyclase. The B2 and B3 neurons are hyperpolarised by octopamine – their thresholds are 5 and 0.5 μM respectively. Their responses are not affected by GDP-β-S, IBMX, 8-bromo-cAMP, Rp-cAMPS or SQ 22536. Forskolin has an excitatory effect on the B2 and B3 motoneurons; no inhibition was seen. In fact forskolin excites all the buccal neurons tested so far.

We conclude that the inhibitory octopamine receptor in the B2 and B3 neurones does not use cAMP for signalling, and must use a different pathway.


Heterogeneous actions of cocaine and amphetamine regulated transcript (CART) peptides on rat sympathetic preganglionic neurones in vitro

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The wide CNS distribution, neuronal co-localisation and profound actions following administration indicate that cocaine and amphetamine regulated transcript peptides (CART) play an important neuromodulatory role in the CNS. However the cellular actions of CART peptides remain largely unknown. Therefore we have begun to investigate the actions of CART peptides on sympathetic preganglionic neurones (SPNs) which receive direct inputs from both brainstem (Dun et al. 2002) and hypothalamic (Elías et al. 1998) neurones co-expressing CART.

Whole cell patch clamp recordings were made from neurones in transverse spinal cord slices from humanely killed neonatal Wistar rats (P7–14). SPNs were identified by their position in the lateral horn and characteristic electrical properties. The mean resting potential of SPNs was −54.5 ± 1.7 mV and the mean input resistance was 753 ± 39 mΩ, n = 44. In 4 SPNs, application of 200 nM rCART (55–102) depolarised the membrane potential (from −48 ± 3.5 to −40.6 ± 4.1 mV) and reduced the neuronal input resistance. In a further 4 cells, 200 nM rCART (55–102) produced a hyperpolarisation of the membrane potential (from −50.8 ± 3.5 to −55.3 ± 3 mV) and a reduction in input resistance (by 21.3 ± 5 %). The hyperpolarisation and decrease in input resistance were blocked by bicineuline (10 μM; n = 2) suggesting rCART (55–102) activated GABAA receptors. In a further 30 SPNs, rCART (55–102) had no action on the membrane potential or input resistance. The heterogeneous actions of CART peptides on SPNs may reflect the different CART inputs that these neurones receive: from the arcuate nucleus in the hypothalamus colocalised with POMC (α-MSH) and from the brainstem colocalised with noradrenaline. Thus we have compared the actions of α-MSH with those of rCART (55–102). In 6 SPNs,
200 nM α-MSH depolarised the membrane potential (from $-57.5 \pm 2.7$ to $-47.5 \pm 1.1$ mV). However, unlike rCART (55-102), the α-MSH induced membrane depolarisation was associated with an increase in input resistance (by $27.5 \pm 7.5$%) suggesting the closure of resting conductances. In cells in which rCART (55-102) induced membrane hyperpolarisation, α-MSH was without effect ($n = 3$) or produced a membrane depolarisation ($n = 1$).

These data suggest CART peptides differentially regulate electrical excitability of SPN. Further studies are required to clarify the functional significance of these observations and if these effects reflect differential CART inputs arising from the brainstem and hypothalamus.


All procedures accord with current UK legislation

C58

Effects of administration of SKF 38393, a D1 agonist, on methamphetamine-induced changes in shape of Zebrin bands in rat cerebellum

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The cerebellum is involved in emotional stress response and has parasagittal Zebrin (Aldolase C; Aldoc) bands expressed in Purkinje cells, which is thought to be a modular computing system (Brochu et al. 1990). Zebrin compartment has plasticity after external stimuli (Dusart et al. 1994; Zagrebelsky et al. 1997). We have reported by in situ hybridization study that daily methamphetamine administration (5 mg kg$^{-1}$, i.p.) for 3 days into rats induced fragmentation of Aldoc mRNA stripes and lateral shift of those stripes (Hamamura M et al. 1999). To know if these shape changes of Aldoc mRNA stripes are correlated with hyper locomotive behaviour induced by methamphetamine injections, we measured Aldoc mRNA in rats injected with a D1 receptor agonist SKF38393 (1 mg kg$^{-1}$, i.p.) for 1 week after prior injections (once 3 days for 2 weeks) of the methamphetamine (1 mg kg$^{-1}$, i.p.), whose treatment induced loss of hyper locomotion (Shuto et al. 2003). The rats were killed humanely.

The rats with the five injections of methamphetamine showed fragmentation of Aldoc mRNA stripe in the lobules VII and VIII. In addition, in the rats with the same repeated injections followed by the seven daily SKF38393 injections the number of stripes of Aldoc mRNA was not significantly different from those with repeated methamphetamine injections. On the other hand the shift of horizontal location of Aldoc mRNA stripes from the meridian in rats with repeated methamphetamine administration ($n = 4$ for each) was significantly different from either that in the saline control or that in the rats with methamphetamine plus SKF38393 treatment ($P < 0.05$, $n = 4$ for each, Wilcoxon’s signed rank test). Since the SKF treatment rescued the horizontal shift of the Aldoc mRNA stripes induced by repeated methamphetamine injections, we conclude that the horizontal location change in Aldoc mRNA stripe but not the fragmentation is well correlated with behaviour in amphetamine psychosis.


All procedures accord with current national and local guidelines

PC19

Dopamine D1 receptor modulation of rat striatal NMDA receptors

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Striatal dopamine D1 receptors have been reported to increase (Flores-Hernandez et al. 2002), or attenuate NMDA receptor currents (Lee et al. 2002). In our experiments we have used whole cell patch-clamp recordings from medium spiny neurons in 300μm thick striatal slices from humanely killed 7 day old rats to investigate the mechanism of NMDA receptor modulation. NMDAR responses were evoked by 10 μM NMDA and 10 μM glycine. In the presence of TTX (100 nM) to block action potentials and the D2 receptor antagonist spiperone (2 nM) with ATP (1 mM) and GTP (1 mM) in the pipette solution. The D1 receptor agonist, SKF-82958 (20 μM), significantly (paired t test, $P < 0.05$) decreased the NMDA whole-cell current from $249 \pm 36$ pA to $153 \pm 31$ pA (mean $\pm$ s.e.m., $n = 10$ cells). Replacement of intracellular GTP with GDP-β-S (0.5 mM) did not prevent inhibition (control: $162 \pm 34$ pA, SKF-82958: $74 \pm 12$ pA, $n = 9$ cells) suggesting D1 inhibition is not dependent on G protein activation. These results are consistent with those of Lee et al. (2002) who demonstrated a direct inhibitory interaction between the C-termini of the D1 receptor and the NMDA receptor NR2A subunit. However protein immunohistochemistry (Porter-Cailliau et al. 1996) and in situ mRNA hybridization (Monyer et al. 1994) show a lack of NR2A expression in 7 day old rat striatum. We therefore tested for the presence of functional NR2A-containing receptors (Paoletti et al. 1997) using ZnCl2 (100 nM) or for the presence of tonic Zn inhibition using the Zn chelating agent TPEN (1 μM) (control: $194 \pm 20$ pA, ZnCl2: $198 \pm 15$ pA, TPEN: $181 \pm 11$ pA, $n = 5$ cells).

These results suggest an absence of functional NR2A receptors in 7 day old rat striatum and therefore that there may be an NR2A-independent mechanism mediating direct, G protein-independent, inhibition of NMDA receptors in the neonatal rat striatum.


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All procedures accord with current UK legislation


All procedures accord with current UK legislation
Functional effects of co-transfecting β-subunits 1, 1A and 3 with Na\textsubscript{v}1.8 α-subunit in a COS-7 heterologous system

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Na\textsubscript{v}1.8 is a tetrodotoxin-resistant (TTX-r) Na\textsuperscript{+} channel that normally functions only in small diameter primary sensory neurones, and it is known to play a role in pain-pathways. The heterologous expression of peripheral nerve TTX-r Na\textsuperscript{+} channels in mammalian systems has proved problematic, with low probabilities of functional expression and abnormal channel voltage-dependence and kinetics. We now know that poor expression may result from a requirement for accessory proteins, including the chaperone protein p11 (Okuse et al. 2002). However, Na\textsuperscript{+} channel β-subunits in nerve are normally associated with β-subunits (e.g. Chen et al. 2002), and there is evidence that co-expression of Na\textsubscript{v}1.8 with the β1 subunit increases functional expression when mRNA is injected into Xenopus oocytes (Vijayaragavan et al. 2001).

We have used lipofectamine to co-transfect green fluorescent protein (GFP, 0.4 µg) and an excess of cDNA for Na\textsubscript{v}1.8 in pRK7 vector (0.7 µg), with and without one of 3 different β-subunit clones. This work was supported by the MRC and the Wellcome Trust.

We found functional expression of Na\textsubscript{v}1.8 in only 15.6 % of fluorescent cells (total n = 96), when the α-subunit alone was transfected with GFP. There was no significant change in the probability of functional expression with β3 and β1A subunits, but co-transfection with β1 reduced the probability to 2.2 % (total n = 45, P < 0.05 Chi Square test, Yates corrected). The maximal Na\textsuperscript{+} current density was unaffected by co-transfection of β3 (17.9 ± 5.73 pA pF\textsuperscript{-1} α-subunit alone, n = 7, versus 16.85 ± 1.85 pA pF\textsuperscript{-1} α + β3 subunits, n = 6, means ± s.e.m.) however the average maximal current density was reduced by β1A to 5.84 pA pF\textsuperscript{-1} (n = 3), although the effect was not statistically significant. The current voltage-dependence and kinetics appeared unchanged by the co-transfection of β-subunits, although the slope of the voltage-dependence of inactivation was non-significantly increased.

Our findings suggest that co-transfection of Na\textsuperscript{+} channel β-subunits does not appear to be sufficient to recapitulate neuronal Na\textsubscript{v}1.8 channel behaviour in a COS-7 heterologous expression system.

Okuse K et al. (2002). Nature 417, 653–656.

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Molecular and biophysical identity of orexin/hypocretin neurons

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The orexin/hypocretin peptides are produced in neurons of the lateral hypothalamus which send widespread projections throughout the brain. Orexins are implicated in the maintenance of wakefulness and disruption of their function in animal models leads to narcolepsy (Hungs and Mignot, 2001). Recent in vitro experiments suggested that orexin neurons have intrinsic electrical pacemaker activity (Eggermann et al. 2003). Because orexin neurons are active principally during wakefulness, this intrinsic firing may be critical for the normal sleep-wake cycle. It is therefore of interest to elucidate the biophysical and molecular mechanisms responsible for the pacemaker activity of orexin neurons. Studies of other intrinsically firing neurons show that pacemaker activity arises from a complex interplay between plasmalemmal ionic conductances. Thus our aim was to characterise the molecular and biophysical determinants of ionic currents expressed by orexin neurons.

We performed standard whole-cell patch-clamp recordings in living brain slices of humanely killed mice (C57BL/6, male, P12–20) to elucidate the biophysical identity of these currents, by measuring the voltage-dependence of activation and the time- and voltage-dependence of inactivation, and their sensitivity to pharmacological agents. We also used multi-label immunocytochemistry combined with confocal microscopy to determine the molecular identity of the ion channel subunits which co-localise with orexin in lateral hypothalamic neurons. Together, these data allowed identification of currents which determine the firing properties of orexin neurons.


*The first two authors contributed equally to this work. This work is supported by the Wellcome Trust.

All procedures accord with current UK legislation

Targeting GABAergic neurons using an adeno viral vector

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GABAergic neurons are involved in a vast variety of brain functions, including central control of arterial blood pressure. Visualisation of these cells is very difficult because of the lack of reliable antibodies. Moreover, none of the available stains are suitable for high quality fluorescence imaging of the fine structure of these cells. Visualisation of these neurons in live preparations has been attempted in a transgenic mouse where a short piece of the promoter of the GABA-synthesising enzyme GAD67 was used to drive EGFP expression in a subset of GABAergic cells in the hippocampus (Oliva, J. et al. 2000). Since the expression in other parts of the brain has not been well characterised and these animals are not readily accessible, we report here an alternative strategy for visualising living GABA interneurones within the brainstem.

We have generated an adeno viral vector that incorporates 3.7kb of the GAD67 promoter and the first exon-intron sequence to drive the expression of EGFP and tested the expression profile in brainstem and hippocampus. Male Wistar rats (75–150g) were anaesthetised (ketamine, 60 mg kg⁻¹ and medetomidine, 250 µg/kg, ip) and bilateral injections of adeno viral vectors were made using fine glass capillaries. The rats were allowed to recover for 5–7 days and were then deeply anaesthetised (pentobarbitone 100 mg kg⁻¹, ip), perfused and brainstem sections processed for GABA using primary anti-GABA polyclonal antibodies and secondary rhodamine-coupled antibodies. In the hippocampus and nucleus tractus solitarii (NTS) this vector resulted in medium-intensity EGFP expression in numerous neurones (soma size ~ 15–20 µm). In the hippocampus their location was consistent with the distribution of inter-neuronal layers. The absolute majority of EGFP-positive cells (> 90 % in NTS) were immunopositive for GABA. In addition, we have never observed EGFP expression in glial cells (< 10 µm) although some of these cells brightly stained for GABA presumably reflecting GABA uptake from the extracellular space. This vector has also been used to transfect putative GABAergic neurones in organotypic slice cultures of hippocampus and NTS. Fine details of these neurones were visualised using live cell confocal imaging. In conclusion, our novel vector is selective for GABAergic neurones, at least in certain brain areas. This approach will greatly facilitate imaging and electrophysiological studies centred on the role of GABAergic inhibition in physiological and pathological states.


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All procedures accord with current UK legislation

Syntactin-1A is excluded from recycling synaptic vesicles in cultured rat hippocampal neurons

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SNAREs (soluble NEM-sensitive factor attachment protein receptors) mediate the fusion of synaptic vesicles at the plasma membrane. The v-SNARE syntaptobrevin is localised primarily to synaptic vesicles, although it redistributes onto the plasma membrane during vesicle recycling (Sankaranarayanan & Ryan 2000). Inversely, the t-SNARE syntactin is thought to be anchored in the target plasma membrane. However, a cofractionation study showed that syntactin-1 is also present in vesicular membrane (Walch-Solimena et al. 1995). We have investigated whether the subcellular distribution of syntactin-1A is regulated during synaptic vesicle recycling.

Neurons were dissociated from hippocampal CA1-CA3 regions of 2- to 4-day-old Sprague-Dawley rats, which were killed humanely, and maintained in culture for 2–3 weeks prior to experiments. To monitor the distribution of syntactin, we expressed super-epcliptic pHluorin (SE; Sankaranarayanan et al. 2000) fused to the C-terminus of syntactin-1A (stx-SE). Data are presented as means ± S.E.M.

Under steady-state conditions, stx-SE was localised in somatic, dendritic and axonal regions. We quantified the subcellular localisation of stx-SE by monitoring fluorescence changes in stx-SE during either low pH or NH₄Cl application. We found that
9 ± 3% (n = 9) of stx-SE at synaptic boutons (identified by FM 4–64 loading) was localised in an acidic compartment, which may correspond to synaptic vesicles (Walch-Solimenta et al. 1995), while the remainder was on the presynaptic surface. At non-synaptic axonal regions, 100 ± 2% (n = 9) of stx-SE was present on the plasma membrane.

We next investigated whether intracellular presynaptic syntaxin was localised to synaptic vesicles that recycle during action potentials (AP). Repetitive AP stimulation at 10–100 Hz failed to induce accumulation or redistribution of stx-SE on the plasma membrane. Similar results were found when the signal-to-noise level was enhanced using prior photobleaching of baseline fluorescence and application of bafilomycin-A1, which blocks reacidification of recycling vesicles thereby trapping recycling stx-SE in a fluorescent form. These data indicate that syntaxin-1A is present in an internal compartment that does not participate in AP-evoked vesicle recycling.

Our results show that most syntaxin-1A is localised to the plasma membrane and remains excluded from synaptic vesicles during endocytosis. These data suggest that sorting of synaptic vesicle cargo from an abundant plasma membrane component operates with high fidelity.

PC25

Voltage-dependent sodium channel as a novel target controlled by metabotropic glutamate receptor via cAMP/PKA cascade in insect pacemaker neurones

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Apart from the recently cloned metabotropic glutamate receptor (DmGluRA) from Drosophila melanogaster head cDNA library, the characterisation of the native neuronal mGluRs in insect is still in its infancy. Whole cell patch-clamp studies performed on the cockroach Periplaneta americana cultured adult dorsal unpaired median (DUM) neurones revealed that bath application of glutamate (10 μM), applied alone, decreased I\textsubscript{Na} by 32 ± 4.5% (n = 5). In this condition, we never observed any effect of L-AP4. Increasing intracellular cAMP level, by perfusing the cell body with 0.3 mM cAMP through the patch pipette, also reduced I\textsubscript{Na} (28.44 ± 4% (n = 19) like L-AP4. In this case, glutamate still produced an additional effect on I\textsubscript{Na} as observed in control condition. Finally, because both glutamate and L-AP4 effects were completely suppressed in the presence of the PKA inhibitor H89 (0.3 mM), it was strongly suggested that glutamate regulated I\textsubscript{Na} via a cAMP/PKA cascade. This was confirmed by immunocytochemistry using cAMP antibodies. cAMP immunoreactivity exhibited by DUM neurone cell body was observed before and after stimulating mGluRIII by glutamate and L-AP4. The intensity of the cAMP-immunofluorescence was higher with glutamate compared to L-AP4 demonstrating that the stronger effect observed with glutamate occurred via a higher elevation in cAMP level and suggested the implication of a second intracellular regulatory pathway.

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PC26

Auditory pattern recognition based on instantaneous spike rate coding in the cricket (Gryllus bimaculatus)

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Auditory pattern recognition by the CNS is fundamental to acoustic communication. For the neural processing of amplitude modulated sounds the instantaneous spike rate rather than the mean neural activity may be the appropriate coding principle. We compared both coding parameters in the simple auditory system of the cricket Gryllus bimaculatus. Since crickets communicate with stereotyped sound pulses (syllables) of constant frequency, they are established models to investigate the neuroneal mechanisms of auditory pattern recognition. We demonstrate that the primary auditory afferents are not tuned to syllable patterns of certain repetition rates but they act as syllable onset detectors, which preferentially respond to the beginning of sound pulses. This explains why the duration of syllables is not relevant for phonotactic behaviour. When stimulated with different temporal sound patterns the response of a thoracic low order interneurone (Omega neurone ON1) is very similar when the average discharge rate is considered. However, analysing the instantaneous discharge rate, demonstrates that the neuron responds with prominent peaks in its instantaneous discharge rate to syllable rates close to the species-specific sound pattern. The occurrence and repetition rate of these peaks in the neuronal instantaneous discharge are sufficient to explain temporal filtering in the cricket auditory pathway since they closely match the tuning of phonotactic behaviour to different sound patterns. Temporal filtering or “pattern recognition” may not happen as previously proposed in the brain but may already occur at the earliest stage in the auditory pathway. This is in good agreement with robotic models of cricket phonotaxis, where pattern recognition results from simple low level auditory processing. Our data emphasize the importance of instantaneous discharge rate coding in auditory processing rather than integration of neural activity over long time periods.

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Characterisation of electrophysiological consequences of traumatic brain injury in the rat using a novel in vitro model

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White matter regions of the brain, which contain axonal tracts, have differing susceptibility to the consequences of traumatic brain injury (TBI) than regions containing predominantly neuronal cell bodies. Even relatively mild trauma, which does not immediately disrupt the axonal structure, can initiate processes that ultimately lead to the loss of axonal integrity and function. The changes in electrophysiological properties of neurons accompanying this degeneration remain to be fully understood. The work described here forms part of a European Union initiative aimed at reduced the number of falls or road traffic accidents. To this end, organotypic slice cultures of white-matter from 300–400 µm thick brain slices were obtained from 21–40 day old CB57BL/6JCrL mice. The animals were anaesthetised with fluothane 4% in O₂ and humanely killed. Slices were continuously superfused with ACSF at 32–34°C. Pellets of biocytin were implanted in either the GP or STN in slices prepared in the horizontal, sagittal or parasagittal plane. After 8–10 h slices were fixed and processed to reveal the reaction product.

Extensive labelling, including fibres in the internal capsule and cell bodies in the GP and STN, together with labelling in substantia nigra and striatum, was best observed in parasagittal slices, reflecting both retrograde and anterograde biocytin transport. To show functional connectivity, whole-cell recordings were made in parasagittal slices cut 20° to the midline. Single shock stimulation in the GP evoked GABA__A receptor-mediated IPSCs in the STN (44/59 cells) that reversed around ECl and were blocked by bicuculline (10 µM, n = 7). Stimulation of the STN evoked EPSCs in the GP (22/33 cells), which were blocked by CNQX (10 µM) and DL-AP5 (100 µM, n = 5). In 2/2 slices, both GP to STN and STN to GP connectivity was demonstrated. In current clamp, evoked EPSPs (from the STN) were able to trigger action potentials in the GP (n = 3), while evoked IPSPs (from the GP) caused a cessation of STN neuronal activity observed in several basal ganglia nuclei seen both in idiopathic Parkinson’s disease and animal models thereof. Indeed, the frequency and pattern of this activity may be related to resting tremor. Here, we describe a brain slice preparation that maintains the functional connectivity between the GP and STN.

Reciprocal connectivity between the glutamatergic subthalamic nucleus (STN) and GABAergic globus pallidus (GP) appears essential for oscillatory activity and rhythmic bursting in the GP–STN network (Plenz and Kitai. 1999). This central pattern generator could play a prominent role in driving the oscillatory neuronal activity observed in several basal ganglia nuclei seen both in idiopathic Parkinson’s disease and animal models thereof. Indeed, the frequency and pattern of this activity may be related to resting tremor. Here, we describe a brain slice preparation that maintains the functional connectivity between the GP and STN.

In summary, the parasagittal mouse brain slice maintains functional GP–STN connectivity and provides a suitable model for future pharmacological and mechanistic studies of this basal ganglia neuronal network.


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All procedures accord with current UK legislation
Inhibition of Ih reduces epileptiform activity in rodent hippocampal slices


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The cationic anomalous inward rectifier current Ih has been suggested to be a pacemaker current controlling oscillatory activity in certain brain regions and recent evidence has proposed a role for this channel in epileptic states. We have therefore investigated whether Ih is critical in the generation of epileptiform discharges recorded from the adult hippocampal slice preparation, using extracellular electrophysiological techniques. The effect of the Ih blocker ZD-7288 was examined in a number of in vitro models of epileptiform activity, induced by a range of pharmacological manipulations.

Male Lister Hooded rats were humanely killed by cervical dislocation. Brain slices were prepared using standard methods and extracellular recordings were made from stratum pyramidale in hippocampal area CA3. Data are presented as mean ± S.E.M. ZD-7288 produced a concentration-dependent inhibition of both non-synaptic and synthetically-mediated epileptiform activity. Removing extracellular Ca2+ and elevating K+ to 6 mM resulted in epileptiform activity which is independent of action potential driven activity. This non-synaptic bursting was partially inhibited by 10 μM ZD-7288 (32 ± 8% reduction; n = 5) and completely abolished by 100 μM ZD-7288 (n = 5). Similarly, ZD-7288 produced a concentration-dependent inhibition of synaptically-mediated epileptiform activity in the following experimental models: removal of extracellular Mg2+ (IC50 = 23 ± 4 μM; n = 6), elevation of extracellular K+, from 3 to 8.5 mM (IC50 = 27 ± 7 μM; n = 3–11) and the addition of 10 μM bicuculline to the extracellular medium (IC50 = 18 ± 7 μM; n = 5). It has recently been reported that prolonged ZD-7288 application exerts a non-specific depression of glutamatergic synaptic transmission. Therefore we compared the time course of ZD-7288 inhibition of fEPSPs (recorded in area CA3 in response to stimulation in the dentate hilus) and epileptiform bursting induced by 10 μM bicuculline. At 30 and 60 mins after 100 μM ZD-7288 application a significant reduction in burst frequency was observed without any significant change in fEPSP amplitude. A clear reduction in fEPSP amplitude was only observed after 120 mins, by which time epileptiform bursting had been abolished. Furthermore, capsazepine which exhibits similar potency to ZD-7288 at inhibiting Ih, failed to inhibit glutamatergic synaptic transmission per se (100 μM) but produced a significant inhibition of bicuculline-induced epileptiform activity (61 ± 8%; n = 7 at 10 μM).

These data suggest that broad spectrum inhibition of Ih reduces neuronal hyperexcitability in the hippocampus and that molecules possessing this profile may be useful in treating temporal lobe epilepsy.

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