O44

Electrical synapses between Bergmann glial cells and Purkinje neurones in rat cerebellar slices

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The existence of gap junctions coupling neurones and astroglial cells was initially suggested by Nedergaard (1994), who observed propagated Ca^{2+} waves between astrocytes and neurones in mixed cultures. This observation was somehow neglected until very recently, when gap junctional coupling between co-cultured embryonic neurones and astrocytes was confirmed by both dye-transfer assay and direct measurement of junctional currents (Froes *et al.* 1999). Direct coupling between astrocytes and neurones was further substantiated by experiments *in situ*, in brainstem slices, which found dye transfer between astrocytes and neurones and demonstrated electrical synchronisation between spontaneously firing neurones and astroglial cells (Alvarez-Maubecin *et al.* 2000). In the present study we directly demonstrated electrical coupling between Bergmann glial cells (BG) and Purkinje neurones (PN) in acutely isolated cerebellar slices. Electrical coupling between these two cells was identified by dual whole-cell voltage clamp (Clark & Barbour, 1997), which allowed direct recording of junctional current.

Sagittal cerebellar slices (250 μm) were prepared from 14- to 21-day-old Sprague-Dawley rats as described previously (Kirischuk *et al.* 1995). Animals were humanely killed according to UK legislation. Whole-cell recordings from PN–PN, PN–BG and BG–BG pairs were made using Nomarski optics and infrared visualisation, which allowed precise morphological identification of cells. The pipettes used had a resistance of 3–4 mΩ when filled with intracellular solution (mM: KCl 130, $MgCl_2$ 2, Hepes/KOH 10, $ATPNa_2$ 4, Fura-$2K_5$ or Fluo-$3K_5$ 0.2, pH 7.3). Slices were continuously superfused by artificial extracellular solution containing (mM): NaCl 130, KCl 2.5, $CaCl_2$ 2.5, $MgCl_2$ 1, $NaHCO_3$ 26.3, KH_2PO_4 1.6, glucose 10, pH 7.4 when equilibrated with 95 % O_2–5 % CO_2 at 22–24 °C. Current recordings were made with EPC-9/2 amplifier (HEKA, Lambrecht, Germany); electrical stimulation, data aquisition and off-line analysis were controlled by PULSE software (HEKA, Lambrecht, Germany). After establishing dual whole-cell configuration cells were held at −80 mV. Junctional currents were recorded by applying hyper- and depolarising voltage sequences ranging from −120 to +40 mV (voltage step 10 mV) to one of the cells in the pair, while ion currents were measured from both cells.

As has been shown before (Clark & Barbour, 1997), junctional currents were frequently observed in BG–BG pairs: we found electrical coupling in 27 out of 34 pairs analysed. When the similar protocol was applied to the PN–BG pairs junctional currents were found in 32 out of 48 pairs analysed. The electrical coupling was bi-directional as similar junctional currents were observed in PN when the voltage step protocol was applied to BG. To correlate the appearance of these currents with gap junctions we treated slices with octanol (0.2 mM) or halotane (0.1 mM) – known inhibitors of gap junction conductance. Both agents applied for 5 min resulted in a complete inhibition of junctional currents in PN–BG pairs ($n = 5$ for octanol and 5 for halotane). The washout (15 min) led to a complete recovery of junctional currents after treatment with octanol ($n = 5$ out of 5); the action of halotane was irreversible.

We conclude therefore that cerebellar neurones and glial cells are directly connected via gap junctions.

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All procedures accord with current UK legislation.

O45

Synaptic plasticity at superficial synapses in mouse perirhinal cortex *in vitro*

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The perirhinal cortex is implicated in the discrimination of novel and familiar stimuli. The firing rate of perirhinal cortical neurones decreases as a novel object becomes familiar after repeated presentation. Whilst the mechanism underlying the decrease in firing rate is not fully understood, there is growing evidence that a reduction in perirhinal synaptic efficacy may be involved. Rat perirhinal synapses have recently been shown to support a robust form of activity-dependent long-term depression (LTD) (Cho *et al.* 2000). We report here, that a similar form of LTD is also a feature of the mouse perirhinal cortex.

Coronal cortical slices (400 μm thick) were prepared from mice (B6xCBA) aged 1–2 months, killed humanely by cervical dislocation. Slices, containing the perirhinal cortex, were maintained at 25°C in an interface chamber. Recording microelectrodes (2–5 MΩ)were placed in layer II/III of the perirhinal cortex and field EPSPs evoked by monopolar stimulation of the superficial layer. Short-term plasticity (paired-pulse) was examined. LTD was induced using a low-frequency stimulation (LFS) protocol (900 shocks at 1 Hz) and data are expressed as mean percent change in EPSP amplitude ± S.E.M., measured 1 h post conditioning (P values obtained using an unpaired t test).

Paired-pulse stimulation (interpulse interval, 20−2000 ms) produced paired-pulse profiles that favoured depression of the second response, a result which is consistent with the predominance of paired-pulse depression seen at rat perirhinal synapses. LFS of superficial synapses induced homosynaptic LTD -11.7 ± 3.2 %, $n = 19$).

In the rat, perirhinal LTD has been shown to be dependent on both the activation of NMDA receptors and metabotropic glutamate (mGlu) receptors. In agreement, LTD at mouse perirhinal synapses was blocked by group I (500 μM AIDA) and group II (1 μM LY341495) mGlu receptor antagonists, 2.6 ± 1.7 % ($n = 10$, $P < 0.001$) and -1.1 ± 2.7 % ($n = 10$, $P < 0.02$) respectively, but was unaffected by a group III antagonist (10 μM CPPG), -11.2 ± 3.1 % ($n = 6$, $P > 0.5$). In contrast, application of the NMDA receptor antagonist, 50 μM D-AP5, failed to block the induction of LTD at mouse perirhinal synapses, -10.3 ± 5.9 % ($n = 10$; $P > 0.5$).

We also examined the action of these drugs on perirhinal paired-pulse depression. Interestingly, the drugs that blocked the induction of LTD also reduced paired-pulse depression whereas those drugs that had little effect on LTD induction did not markedly alter the paired-pulse profile.

The data presented here suggest that LTD is a feature of both rat and mouse perirhinal cortices. However, in the mouse, NMDA receptors do not appear to be involved in the induction of LTD at superficial synapses.

Cho *et al.* (2000). *Nat Neurosci* **3**, 150–156

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O46

Characterisation of high-frequency activity in the medial entorhinal cortex *in vitro*

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§ In memory of Eberhard H. Buhl

Fast network oscillations in the hippocampus that range from approximately 150 to 250 Hz are termed ripples (O'Keefe & Nadel 1978; Buszáki *et al.* 1992). These ripples are thought to be due to simultaneous excitation of pyramidal cells and interneuronal networks and represent IPSPs on the somata of the pyramidal cells due to feedback from basket cells (Buszáki *et al.* 1992; Chrobak & Buszáki, 1996). However, Draguhn *et al.* (1998) hypothesized that gap junctions located between axons of principal neurones mediate ripples observed *in vitro*. High frequency oscillations have been recorded in the entorhinal cortex (EC) of both epileptic humans (Bragin *et al.* 1999) as well as epileptic and non-epileptic rodents (Chrobak & Buszáki, 1996; Bragin *et al.* 2002). Using slices from non-epileptic rats, Dhillon & Jones (2000) have demonstrated that neurones in layer III of the medial EC (mEC) exhibit electrical coupling, most likely mediated by gap junctions.

Combined entorhinal–hippocampal slices (~450 μm) were taken from adult (200–250g) 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 gas (95 % O_2 and 5 % CO_2) at 36 °C

Using conventional extracellular recording techniques, we are now able to demonstrate ripples only in layer III of the mEC. The ripples had a mean (± S.E.M.) frequency of 192.5 ± 7.2 Hz and were approximately 100–200 μV in amplitude ($n = 3$). These ripples were observed in the absence of synaptic transmission (ACSF $[Ca^{2+}]_o$), they had a mean frequency of 215.5 ± 11.8 Hz ($n = 5$) and also displayed similar amplitude range to those observed in physiological ACSF. There was no significant difference in the frequency in either condition ($P > 0.05$, Student's paired t test). In the presence of gap junction blockers such as octanol (500 μM) and carbenoxolone (100–200 μM), spontaneous ripples were significantly suppressed ($P < 0.05$, Student's paired t test).

Thus, we can demonstrate high frequency activity in an *in vitro* slice preparation of the mEC, and in agreement with previous work these ripples are located in the only lamina of the mEC that exhibits electrical coupling, supporting the notion that gap junctions are crucial in the generation of these ripples.

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All procedures accord with current UK legislation.

O47

Involvement of JNK in β-amyloid peptide mediated depression of LTP in the CA1 *in vivo*

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Alzheimer's disease is associated with increased levels of β-amyloid peptide (Aβ), which has been shown to mediate at least some of its cellular effects by activation of mitogen activated protein (MAP) kinases. Here we have examined the effects of Aβ and D-JNK1, an inhibitor of c-Jun-N-terminal kinase (JNK) on long-term potentiation (LTP) in the CA1 region *in vivo*. We also examined phosphorylation of JNK2, JNK3 and the transcription factor, c-jun.

Following experimental procedures animals were humanely killed. Tissue was prepared from the CA1 region of electrically stimulated hippocampi from urethane-anaesthetized (1.5g kg^{-1}, I.P.) male Wistar rats injected intracerebroventricularly (I.C.V.) 2 h and 6 h previously with water or $Ab_{[1–40]}$ (1 nmol in 5ml). Phosphorylation of JNK2, JNK3 and c-jun was assessed by gel electrophoresis and immunoblotting using phospho-specific antibodies.

JNK2 phosphorylation was increased significantly 2 h post Aβ-injection (12.72 ± 1.0 $n = 6$) compared with controls (9.2 ± 0.2, $n = 6$) and at 6 h (43.6 ± 4.3) compared with controls (29.0 ± 1.6 $n = 5$) (arbitrary units; means ± S.E.M.; $P < 0.05$, Student's unpaired t test). JNK3 phosphorylation was also increased significantly 2 h post Aβ-injection (10.13 ± 0.44) compared with control tissue (6.76 ± 0.37, $n = 5$) ($P < 0.05$), while 6 h post injection (27.82 ± 4.33) levels were similar to the control level (22.43 ± 2.58, $n = 6$). In addition, phosphorylation of the JNK substrate, c-jun, was increased significantly 2 h post Aβ injection (14.97 ± 2.64) compared with the control value (8.35 ± 1.19, $n = 6$; $P < 0.05$, unpaired t test).

To examine the effects of Aβ and D-JNK1 on LTP, water vehicle, $A\beta_{[1–40]}$ (1 nmol) and/ or D-JNK1 (1 or 2.5 nmol in 5ml) was injected 1 h prior to high frequency stimulation (HFS). HFS comprised 10 trains of 10 pulses at 200 Hz at 20 s intervals delivered 3 times. LTP was measured 60 min post HFS. $A\beta_{[1–40]}$ (1 nmol) significantly reduced LTP (115 ± 6 %, $n = 12$, $P < 0.001$) (mean ± S.E.M., ANOVA) compared with control (160 ± 9 %, $n = 15$). Following injection of D-JNK1 (1 nmol) there was no significant change in LTP (155 ± 10 %, $n = 7$). A higher concentration of D-JNK1 (2.5 nmol) caused a significant depression of LTP (135 ± 9 %, $n = 7$, $P < 0.05$), compared with control values (160 ± 9 %, $n = 15$). Combined injection of $A\beta_{[1–40]}$ and D-JNK1 at 1 or 2.5 nmol attenuated the Aβ-mediated depression of LTP observed previously; the mean percentage changes were 133 ± 7 % ($n = 10$) and 140 ± 11 % ($n = 5$), respectively. These values are significantly different from those recorded in the presence of Aβ alone ($P < 0.05$).

These results demonstrate that Aβ causes an increase in JNK2, JNK3 and c-jun phosphorylation, while changes in phospho-JNK3 are time dependent. The inhibitor D-JNK1 attenuated the depressive effects of $A\beta_{[1–40]}$ on LTP suggesting that the JNK signalling pathway may be involved in mechanisms associated with the Aβ mediated depression of LTP.

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All procedures accord with current National guidelines.

O48

Junctional fibroblasts restricted to neuromuscular synapses and their responses to nerve injury

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We raised a novel antibody (code-numbered 2166) and used immunostaining to study a hitherto neglected cell type located at neuromuscular junctions.

Mice and rats were humanely killed by cervical dislocation and standard techniques were used for immunostaining and confocal microscopy of isolated whole-mount preparations of triangularis sterni and/or transverse sections of hind limb muscles.

The 2166-positive cells exhibit a distinct morphology, and screening against a panel of antibodies showed an immunohistochemical profile different from those of terminal Schwann cells (tSC) and muscle satellite cells. The identity of junctional 2166-positive cells as fibroblasts was confirmed by immunostaining for prolyl-4-hydroxylase. Although perijunctional fibroblasts have been described before (Gatchalian *et al.* 1989), immunohistochemistry using the 2166 antibody suggests that these junctional cells represent a distinct sub-population of fibroblast. Previous studies identified tSC as the ones at neuromuscular junctions expressing neuregulin GGFIIβ3 (Trinidad *et al.* 2000). However, immunostaining with HM-24 antibody shows that 2166-positve junctional fibroblasts express this neuregulin isoform and terminal Schwann cells do not. The junctional fibroblast population arises from a more diffuse, larger population during postnatal development. To test for a possible functional role, we performed partial denervation of adult mouse triangularis sterni muscles under ketamine–xylazine anaesthesia (86 and 13 mg kg^{-1}, respectively, I.P.). One day after denervation, junctional fibroblasts had proliferated and extended processes between the motor endplates of adjacent fibres, while terminal Schwann cells remained in an inactivated state on the denervated endplates. By 3 days post denervation, tSC became activated (identified by nestin protein expression; Kang *et al.* 2001) and extended processes which associated with the pre-formed junctional fibroblast sprouts.

These observations suggest that reinnervation following partial denervation may not be completely dependent on terminal Schwann cells (Son *et al.* 1996), and that junctional fibroblasts provide cellular bridges for denervation-induced terminal Schwann cell sprouting. We propose that junctional fibroblasts are a specific cellular component, with possible roles in development, maintenance and plasticity of neuromuscular synapses.

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O49

Asynchronous synapse withdrawal induced by axotomy in Wld mutant mice expressing fluorescent protein in single motor units

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Synapse elimination from polyneuronally innervated muscle fibres is regulated by activity-dependent and -independent mechanisms (Costanzo *et al.* 1999, 2000). An unresolved issue is whether synapse withdrawal is prompted wholly by local interactions at motor endplates, or whether mechanisms intrinsic to motor axons also initiate synapse withdrawal (Brown *et al.* 1976). We have addressed this by examining the complement of motor nerve terminals in single motor units expressing Yellow Fluorescent Protein (YFP), in mice with slow Wallerian degeneration. Axotomy induces withdrawal – rather than degeneration – of synapses from motor endplates in these mice, but hitherto it was unclear whether this occurred synchronously or asynchronously within motor units (Gillingwater *et al.* 2002).

We mated double-mutant Wld^S/Tg-4836 line mice with mice expressing YFP in a sub-set of motor axons (Mack *et al.* 2000; Keller-Peck *et al.* 2001). Mice in the F1 generation show the same phenoype as Wld^S homozygous mutant mice. At age 4–6 weeks, six of these mice were anaesthetized with ketamine–xylazine (100 mg kg^{-1} Ketanest; 5 mg kg^{-1} Rompun; I.P.) and the sciatic nerve sectioned unilaterally. After 5 days, the mice were humanely killed by cervical dislocation and hind-foot lumbrical muscles were isolated and examined for fluorescence of YFP. A single YFP-expressing motor unit was present in four lumbrical muscles out of 24 dissected from these six mice. One of these units was reconstructed from a complete confocal *z*-series taken through the muscle. Out of 58 motor end-plates supplied by this unit, 19 were almost fully occupied (> 80 % occupancy), whereas five terminals occupied less than 20 % of their motor end-plates. The distribution of fractional occupancies was not statistically different from a random distribution ($P > 0.05$; Kolmogorov-Smirnov test). We therefore conclude that a single, synchronised disruption of connectivity between a motoneurone cell body and its terminals can result in asynchronous nerve terminal withdrawal. This suggests that asynchronous withdrawal of synapses from motor units undergoing postnatal remodelling could reflect selective trafficking of soma-derived maintenance factors into nerve terminals, as well as competition for extracellular factors in the vicinity of motor end-plates.

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O50

The role of astrocytes in early ischaemic injury of developing white matter oligodendrocytes

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Ischaemic injury of developing white matter is thought to be the major cause of cell damage in the lesions that underlie cerebral palsy (CP). Typically, these lesions may include an infarcted core and/or an extensive region of hypomyelination. Immature (O4+/GalC−) oligodendroglia appear to be selectively damaged by ischaemia, with activation of non-NMDA type glutamate receptors central to ischaemic injury in these cells. We here examine the role that neighbouring astrocytes may have in immature oligodendroglial injury, focusing upon early astrocyte swelling and the putative release of glutamate.

Neonatal rats were humanely killed and the optic nerves were dissected. Neonatal rat optic nerve (nRON) glial cells were then AM-loaded with Fura-2 and imaged *in situ* for changes in $[Ca^{2+}]_i$ prior to and during perfusion with artificial CSF (aCSF) absent in oxygen and glucose (to mimic ischaemia). Post-natal day 8–12 nRONs were used since at this age the nRON is close developmentally to the human white matter that is subject to injury in CP. The nRONs were continuously perfused and maintained throughout at 37 °C. Cells of the oligodendrocyte and astrocyte lineage were differentiated by rapid-live staining using RAN-2 and platelet derived growth factor α antibodies (Wilke *et al.* 2002).

The onset of ischaemic conditions produced a rapid elevation in $[Ca^{2+}]_i$ and a degree of cell death in both cell types. Astrocyte death was independent of the $[Ca^{2+}]_i$ rise, and dependent upon the presence of Na^+ and Cl^- in the aCSF. Astrocyte death was prevented by blocking the Na^+–K^+–Cl^- cotransporter (NKCC) with 50 mM bumetanide and therefore had characteristics consistent with cell swelling-mediated lysis. Oligodendroglial death during ischaemia was dependent upon $[Ca^{2+}]_i$ elevation and was eliminated by blocking non-NMDA glutamate receptors with 30 μM CNQX. However, both the $[Ca^{2+}]_i$ rises and cell death seen in oligodendroglia during ischaemia were blocked by bumetanide, even though NKCC expression was restricted to astrocytes (determined by immuno-histochemistry). We hypothesise that early astrocyte swelling and death is associated with glutamate release that gates non-NMDA receptors on oligodendroglia resulting in their injury.

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All procedures accord with current UK legislation.

O52

Glial gap junctions do not control synchronous activity in cultured central neurons

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Neural network activity in the CNS is abolished or strongly reduced by gap junction (GJ) blockers. Indeed neural GJs are thought to contribute to network activity. However, when a GJ blocker is applied it exerts its action on neurons but also on astrocytes. Astrocytes are extensively coupled through GJs and have been recently shown to modulate several neuronal properties including synaptic transmission. We therefore tested whether GJ communication in astrocytes affects neural network activity. To this end, we used different primary culture models of hippocampal or cortical neurons and astrocytes, where neuronal GJs are not thought to be present.

Primary cultures were prepared from rat or mouse embryos, plated on astrocytes and grown for 8–15 days. Animals were humanely killed by cervical dislocation. Single or double whole-cell patch recordings from neurons were performed at −60 mV. Calcium signals were monitored in cells loaded with Fluo-4 AM (3 μM). Data were averaged between different experiments and presented as means ± S.E.M. Statistical analysis was carried out by Student's paired *t* test.

Using patch clamp and calcium imaging techniques, we investigated the effects of the GJ blocker carbenoxolone (CBX) on spontaneous and bicuculline-induced (10 μM) network activity. In co-cultures of neurons and astrocytes, application of 100 or 20 μM CBX induced a strong and reversible decrease in the frequency of synaptic activity and calcium oscillations associated with spontaneous (from 3.3 ± 0.3 to 0.2 ± 0.1 min^{-1}) or bicuculline-induced (from 2.1 ± 0.2 to 0.5 ± 0.2 min^{-1}) burst activity, without altering the frequency or amplitude of miniature events recorded in the presence of TTX. Dye or electrical coupling between neurons was not detected in pair of neurons and immunocytochemical staining for major neuronal connexins was faint or absent, suggesting the effect of CBX is not mediated through neuronal GJs. To further test the involvement of astrocyic GJs we used other mice culture models where astrocytes were not coupled. Since neurons do not express receptors for endothelin 1 (ET-1), we used this peptide to block GJs in astrocytes. ET-1 (200 nM) did not mimic or occlude the effect of CBX, which reduced burst frequency from 11.3 ± 1.5 to 0.6 ± 0.2 min^{-1}. Furthermore CBX exerted its effect (from 10.3 ± 2.4 to 1.1 ± 0.2 min^{-1}) in enriched neuronal cultures where only a few, isolated astrocytes were present. We then tested if CBX affected basic neuronal properties and we found that 100 μM CBX increased the spike threshold and strongly decreased the firing rate in response to depolarising stimuli, suggesting it could affect some neuronal currents.

Thus, since astrocytic GJs are not involved in the generation of spontaneous network activity and neuronal GJs are not detected in culture, it is likely that CBX exerts its action through other mechanisms.

All procedures accord with current National guidelines.

O63

Increased numbers of neurones express α4, β1 and δ $GABA_A$-receptor subunits in the periaqueductal grey matter during late dioestrus in the rat: relationship to panic behaviour

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In women, susceptibility to panicogenic challenge increases when progesterone levels fall sharply during the late luteal phase of the menstrual cycle (Follesa *et al.* 2001; Le Melledo *et al.* 2000). In rats, the dorsal half of the periaqueductal grey matter (PAG) contains neural circuitry that can initiate panic-like behaviour (Lovick 2000). In female rats, we found that the fall in

progesterone levels during late dioestrus is associated with increased numbers of $\alpha4$ and δ $GABA_A$-receptor subunit immunoreactive neurones in the PAG (Griffiths *et al.* 2002). In other regions of the brain $\alpha4$ subunits are associated with receptors containing $\beta1$ subunits (Bencsits *et al.* 1999). We have therefore investigated whether plasticity of $\beta1$ receptor subunit expression occurs in the PAG during the oestrous cycle.

Urethane-anaesthetised female rats (1 g kg^{-1} I.P.) were humanely killed by perfusion with 100 ml heparinised (100 U ml^{-1}) saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The stage of the oestrous cycle was determined from vaginal cytology. Frozen sections of midbrain 40 μm thick were processed to reveal immunoreactivity for $\beta1$ subunits using antibody sc-7361 N-19 (Santa Cruz Biotechnology). Immunostaining was revealed using a biotinylated secondary antibody and diaminobenzidene as the chromogen.

During the early stages of the oestrous cycle the number of $\beta1$ immunoreactive cells in the PAG remained relatively constant, ranging from 32.3 ± 2.0 cells $(0.05\ mm^2)^{-1}$, mean ± S.E.M., in proestrus to 28.4 ± 1.2 cells $(0.05\ mm^2)^{-1}$ in early dioestrus (5 rats per stage). However there was a 67% increase to 47.4 ± 1.8 cells $(0.05\ mm^2)^{-1}$ in late dioestrus compared to early dioestrus ($n = 5$, $P < 0.0001$ ANOVA with Bonferroni-Dunn).

These findings are in line with our previous data on plasticity of $\alpha4$ and δ receptor subunit expression in the PAG (Griffiths *et al.* 2002). Moreover, they raise the possibility that falling progesterone levels during late dioestrus lead to increased expression of an aberrant receptor within the PAG in which $\alpha4$, $\beta1$ and δ subunits co-assemble. Recombinant $\alpha4\beta1\delta$ $GABA_A$ receptors show an atypical pharmacology (Dunn, Tancowny & Martin, this meeting). In the PAG, a disturbance of GABAergic control associated with the expression of this receptor subtype may contribute to an increased susceptibility to panicogenic challenge.

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O64

Characterisation of $GABA_A$ receptors containing rat $\alpha4\beta1\delta$ subunits

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The functional characteristics of heteropentameric $GABA_A$ receptor subtypes are defined by their precise subunit composition. Anxiolytic benzodiazepines and endogenous neuroactive steroids allosterically modulate many subtypes, where acute exposure generally facilitates GABA-mediated transmission. Chronic exposure results in time-dependent and cell-specific changes in the expression of several receptor subunits, particularly the less common subunits.

We have shown that in rats exposed to diazepam for 28 days, changes in $\alpha4$ RNA were mirrored by a change in the level of $\beta1$ RNA (Holt *et al.* 1996). Since the $\alpha4$ subunit is often associated with the δ subunit, the present study has focused on the properties of $\alpha4$, $\beta1$ and δ containing receptors. In a companion study reported at this meeting Griffiths *et al.* show that during the rat oestrous cycle the $\alpha4$, $\beta1$ and δ subunits undergo parallel changes in expression in the periaqueductal grey matter, an area associated with initiating panic-like behaviour. Women suffering from premenstrual dysphoric disorder (PMDD), when challenged with flumazenil in the late luteal phase of their cycle, exhibit panic-like behaviour (Le Melledo *et al.* 2000). These observations suggest that flumazenil, an antagonist at the most common $GABA_A$ receptor subtypes, may act as an inverse agonist at the aberrant receptors expressed under these conditions.

Recombinant $GABA_A$ receptors, expressed in *Xenopus* oocytes by injection with 50 ng of total cRNA containing equal concentrations of each subunit-encoding RNA, were characterised by standard two-electrode voltage clamp procedures. The concentration dependence for GABA-mediated currents were measured for each receptor combination and the effects of either 100 nM flumazenil or flunitrazepam were measured at the GABA EC_{50}.

The agonist response of the ubiquitous $\alpha1\beta2\gamma2L$ (EC_{50} 32.8 ± 2.5 μM, mean ± S.E.M.) was enhanced by flunitrazepam (42.1+ 3.2%, $n = 7$) but unaffected by flumazenil. After sequentially changing a single subunit in the expressed receptors we found that the agonist response of the $\alpha4\beta1\delta$ receptor (EC_{50} 2.02 ± 0.33 μM) was insensitive to flunitrazepam but flumazenil exhibited significant inverse agonist activity, decreasing the response to the GABA EC_{50} by 32.8 ± 6.6% ($n = 9$). This receptor also displayed unusually slow desensitisation kinetics.

These results lend credence to the suggestion that aberrant $GABA_A$ receptors expressed during the late luteal phase of the menstrual cycle may explain the panicogenic effects of flumazenil challenge in PMDD.

Holt RA *et al.* (1996). *Neuropharmacol* **35**, 1457–1463.
Le Melledo J *et al.* (2000). *Am J Psychiatry* **157**, 821–823.

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All procedures accord with current National guidelines.

O65

The role of $GABA_B$ autoreceptors in control of neurokinin-induced GABA release in the rat entorhinal cortex

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We have recently shown that neurokinin 1 receptor (NK1r) agonists provoke an increase in GABA release onto pyramidal cells in the entorhinal cortex (EC) *in vitro* (Stacey *et al.* 2002). This effect is due to excitation of inhibitory interneurones, and is extremely robust, with low nanomolar concentrations provoking a 3–4-fold increase in frequency of spontaneous inhibitory postsynaptic currents (sIPSCs). In the present study we have examined (1) whether the NK1r-mediated increase in release of GABA might be self-limiting via activation of presynaptic $GABA_B$ autoreceptors and (2) whether such feedback could act to reduce electrically evoked IPSCs (eIPSCs).

Wistar rats were anaesthetized by intramuscular injection of ketamine (120 mg kg^{-1}) plus xylazine (8 mg kg^{-1}) and then decapitated. Slices of the EC were prepared and maintained *in vitro* by conventional techniques. Whole cell patch clamp

recordings were made from pyramidal neurones in layer V visually identified using differential interference contrast optics and infra-red video microscopy. The patch pipette solution was Cs^+-based and contained QX-314 to block postsynaptic $GABA_B$-receptors. Intra- and extracellular Cl^- concentrations were symmetrical and the extracellular medium contained NBQX and 2-AP5 to block ionotropic glutamate receptors. Under these conditions we recorded pure $GABA_A$ receptor-mediated IPSCs. eIPSCs were electrically elicited at 0.1 Hz via an extracellular electrode placed lateral to the recording site in layer V.

Mean (± S.E.M.) control baseline frequency of sIPSCs was 4.4 ± 1.3 Hz and this was doubled to 8.7 ± 1.6 Hz on addition of the NK1r agonist, GR73632 (50 nM, $n = 10$). Perfusion with the $GABA_B$-receptor antagonist, CGP55845 (1 μM), alone had no effect on baseline frequency (4.6 ± 0.6 Hz, $n = 10$), but addition of GR73632 in the presence of CGP55845 increased sIPSC frequency to 15.5 ± 3.2 Hz, a significant ($P > 0.05$, paired t test, $n = 10$) increase compared to the agonist alone. Under control conditions the peak amplitude of eIPSCs ($n = 7$) was 215.8 ± 45.6 pA. During the increase in release induced by GR73632 the amplitude decreased to 151.6 ± 39.7 pA (i.e. by around 30 %). CGP55845 (1 μM), alone increased eIPSC amplitude to 383.1 ± 72.9 pA. However, in the presence of CGP55845, GR73632 still decreased the amplitude (276.7 ± 62.1 pA) to around the same extent (28 %).

These results indicate that feedback activation of $GABA_B$ receptors does not limit spontaneous GABA release under baseline conditions, but that the increased release provoked by NK1r activation is sufficient to access the presynaptic inhibitory autoreceptor. The increase in amplitude of eIPSCs by CGP55845 indicates that $GABA_B$ receptors were acting to limit the eIPSC amplitude. However, the reduction in the eIPSC amplitude still occurred during NK1r agonist-induced GABA release, suggesting that a mechanism other than feedback via the $GABA_B$ autoreceptor might underlie this effect.

Stacey AE *et al.* (2002). *Neuroscience* **115**, 575–586.

A.E.S. was an MRC Scholar.

All procedures accord with current UK legislation.

O66

The glutamine transporter SAT1 is localized to a subpopulation of GABAergic, as well as glutamatergic, neurones in the rat brain

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The recently cloned system A transporter SAT1 (formerly known as GlnT) has been shown to mediate high affinity glutamine uptake and to be expressed by many glutamatergic neurones in the rat brain (Varoqui *et al.* 2000). Glutamine accumulated via SAT1 may act as a precursor for glutamate synthesis in excitatory neurones using glutamate for transmission, but since inhibitory neurones are known to require glutamate for synthesis of GABA, the transporter may also play a role in supplying glutamine to some GABAergic neurones. We have therefore examined the distribution of SAT1 immunoreactivity in the rat brain, using an affinity-purified antibody directed against an N-terminal sequence of SAT1, and performed immunolabelling and *in situ* hybridisation studies to examine the expression of SAT1 immunoreactivity in populations of glutamatergic and GABA neurones.

Adult rats were humanely killed by perfusion with paraformaldehyde fixative under halothane anaesthesia (5 % in O_2) and coronal sections cut using a vibratome. Labelling with the specific antibody detected SAT1 immunoreactivity in widespread and numerous neurones throughout the brain that displayed granular or punctate labelling of their somata. At the ultrastructural level, immunoreaction was associated with presumptive endosomal inclusions and in some cases plasmalemma, in somata, dendrites, pre-terminal axons and synaptic boutons. Glial cell labelling was only rarely observed. The well-labelled somata in areas such as olfactory bulb, neocortex, hippocampus, thalamus, pontine and medullary reticular formation, vestibular, cochlear and deep cerebellar nuclei, dorsal column and spinal trigeminal nuclei suggested SAT1 expression in excitatory and inhibitory neurones. This notion was supported by multiple fluorescence labelling with antisera to various known markers for GABA and glutamate neurones, and RNA probes for glutamic acid decarboxylase (GAD65 and GAD67) and vesicular glutamate transporter (VGLUT2) transcripts. However, some known groups of glutamatergic neurones, such as the cerebellar granule cells, were only modestly labelled for SAT1. The presence of SAT1 immunoreactivity in subpopulations of GABA neurones that were reactive for parvalbumin, but rarely for nitric oxide synthase, calbindin D28k, calretinin, somatostatin or neuropeptide Y, could indicate expression of the transporter in fast spiking inhibitory interneurones.

Our observations suggest that glutamine uptake mediated by SAT1 may be a characteristic of subsets of both glutamatergic and GABAergic neurones in the brain.

Varoqui H *et al.* (2000). *J Biol Chem* **275**, 4049–4054.

All procedures accord with current UK legislation.

O67

Evidence that parvalbumin GABAergic neurons play a role in pacing kainate-induced theta-frequency activity in the rat medial septum/diagonal band of Broca *in vitro*

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We have previously demonstrated (unpublished data) that extracellular field activity at theta frequency (4–15 Hz) can be evoked in the medial septum/diagonal band of Broca (MS/DB) slice *in vitro* in the presence of kainate. To investigate the involvement of the neuronal populations of the MS/DB in this activity, we carried out simultaneous intracellular and extracellular field recordings.

MS/DB slices were prepared from male Wistar rats (21 days) which were terminally anaesthetised with pentobarbitone sodium (120 mg kg^{-1}, I.P.). The animals were transcardially perfused with ~25 ml of modified ACSF and rapidly decapitated. Longitudinal slices (450 μm) were placed in an interface recording chamber and maintained at 32°C. Persistent theta oscillations were induced by bath application of 100 nM kainate.

Previously we have demonstrated that the kainate-induced theta frequency activity in the MS/DB is significantly reduced upon application of the $GABA_A$ receptor antagonist bicuculline. Here we show that there is also a significant reduction in the peak power at theta range following application of the AMPA/kainate

receptor antagonist NBQX (10 μM, $n = 4$, $P < 0.05$, Man-Whitney rank sum test), but not after application of the specific AMPA antagonist SYM 2206 (10 μM, $n = 12$) or the NMDA receptor antagonist D-AP5, (50 μM, $n = 12$). Moreover, we demonstrate that the regions where we find extracellular field recordings of kainate-induced theta frequency activity correspond to areas of the MS/DB that have parvalbumin immunopositive somata. Therefore these data suggest that the theta activity is dependent upon both kainate receptor and $GABA_A$ receptor activation. Intracellular recordings were made from three types of MS/DB neuron during extracellular field recordings of kainate-induced theta-frequency activity. These neurons were identified as fast-spiking, slow-firing and regular-spiking according to previous criteria (Gorelova & Reiner, 1996; Morris *et al.* 1999). The fast-spiking type ($n = 5$) displayed spontaneous and persistent rhythmic single spiking activity at theta frequencies. The slow-firing ($n = 5$) and regular-spiking ($n = 1$) types displayed non-rhythmic single spiking activity. Previous studies have shown that fast-spiking and regular-spiking neurons in the MS/DB are GABAergic and that parvalbumin is localised selectively in the fast-spiking neuron type; conversely the slow-firing neurons were shown to be cholinergic. We conclude that the parvalbumin GABAergic neurons may pace rhythmic theta activity in the MS/DB.

Gorelova N & Reiner PB (1996). *J Neurophysiol* **75**, 695–706.

Morris NP *et al.* (1999). *Neuroscience* **92**, 589–600.

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O68

Pharmacology of the group I metabotropic glutamate receptor-mediated responses in rat CA1 hippocampal neurones

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Group I metabotropic glutamate receptors (mGluRs) play a key role in the mediation of synaptic plasticity in the hippocampus. We have demonstrated previously that, in combination with depolarisation, the specific group I mGluR agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) evokes a somatic supralinear $[Ca^{2+}]_i$ response and a simultaneous, but independent, inward current in rat CA1 hippocampal neurons (Rae & Irving, 2001). To date however, the specific role(s) that the group I mGluR subtypes, mGluR1 and mGluR5, play in mediating this combined response has yet to be fully elucidated. Therefore, utilising specific group I mGluR agonists and antagonists, the aim of the present study was to determine the pharmacology underlying these DHPG-evoked supralinear responses.

Experiments were carried out at room temperature on transverse hippocampal slices (350–400 μm) prepared from 14- to 21-day-old rats killed humanely by cervical dislocation. $[Ca^{2+}]_i$ measurements were made using conventional epifluorescence Ca^{2+} imaging combined with whole-cell patch-clamp recording by including the calcium-sensitive dye, bis fura-2 (150 μM), in a potassium gluconate-based pipette solution. All experiments were carried out in standard Krebs solution containing TTX (0.5 μM), at −30 mV in order to maximise mGluR responses. Data are expressed as means ± S.E.M.

At −30 mV, DHPG (100 μM; 2 min) evoked a large $[Ca^{2+}]_i$ signal in conjunction with a relatively slow inward current. In the presence of the mGluR5 antagonist, 6-methyl-2-(phenylethynyl)-pyridine (MPEP; 10–50 μM), the size of the DHPG-evoked $[Ca^{2+}]_i$ signal was reduced by 88.4 ± 4.1 % ($P < 0.001$; Student's paired *t* test) in 14/19 cells. The amplitude of the inward current was also reduced by 43.9 ± 4.5 % ($n = 17$; $P < 0.001$). The mGluR1 antagonist, 2-methyl-4-carboxyphenylglycine (LY367385; 100 μM), reduced both the size of the DHPG-evoked $[Ca^{2+}]_i$ signal (75.9 ± 6.2 %; $n = 11/13$; $P < 0.001$) and the amplitude of the inward current (50.7 ± 6.5 %; $n = 15/16$; $P < 0.001$). However, MPEP and LY367385 together reduced the inward current by 70.6 ± 6.8 % ($n = 4$; $P < 0.05$) and abolished the $[Ca^{2+}]_i$ signal. The specific mGluR5 agonist (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG; 0.5–1 mM) activated an inward current (53.4 ± 11.7 pA, $n = 8$) in the absence of an increase in $[Ca^{2+}]_i$.

These results suggest that both mGluR1 and mGluR5 contribute to the combined electrical and $[Ca^{2+}]_i$ response evoked by DHPG.

Rae MG & Irving AJ (2001). *Soc Neuroscience Abs* 493.11.

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All procedures accord with current UK legislation.

O69

Group I mGluR-induced calcium signalling and electrophysiological responses in the rat superficial superior colliculus

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Group I metabotropic glutamate receptors (mGluRs) are linked to the release of Ca^{2+} from intracellular stores, as well as affecting a number of membrane conductances. In the superficial superior colliculus (SC) presynaptic group I mGluRs are thought to modulate synaptic transmission (Cirone *et al.* 2002), but postsynaptic actions remain to be demonstrated. In this study we have investigated the actions of the selective group I mGluR agonist dihydrophenylglycine (DHPG) on Ca^{2+} signalling and membrane conductances in cultured SC neurones and SC slices.

Primary cultures of rat superficial SC neurones were loaded with the cell-permeant Ca^{2+} indicator fura-2-AM (2 μM). $[Ca^{2+}]_i$ was monitored using digital epifluorescence microscopy. SC slices (350 mm), obtained from 3- to 4-week-old rats were used for combined whole-cell voltage clamp recording and Ca^{2+} imaging. The Ca^{2+} indicator bis fura-2 (150 μM) was included in the pipette solution. Cultured cells or slices were perfused with standard media containing TTX (0.5 μM) at room temperature. All animals were killed humanely by cervical dislocation.

In 47 % of cultured SC neurones, exposure to DHPG (50–100 μM; 90 s) resulted in reproducible, transient rises in $[Ca^{2+}]_i$. This reflected the release of Ca^{2+} from intracellular stores as it was blocked following prior incubation with thapsigargin (2 μM), a Ca^{2+} store depletory agent. The DHPG response was reduced both by the mGluR5 antagonist MPEP (3 μM) and the mGluR1 antagonist LY367385 (40 μM) (81.1 ± 5.7 % and 79.0 ± 9.2 %, respectively; $n = 19$ and 13; means ± S.E.M.; $P < 0.01$; Student's unpaired *t* tests). Exposure of SC slices to DHPG (100 μM for 2 min at −60 mV) was also associated with a transient increase in $[Ca^{2+}]_i$ levels in 8/26 (32 %) neurones. Increasing Ca^{2+} store loading by depolarisation to between −35

and −20 mV enhanced DHPG responses in 6/8 neurones (7.4 ± 1.5 control, 19.7 ± 6.1 depolarised; total area of responses measured; $P < 0.05$). Under control conditions, DHPG exposure was often associated with an inward current (18.1 ± 4.3 pA; 12/26 cells), which was much more prolonged than the intracellular Ca^{2+} elevation. In 7/26 of cells held at −60 mV, an inward current was evoked in the absence of any Ca^{2+} response (18.6 ± 7.5 pA).

In conclusion, activation of Group I mGluRs in superficial SC neurones causes both the release of Ca^{2+} from intracellular stores and an inward current in SC neurones, which are likely to reflect different signalling mechanisms. In addition to the proposed presynaptic actions of mGluRs in the SC, postsynaptic effects may also exert a modulatory role on SC function.

Cirone J *et al.* (2002). *J Physiol* **541**, 895–903.

All procedures accord with current UK legislation.

O70

Presynaptic metabotropic glutamate receptors have dual effects on glutamate release at terminals in the rat entorhinal cortex

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We have shown that activation of a metabotropic glutamate receptor (mGluR), specifically mGluR4a, enhances glutamate release onto neurones in layer V of the rat entorhinal cortex (EC) (Evans *et al.* 2000). Glutamate release occurs by both action potential (AP)-dependent and -independent means. By simultaneously recording predominantly action potential-independent spontaneous excitatory postsynaptic currents (sEPSCs) and AP-evoked (e)EPSCs we were able to assess the ability of mGluR4a to differentially modulate these forms of glutamate release.

Slices (450 μm) of EC were prepared from Wistar rats (45–60g) anaesthetised with ketamine (120 mg kg^{-1} I.M.) and xylazine (8 mg kg^{-1} I.M.). Whole-cell voltage clamp recordings (V_h −60 mV) of sEPSCs were made from visually identified neurones in layer V. An extracellular electrode was placed in the white matter and used to generate eEPSCs at 0.033 Hz. Drugs applied by bath perfusion included (1*S*,3*R*,4*S*)-1-aminocyclopentane-1,2,4-tricarboxylic acid (ACPT-1, 20 μM), forskolin (10 μM) and SQ22536 (50 μM). Mean eEPSC amplitudes (± S.E.M.) were compared using Student's paired *t* test (95% confidence limit) on samples of 10 eEPSCs. Interevent intervals (IEI) of sEPSCs were compared using with the Kolmogorov-Smirnov test (again at 95%) applied to cumulative probability distributions (at least 200 events sampled from each neurone in each situation).

The specific mGluR4a agonist ACPT-1 depressed eEPSC amplitude (29.4 ± 2.1 pA to 18.0 ± 1.4 pA, $n = 10$, $P < 0.05$) and simultaneously reduced mean IEI (i.e. increased frequency) of sEPSCs (256 ± 50 ms to 151 ± 25 ms, $n = 10$, $P < 0.01$). As we have previously reported that the latter effect was mediated through protein kinase A (PKA, Evans *et al.* 2001), we examined whether the reduction of eEPSC amplitude depended on the same effector. However, perfusion with the PKA activator, forskolin, decreased IEI (483 ± 71 ms to 221 ± 111 ms, $n = 3$ $P < 0.01$), but increased the amplitude of eEPSCs (49.2 ± 4.4 pA to 98.6 ± 8.3 pA, $n = 3$ $P < 0.05$). When PKA activity was blocked with the specific adenylyl cyclase inhibitor, SQ 22536, ACPT-1 still depressed eEPSC amplitude (35.4 ± 6.1 pA to 21.9 ± 1.2 pA, $n = 6$ $P < 0.01$), but the decrease in sEPSC interval was abolished (212 ± 47 ms v 219 ± 49 ms in ACPT-1). Finally we indirectly assessed whether ACPT-1 could alter presynaptic Ca^{2+} influx. ACPT-1 reduced the paired-pulse ratio of eEPSC amplitude (50 ms interval) from 1.65 ± 0.2 to 1.2 ± 0.2 ($n = 6$, $P < 0.05$ *t* test), suggesting that mGluR4a activation reduces presynaptic Ca^{2+} influx.

Thus, presynaptic mGluRs may simultaneously enhance spontaneous glutamate release and depress AP-driven release through distinct effector systems.

Evans DIP *et al.* (2000). *J Neurophysiol* **83**, 2519–2525.

Evans DIP *et al.* (2001). *J Neurophysiol* **85**, 575–579.

All procedures accord with current UK legislation.

P99

Glutamatergic transmission modulation in medial septal area

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The medial septum (MS) and the diagonal band (DB) have cholinergic and GABAergic neurons projecting to the hippocampus. Afferent fibres that use diverse neurotransmitters determine its activity, which is concerned with the hippocampal theta rhythm generation and learning and memory processes. Preliminary outcome suggests that some of these connections could be modulated, even though the neurotransmitters involved have not been clearly defined. The aim of the present work is to characterize the glutamatergic neurotransmission in the MS and its modulation through some other neurotransmitters.

Adult Wistar rats were deeply anaesthetized and decapitated, and their brain extracted to prepare slices of 300 μm containing MS and BD (Yajeya *et al.*). After 1–3 h of incubation the slices were transferred to an immersed recording chamber. The perforated patch clamp technique was used with gramicidin or anfotericin in the patch pipette to obtain the whole cell configuration. Excitatory evoked postsynaptic currents (EPSC) generated by DB stimulation were recorded at −60 or −70 mV voltage clamp potential. Analysis was done by the Mini Analysis program 5.2 of Synaptosoft.

Forty-five neurons were recorded. DB stimulation evoked an inward current of variable amplitude depending on the stimulus intensity. Perfusion with D-AP5 (50 μM) generated a significant decrease ($P \leqslant 0.05$) in the amplitude ($n = 10$) of the current, which was completely blocked by CNQX (10 μM) suggesting that its was mediated through glutamic acid acting on AMPA/kainate and NMDA receptors. Perfusion with GABA (20 μM) also decreased the excitatory current ($n = 6$), an effect which was reversed or even inverted by bicuculline (10 μM) perfusion. This result suggests a modulatory effect through $GABA_A$ receptors. On the other hand, the current amplitude was decreased under perfusion with D-tubocurarine (2 μM) ($n = 6$) or atropine (5 μM) ($n = 5$), indicating that acetylcholine acting on nicotinic or muscarinic receptors could control glutamic neurotransmission in the MS. In order to determine the pre- or postsynaptic location of acetylcholine effect, a paired pulse protocol was used. PP-ratio for control (S_2/S_1) was 1.283, meanwhile PP-ratio for atropine was 1.056. The observed results support that the

acetylcholine could act in both locations. The different possibilities described concerning the modulation of glutamic neurotransmission in the MS could be related to the topographic projections of the septum towards other areas of nervous system.

Yajeya I *et al.* (1997). *Neuroscience* **78**, 731–743.

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All procedures accord with current National guidelines.

P100

Two classes of CA1 pyramidal cell characterised by distinct afterhyperpolarisation properties

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Observations of heterogeneity among the principal cell populations of the neocortex and the subiculum have been described previously in the literature (Behr *et al.* 1996; Pineda *et al.* 1999). Heterogeneity among hippocampal principal cells has been suspected for some time, but has not been conclusively proven. Here we describe two populations of CA1 pyramidal cell distinguished on the basis of their afterpotential profiles.

Intracellular recordings of CA1 pyramidal cells were made from 450 μm hippocampal slices, obtained from male adult Wistar rats weighing approximately 150 g. Animals were killed humanely after being anaesthetised with inhaled isoflurane, before intramuscular injection with xylazine (> 10 mg kg^{-1}) and ketamine (> 100 mg kg^{-1}). Rats were cardioperfused with sucrose ACSF. Data are expressed as mean amplitude ± standard error of the mean.

Recordings revealed that pyramidal cells expressed one of two types of slow AHP after short (80 ms duration) depolarising current steps. Type I sAHP was preceded by a medium afterhyperpolarisation (mAHP) and was almost completely inhibited by application of 20 μM 5-HT (control = 4.97 ± 1.3 mV, 5HT = 0.80 ± 0.3 mV, $n = 6$, $P = 0.016$, paired t test). Cells that expressed type I sAHP usually fired single spikes during depolarising steps, although not exclusively. Type II sAHP had a shorter duration than type I and was not preceded by a mAHP. Cells expressing this form of sAHP generally fired bursts of action potentials. Type II sAHP was significantly attenuated by 5HT application (control = 8.8 ± 2.0 mV, 5HT = 4.8 ± 1.9 mV, $n = 5$, $P = 0.04$ paired t test). The ratio of hyperpolarisation amplitude to number of action potentials per burst was greater for type II sAHP than type I sAHP. The two forms of sAHP showed varying sensitivity to nifedipine. Type I sAHP was largely insensitive to 20 μM nifedipine (control = 3.2 ± 0.31 mV, nifedipine = 3.19 ± 0.55 mV, $n = 5$, $P = 0.9$ paired t test), whereas type II sAHP was significantly reduced by nifedipine (control = 8.2 ± 0.78 mV, nifedipine = 4.1 ± 1.3 mV, $n = 3$, $P = 0.002$ paired t test).

We suggest that calcium entry through L-type calcium channels is required to activate type II sAHP, but not essential to activate type I sAHP. These results suggest that there is heterogeneity among the principal cell population of the CA1 area of the hippocampus.

Behr J *et al.* (1996). *Neuroscience Letters* **220**, 41–4.

Pineda JC *et al.* (1999). *Neuroreport* **10**, 1951–6.

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All procedures accord with current UK legislation.

P101

The muscarinic agonist carbachol has effects on the prepositus hypoglossi neurons

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The prepositus hypoglossi nucleus (PHN) is involved in the maintenance of eye position following horizontal eye movements. Previous studies show that neurons belonging to this nucleus are able to transform eye velocity signals coming from paragigantocellular formation (RDPG) to a position signal, in order to send this information to the external ocular motor nucleus. This transformation could be mediated by acetylcholine. In the present work we try to determine if the above-mentioned neurotransmitter could be a modulator in the signal transformation process.

For this purpose, we made a current-clamp intracellular recording of neurons from brain stem slices obtained from Wistar rats (60–80 g) (Yajeya *et al.* 2000). Rats were deeply anesthetized with halothane and humanely killed. All the experiments strictly conformed to National guidelines.

The recorded neurons ($n = 70$) were classified according to their type of spike as type A (80 %) and type B (20 %). External stimuli in RDPG were used to study postsynaptic potentials, resulting in EPSPs in all cases, which were totally blocked with perfusion of CNQX ($n = 10$). After TTX blockage of evoked synaptic activity, the administration of carbachol (Cch, 10 μM) produced a significant depolarization (10.22 ± 1.2 mV, $P < 0.01$, Student's paired t test), suggesting a postsynaptic effect ($n = 10$). On the other hand, variations in the amplitude of postsynaptic potentials were also studied under the action of Cch, showing a 56.6 % reduction ($n = 10$). Paired-pulse stimulation was used to determine the possible site of Cch action and the differences observed in paired-pulse depression indicate a presynaptic effect ($n = 5$) (S_2/S_1, control 60%, problem 95%).

Our results show that afferents fibres to NPH arriving from RDPG are glutamatergic and muscarinic agonist Cch acts as modulator at pre- and postsynaptic sites.

Yajeya J *et al.* (2000). *Synapse* **38**, 151–160.

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All procedures accord with current National and local guidelines.

P102

Acute hypoxia stimulates Ca^{2+} release from an intracellular, bradykinin-sensitive Ca^{2+} pool in cultured rat type I cortical astrocytes

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Numerous studies have indicated that acute hypoxia modulates plasmalemmal ion channel activity, thereby regulating Ca^{2+} entry in a variety of cell types (Lopez-Barneo *et al.* 2001). Hypoxic modulation of intracellular Ca^{2+} stores may also be involved in the overall cellular response to hypoxia in some (Dipp *et al.* 2001) but not all (Vicario *et al.* 2000) tissue types. Here, we report the effects of acute hypoxia ($P_{O_2} \approx 25$ mmHg) on Ca^{2+} stores in rat type I cortical astrocytes isolated from animals that had been humanely killed.

$[Ca^{2+}]_i$ was monitored in Fura-2 loaded cells (Smith *et al.* 2001). Transient rises of $[Ca^{2+}]_i$ above baseline were integrated and results are presented as mean (± S.E.M.) ratio unit seconds (r.u.s.). Statistical significance was determined using Student's unpaired *t* tests. All experiments were conducted in a Ca^{2+}-free perfusate containing 1 mM EGTA (Smith *et al.* 2001).

Bath application of 100 nM bradykinin (BK) evoked transient rises of $[Ca^{2+}]_i$ (mean 9.84 ± 0.66 r.u.s., $n = 35$ cells). Exposure of cells to acute hypoxia evoked smaller rises, of 2.7 ± 0.39 r.u.s. ($n = 20$ cells, $P < 0.01$), and following such challenges, subsequent exposure to 100 nM BK evoked significantly smaller responses (mean 4.7 ± 0.5 r.u.s., $n = 20$ cells, $P < 0.01$) than were seen in the absence of hypoxia. Hypoxia failed to elicit rises of $[Ca^{2+}]_i$ following exposure to BK ($n = 12$). Pretreatment of cells for 20 min with 1 mM thapsigargin prevented any rises of $[Ca^{2+}]_i$ when cells were exposed either to hypoxia ($n = 12$) or 100 nM BK ($n = 12$). When cells were not exposed to either hypoxia or BK, but Ca^{2+} (2.5 mM) was readmitted to the perfusate in place of EGTA, no rises of $[Ca^{2+}]_i$ were observed ($n = 12$). However, when Ca^{2+} was readmitted following BK application, a rise of $[Ca^{2+}]_i$ was consistently observed (mean plateau level 0.15 ± 0.01 r.u., $n = 13$). Similarly, following exposure to hypoxia, readmission of Ca^{2+} to the perfusate evoked rises of $[Ca^{2+}]_i$ (mean plateau level 0.12 ± 0.02 r.u., $n = 11$).

Our results indicate that acute hypoxia can evoke Ca^{2+} release from intracellular store(s) in type I cortical astrocytes. This store can also be liberated by BK, and is depleted by prior treatment with thapsigargin. Furthermore, hypoxic mobilization of Ca^{2+} from intracellular stores appears sufficient to trigger capacitative Ca^{2+} entry. These data imply a possible role for altered calcium signalling in the pathology of central ischaemia.

Dipp M *et al.* (2001). *Am J Physiol* **281**, L318–L325.

Lopez-Barneo J *et al.* (2001)). *Annu Rev Physiol* **63**, 259–287.

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

Vicario I *et al.* (2000). *Am J Physiol* **279**, C51–C61.

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All procedures accord with current UK legislation.

P103

Modulation of action potential-dependent Ca^{2+} transients in hippocampal mossy fibres by granule cell membrane potential and ionotropic receptors

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Whether presynaptic ionotropic GABA receptors can modulate neurotransmitter release in the cerebral cortex is not known. We have recently shown that $GABA_A$ receptor activation modulates the excitability of rodent hippocampal mossy fibres in response to extracellular stimulation (Ruiz, Fabian-Fine, Scott, Rusakov and Kullmann, submitted). We also found that blocking $GABA_A$ receptors alters the threshold for antidromic recruitment of mossy fibres, implying that they are tonically active. In order to determine whether presynaptic $GABA_A$ receptors can modulate orthodromic signalling, we performed fast multi-photon imaging of action potential-dependent Ca^{2+} transients in individual mossy fibre varicosities.

Hippocampal slices were obtained from guinea-pigs killed by cervical dislocation. Granule cells were held in whole cell voltage clamp, with pipettes filled with a low $[Cl^-]$ solution containing the Ca^{2+}-sensitive dye Fluo-4 (in some experiments the morphological tracer Alexa 594 was also added). Antidromic action potentials were delivered via an extracellular stimulating electrode positioned in stratum lucidum. These evoked a rapid Ca^{2+} transient in hilar varicosities, which exhibited a biphasic dependence on somatic holding potential that peaked at approximately −85 mV. This biphasic dependence on membrane potential is qualitatively consistent with the effects of K^+ and kainate on orthodromic transmission reported by Schmitz *et al.* (2001). The amplitude of the Ca^{2+} transient when the cell was held at −70 mV was attenuated by blocking $GABA_A$ receptors with 10 μM SR95531 (reduction to 66 ± 4 % of baseline; $P < 0.001$, *t* test, $n = 9$).

These results imply that axonal $GABA_A$ receptors play a potentially important role in modulating information flow to the hippocampus.

All procedures accord with current UK legislation.

P104

Neuromodulation of thalamic response modes in the lateral geniculate nucleus of the cat

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Burst responses in thalamic neurons have been typically associated with sleep states and not linked to sensory transmission. However, it has been demonstrated that bursts occur also during the wake state and could be implicated in high level processes such as attention. Bursts are due to a hyperpolarization of the membrane potential in relay cells linked to activation of specific ionic currents. We asked if this switch from tonic to burst mode is gradual or occurs abruptly upon reaching a threshold and how it is regulated by neurotransmitters acting at the thalamus.

Experiments were carried out in adult cats anaesthetized (with halothane, 0.1–5 % in nitrous oxide–oxygen 70 %–30 %), and neuromuscularly blocked (gallamine 10 mg kg^{-1} h^{-1}). EEG, ECG, CO_2 and temperature were monitored (all procedures

conformed to the Spanish Physiological Society, and the European Union (Statute No. 86/809). Extracellular recordings and iontophoretic ejection of drugs were made in A laminae of the lateral geniculate nucleus (LGN) using multibarrelled pipettes containing NaCl for recording and a selection of the following drugs: ACh, GABA, isoguvacin ($GABA_A$ agonist), baclofen ($GABA_B$ agonist), L-NitroArg (NOS inhibitor), atropine and scopolamine (ACh receptor antagonists). Spikes were collected during presentation of visual stimulus in control and during ejection of drugs. Spikes in tonic or burst mode were selected offline and the ratio in the different conditions compared. At the end of the experiment all animals were painlessly killed.

Our results show that (i) the percentage of spikes in bursts increases under conditions that supposedly hyperpolarize the cell, such as application of GABA agonists or ACh antagonist ejection; (ii) the increase in the percentage of bursts is not a regular process, bursts appearing suddenly at a particular level of drug ejection; and (iii) burst number is not directly related to cell responses: ejection of L-NitroArg, blocking NO synthesis, reduces thalamic activity presumably without modifying the membrane potential, since it did not affect the burst ratio. In a number of cells we could not increase the number of bursts; these cells form a group that in control had an extremely low percentage of burst (less than 2 %). These cells could confirm previous results showing a population of LGN cells which are unable to respond in burst mode.

These results fit with the existence of two different states in relay cells, associated with awake and sleep, the switch from one to the other under control of the modulatory systems to the thalamus. However, they also support the idea that an important number of bursts remain in the awake state, suggesting that bursts can play a role in visual processing.

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All procedures accord with current National and local guidelines.

P106

Pharmacological manipulation of the slow after-hyperpolarization modulates kainate-evoked gamma frequency oscillations in the mouse hippocampus *in vitro*

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The slow afterhyperpolarization (sI_{AHP}) is a Ca^{2+}-dependent K^+ current that regulates hippocampal neuronal firing properties. The sI_{AHP} is responsible for accommodation of action potential firing during bursts of activity. Although the effects of the sI_{AHP} on cell firing properties is well characterised its effect on the behaviour of an oscillating network is unclear. The influence of the sI_{AHP} on gamma frequency oscillations (30–80 Hz) 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 ($\geqslant 100$ mg kg^{-1}) and xylazine ($\geqslant 10$ mg kg^{-1}). When all responses to noxious stimuli had terminated the animals were intracardially perfused with ~25 ml of modified artificial cerebrospinal fluid (ACSF) in which 126 mM NaCl was replaced with equiosmolar sucrose. Gamma frequency oscillations were induced by bath application of kainate (50–200 nM). Extracellular recordings were made from stratum radiatum/lacunosum–moleculare in the CA3 region. The power of the oscillation (the area from the power spectrum between 20–80 Hz) is expressed as mean percentage change ± S.E.M. Statistical significance was tested using either a paired *t* test or signed rank test.

The L-type calcium channel antagonist, nifedipine (10 μM), was bath applied to the established kainate-induced oscillation to decrease the sI_{AHP}. Nifedipine caused a trend to gradually increase the power of the gamma frequency oscillation, although this was not significant. After 15, 30 and 45 min application of nifedipine the power increased by 55 ± 28.3 % ($n = 12$; $P > 0.05$), 79 ± 23.7 % ($n = 12$; $P > 0.05$), and 101 ± 36.6 % ($n = 10$; $P > 0.05$) respectively. The frequency of the oscillation showed a significant reduction of 5 ± 2.6 % ($n = 12$; $P < 0.05$) after 30 min. The L-type calcium channel agonist, Bay K 8644 (1 μM) was bath applied to the established kainate-induced oscillation to increase the sI_{AHP}. After 15 min application there was a significant decrease in the power of the gamma frequency oscillation of 23 ± 5.1 % ($n = 8$; $P < 0.01$). A further significant decrease in power of 53 ± 6.5 % ($n = 8$; $P < 0.01$) was seen after 30 min application. However, there was no significant change in the frequency of the oscillation ($P > 0.05$).

These results suggest that modulation of the sI_{AHP} can affect kainate-induced gamma frequency activity. In particular the L-type calcium channel agonist Bay K 8644 significantly disrupted gamma oscillations. These results suggest that the sI_{AHP} has a direct effect on the behaviour of an oscillating network.

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All procedures accord with current UK legislation.

P107

Neurotransmission in the amygdala basolateral complex: effect of β-amyloid peptide (25–35)

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Alzheimer's disease (AD) is a common progressive neurodegenerative disorder with still largely unknown etiopathology. Several lines of experimental data support a strong relationship between the disorder and an excessive brain accumulation of β-amyloid peptides (βA) in extracellular senile plaques. In the present study, effects of βA 25–35, which is thought to be the active site of βA, on membrane and action potentials in addition to postsynaptic potentials evoked by stimulating the external capsule were studied in basolateral amygdaloid slices from halothane-anaesthetized Wistar rats' (80–90 g), using an *in vitro* intracellular recording technique (Yajeya *et al.* 2000). All experiments strictly conformed to national guidelines and the rats were humanely killed. Results were compared using Student's paired *t* test and all changes found were statistically significant ($P < 0.05$).

Bath application of βA 25–35 (800 nM, 1.2 μM) resulted in depolarization or hyperpolarization of membrane potential averaging 9.18 ± 4.18 mV and 6.5 mV, respectively. No changes occurred when the experiments were performed on slices previously perfused with the selective Na^+ channel blocker tetrodotoxin (TTX). On the other hand, application of the non-NMDA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-

dione (CNQX), the selective antagonist for NMDA receptors 2-amino-5-phosphopentanoic acid (APV), followed by βA 25–35 did not affect the membrane potential. The aforementioned results suggest that βA 25–35 actions were not generated through its effect on the postsynaptic membrane. As far as postsynaptic potentials (elicited by stimulating the external capsule) are concerned, we found that perfusion of βA 25–35 (800 nM) induces a significant decrease (75 ± 2% reduction in relation to control) of the amplitude of postsynaptic potentials, especially the excitatory postsynaptic potentials (EPSP), in a time-dependent manner. With the aim of determining the location of all βA 25–35 actions, we used a paired-pulse protocol measured at 60 ms intervals and compared the ratio of the first pulse in relation with the second (S_2/S_1). The fact that βA 25–35 decreased PPF (from 33.24% control to 5.37%) through bath application is concomitant with a presynaptic mode of action. The contribution of α-7 nicotinic receptors was evaluated through application of methyllycaconitine (100 μM) together with β-amyloide (800 nM). β-Amyloide depressed the amplitude of EPSP indicating that α-7 nicotinic receptors are not involved in the action mechanisms of the peptide.

The results obtained so far provide evidence that βA 25–35 actions are fundamentally related to modulatory effects on neurotransmission within amygdala.

Yajeya *et al.* (2000). *Synapse* **38**, 151–160.

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All procedures accord with current National and local guidelines.

P108

17β-Oestradiol and a hydrophilic derivative of vitamin E, but not vitamin C, attenuate the amyloid β-peptide-mediated toxicity in rat hippocampal neurons

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Oxidative stress has been postulated as a key pathophysiological event in the amyloid β peptide (Aβ)-mediated neurotoxicity and provides the rationale for the testing of antioxidant molecules to reduce neurotoxicity of Aβ. In the present work, we have studied (1) the production of endoperoxide in primary cultures of rat embryo hippocampal neurons challenged with 10 μM amyloid-β peptide (Aβ) fibrils; (2) the effect of the antioxidants 17β-oestradiol (E2, 10 μM), trolox (100 μM; a hydrophilic derivative of vitamin E) and vitamin C (100 μM) on endoperoxide production elicited by Aβ, and (3) cell viability in response to Aβ or 100 μM H_2O_2 (used as a stimulus of oxidative stress) in the presence or absence of antioxidants.

Hippocampal neurons were obtained from 18-day-old Sprague-Dawley rat embryos anaesthetised by I.P. injection of ketamine and killed by cervical dislocation. Cell culture, cell viability (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method), enzymatic assays (superoxide dismutase activity, SOD) and reactive oxygen species measurements were carried out as described previously (Muñoz *et al.* 1999, 2002).

Cells treated with Aβ fibrils showed an increased production of endogenous peroxide ($P < 0.001$) (Fig. 1*A*), and this increase was inhibited when cells were preincubated with trolox or with E2. No effects were observed in cells treated with Vit C in the presence of Aβ fibrils.

Our results also showed that E2 and trolox attenuated the neurotoxicity mediated by Aβ fibrils (Fig. 1*B*) while vitamin C failed to increase the cell survival of cells challenged with Aβ.

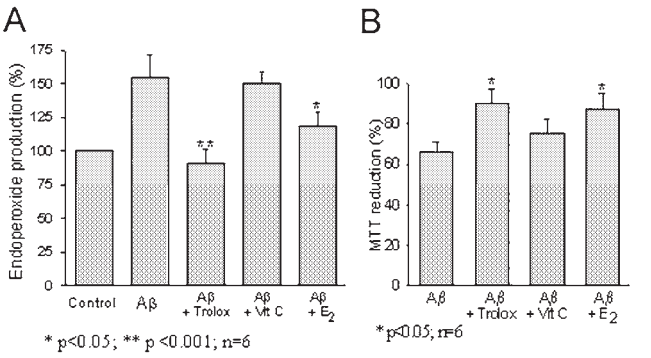

Figure 1. Mean (± S.E.M.) increase in endoperoxide production (*A*) and MTT reduction (*B*) Control value in untreated cells 100% obtained in 6 experiments. ANOVA analysis revealed significant differences between antioxidant treated and non-treated cultures.

Muñoz FJ *et al.* (1999). *Neuroreport* **10**, 3621–3625.

Muñoz FJ *et al.* (2002). *J Neurosci* **22**, 3028–3089.

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All procedures accord with current local guidelines.

P109

Buridan's test as an experimental tool for the study of stable and oscillatory magnetic field effects on the motor behaviour of *Drosophila melanogaster*

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Because of its putative harmful actions on humans, increased attention has been recently paid to stable and pulsatile magnetic field effects on the structure and functioning of living organisms. Available information regarding the mechanisms of action of magnetic fields on living beings (and on adaptive responses developed by the latter) is rather scarce and fragmentary. Here we have taken advantage of the available information on the physiology and genetics of the visual system of *Drosophila* to design a test for the study of magnetic field effects on animal motor behaviour.

Drosophila is highly conditioned to orient its behavior according to available visual information. Thus we have developed a test to determine the sensitivity of this fly to the presence of different types of stable or pulsatile magnetic fields (fixed or variable in relation to the animal's location in the space). We have used a modified version of Buridan's test, a very well known behavioural paradigm. The classic description of Buridan's test is supported on three basic characteristics; for its implementation we have developed the following instrumentation: (1) the animal was placed on a circular arena (11.5 cm in diameter). The arena

was located inside a larger (15 cm) cylinder widely illuminated. Under these conditions, the animal maintains a strong walking behaviour: the fly walks back and forward between two vertical black stripes, identical in size and shape. Home-made computer programs allowed the determination of animal position, trajectory and speed. When the black stripes were removed, and the arena illuminated faintly, the animals selected resting positions at random. (2) Experimental animals were unable to reach the two black stimuli, because the arena was surrounded by a water pit. Besides, the flies were unable to fly, since their wings were carefully cut under a dissecting microscope. (3) With this experimental design, the evoked behaviour was proved to last for hours, making the test very suitable for quantitative purposes.

Until now we have tested the effects of a fixed magnetic field (24–48 mT) on flies location on the arena and on their performance during Buridan's test. Preliminary results indicate that fixed magnetic fields (orthogonal to the animal's trajectory) are able to modify spontaneous location of the flies on the area and their linear trajectories during Buridan's test performance. Flies move less when a magnetic field is present (females, $n = 24$; males $n = 21$; Student's t test, $P < 0.05$). The effects are different for males and females. At present, we are checking many different *Drosophila* mutants in a search for a strain insensitive to these magnetic field signals.

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P110

Possible mechanism of action of $HgCl_2$ on *in vivo* striatal dopamine release

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Mercury is a neurotoxin that exists in a number of physical and chemical forms, producing different effects in the brain. Methylmercury (MeHg) produces significant increases in the spontaneous output of dopamine (DA) from rat striatal tissue (Faro *et al.* 2000). Because the organic (MeHg) and inorganic (HgCl, $HgCl_2$) forms of mercury are neurotoxic to different degrees, in this work some standard pharmacological agents to determine the neurochemical basis for $HgCl_2$-induced DA release were used.

All animals were humanely killed. Animals were anaesthetized I.P. with choral hydrate (400 mg kg^{-1}) and a microdialysis probe was stereotaxically implanted in the striatum. The day after surgery, a Ringer solution (147 mM NaCl, 4 mM KCl, 3.4 mM $CaCl_2$ pH 7.4) was pumped through the microdialysis probe at a flow rate of 2 μl min^{-1}. Dialysates were collected every 15 min, and after three basal samples 1 mM $HgCl_2$ was infused during 60 min. The perfusate was then switched back to the unmodified perfusion medium and the infusion was carried out for an additional period of 120 min. In order to determine the possible mechanisms of dopamine release induced by $HgCl_2$, we analysed the *in vivo* DA release following the administration of nomifensine (NOM), and reserpine (RES) together with $HgCl_2$. Dopamine striatal levels were analysed by HPLC with electrochemical detection (Duran *et al.* 1998). The results are shown as the means ± S.E.M. of 5–6 experiments expressed as a percentage with respect to basal levels. Statistical analysis of the results was performed by means of repeated measures ANOVA and the Student-Newman-Keuls multiple range test. The experiments were performed according to the guidelines of the European Union Council (86/609/EU) for the use of laboratory animals.

$HgCl_2$ induced an increase of striatal DA levels to 1835 ± 410 % of basal values. Co-infusion of $HgCl_2$ and NOM (50 μM) produced increases in the release of DA (4824 ± 1038 %) ($n = 5$, $P < 0.01$) higher than those produced by NOM alone (2870 ± 319 %) ($n = 5$, $P < 0.01$). Three hours after I.P. reserpine injection (15 mg kg^{-1}), extracellular DA levels decreased to 17.2 ± 7.0 % of basal levels. Infusion of $HgCl_2$ in RES-pretreated animals increased striatal DA levels to 197 ± 45 % ($n = 5$, $P < 0.001$) of reserpinized levels, this effect of $HgCl_2$ on striatal DA levels being significantly lower than that observed in non-reserpinized animals. The effect on the extracellular DA levels in the group treated with $HgCl_2$ and NOM seems to be additive compared with those induced by $HgCl_2$ or NOM alone. This could indicate that $HgCl_2$ acts in a different way from NOM in order to increase the striatal DA levels. Because NOM increases DA extracellular levels acting on the DA transporter (DAT), our results could indicate that $HgCl_2$ induced an increase in DA striatal levels by means of exocytotic vesicular release. This seems to be confirmed by the results obtained in the group of RES-pretreated animals, in which the $HgCl_2$-induced DA release is avoided by the depletion of vesicular DA produced by reserpine. This mechanism of action of $HgCl_2$ on striatal DA release is different from that previously found for the MeHg, which seems to act by a mechanism mediated by DAT (Faro *et al.* 2002).

Duran *et al.* (1998). *J Liq Chromatogr Relat Technol* **21**, 2799–2811.

Faro *et al.* (2000). *Nerochem Res* **25**, 225–229.

Faro *et al.* (2002). *Neurochem Int* **40**, 455–465.

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All procedures accord with current National and local guidelines.

P111

Effect of anatoxin-α, a cianobacterial neurotoxin, on striatal dopamine release. An *in vivo* microdialysis study

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Anatoxin-α is a neurotoxic alkaloid produced by the cyanobacterium *Anabaena flos-aquae* (Carmichael *et al.* 1994). The effect of anatoxin-α on striatal dopamine release was investigated in conscious and freely moving rats using the microdialysis technique.

Animals were anaesthetized I.P. with choral hydrate (400 mg kg^{-1}) and a microdialysis probe was stereotaxically implanted in the striatum. The day after surgery, a Ringer solution (147 mM NaCl, 4 mM KCl, 3.4 mM $CaCl_2$, pH 7.4) was pumped through the microdialysis probe at a flow rate of 1.5 μl min^{-1}. Dialysates were collected every 20 min, and after three basal samples anatoxin-α (1, 2, 3.5 and 7 mM) was infused over 20 min. The perfusate was then switched back to the

unmodified perfusion medium and the infusion was carried out for an additional period of 120 min. Dopamine striatal levels were analysed by HPLC with electrochemical detection (Duran *et al.* 1998). The results are shown as the mean ± S.E.M. of five experiments expressed as a percentage of basal levels. Statistical analysis of the results was performed by means of repeated measures ANOVA and the Student-Newman-Keuls multiple range test. The experiments were performed according to the guidelines of the European Union Council (86/609/EU) for the use of laboratory animals. All animals were humanely killed.

Anatoxin-α caused a concentration-dependent increase in dopamine output in striatum. The infusion of 1 mM anatoxin-α induced no significant changes in striatal dopamine release The administration of 7 mM and 3.5 mM anatoxin-α produced a maximum increase in striatal dopamine levels, 40 min after the anatoxin-α perfusion (1461 ± 396 and 454 ± 165 % of basal levels, respectively). Basal values were restored 80 min after administration of anatoxin-α. Administration of 2 mM anatoxin-α increased the striatal dopamine levels to 202 ± 25 % 20 min after toxin administration and returned to basal values 20 min later. The increase in striatal dopamine levels was smaller when the flow rate was changed to 2 μl min^{-1} or when the time of infusion was reduced to 10 or 5 min (254 ± 30 and 132 ± 56, respectively).

Anatoxin-α has been found to act as a potent agonist at the neural nicotinic acetylcholine receptor (Thomas *et al.* 1993). It is well known that the striatal dopamine release is modulated by such cholinergic receptor agonists as nicotine (Calabresi *et al.* 2000). Several *in vitro* reports have indicated a stimulatory effect of anatoxin-α on the striatal dopamine release (Soliakov *et al.* 1995); however, no data of microdialysis studies on the effects of anatoxin-α on dopamine release have been published. The present results are the first *in vivo* evidence indicating that the local application of anatoxin-α by microdialysis produced an increase in striatal dopamine levels and they provide another piece of evidence that the striatal dopaminergic system may be under cholinergic modulation.

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Carmichael *et al.* (1994). *Sci Am* **270**, 78–86.

Duran R *et al.* (1998). *Chromatogr Relat Technol* **21**, 2799–2811.

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Thomas P, J *et al.* (1993). *J Neurochem* **60**, 2308–2311.

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All procedures accord with current National and local guidelines.

P112

Acute effects of cannabinoids on noradrenaline synthesis in rat brain *in vivo*

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Psychoactive cannabinoids produce several effects on the central nervous system attributable to specific interactions with their receptors (CB). Two subtypes of CB receptors have been identified: the CB1 receptor is widely distributed in the brain and CB2 receptor is found predominantly in the spleen and haemopoietic cells. The aim of the present study was to assess the acute effects of the primary psychoactive constituent of cannabis, delta9-tetrahydrocannabinol (THC), on noradrenaline (NA) synthesis in the rat brain *in vivo*.

Groups of four male Sprague-Dawley rats (220–300 g) were treated with vehicle (ethanol:cremofor:water; 1:1:18; 1 ml kg^{-1}, I.P.) or different doses of THC (5, 10 and 20 mg kg^{-1}, I.P.) and animals were killed by decapitation 1 h later. All animals were injected with the inhibitor of the aromatic L-amino acid decarboxylase NSD 1015 (3-hydroxybenzyl-hydrazine HCL, 100 mg kg^{-1}, I.P.) 30 min before killing and the accumulation of 3,4-di-hydroxyphenylalanine (DOPA) after decarboxylase inhibition was used as a mesure of the rate of tyrosine hydroxylation. The brain was rapidly removed and different regions were dissected (a piece of brainstem containing the locus coeruleus, hippocampus, hypothalamus, parieto-occipital cortex and frontal cortex) and they were stored at −80 °C. Brain regions were homogenized and DOPA and NA were detected by HPLC with electrochemical detection. ANOVA followed by Scheffé's test was used for statistical evaluation. This study was approved by the research and ethical review board of the Dirección General de Investigación (MCT, Madrid) and the experiments were performed according to the guidelines of the Univesity of Balearic Islands.

THC induced dose-dependent increases in DOPA accumulation in locus coeruleus (71 ± 17 %, $P < 0.05$), hippocampus (70 ± 11 %, $P < 0.01$), hypothalamus (56 ± 5 % $P < 0.001$) and parieto-occipital cortex (56 ± 7 %, $P < 0.005$). Also THC induced a dose-dependent decrease in total content of NA in hippocampus and hypothalamus (39 ± 9 %, $P < 0.05$) probably as a result of NA release. In contrast, no changes were observed in frontal cortex. Moreover, the acute administration of the selective CB1 agonist WIN 55,212-2 (2 mg kg^{-1}, I.P.) also increased DOPA accumulation and decreased NA content in hippocampus and hypothalamus.

In summary, these results indicate a stimulating effect of cannabinoids on NA synthesis and suggest an implication of CB1 cannabinoid receptor in these effects.

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All procedures accord with current National and local guidelines.

P113

Region-specific regulation of the extracellular signal-regulated kinase signalling pathway after acute sufentanil administration in rat brain

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The mitogen-activated protein kinases (MAPKs), such as the extracellular signal-regulated kinases ERK1 and ERK2, are activated by a wide variety of G protein-coupled receptors (GPCRs), including the opioid receptors (Ortiz *et al.* 1995). GPCR-mediated activation of MAPKs has been studied extensively *in vitro*, although little is known about GPCR-mediated activation of MAPKs *in vivo* (Bhat *et al.* 1998). Recently, we described that acute sufentanil administration (a potent μ-opioid agonist) in rats increased the phosphorylated form (active) of MEK (ERK kinase) by a naloxone-sensitive mechanism. The aim of this work was to compare the effect of

sufentanil administration on MEK1/2 and ERK1/2 in various regions of the rat brain.

Male Sprague-Dawley rats (250–300 g) received a single 0.9 % saline vehicle S.C. (control rats, $n = 7$–9) or sufentanil (1, 2.5, 5, 15 or 30 μg kg^{-1}, S.C., $n = 4$) and were killed by decapitation 30 min later. The cerebral frontal cortex, caudoputamen and hippocampus were dissected, immediately frozen in liquid nitrogen, and stored at −80 °C. The experiments were performed according to the guidelines of the University of the Balearic Islands. The phospho-MEK1/2, MEK1/2, phospho-ERK1/2, and ERK1/2 proteins were immunodetected with specific antibodies by Western blot. The immunoreactivity was detected with an enhanced chemiluminescence (ECL) Western blot detection system and quantified by densitometric analysis. Results are given as means ± S.E.M.; ANOVA followed by Scheffé's test was used for the statistical evaluations.

High doses of sufentanil increased the density of p-MEK1/2 in both frontal cortex (15 μg kg^{-1}; 206 ± 21 %, $P < 0.001$, and 30 μg kg^{-1}; 207 ± 7 %, $P < 0.001$) and caudoputamen (15 μg kg^{-1}; 213 ± 32 %, $P < 0.05$, and 30 μg kg^{-1}; 277 ± 28 %, $P < 0.001$), and decreased the density of p-MEK1/2 in the hippcampus (15 μg kg^{-1}; 78 ± 0.5 %, $P < 0.05$, and 30 μg kg^{-1}; 77 ± 5 %, $P < 0.05$). The density of p-ERK1/2 was decreased in hippocampus (p-ERK1: 15 μg kg^{-1}; 77 ± 6 %, $P < 0.05$, and 30 μg kg^{-1}; 69 ± 2 %, $P < 0.01$; p-ERK2: 68 ± 6 %, $P < 0.01$), whereas only p-ERK1 decreased in frontal cortex (30 μg kg^{-1}; 77 ± 3 %, $P < 0.01$), and no changes were observed in caudoputamen. No changes were observed in the density of total MEK1/2 and total ERK1/2 in any brain region studied after sufentanil treatment. The results indicate the existence of a region-specific regulation of the ERK pathway in rat brain after sufentanil treatments.

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Bhat RV *et al.* (1998). *J Neurochem* **70**, 558–571.

Ortiz J *et al.* (1995). *J Neurosci* **15**, 1285–1297.

All procedures accord with current National and local guidelines.

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Freely moving rat spectroscopy: NO determination

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Under physiological conditions, NO reacts with oxyhaemoglobin to form methaemoglobin, which levels can be used as indirect index of NO (Kelm *et al.* 1997). We have previously reported a method for *in vivo* spectroscopic recordings of methaemoglobin in anaesthetized rats (Gonzalez-Mora *et al.* 2002). The present work describes a method for using this technique to estimate NO levels in the brain of the freely moving rat.

Adult Sprague-Dawley rats were anaesthetized (ketamine–xylazine I.M.) for surgical procedure (approved by the local Animal Care and Use Committee). At the end of the experiment, animals were humanely killed by an overdose of anaesthetic. Two optical fibres and an attached polymicro tubing were placed together as previously described (Gonzalez-Mora *et al.* 2002). The stereotaxic surgical procedures consisted of the attachment to the skull of a carrier assembly that allows the fixation of the optical fibre tips into the striatum of a freely moving rat. Light from a halogen lamp was passed through an optical fibre and the scattered light was collected by another optical fibre and sent to a miniature spectrometer. The optical absorption spectra were obtained as previously described (Gonzalez-Mora *et al.* 2002).

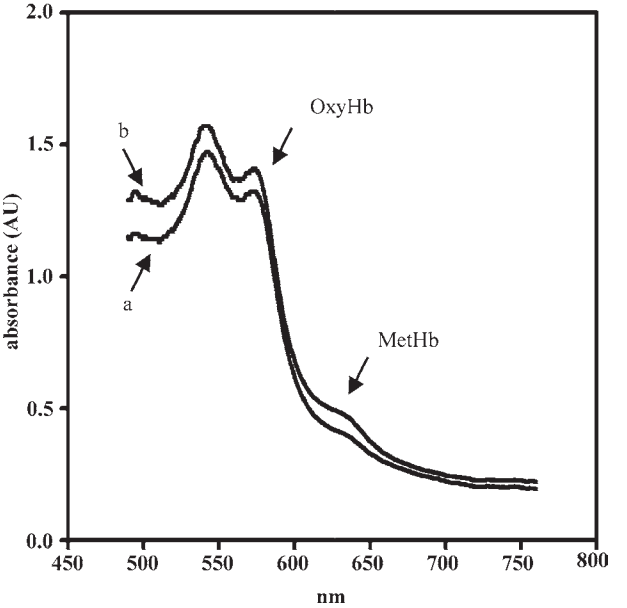

Figure 1. Representative spontaneous changes in absorbance units of the striatum. Spectrum a was taken an hour before spectrum b.

In order to attribute *in vivo* changes in absorbance at 635 nm to NO, we studied the absorbance changes in response to manipulations of basal NO concentration in the tissue by the direct injection of NO solution close to the spectroscopic sensor.

The injection of NO solution drammatically increased the absorption band with a maximum at 635 nm, as reported *in vitro* (Kelm *et al.* 1997) and in anaesthetized rats (Gonzalez-Mora *et al.* 2002). To evaluate if this technique is sensitive enough to record basal levels of methaemoglobin and the spontaneous changes due to physiological activity, spectra were recorded every 10 min during 5 h. As illustrated, spontaneous changes in the absorption of the methaemoglobin band (maximum at 635 nm) were observed. This indicates that this technique is able to detect not only the basal levels but also their spontaneous modifications. Finally, as can be seen in Fig. 1, this technique allows one to record simultaneously changes at oxyhaemoglobin band (maximum at 577 nm). Thus, the method described here is a useful tool to gain insight in the interaction between NO and microcirculation and behaviour.

Kelm *et al.* (1997). *J Biol Chem* **272**, 9922–9932.

Gonzalez-Mora JL *et al.* (2002). *J Neurosci Meth* **119**, 151–161.

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All procedures accord with current National and local guidelines.