C130

Mitochondrial calcium uptake during brief depolarisation of chick sensory neurones

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The uptake of calcium by mitochondria has traditionally been regarded as a pathological rather than a physiological phenomenon. However, recent work from a number of laboratories demonstrates that mitochondria will accumulate calcium during normal cell signalling processes. We have combined the whole cell patch clamp and confocal imaging techniques to study calcium dynamics in sensory neurones isolated from humanely killed E12 chick embryos. Neurones were acetoxymethyl ester preloaded with one of the mitochondrial calcium indicators Rhod-2, Rhod-FF or Rhod-5N (5μM for 30 min), and then whole cell patch clamped through a pipette containing the cytosolic calcium indicator Oregon Green 488 BAPTA-1 dextran (100μM OGBD, 10kD). Whole cell patch clamping allowed cytosolic Rhod dye to leave the cell at the same time as the cytosol loaded with OGBD. Depolarisation of the plasma membrane to +10mV for 1 s evoked a large Rhod-2 intensity increase in discrete organelles (N=11 cells). Colabelling with mitotracker green FM confirmed the identity of these organelles as mitochondria (N=7 cells). 50ms depolarizations evoked smaller but still significant signals from mitochondrial Rhod-2 (N=10 cells). When the lower affinity mitochondrial calcium indicator Rhod-FF (Kd = 19μM) was used, 1 s plasma membrane depolarisations evoked significant mitochondrial signals (N=5 cells), indicating that mitochondrial calcium elevations of micromolar amplitude are evoked by relatively brief episodes of calcium influx across the plasma membrane. To our surprise, inclusion of 100μM ruthenium red in the patch pipette did not significantly inhibit mitochondrial calcium uptake under these conditions (N=6 cells), suggesting that uptake occurs through a pathway distinct from the classical ruthenium red-sensitive calcium uniporter. These results suggest that mitochondrial calcium uptake will occur during normal electrical activity in sensory neurones and raise interesting questions about the mechanism by which the uptake occurs.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C131

Calcium-dependent inhibition of N-methyl-D-aspartate receptors by P2X receptors in the pyramidal neurons of mouse neocortex

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Evoked excitatory postsynaptic currents (EPSCs) were recorded from the pyramidal neurons of layer 2/3 of the somatosensory cortex in the slices obtained from the brain of humanely killed 17- to 22-day-old mice. EPSCs were elicited by electrical stimulation of vertical axons originating from layer 4-6 at 0.1 Hz, in the presence of picrotoxin (100 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (30 μM). The current measured at −80 mV was mediated mostly by P2X receptors, whereas current through NMDA receptors was the dominant component of synaptic currents measured at −40 mV or 40 mV [1]. Statistical significance of data obtained was verified using Student's paired t test with $P < 0.05$.

Low frequency stimulation at −80 mV caused a strong inhibition of NMDA component of EPSC that was subsequently measured when switching the holding potential to −40 or 40 mV. This suppression of the NMDA component of the EPSC was abolished when intracellular solution was supplemented with BAPTA (3 mM). The inhibition of the NMDA receptor-mediated component of the EPSC was also decreased by the P2X receptor antagonist PPADS (63 ± 22%, n = 10), suggesting that NMDA receptors were inhibited following activation of P2X receptors.

Direct application of P2X receptor agonists to the cortical slice also caused inhibition of current through NMDA receptors. When ATP (10 μM) or αβ-methylene-ATP (10 μM) was applied for 60 s when holding the cell at −80 mV, subsequently elicited NMDA receptor-mediated EPSCs measured at −40 mV were inhibited. ATP inhibited the NMDA receptor-mediated EPSCs by 43 ± 24% (n = 12), whereas αβ-methylene-ATP inhibited 26 ± 10% (n = 9). When these P2X receptor agonists were applied at 40 mV, they did not inhibt the subsequently elicited NMDA receptor-mediated EPSC.

The inhibition of the NMDA current following activation of P2X receptors was also observed in acutely isolated pyramidal neurons. ATP (10 μM, 5 s) or αβ-methylene-ATP (10 μM, 5 s) reduced the current evoked by subsequent application of NMDA (100 μM) by 25 ± 13% (n = 5). The suppression of evoked NMDA currents did not occur when Ca^{2+} in the extracellular solution was substituted by Ba^{2+}.

Taken together these results suggest that postsynaptic P2X receptors can control the activity of NMDA receptors via a calcium-dependent mechanism. This may imply a modulatory role for the purinergic component of excitatory synaptic transmission.

Pankratov Y, Lalo U, Krishtal O & Verkhratsky A (2003). Mol Cell Neurosci 24, 842-849.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C132

Group II and group III metabotropic glutamate receptors have an enhanced depressive effect on glutamate release in the entorhinal cortex of chronically epileptic rats

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Activation of group II and III metabotropic glutamate receptors (mGluRs) facilitates spontaneous glutamate release in the entorhinal cortex (EC) 4-6 week old rats (Evans et al. 2000;

Ayman et al. 2003). In an accompanying presentation at this meeting (Jones, Ayman & Woodhall) we have shown that this effect of the mGluRs is reversed to depression in older animals (5-6 months). A large body of literature has shown diverse changes in function of mGluRs in animal models of epilepsy. In the present experiments we have looked for adaptive changes in group II and group III mGluR function in the EC of chronically epileptic rats.

Male Wistar rats, previously rendered chronically epileptic, were humanely killed and slices of EC were prepared using conventional techniques (Glien et al. 2001) and compared to age-matched control (AMC) animals (5-6 months). At the time of experiments, all epileptic animals were exhibiting spontaneous recurrent seizures. Glutamate release was monitored by recording spontaneous excitatory postsynaptic currents (sEPSCs) from neurones in layer V. All recordings were made in the presence of the NMDA receptor blocker, MK-801 (10 μM), so EPSCs reflect activation of AMPA receptors. Data are expressed as mean±s.e.m. Statistical comparisons were made using either the Wilcoxon rank sum test (WRST) or the Kolmogorov-Smirnov test (KS).

In 14 cells from the AMC group mean interevent interval (IEI) was 246.5±6.6 ms, and in 17 cells from the epileptic group mean IEI was slightly less at 220.9±5.1 ms. Mean amplitude of sEPSCs was also slightly larger in the cells from the epileptic group (13.4±0.2 pA v 14.6±0.1 pA). However, neither change was significant (WRST, $P>0.1$). Many cells from the epileptic group displayed burst-like activity and this was not observed in any cells from the AMC group.

In 8 layer V neurones from AMC animals, the group III mGluR agonist, ACPT-1 (20 μM), increased the mean IEI of sEPSCs from 265.7±9.5 ms to 357.3±10.3 ms (KS, $P<0.001$), reflecting a 28% decrease in frequency. In 9 cells from the epileptic group mean IEI increased from 222.7±6.5 ms to 740.2±204.1 ms (KS; $P<0.001$), in the presence of ACPT-1, a 70% decrease in frequency. Mean amplitudes were not significantly altered in any situation (WRST, $P>0.1$). In AMC animals the group II agonist, DCG-IV (5 μM), in the presence of CPPG to block group III mGluRs, decreased frequency by approximately 30% (KS, $P<0.001$), whereas, in neurones from epileptic animals the reduction was over 50%.

Thus, the depression of glutamate release by both groups of receptor appears to be enhanced in chronically epileptic animals. This might reflect an adaptive change in an attempt to dampen hyperexcitability in cortical networks.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C133

LTD in the rat perirhinal cortex: the role of AMPA receptor trafficking

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A primary function of the perirhinal cortex is in the process of recognition memory, particularly familiarity discrimination. Evidence that there is a decremental response in perirhinal neurones upon exposure to familiar objects (Brown & Xiang, 1998), has led to the proposal that long term depression (LTD) at perirhinal synapses could be a plastic mechanism underlying recognition memory (Brown & Bashir 2002). The role of AMPA receptor recycling within recognition memory and LTD expression in the perirhinal cortex has yet to be investigated. The trafficking protein AP2 acts as an adapter for clathrin-mediated endocytosis of AMPA receptors. It has an overlapping binding site on the C-terminus of the GluR2 subunit with NSF, an ATPase associated with maintaining AMPA receptors at the synapse (Lee et al. 2002). In this report we have investigated the role of GluR2 and its interactions with AP2 and NSF upon LTD in the perirhinal cortex.

Perirhinal cortex slices were prepared from humanely killed adult male DA rats. Whole-cell voltage clamp recordings were made from layer II/III neurones. Low-frequency stimulation (LFS; 200 stimuli, 1Hz) combined with depolarisation to -40mV resulted in LTD (43 ± 5% of baseline, n=7). This form of LTD is NMDAR-dependent since bath application of 50μM AP5 (NMDAR antagonist) inhibited LTD induction (91 ± 5%, n=6). Antagonism of GluR5 and mGluR receptors with 5μM UBP302 and 100μM LY341495, respectively, had no effect upon LTD induction (46 ± 6%, n=3; 44 ± 11%, n=3).

Specific inhibitory polypeptides of the AP2- or the NSF-GluR2 interaction were introduced, via the intracellular whole-cell solution. In line with results from the hippocampus, LTD expression was blocked by the AP2 specific inhibitory peptide pep-ΔA849-Q853 (94 ± 5%, n=6) but was unaffected by pep-R845A (44 ± 5%, n=5) (Lee et al. 2002). Pep2m, which inhibits NSF and AP2, also blocked LTD expression (95 ± 1%, n=3). However, the specific NSF inhibitory peptide pep-R845A and pep2m, which cause run-down of hippocampal baseline EPSCs, had no effect on baseline transmission in perirhinal neurones. These results suggest that AP2 is vital for LTD expression but that NSF may potentially have a different role in AMPA receptor trafficking in the perirhinal cortex, compared with the hippocampus.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

C134

Non-synaptic function of local GABAergic system in isolated rat trigeminal ganglion

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Gamma-amino butyric acid (GABA) is a major inhibitory neurotransmitter in the adult mammalian central nervous system. Immunohistochemical studies (Roy *et al.* 1991; Szabat *et al.* 1992; Stoyanova *et al.* 1998) have reported GABA immunoreactivities in the peripheral sensory systems, including sensory ganglia. However, the exact localization of the GABAergic system and

its physiological functions in the sensory ganglia are still unclear. In this study, we investigated the function and mechanism of the local GABAergic system in isolated rat trigeminal ganglion (TG) from humanely killed rats.

Rat TGs consist of different sized neural cell bodies and satellite cells. Using the patch-clamp technique, we observed GABA-induced Cl^- currents in the neuronal cell bodies. We used a home-made rapid superfusion system (10-90% rise time: ~80 ms) for drug application (Murase *et al.* 1990). In all TG neurons tested (n = 58), application of GABA induced an inward Cl^- current which desensitized. Muscimol, a $GABA_A$ receptor agonist, mimicked the GABA-induced Cl^- current. A $GABA_A$ receptor-selective antagonist, bicuculline methiodide (BMI), attenuated the GABA-induced Cl^- current. Therefore, we conclude that TG neurons express functional $GABA_A$-receptor channels on their cell bodies. The EC_{50} for GABA varied widely from 5.29 to 242 μM and the Hill coefficient (n_H) from 0.98 to 2.6 at -60 mV among TG neurons. Immunohistochemical analysis showed that $GABA_A$ receptor subunits α1, α5, β2/3 and γ1/2/3 were homogeneously expressed in almost all neurons whereas expressions of α3, α4, α6 and δ subunits were heterogeneous, which is consistent with the variable EC_{50} and n_H data. *In situ* hybridization and immunohistochemical analysis revealed that glutamate decarboxylase 65 (GAD65) and its mRNA were expressed in all neuronal cell bodies and approximately 70% of neurons were GABA immunopositive.

All satellite cells were strongly immuno-positive for GABA whereas GAD65/67 and their mRNAs were undetectable, which suggests that satellite cells take up GABA from the extracellilar environment. Moreover, using HPLC coupled with *o*-phthaldialdehyde precolumn derivatization and fluorometric detection, we found that GABA was released from the neuronal cells and/or satellite cells when the TG cells were depolarized with increasing external K^+ concentration (up to 100 mM).

These results suggest that GABA acts as a non-synaptically released diffusible neurotransmitter that may modulate somatic inhibition of neurons within TG. We speculate that a functional cross-talk exists between neuronal cell bodies and satellite cells in the rat TG, via the local $GABA_A$ergic system.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC126

Three types of extrasynaptic $GABA_A$ receptors in dentate gyrus granule neurons

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We used the patch-clamp technique to examine single-channel currents activated by GABA in dentate gyrus granule neurons in rat hippocampal slices from humanely killed rats. We found that GABA activated three types of extrasynaptic GABAA receptors (GABARex I, II and III). These receptors were activated with a delay, varied in their affinity for GABA and their maximal conductance.

The maximal conductances at saturating concentrations were 61 ± 3 pS (n=70), 85 ± 8 pS(n=56) and 43 ± 3 pS (n=42) for GABARex I, II and III, respectively. The three receptor types were differently modulated by GABAA receptor specific drugs. The different properties of GABARex I, II and III allow neuronal inhibition to be graded and precisely set by the receptors expressed, the GABA concentration and other drugs that may be present.

Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC127

Loss of inhibitory postsynaptic currents following selective ablation of the $GABA_A$ receptor γ2 subunit in cerebellar granule cells of mice

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Mature cerebellar granule cells express multiple $GABA_A$ receptor subunits. The resulting receptor subtypes are differentially distributed between synaptic and extrasynaptic sites, and are thought to participate in distinct forms of inhibitory signalling. Extrasynaptic α6δ-containing receptors are responsible for a persistent background conductance causing a tonic inhibition (Brickley et al. 2001; Stell et al. 2003), whereas synaptic γ2-containing receptors underlie phasic IPSCs. In granule cells (Brickley et al. 1999) and other cell types (Schweizer et al., 2003), the γ2 subunit is thought to anchor $GABA_A$ receptors at synapses.

We have selectively inactivated the γ2 subunit gene in cerebellar granule cells, using mice with Cre recombinase driven from the granule cell-specific α6 promoter (BACα6Cre) crossed with a line harboring a floxed γ2 subunit allele (fγ2) (Aller et al. 2003). *In situ* hybridization on adult brain sections with an exon 8-specific probe confirmed the selective absence of exon 8-containing γ2 subunit mRNA from BACα6Cre x fγ2/fγ2 granule cells.

We made whole-cell recordings from granule cells in acute cerebellar slices. Mice anaesthetized with isoflurane were humanely killed. We prepared 250μm thick sagittal cerebellar slices and made recordings from visualized neurons at 35-37°C. The vast majority of granule cells from wild-type mice (38 of 46; 83%) displayed $GABA_A$ receptor-mediated spontaneous inhibitory postsynaptic currents (sIPSCs), whereas 49 of 50 cells (98%) from BACα6Cre x fγ2/fγ2 mice were without sIPSCs. This loss of synaptic currents was selective to granule cells, as stellate cells from both groups of mice displayed sIPSCs.

For granule cells from BACα6Cre x fγ2/fγ2 mice (n=21) and their wild-type littermates (n=16), we also determined the magnitude of $GABA_A$ receptor-mediated tonic conductance (as blocked by 100μM SR95531 (Brickley et al, 2001)). The normalized tonic conductance in the two groups (273±41 and 327±43 pS/pF, respectively) was not different (Kolmogrov-Smirnov, P=0.59).

Our results support the idea that γ2 subunits are needed for targeting of $GABA_A$ receptors to synaptic sites. In the absence of normal phasic inhibition, there was no clear change in the magnitude of tonic inhibition.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC128

Ultrastructural localisation of ryanodine receptors in hair cells of the mammalian cochlea

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Calcium-induced calcium release (CICR), mediated by ryanodine receptors (RyRs), has been shown to affect calcium signals in many cell types including hair cells (Kennedy & Meech, 2002; Lioudyno *et al.* 2004; Marcotti *et al.* 2004). The subcellular location of RyRs has been examined in this study to provide further information about the possible role of CICR in the sensory cells of the cochlea of humanely killed rats.

We performed post-embedding immunogold labelling with 3 polyclonal and 2 monoclonal antibodies to determine the distribution of RyRs. Labelling was carried out with each antibody on ultrathin sections of post-hearing rat cochleas embedded in two different resins. Cochlear segments were embedded in Lowicryl HM20 resin using a freeze substitution technique and in LR White using a standard ethanolic dehydration procedure. Better preservation of membranes and intracellular structures was obtained with freeze substitution although the LR White embedded tissue retained greater RyR antigenicity. Positive controls carried out on cardiac and skeletal muscle embedded in both resins gave some indication of antibody specificity for the different receptors, RyR1 and RyR2, the former occurring predominantly in skeletal muscle and the latter in the heart.

Polyclonal antibodies against RyRs 1, 2 & 3 (kindly provided by Vincenzo Sorrentino, University of Siena, Italy) did not appear to be strictly specific for their respective receptors under our conditions although they did appear specific for ryanodine receptors. Monoclonal antibody 2142 (kindly provided by Tony Lai Cardiff, UK), preferentially labelled RyR1, detecting it 4 times more readily than RyR2 in LR White embedded tissue. Monoclonal antibody C3-33 (Affinity Bioreagents), although RyR specific, did not appear to preferentially label receptor 1 or 2. Labelling was found with all the antibodies on both the inner and outer hair cells. The heaviest labelling in IHCs was found towards the base of the cell. In outer hair cells, labelling was seen throughout the cell, with the heaviest labelling occurring in the region between the nucleus and cuticular plate. Labelling was also associated with the sub-surface cisternae. These findings confirm the presence of RyRs in regions of hair cells where calcium signalling is thought to occur and add to the evidence that CICR plays an important role in calcium regulation in mammalian hair cells.

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PC128a

Exercise upregulates gene expression of dopamine receptor D1 and plexin A1 in mouse hippocampus

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Previous studies have demonstrated that voluntary wheel running improves learning and memory capabilities in rodents. In this study, we further investigated whether chronic treadmill exercise improved hippocampus-dependent learning/memory capabilities and upregulated neural plasticity-related genes in the hippocampus. Male C57BL/6 mice (3 months old) were divided into control and exercise groups. The exercised animals received treadmill running for 60 min/d, 5 d/wk for 4 or 12 weeks (speed: 10-12 m/min). At the end of the experiments, one-trial passive avoidance tasks or Y maze tests were executed to evaluate learning and memory behaviors. The animals were then humanely killed. The mRNA and protein expressions of dopamine receptor D1 and plexin A1, an axon guidance molecule, in the hippocampus were determined by real-time PCR and Western blotting techniques, respectively. The results were analyzed by unpaired Student's t test (statistically significant if $P<0.05$). Our preliminary results showed that 1) 4- or 12-week treadmill running improved learning and memory capabilities, indicated by increases in passive avoidance retention latency and the novel arm entry probability of the Y maze task; 2) exercise tended to elevate gene expressions of D1 and plexin A1 in the mouse hippocampus. In conclusion, chronic treadmill exercise improves the capabilities of learning and memory, possibly due to an increase in hippocampal neural plasticity-related gene expression.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC128b

Investigation of synaptic transmission in hippocampal brain slice using multielectrode array (MEA) system

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Researchers have developed many innovative ways to investigate the neural circuitry of hippocampus. However, it is still a difficult task because of its complexity and inaccessibility. Recently, a multi-electrode array system has been adapted to deal with the hippocampal neural circuit. With this tool, we have studied the spatiotemporal synaptic transmission changes in hippocampal slice culture.

Three to five weeks old Sprague-Dawley mail rats were humanely killed by instant decapitation using a Guillotine and then whole brain was removed. Acute hippocampal slices were obtained by conventional methods. The experiments were performed in accordance with the animal care guidelines of the Korean Academy of Medical Sciences and planned to minimize the number of animals and their suffering. MEA system (multielectrode array; MCS GmbH, Germany) were used in the study. Train pulse (140μs biphasic, 3Hz for 4min and 65μA current) was applied to a selected channel at every hour for 6 hours (STG1004; MCS GmbH) and the responses were recorded in other 59 channels using Recorder-Rack software (MCS GmbH). Analysis of data was carried out by a histogram and pseudocolor imaging analysis methods that were developed in our laboratory.

Ensemble of acute hippocampal slice and the MEA system has been successfully adapted to reveal the high-resolution spatiotemporal synaptic transmission changes in hippocampal brain slice.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC182

Creatine transport in rat telencephalum, diencephalum and cerebellum

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Oral creatine supplementation may be useful to prevent and/or treat neurodegenerative pathologies. Creatine transporter (CRT) is widely distributed in the central nervous system and it has been functionally characterized in cells transfected with CRT cDNA. However, information regarding brain creatine transport is lacking. In the present study we investigate the transport of creatine in synaptosomes isolated from the rat telencephalum, diencephalum and cerebellum, and its variation with age.

The experiments were performed in accordance with the current national and local guidelines for the care and use of laboratory animals. Six Wistar rats of either 18-day (suckling) or 2-month-old (adult) were anaesthetized with ether and then humanely killed. Whole brains were removed and crude synaptosomal fractions of telencephalum and diencephalum were prepared using ficoll gradient (1). [^{14}C]-creatine uptake was measured in brain synaptosomal preparations using a rapid filtration technique (2) and expressed as pmol/mg protein/3 min. ANOVA followed by Newman-Keuls' test was used for statistical analysis.

Brain synaptosomes accumulate creatine against its concentration gradient. This accumulation was Na^+-dependent (5.0 ± 0.4 and 7.2 ± 0.6 in the telencephalum, and 12.3 ± 1.3 and 14.0 ± 1.5 in the diencephalum, of suckling and adult rats, respectively) and antagonized by the specific creatine transport inhibitor guanidinopropionic acid (2.66 ± 0.24, $p<0.001$ as compared with Na^+-dependent creatine uptake). Na^+-independent creatine uptake ranged from 1.9 ± 0.4 to 2.6 ± 1.3. In both suckling and adult rats, creatine transport activity was lower in the telencephalum than in the diencephalum ($p<0.001$), and it was significantly ($p<0.05$) increased with age in the telencephalum. Northern assays revealed two CRT mRNA transcripts, of 4.2-kb and 2.7-kb in the brain isolated from suckling rats. The expression of the 4.2-kb transcript was 5 to 6 times higher than that of the 2.7-Kb transcript and it was significantly ($p<0.05$) less abundant in the telencephalum than in the other brain regions examined. The 2.7-Kb transcript was equally distributed in the three brain regions examined.

The Western blots revealed immunoreactive bands of 57-, 60- and 71-kDa in the three brain regions studied. In suckling rats, the three proteins were more abundant in the diencephalum and cerebellum than in the telencephalum. In adult rats, however, only the 71-kDa protein was more abundant in the diencephalum and cerebellum than in the telencephalum, whereas the abundance of the other two proteins were lower in the diencephalum and cerebellum than in the telencephalum.

In conclusion, creatine transport in the brain appears to be mediated by the 71-kDa protein.

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PC183

The role of osteopontin in the peripheral nervous system

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Oldberg A, Franzen A & Heinegard D (1986).

Osteopontin (OPN) is a 39 kDa highly acidic cytokine phosphoprotein, encoded by a single gene which is highly conserved in vertebrates. OPN was originally isolated in bone in 1986(1) and has subsequently been isolated in many other tissues, including the kidney, lung, liver and central and peripheral nervous tissue. OPN plays a variety of roles within different cell types, including adhesion, cell survival, wound healing and tumorigenesis. Studies have shown that, within the dorsal root ganglia (DRG), OPN is expressed in 25% of total neurons, and is largely confined to parvalbumen expressing neurons(2). However, to date few studies have analysed its role in the peripheral nervous system. Previous work in our laboratory has demonstrated a sustained 36% upregulation in OPN mRNA within the DRG 1 and 3 weeks after axotomy. This may suggests a role in cell survival and/or the regeneration of damaged neurons. To further elucidate the role played by OPN within the peripheral nervous system a range of experiments has been conducted.

Mice were anaesthetised with 1 part Hypnorm and 1 part Hypnovel to 2 parts water. For axotomy, the dose was 150 - 200ul I.P. per mouse. For immunohistochemical perfusion, the dose was 300ul per mouse. All animals were humanely killed at the end of the experiments.

Following immunohistochemistry performed 3 days, 1 week and 3 weeks after axotomy, no change in the pattern or levels of OPN expression were noted.

To investigate the possibility that OPN may play a role in pain processing, thermal and mechanical nociceptive testing was performed on intact OPN knockout and strain matched wild-type mice. Further, mechanical allodynia was measured after a partial sciatic nerve injury (SNI, a model of neuropathic pain behaviour(3)) in mice of both genotypes. No difference in the responses of knockout and wild-type animals were noted in the intact animals nor after SNI.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC184

ANNURCA APPLE-RICH DIET REVERSES AGING IMPAIRMENT OF LONG TERM POTENTIATION IN AGED RATS

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There is a general agreement that maintenance of long term potentiation (LTP) is markedly impaired with age (3). The

impairment in LTP in aged rats was associated with an increase in accumulation of reactive oxygen species (ROS) that appears to be due to an increase in superoxide dismutase (SOD) activity in the absence of concomitant changes in activities of glutathione peroxidase or catalase (3). Diet supplements with antioxidants can reverse age-impaired ability to sustain long term LTP. There is evidence that natural antioxidants from fresh fruit could be more effective than a dietary supplement (5). An apple of the variety annurca is a typical product cultivated in the area around Naples and it has been previously characterized for a high antioxidant power (1,2). The aim of the present work was to study the effects of annurca apple in young and old rats on LTP induced in the dentate gyrus. Furthermore we studied the effect of this diet on the activity of superoxide dismutase on frozen brain sections (4). Groups of aged and young rats were fed with a control diet or a diet supplemented with fresh apple of the annurca variety for 10 weeks. After this period, rats were anaesthetized with intraperitoneal injection of urethane (1.0 g/kg) and assessed for the ability to sustain LTP. The responses were quantified by the analysis of fEPSP slope expressed as a percentage of the pre-tetanus mean value. At 50 min after high frequency stimulation, the mean value of fEPSP ± SEM was 129.8 ± 5.3 for the aged treated group, 112.0 ± 1.7 for the aged control group, 129.8 ± 6.2 for the young treated group and 124.8 ± 2.1 for the young control group. One-way analysis of variance showed significant differences among the groups. Post hoc test analysis showed that fEPSP in the aged control group was significantly smaller than the other groups. Therefore, aged rats fed with the supplemented diet sustained LTP at the level of young rats. An age-related increase in SOD activity, quantified by histochemical method, was also reversed by the apple-supplemented diet. This result demonstrates that annurca apple in the diet could have an important health care role in aging. All animals were humanely killed at the end of the experiment.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC185

Adaptive plasticity after disruption of the lamprey locomotor network

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The lamprey is a lower vertebrate model system for studying locomotor network function. Neural networks in the spinal cord, which generate basic locomotor outputs, are activated and modulated by descending inputs from the brain. The lamprey recovers locomotor function after being paralysed by complete spinal lesions that remove these descending inputs, and thus prevent the activation of locomotor networks (Cohen *et al.* 1988). As in mammalian systems, the analysis of functional recovery in the lamprey has focused on regrowth of ascending and descending axons across lesion sites. However, there is evidence that regrowth alone cannot account for the recovery (Mackler & Selzer, 1985; McClellan, 1994).

A complementary approach is to investigate how plasticity may alter network properties in locomotor networks to optimise function below lesion sites after injury. This plasticity could be evoked as a result of neuromodulator or activity-dependent effects on network cellular or synaptic properties, or through intrinsic changes in cellular or synaptic properties i.e. homeostatic or adaptive plasticity (Turrigiano, 1999).

A potential role for adaptive plasticity has initially been examined by pharmacologically disrupting network function. Larval animals were anaesthetised by immersion in Tricaine methane-sulphonate (100-200mg/l) and the trunk region of the spinal cord was isolated. Spinal cords were then incubated in tetrodoxin (TTX, 1.5μM), 6-cyano-7-nitroquinoxaline-2,3-dione plus DL-2-amino-5-phosphonopentanoic acid (CNQX, 10μM; AP5, 50μM respectively), or CNQX (10μM) for 24 hours. TTX abolishes all action potential-evoked synaptic transmission in the network by blocking presynaptic Na^+ channels, while CNQX and AP5 block postsynaptic AMPA and NMDA receptors, respectively. Intracellular recording was used to investigate several electrophysiological parameters to identify potential changes in presynaptic and postsynaptic properties, including the resting membrane potential, input resistance and the amplitude and frequency of spontaneous miniature EPSPs and IPSPs.

The resting membrane potential of cells in TTX (-61.50±2.28mV, n=10) and CNQX/AP5-treated cords (-56.17±3.45mV, n=6) was significantly depolarised relative to control (-72.71±2.29mV, n=7; $p<0.05$, ANOVA with post-hoc Fishers pairwise comparison). There was also an increase in the frequency of spontaneous miniature EPSPs in TTX (3.23±0.29Hz, n=12) and CNQX/AP5 (3.01±0.41Hz, n=7) when compared with control (1.66±0.32Hz, n=10; $p<0.05$, ANOVA with post-hoc Fishers pairwise comparison). These changes were not seen when only postsynaptic AMPA receptors were blocked. Together, these effects suggest changes in presynaptic and postsynaptic properties.

Disrupting network activity can thus evoke adaptive plasticity. This now provides a basis for examining the role of adaptive changes in locomotor networks below lesion sites in the recovery of locomotor function after complete spinal lesions.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC186

Variable Depth Total Internal Reflectance Fluorescence (TIRF) Microscopy in COS Cells

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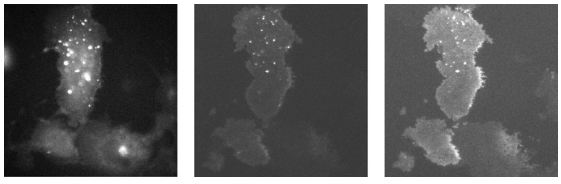
Many facets of cell physiology are now accessible for study by live cell imaging. To a large extent this is attributable to the growth in availability of cellular and sub-cellular fluorescent probes. These probes take many different forms; they may localize to specific environments within a cell, they may detect changes in ion concentration or they may be used to tag specific proteins, thereby enabling tracking and localization of the tagged protein. Whilst fluorescence detection technology has also developed rapidly in recent years, one major limitation of conventional imaging remains the lack of depth resolution. The comparatively new technique of total internal reflection fluorescence (TIRF) microscopy overcomes this limitation, using the principle of total internal reflection at a glass/water interface to generate an evanescent wave and excite a very thin fluorescent volume immediately above the interface (Axelrod, 2003). The illumination depth using this method is extremely small, allowing fluorescence excitation up to only a few hundred nanometres into the sample, typically a small fraction of the thickness of a cell. Small changes to the angle of incidence of the incoming light beam to the interface alter the penetration depth of the evanescent wave, and this effect can be utilised to probe changes in fluorescent signal pattern at a range of depths within the TIRF interface.

In this study we devised a new TIRF system that allows rapid changes in the depth of measurement into the cell. This enables distinctions to be made between fluorescence arising due to changes at the cell membrane and those arising from changes within the cell. The rapid depth changes are achieved by use of a galvanometer mounted mirror deflecting the beam at a variable angle into a prism below the cell via a combination of lenses. Depth changes can be achieved on a millisecond timescale allowing a dynamic event to be tracked almost simultaneously at different depths. The images shown are those of COS cells transfected with YFP-e-sarco-glycan. The epifluorescence image (panel 1) shows general cell wide labelling whereas the TIRF images (panels 2 and 3 taken with calculated illumination depth constants of 62 and 80nm), show that the protein is differentially expressed within the cell. Images were acquired sequentially with 50ms intervals between frame starts.

These results illustrate that variable angle TIRF microscopy can be used to collect multiple thin optical sections close to the cell membrane surface. This has the considerable advantage over conventional TIRF microscopy that dynamic changes, such as the movement of structures towards or away from the cell membrane, can be discriminated in near real time.



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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC187

Projections from the olfactory bulb in rat: a study using triple fluorescent procedure

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In mammals the main olfactory bulb (MOB) receives afferent sensory glutamatergic receptor neurons and sends efferent fibres direct to different structures. Recent physiological properties, involving MOB, in particular regeneration research and neurogenesis have been shown (1).

In addition, the MOB receives a large number of projections from different CNS zones. A dendrodendritic reciprocal synapse is proposed to understand the olfactory mitral cell and granular cell syncronization. A dendrodendritic reciprocal synapse provides a recurrent excitatory connection in the olfactory bulb (2). Previous studies have demonstrated different interactions between the olfactory bulb and different cortical and subcortical zones in relation to discrimination and integration of olfactory inputs with monosynaptic and polysynaptic pathways: this determines the access to hippocampal formation. For example, the olfactory lobes of Alzheimer's patients exhibit oxidative stress and it is well known that olfactory dysfunction frequently accompanies neurodegeneration. Different neurotransmitters are involved in MOB projections: cholinergic projections to the hippocampus have been proposed to serve as the pacemaker for the hippocampal theta rhythm (3).

Our electrophysiological and neuroanatomical studies have demonstrated that interpeduncular neurons (IPN) are influenced by olfactory bulb neurons (4) with monosynaptic pathway. This projection is evoked by natural olfactory stimulation of the olfactory receptor and is monosynaptically connected to the hypoglossal nucleus.

In the present study, we used a fluorescent retrograde technique in the adult rat to identify direct branching axonal projections from the olfactory bulb to the IPN and to amygdaloid nucleus; immunohistochemical methods were used to identify which neurotransmitters or neuropeptides are involved in these projections. Ten male Wistar rats were anaesthetized with chloral hydrate (400 mg/kg i.p.). Two fluorescent tracers were injected into the same rat: fluoro gold (FG) was injected into the basomedial amygdaloid nucleus on one side, and rhodamine-labelled beads (RLB) into the interpeduncular nucleus of the same side.

Four series of MOB coronal sections (35-40μm) were cut on a Reichert cryostat and observed with a fluorescent microscope and immunocytochemically processed with specific antibodies. Vasoactive intestinal peptide (VIP), substance P (SP) and neuropeptide Y (NPY) neurons were revealed by immunocytochemical detection of polyclonal antibodies. In the MOB small (10-25μm) single FG-mitral cells and single RBL mitral cells were

found. Double-projecting neurons were distributed in the entire mitral layer. The results demonstrate that pools of neurons in the MOB show collateral projections to the amygdaloid and IPN expressing different neuropeptides.

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PC188

Comparison of electrophysiological properties of excitatory and inhibitory neurons in rat hippocampal culture

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Voltage-gated K^+ (Kv) channels play a major role in regulating the excitability of mammalian hippocampal neurons. In these neurons Kv channels control neuronal spike properties and firing frequency, and modulate neurotransmitter release. Kv channels have been divided into four subfamilies, Kv1-Kv4, which differ in their primary structure, biophysical properties, and subcellular localization.

The aims of the present study were to compare the spiking parameters of excitatory and inhibitory hippocampal interneurons and to examine the expression of some types of voltage-gated potassium channels on neuronal membranes.

Regular spiking (RS) parameters were studied in inhibitory and excitatory hippocampal cultured neurons (prepared from hippocampi isolated from 1-day old humanely killed Wistar rats) using combined whole cell current and voltage clamp recordings to examine action potential waveforms and postsynaptic responses correspondingly. The experiments were carried out on 18- to 28-days-old cultured neurons. GABAergic neurons were identified by the presence of monosynaptic inhibitory currents in the postsynaptic cell in response to action potentials generated in the stimulated cell in the presence of the excitatory transmission blockers D_L-AP5 (20 μM) and DNQX (20 μM). Dentate gyrus granule cells (GCs) were chosen as examples of excitatory neurons. The electrical activity of GCs was studied in presence of inhibitory transmission blocker bicucculine (10 μM). The RS parameters (i.e. spike amplitude, after-hyperpolarization amplitude, depolarization slope, repolarization slope, spike frequency adaptation) of inhibitory and excitatory neurons were reliably distinguished (Student's *t* test, $P<0.05$). The distribution of voltage-gated potassium channels (Kv1.2, Kv3.1 and Kv4.2) was determined by immunocytochemistry. It was shown that expression of Kv4.2 potassium channels can determine regular spiking phenotype (Birnbaum *et al.* 2004). We found that expression of Kv4.2 was detected only in excitatory interneurons. No Kv4.2-specific fluorescence was observed in inhibitory interneurons. Kv1.2 immunoreactivity was detected in both GCs and RS inhibitory interneurons.

Excitatory RS interneurons belonged to one population of GCs and were immunopositive for somatostatin. Inhibitory RS interneurons were divided onto two groups (k-means algorithm for data clusterization) according to morphology and peptide expression. The first group of cells demonstrated good immunoreactivity to somatostatin and had a soma size ~27.9 μm and an average of 5.1 neurites (n=20). The second group did not stain for somatostatin and had a soma size ~17.3 μm and an average of 3.3 neurites (n=15).

In conclusion, different mechanisms appear to be responsible for the maintenance of regular spiking electrical activity in these inhibitory and excitatory interneurons.

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This work was partly supported by a Wellcome Trust (069663) grant to S.F.

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PC189

5-Hydroxytryptamine (5-HT) induced excitation in the subthalamic nucleus: Action at $5HT_{2C}$ receptors

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Receptor binding and mRNA for many of the characterised 5-HT receptors have been found in basal ganglia. Of particular interest are $5\text{-}HT_{1A}$ and $5\text{-}HT_{2C}$ receptors, which have previously been reported to have potential therapeutic benefit in Parkinson's disease (Nicholson & Brotchie, 2002). Thus, antagonists of the $5\text{-}HT_{1A}$receptor suppress L-DOPA induced dyskinesia in animal models and Parkinson's patients while antagonist action at $5\text{-}HT_{2C}$ receptors reduce motor side-effects, especially orofacial dyskinesias and turning behaviour. Furthermore, there appears to be up-regulation of $5\text{-}HT_{2C}$ receptors and down-regulation of $5\text{-}HT_{1A}$ receptors in the dopamine depleted state.

Here, we use single unit extracellular recordings in a brain slice preparation to determine the effects of exogenously applied 5-HT on single STN neurones and to pharmacologically characterise the 5-HT receptor subtypes involved. 300μm thick sagittal and parasagittal slices were obtained from 30- to 40-day-old CB57BL/6JCrL mice. The animals were anaesthetised with 4% fluorothane in O_2 and humanely killed. Slices were perfused at 2-3ml/min with aCSF containing (mM) NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, glucose 10, buffered to pH 7.4 with $NaHCO_3$(26mM) saturated with 95% O_2-5% CO_2. Single unit extracellular recordings were made using glass microelectrodes filled with 2M NaCl of resistance ~10MΩ. All data are expressed as mean±S.E.M. and a paired t test used to test for statistical significance.

Recordings were made from 35 STN neurones. These cells fired action potentials at a rate of 8.4 ±0.7Hz in a mostly regular manner. In 28 cells (80%), 5-HT application increased STN firing rate (10μM, 172 ± 18.6%) with an estimated EC_{50} value of

6.5μM. No changes in firing pattern were observed. The excitation was mimicked by the non-specific 5-HT_2 receptor agonist α-methyl 5-HT (1-10μM, 11 cells). Excitations were significantly reduced by prior 15-min perfusion with the specific 5-HT_{2C} receptor antagonist RS 102221 (500nM). RS 1002221 (500nM) also reduced the excitatory effect of α-methyl 5-HT (3μM). In 5 cells (14%), 5-HT induced a biphasic response of excitation followed by inhibition which also appeared concentration-dependent, while in 2 cells (6%) inhibition alone was observed. No inhibitions were observed on α-methyl 5-HT applications. Both the excitations and inhibitions were unaffected by CNQX (10μM) and picrotoxin (50μM).
In summary, the excitation induced by 5-HT in STN neurones appears to be a direct effect mediated by 5-HT_{2C} receptors although inhibitions possibly mediated by the 5-HT_{1A} receptor subtype are evident.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC190

Proteomic analysis of dorsal root ganglia from wildtype and galanin knockout mice

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The mechanisms which regulate the regeneration of dorsal root ganglia (DRG) sensory neurons following injury remain poorly understood. Damage to a peripheral nerve causes major changes within the cell bodies of the DRG neurons which are thought to promote regeneration, by stimulating neurite outgrowth, and enhance survival of the damaged neurons. Within the intact adult DRG the neuropeptide galanin is expressed at low levels in less than 5% of neurons. However, following peripheral nerve injury there is a rapid and robust upregulation of both galanin mRNA and peptide leading to approximately 50% of all DRG neurons expressing the peptide. Previous work from our laboratory has shown that galanin plays an important role in both neuronal survival and regeneration (1,2,3).
In this study we used a proteomic approach to compare protein expression within the DRG of wildtype and galanin knockout mice, both after acute isolation and following an *in vitro* model of axotomy. Adult mice were killed humanely and their DRGs removed. For acutely isolated samples, DRGs were collected on ice and homogenised. As an *in vitro* model of nerve injury DRGs were cultured as free floating explants for a period of 3 days (3DIV) (4).
DRG extracts were analysed by two-dimensional gel electrophoresis. Differences in protein expression were compared between (a) acutely isolated wildtype and acutely isolated galanin knockout DRG, (b) post-injured (3DIV) DRG from wildtype and galanin knockout mice and (c)acutely isolated and post-injured wildtype DRG. Following tryptic digestion and mass spectrometric analysis several differentially expressed proteins have been identified. These include proteins involved in cytoskeletal reorganisation, metabolism and ubiquitination.

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PC191

5-HT modulation of glutamatergic synaptic inputs to neonatal rat facial motoneurones in vitro

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Serotonergic modulation of ion channels in the dendrites of facial motoneurones (FMs) can markedly influence the integration of EPSPs. EPSPs may also be subject to modulation through mechanisms that influence glutamate release. We have used whole-cell, patch-clamp recordings and neonatal rat brainstem slices, prepared from humanely killed 4 to 14-day-old rats, to characterise excitatory glutamatergic transmission onto FMs and investigate its modulation by serotonin (5-HT). Effects were statistically significant ($P \leq 0.05$) using an unpaired Student's t test, unless otherwise stated.
Minimal stimulation with an electrode placed close to the dendrites of a FM evoked EPSCs that fluctuated in amplitude and occasionally failed to generate a response. At a holding potential of −70 mV, EPSCs had mean amplitudes, rise and decay time constants of −80 ± 33 pA, 1.8 ± 0.6 and 11.1 ± 3.0 ms, respectively (n = 12, mean ± S.D.). EPSC current-voltage relationships displayed outward rectification with a V_{rev} of −17 ± 11 mV (n = 4). Application of NBQX (20 μM) reduced mean EPSC amplitude to 16 ± 11 % of control (n = 12). In the presence of NBQX, holding at +20 mV revealed an EPSC with a mean amplitude of 83 ± 20 pA while at −70 mV removal of external Mg^{2+} uncovered an EPSC −42 ± 16 pA in amplitude. Both EPSCs were inhibited by AP-5 (50 μM, n = 4). EPSCs displayed paired pulse facilitation which when averaged after excluding failures occasionally resulted in paired pulse EPSCs of equal amplitude suggesting stimulation of a single release site.
Bath-application of either 5-HT (10 μM) or the 5-HT_{1B}-receptor agonist, CP93129 (10 μM), significantly reduced the mean EPSC amplitude at −70 mV to 31 ± 26 % (n = 15) and 20 ± 15 % (n = 4) of control, respectively (one sample Student's t test). The 5-HT_{1A}-receptor agonist, 8-OH-DPAT (10 μM), failed to significantly alter EPSC amplitude (85 ± 14 %, n = 3, one sample Student's t test). Application of the 5-HT_{1B}-receptor antagonist, isamoltane (1 μM), reduced the 5-HT-mediated inhibition of EPSC amplitude from 26 ± 15 % to 49 ± 18 % (n = 4), whereas the 5-HT_{1A}-receptor antagonist, WAY-100635 (1 μM), had no effect on the response to 5-HT (20 ± 13 % of control, n = 3). The reduction in EPSC amplitude was associated with a significant increase in the EPSC failure rate from 25 ± 19 % to 65 ± 27 % for 5-HT (n = 15) and 26 ± 25 % to 88 ± 14 % for CP93129 (n = 4). Examination of miniature EPSCs in the presence of TTX (0.3 μM) showed a reduction in frequency from 3.1 ± 1.6 to 2 ± 1.2 Hz in the presence of 5-HT, while amplitude was

not significantly different (69 ± 19 to 55 ± 11 pA) (n = 3, Kolmogorov-Smirnov two sample test).
In summary, both non-NMDA and NMDA receptors contribute to excitatory synaptic transmission in the facial nucleus. Our data indicate that in addition to altering the integrative properties of FMs, 5-HT also regulates excitatory synaptic transmission through mechanisms that modulate glutamate release.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC192

Dopamine regularises firing in subthalamic nucleus neurones from 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine lesioned mice *in vitro*

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The subthalamic nucleus (STN) is a key component of the basal ganglia, thought to play a central role in the control of normal movement. In Parkinson's disease (PD) and in dopamine depleted animal models of PD, STN neurones are overactive and show synchronised oscillatory activity. As lesion or deep brain stimulation of STN can alleviate symptoms, this aberrant neuronal activity may be fundamental to expression of PD.
Single unit extracellular recordings were made from up to 3 spontaneously firing STN neurones simultaneously, in 300μm thick parasagittal slices of mouse ventral midbrain, perfused with aCSF at 32°C. Eight mice were treated 10-21 days previously with the dopamine neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) i.p., using the regime described by Araki et al. (2001). All animals were humanely killed. Statistical comparisons were made using Student's t tests, or one-way ANOVA followed by post-hoc Bonferroni tests. All data are expressed as mean ± S.E.M.
STN neurones in slices from MPTP-lesioned animals fire at 4.73 ± 0.42 Hz (n =54), significantly slower than those from control animals (9.78 ± 1.16, n =20; $p<0.0001$). STN neurones from MPTP lesioned animals fire irregularly, with an average coefficient of variation of inter-spike interval of 78.9% (n =28), significantly higher ($p=0.0007$) than that seen in neurons from control animals (25.8%, n =33). Firing in simultaneously recorded STN neurones is uncorrelated, both in slices from control animals (n =2) and those from MPTP-lesioned animals (n=16).
In cells from control animals, 30μM dopamine increases firing rate by 334.1 ±67.34% without significant effect on the regularity of firing (n=15). In cells from MPTP-lesioned animals, dopamine increases firing rate by 681.4 ±150.9 %, and significantly reduces the coefficient of variation of inter-spike interval (78.9% to 26.2%, $p<0.001$, n =28). Application of CNQX (10μM) AP5 (100μM) and picrotoxin (50μM) cause no significant change in firing pattern or rate in neurons from MPTP-lesioned animals.
In summary, STN neurones in slices from MPTP-lesioned mice fire asynchronously, and significantly more slowly and less regularly than those from control animals. This latter effect can be reversed by applied dopamine, as has been reported in STN neurons *in vitro* from 6-hydroxydopamine-lesioned rats (Zhu et al. 2002).

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PC193

TEMPORAL EXPRESSION AND LOCALIZATION OF GLUCOKINASE IN HYPOTHALAMIC GLIAL CELLS

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Glucokinase plays a fundamental role in glucose homeostasis by catalysing the high Km phosphorylation of glucose in hepatocytes and islet beta cells. In the brain, the hypothalamus is involved in modulating feeding behaviour through its ability to detect changes in circulating glucose. In this area, the expression of the glucose transporter GLUT2, glucokinase (GK), and ATP-sensitive K+ channels, molecules involved in glucose sensing mechanisms, has been demonstrated. Different studies have suggested that GK is expresed in the hypothalamus; however, it has not been clearly determined if glial and/or neuron cells express this protein (Jetton et al. 1994; Roncero et al. 2000). The neurons in the hypothalamus are in close contact with highly elongated ependymal cells, namely tanycytes. In a previous report, we showed that tanycytes are the main glial cells in the periventricular zone of the hypothalamus and they are expressed GLUT2 (Garcia et al. 2003). In these studies, we analysed the GK expression in hypothalamus tissue. The animals used for the slices were humanely killed. Additionally, we used RT-PCR to demonstrate that mRNAs GK is expressed in liver, islet beta cells and hypothalamic tissues. Real time PCR showed that GK expression in the hypothalamus increased gradually from postnatal day 1 to adult rats. Immunocytochemistry indicates that GK is localized principally in tanycytes that are in direct contact with the cerebrospinal fluid and hypothalamic neurons involved in the glucose sensing mechanism. Primary cultures of hypothalamic tanycytes were used to confirm the gene and protein expression of GK. Immunocytochemical studies showed nuclear and cytoplasmic localization of GK cultured tanycytes. Real time PCR demonstrated an upregulation of GK mRNAs when the cells were incubated with 15mM glucose for 12 h. Based on this evidence, we propose that tanycytes are responsible, at least in part, for a mechanism that allows the hypothalamus to detect changes in glucose concentrations.

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PC194

Angiotensinergic mechanisms regulating NaCl and fluid balance in the zona incerta

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The zona incerta (ZI) and the renin-angiotensin system in the brain play a definitive role in the regulation of fluid balance, thirst and hunger for NaCl. Angiotensinergic axon-terminals converge on the surface of the ZI from the circumventricular organs, providing information on the homeostatic state of the periphery, and from brain structures inside the blood-brain barrier. Angiotensin receptors (AT1, AT2) are also found in the ZI. Earlier experiments showed angiotensin II (AII) and III (AIII) microinjected into the ZI have dipsogenic effects, but via different receptors (1,2). This has been further investigated.
Using the two-bottle test procedure (3) intake of water and NaCl (0.15 M) was studied in male Wistar rats (280-330 g) during a 1-week habituation period. Animals were then anasthetized (80 mg ketamine + 20 mg diazepam/kg b wt, i.p.) and bilateral stainless steel guide cannulas (22 gauge) implanted stereotaxically 0.5 mm above the ZI (Paxinos and Watson: AP: -3.3 mm caudal to bregma, ML: 1.2 and DV: 6.4) and attached to the skull. 7 days after surgery, animals underwent the same daily drinking regimen. The volumes consumed (ml/100 g b wt) were determined every 5 min for the 60-min drinking period following injection into the ZI of AII (100 ng, 95.6 pmol) and III (200 ng, 214.8 pmol) or vehicle (Veh: 0.15 M saline solution). Food was available ad libitum, but water and NaCl solution only during the drinking period. Water and NaCl consumption in angiotensin- and Veh-treated rats was compared. Finally angiotensin receptor antagonists were tested on AII- or AIII-induced fluid intake. Animals were humanely killed at the end of the experiment.
During habituation animals always prefered water to NaCl solution. In the first experiment AII increased preference for NaCl compared to water (1.94±0.55 vs. 0.46±0.18) from the 5th min (means±S.E.M., ANOVA). During the first 35 min of the drinking period water intake decreased in AII-treated compared to the Veh-treated rats (0.46±0.18 vs. 1.63±0.25). In the second experiment sodium intake increased in AIII-treated compared to Veh-treated rats but this was significant only after 45 min (4.83±0.98 vs. 1.69±0.58) and was not at the expense of water intake (2.99±0.53 vs. 0.57±0.37 from the 5th min). AIII-injected animals ingested nearly the same amount of water as the Veh-injected ones, AIII-treated animals drank more water only at the 5th min (2.99±0.53 vs. 1.63±0.25). In further experiments, rats were injected with losartan (90 ng, 195.2 pmol), an AT1 antagonist or PD 123319 (180 ng, 244.3 pmol), an AT2 antagonist, 15 min before injection of an agonist. The increase in NaCl ingestion induced by AII was blocked by both antagonists but that invoked by AIII was blocked only by PD 123319 (Figs 1 and 2). The effects of AII, AIII, losartan and PD 123319 on NaCl hunger have not previously been tested in the ZI. The finding that NaCl intake increased after both AII and AIII injections but was blocked differently by the antagonists in two-bottle tests suggests that AT1 and AT2 receptors in the ZI play partially different roles in the regulation of NaCl and water intake.

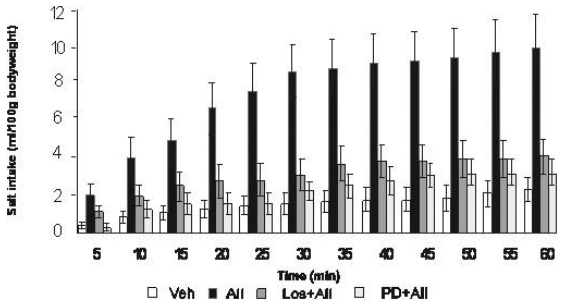

Figure 1. Effects of AII.

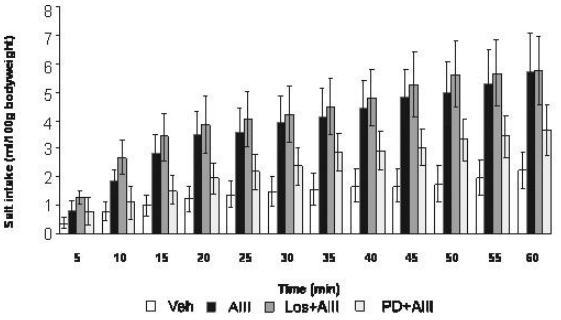

Figure 2. Effects of AIII.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC195

ACUTE EXPOSURE TO NITRIC OXIDE INCREASES EXCITABILITY OF HYPOGLOSSAL MOTONEURONS IN RAT BRAINSTEM SLICES

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Disruption of trophic communication between the motoneurons and their target muscle fibres by axon injury increases excitability of motoneurons and alters the recruitment order

of motor units. These alterations are concomitant with the de novo expression of the neuronal isoform of nitric oxide synthase (NOS-I) which synthesizes the free radical gas nitric oxide (NO). NO is a modulator in certain neurotransmitter systems and recently it has been demonstrated to play a key role in the synaptic remodelling induced in hypoglossal motoneurons (HMNs) by XIIth nerve injury (1). The aim of this work was to determine the role of NO on intrinsic membrane properties and synaptic inputs to the HMN pool. To this end, we studied the effect of acute application of NO donor diethylamine NOnoate (DEA/NO, 10μM) on HMNs. Hypoglossal nuclei-containing brainstem slices were prepared from terminally anaesthetised female Wistar rats (P7, 250-300 μm). Antidromically identified HMNs were recorded in the whole-cell patch clamp mode following the procedures previously established in this laboratory (2). Monosynaptic excitatory postsynaptic potentials (EPSPs) were triggered in HMNs by electrical stimulation within the ventrolateral reticular formation (VRF).

On average, 44 neurones included in this study had membrane potential (Vm) =-53.7 ± 0.6 mV (mean ± S.E.M.), membrane resistance (Rm) = 67.6 ± 3.8 Mohm, threshold current required to trigger an action potential generation, rheobase (Rh) = 0.27 ± 0.035 nA. 20 of 30 HMN tested responded to the stimulation of VRF. A 10 min application of NO donor DEA/NO resulted in a slight but significant depolarisation of the membrane of HMN (7.6 ± 0.9% of control, $p < 0.001$, n = 25, Student's paired t test). Rh decreased after DEA/NO administration by 20.4 ± 7.4% ($p < 0.001$, n = 25), while the apparent membrane resistance did not change significantly ($p > 0.05$). Interestingly, monosynaptic EPSPs were not altered by NO administration (0.53±1.75% of control, n= 11). These findings suggest that NO can induce changes in intrinsic membrane properties of HMN and that similar effects might occur in response to axonal injury.

Sunico et al. (2005). J Neurosci 25, 1448-1458.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC196

A key glutamate residue (E30), conserved in many K channels, regulates the gating of TASK3 two pore domain potassium channels

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Two pore domain potassium (K2P) channels are regulated by a wide variety of pharmacological and physiological mediators, and are responsible for leak K^+ currents in many neurons. Little is known, however, about the regions of mammalian K2P channels that underlie their gating. In this study, we have considered the role of E30 in the K2P channel, TASK3. This conserved glutamate residue has been shown to be important for gating in *drosophila* KCNK0 K2P channels (Zilberberg *et al.* 2001) and for C-type inactivation of *Shaker* voltage-gated K^+ channels (Larsson & Elinder, 2000).

Whole-cell electrophysiological recordings were made from tsA-201 cells, transiently transfected with wild type (WT) and mutated TASK3 channels. TASK3 channel current density, at -40 mV, was significantly reduced from 67 ± 9 pA/pF (mean ± S.E.M., n = 18) to 22 ± 5 pA/pF (n = 13), following mutation E30C ($p < 0.05$, Student's t test). Current through E30C mutated channels could be recovered following treatment with 2.5 mM MTSES, to covalently restore a negative charge at this site (50 ± 5 pA/pF, n = 12). In contrast, MTSES had no effect on the amplitude of current through WT TASK-3 channels.

In addition, the E30C mutation significantly increased the effectiveness of zinc block of TASK3 channels (Clarke *et al.* 2004), with 10 μM zinc producing 75 ± 3% (n = 7) inhibition of E30C channels compared to 29 ± 3% (n = 3) for WT TASK3 ($p < 0.05$). This was not due to the introduction of a novel inhibitory binding site for zinc (C at position 30) since the triple mutation E30C/E70K/H98A (like E70K/H98A, Veale *et al.* 2005) was not inhibited by zinc. The sensitivity of E30C channels to changes in pH_o was unaltered, and there was no significant change in the sensitivity of the channels to methanandamide (3 μM producing an inhibition of 78 ± 3% (n = 6) and 66 ± 8% (n = 4) for WT and E30C channels, respectively, $p > 0.05$).

Thus mutation E30C alters the gating properties of TASK3 channels and, as a result, changes the degree of block produced by some (zinc) but not all (pH, methanandamide) regulators of activity. Our data supports the suggestion (Zilberberg *et al.* 2001) that this conserved glutamate residue is important for gating of K2P channels and that these channels gate in response to conformational changes in the external pore.

Clarke CE *et al.* (2004). *J Physiol* **560**, 51-62.

Larsson HP & Elinder F (2000). *Neuron* **27**, 573-583.

Veale EL *et al.* (2005). *J Physiol Biochem* **61**, 109-110.

Zilberberg N *et al.* (2001). *Neuron* **32**, 635-648.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC199

Electrical coupling among excitatory interneurons in a central pattern generator in the frog tadpole

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Electrical coupling has been found in many brain areas and proposed to be important in generating certain oscillatory or rhythmic activities (Loewenstein *et al.* 2001). *Xenopus* tadpoles at stage 37/38 can swim at a rhythm of 10-25 Hz. Their swimming circuit in the spinal cord and hindbrain has less than 10 types of neurons. Previous study (Perrins & Roberts, 1995) showed electrical coupling between motoneurons in the spinal

cord. Our recent experiments have revealed the existence of extensive electrical coupling among one of the interneuron types: premotor excitatory interneurons with descending axons (dIN).

Paired whole-cell recordings from neurons identified with neurobiotin staining showed that dINs were electrically coupled exclusively to other dINs (35 in 51 dIN-dIN pairs). In contrast, apart from weak coupling in one dIN-motoneuron pair, no clear electrical coupling was found in 29 pairs of one dIN and another central pattern generator neuron of other types (23 motorneurons, 3 commissural interneurons and 3 ascending interneurons). The coupling was bi-directional with a small coupling co-efficient (7.3 ± 2.5%, n=27) and with little evidence for dye coupling. Gap junction blockers carbenoxolone (n=12, 300 - 500 μM) and heptanol (n=3, 2mM) only blocked the coupling at high concentrations and this block was not reversible. Flufenamic acid reversibly blocked the electrical coupling (n=4, 100 - 400μM). All three chemicals produced side effects such as affecting miniature EPSP frequencies, resting membrane potentials, spike shape and dIN firing to current injection.

The lack of specificity of gap junction blockers makes interpreting the role of electrical coupling among dINs during tadpole swimming difficult. The function of this type of gap junctional connection may involve synchronizing dIN activities and produce more accurate swimming activity pattern.

Loewenstein Y, Yarom Y & Sompolinsky H (2001). *Proc Natl Acad Sci U S A* 98, 8095-8100.

Perrins R & Roberts A (1995). *J Physiol* 485, 135-144.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

PC200

The initiation of spinal motor responses in Xenopus tadpoles

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The neuronal mechanisms underlying initiation of motor responses in mammals are still poorly understood. We are exploring these processes in a simpler model system, the Xenopus tadpole. The neurons and connections providing a simple cutaneous crossed flexion reflex have been described (Li et al. 2003). However, the relationship between this reflex, an equivalent ipsilateral reflex (Li et al. 2004), and the mechanism for initiation of locomotion (swimming) is unclear. We have started to analyse the pattern of responses following brief, unilateral stimulation of a known cutaneous sensory pathway, from mechanosensory Rohon-Beard neurons.

Ventral root recordings of motor responses were made in stage 37/38 Xenopus tadpoles immobilised in 10 μM α-bungarotoxin. A suction electrode was used to stimulate (single 0.5 ms stimuli) the sensory neurites of Rohon-Beard neurons in the tail skin on one side. Tadpoles were spinalised close to the hindbrain-spinal cord boundary (caudal to the 2nd post-otic myotome segment).

For stimuli close to threshold for a response, around 76% of tadpoles (13/17) showed a short burst of motor discharge on the contralateral side at a latency in the range 8-15 ms. This would produce a reflex flexion of the body away from the stimulus. In a further four cases, a short latency reflex response was recorded only on the stimulated side. The short latency response was typically followed at a longer latency (25-50 ms from stimulus) by the start of swimming. Here discharge alternated between left and right sides at a frequency of 10-20 Hz. In most cases (10/17), the first swimming burst was on the stimulated side, irrespective of the side of the shorter latency reflex response; in fewer cases (3/17) it was on the opposite side. In the remainder the sidedness was unclear.

Our recordings help define the events occurring shortly after a cutaneous stimulus and indicate that initiation of both reflex and swimming responses show distinct sidedness. At present, the mechanisms that determine this sidedness of response, and the relationship between the neuronal mechanism underlying reflex responses and the initiation of long-lasting swimming remain unclear.

Li W-C, Soffe SR & Roberts A (2003). J Neurosci 23, 9068-9077.

Li W-C, Soffe SR & Roberts A (2004). J Neurophysiol 92, 895-904.

We thank the BBSRC for support.

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