C103

Balance task adaptation with and without arm movement

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Anecdotally arm movements appear to be an intrinsic component of strategies that serve to maintain upright posture. The importance for such arm movements appears to be heightened in situations of balance perturbation or uncertainty. The purpose of this study was to investigate the role of arm movements whilst performing a moderately difficult balance task.

Twenty-four healthy subjects (18 male; mean 34 years old) were asked to maintain their balance when adopting the Romberg position upon a wooden beam with a width of 85mm and a height of 50 mm. The beam was placed on a block of foam (with a width of 270 mm and a height of 85mm) thereby creating balance uncertainty. Subjects were requested to look ahead with their eyes open and use the handrail surrounding them only when their balance was compromised.

The task was performed in 3 trials lasting 60s each, with two conditions (balanced order). Subjects were instructed to either cross (FIXED) or to use their arms (FREE) in order to maintain balance upon the beam. Mean (± SEM) measures of medio-lateral (M-L) and anterior-posterior (A-P) body sway were obtained using a force platform (Amt Inc. USA) and at the level of C7 and the head using Fastrak (Polhemus Inc. USA). Students paired t-tests were performed between population mean sway parameters for each trial and averaged over trials in each condition. The number of touches made by the subject onto the handrail was recorded as task failure.

We found a significant reduction of mean (3 trials) sway path length in the M-L (2859 ± 345 to 2353 ± 198mm) and A-P (2353 ± 197 to 2089 ± 191mm) directions with the FREE condition. Similar trends were observed at the level of C7 and the head, although only the latter in the A-P (1831 ± 353 to 1440 ± 220mm) direction obtained significance. However, the effect of arm movement was predominantly manifest within the first trial - irrespective of parameter. Indeed, sway parameters converged by trial 3 presumably as a result of task adaptation. Further evidence for such a process is gleaned by the fact that task failure becomes less frequent in both conditions, although the rate is consistently reduced in the FREE condition (table 1.).

In conclusion, free arm movements improve overall measures of stability, and perhaps more importantly significantly reduce task failure and speed up balance task adaptation compared to when arms were restricted.

Table 1. Task Failure Frequency (per trial)

Arm Condition	Trial 1	Trial 2	Trial 3
Fixed	0.435	0.217	0.043
Free	0.217	0.000	0.000

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

C105

Massage can increase the passive stiffness of spastic muscle in cerebral palsy

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Spastic muscles in cerebral palsy were thought to be intrinsically stiffer than normal. However, direct assessment of mechanical properties showed that whereas the muscle fibres are stiffer, the surrounding connective tissue is much more compliant (Lieber et al., 2004). Muscle architecture is disordered (Shortland et al., 2002). We tested whether massage stretching spastic muscles mainly transversely rather than longitudinally could increase the range of movement. Calf muscles of 5 adolescents with spastic diplegia were massaged for 14 minutes twice weekly for 5 weeks. The adolescents gave their informed consent; permission for the study was obtained from their parents and doctors, the local Ethics Committee and Education Authority. The ankles were dorsiflexed 3 times before and after massage by an experimenter attempting to apply force at a constant rate of about 8Ns-1 by following a template on a computer screen. The change in angle at the ankle, force applied and EMG of the soleus muscle were recorded. After massage the angles reached by dorsiflexing the ankles were not consistently increased, but surprisingly, the pressure required was significantly incresed in all 10 limbs ($P<0.0003$; paired t-test). Although abnormal stretch reflexes were sometimes induced, EMG activity in a window 2s before the peak of stretch was not significantly greater after massage. Thus the type of massage employed increased intrinsic muscle stiffness rather than reflex excitability. A possible explanation is that massage promoted the repair of damage to sarcomeres by eccentric contractions (see Proske & Morgan, 2001), to which spastic muscles are likely prone.

We assessed whether the adolescents adapted to changes in muscle mechanical properties. All 4 ambulant volunteers increased their Gross Motor Function Measure-66 scores (Russell et al., 2002) tested during the 5th week that massage was applied, and 12 weeks later, compared with one week before (mean increase +/-SEM: 6.4+/-0.7 at 5 weeks; 5.5+/-0.8 at 17 weeks; increase >95% confidence intervals for one participant). The 5th adolescent who cannot move his ankles voluntarily did not improve his score. This suggests that adaptation can occur providing there is some voluntary control.

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This work was supported by the Greater Glasgow Health Board and Boyd Fund.

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

C106

Short- and medium-latency responses to galvanic vestibular stimulation in human lower limb muscles during tonic and rhythmic motor behaviours

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Bipolar binaural galvanic vestibular stimulation induces medium-latency (ML) emg responses in lower limb muscles and sway towards the anode ear. ML responses have a latency of 60-120ms, they are preceded by small short-latency (SL) responses of opposite polarity that have little mechanical effect. Most previous work has studied sway in the sagittal plane when the head is turned to one side. We have studied responses in the coronal plane in seven subjects facing forwards, with informed consent and ethical approval (CO1.057). Subjects were asked to stand, walk on a treadmill or bicycle using previously published methods (Ali et al. 2003; Iles et al. 2000).

In standing subjects facing forward, active lower limb muscles were excited at ML in the cathode side limb and inhibited on the anode side. This was the case for posterior compartment muscles pre-activated by leaning forward (Ali et al. 2003) and anterior compartment muscles pre-activated by leaning backwards. ML responses were preceded by small SL responses of opposite polarity. Identical responses have been reported for the ankle extensors (Day et al. 1997).

During slow walking the vestibular stimulus was triggered at heel strike of the investigated leg. This timing is most effective in changing the footfall of the opposite limb and deviating the path towards the anode side in free walking (Bent et al. 2004). SL and ML responses were observed in all subjects with the same pattern as during standing: in the cathode side limb ML excitation of soleus, tibialis anterior, biceps, semitendinosus, gluteus and iliacus; in the anode side limb ML inhibition. However, ML responses during walking differed in two respects from those during standing: they were very prominent in ankle muscles (doubling the emg level) and occurred at a significantly longer latency (Wilcoxon Matched Pairs Test $P<0.01$; Sign Test $P=0.022$, both two tailed; extra latency averaged over all muscles was 36ms). ML responses during walking were gated by the step cycle: delaying vestibular stimulation by >300 ms abolished them.

During cycling vestibular stimulation was applied at top dead centre for the investigated limb. Very small SL and ML responses were observed in the cathode side limb of 2/7 subjects.

These data suggest that vestibular signals have strong ML actions on the rhythmic motor output to ankle muscles during walking where balance control is essential, but not during bicycling which makes less demand on balance

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Supported by the International Spinal Research Trust.

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

C107

Rotation of human motor units during prolonged firing

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During prolonged contractions, recruitment of additional motor units is considered to be a mechanism that compensates for the decrease in force output of the fatigued muscle units. Not much attention has been paid to the effect of prolonged contractions on the motoneurone part of the motor unit. In the following results we demonstrate substitution and rotation among motoneurones which are active over prolonged periods of time. Intramuscular motor unit activity and surface EMG (electromyogram) were recorded from one of the following four muscles: flexor and extensor carpi radialis, tibialis anterior and soleus. The subject was asked to discharge a discernible unit at a comfortable rate with audio and video feedback. The firing rate of this motor unit was perturbed by various phasic synaptic inputs (from the motor cortex or spindle afferents) during the 2-3 h recording period, which was the main purpose of these experiments. Since each motoneurone discharged for a very long time during such experiments, we took the opportunity to examine the effect of prolonged firing on its discharge behaviour by checking for substitution and rotation among low threshold motoneurones. Results are reported from a total of 10 sets of motor units from all four muscles. When a subject fired a motor unit for a long period, frequently an additional motor unit started to discharge after 10-20 min. When the subject was asked to keep activity down to one unit, very frequently, it was unit 1 that dropped and unit 2 continued to fire. After unit 1 silenced, it was not always possible to bring it back immediately by increasing EMG. After a few minutes, one could re-recruit it by increasing voluntary effort. After 5-10 min, unit 1 came back without any conscious effort by the subject. Now if the subject was again asked to retain just one unit, it was unit 2 that dropped. During long experiments, we observed such back and forth rotation of activity in all four muscles. In addition, it was observed that the dropped motor unit also had difficulty responding phasically. Just after it was dropped, it responded phasically to the inputs quite infrequently. As time passed, it responded more frequently until it became tonic again.

These observations suggest that the threshold of a tonically firing motoneurone increases with time, which may result from sodium inactivation that has a long time constant. At the same time a newly recruited unit may have a decrease in threshold due to persistent inward currents. These two mechanism may allow the observed substitution and rotation among low threshold motoneurones.

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

C108

Increased Magnitude of the Human Long Latency Stretch Reflex Following Timing Signals

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Abrupt unloading of the human forearm can produce muscle activations in the triceps brachii as a result of stretch (Paulignan et al. 1989). The stretch reflex of this muscle occurring at a latency of approximately 50 ms has a transcortical component (Thilmann et al. 1991) and increased response amplitudes may indicate increased levels of cortical excitability (Day et al. 1991).

With local ethics committee approval we investigated the effect of both an external and an internal timing signal on the amplitude of the long-latency stretch reflex of the triceps brachii. Eight healthy male subjects were seated upright with their right upper arm fixed to a support. An electromagnet attached underneath their right wrist supported a 1.5-kg load. They were instructed to maintain their loaded forearm in the horizontal plane, creating an elbow angle of 115°. The subjects performed sixty trials in each of three randomised unloading conditions. First, in the control situation the unloading was imposed and unpredictable in time. Second, the unloading was imposed but signalled by a tone 300 ms prior to release. Third, the unloading was performed voluntarily as the subject pressed a switch that initiated the load release after a delay of 300 ms. The mean rectified electromyographic (EMG) activity from the lateral head of the right triceps brachii muscle was measured in the last twenty trials in the following time bins: 100 to 0 ms prior to the load release and at 50 to 95 ms after the load release. The mean elbow rotation velocity was measured between the moment of load release and the onset of the long latency stretch reflex 50 ms later.

The results are presented as mean ± SD and values significantly different from the unpredictable condition values are shown as * $P<0.03$ (repeated measures ANOVA with LSD post hoc). In the imposed unpredictable condition, the EMG activity at the time of the expected long latency stretch reflex dropped *below* baseline values to 6.6 ± 2.9 μV. However, in the imposed tone and voluntary conditions we observed long latency stretch reflex amplitudes of 14.4 ± 7.9 μV* and 22.4 ± 16.4 μV*. We interpret this as an increase in gain of the reflex as the mean elbow rotation velocity was similar in all three conditions. (Unpredictable 91.8 ± 15.9°.s^{-1}, Tone 89.6 ± 12.1°.s^{-1}, Voluntary 85.6 ± 9.5°.s^{-1}).

We conclude that both an internal (voluntary) and external (tone) timing signal had the effect of increasing the gain of the long latency stretch reflex. This may be due to the afferent signals resulting from the perturbation reaching a motor cortex made more excitable by the previous timing signals.

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

C109

Modulation of primary motor cortex outputs from ventral premotor cortex during visually-guided grasp in the macaque monkey

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The ventral premotor cortex (area F5) plays an important role in the control of hand shape during object grasp. F5 neurones can encode the selection of different grasps, and are excited by vision of graspable objects. However, it is not clear how F5 influences the motor outputs to the hand and arm that control hand shape. In earlier studies in sedated or deeply anaesthetised monkeys, we demonstrated that F5 can exert strong facilitation of corticospinal outputs from primary motor cortex (M1) to the hand (Cerri et al. 2003; Shimazu et al. 2004). We now demonstrate that F5 can give rise to robust modulation of the EMG responses evoked from M1 in the awake monkey performing a visuomotor grasping task.

The study was carried out in 2 adult macaques; all procedures were in accordance with the Animals (Scientific Procedures) Act 1986. An object was presented visually to the monkey, and after a variable delay, the monkey was cued to reach, grasp and displace the object. Single bipolar stimuli (100-200 μA) were delivered to pairs of microwires implanted in contralateral F5 and M1. Surgery was performed under deep anaesthesia, induced with 10 mg. kg-1 ketamine i.m. and maintained with 2-2.5% isoflurane in 50:50 O2:N2O; full aseptic procedures were observed and antibiotics and analgesics given postoperatively. Implantation sites were based on prior electrophysiological mapping. EMG responses were recorded during hand shaping and transport prior to object grasp from 4-10 contralateral hand and arm muscles.

Single conditioning (C) stimuli delivered to F5, that did not by themselves evoke any detectable EMG response, produced a large facilitation (over twofold) of the EMG-response evoked by test (T) stimuli to M1. Facilitation was observed at short C-T intervals of 0 and 1 ms (Wilcoxon signed-rank $p<0.05$) and showed a periodicity compatible with I-wave generation (Ziemann & Rothwell, 2000). In contrast to the anaesthetised/sedated macaque, we also saw some suppression of M1 responses by F5. The pattern of facilitation/suppression was muscle specific.

Thus F5 can exert robust modulation of M1 outputs to the hand during hand transport and shaping prior to grasp that is specific to particular muscles, and therefore unlikely to reflect a general change in excitability. The modulation of responses probably involves F5-M1 cortico-cortical pathways active during visuomotor transformations underlying object grasp. This study supports F5 as having a role in shaping activity of the hand appropriate for grasp.

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C110

Identifying the spinal excitatory interneurons in a vertebrate central pattern generator

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Most vertebrate locomotion central pattern generators (CPG) are presumed to include excitatory interneurons which provide the excitatory drive for the rhythm-generating circuit. However, only in the lamprey have these interneurons been well defined with physiological and anatomical evidence (Parker, 2003). In the *Xenopus* tadpole spinal swimming circuit, a group of interneurons with ipsilateral descending axons (dIN) were proposed to provide glutamatergic excitation to spinal neurons during swimming (Dale and Roberts, 1985). Due to the difficulties in making intracellular paired recordings with sharp electrodes and cell labelling with horseradish peroxidase, the evidence on the anatomical identity of these neurons was very limited. The application of whole-cell patch recording techniques has greatly eased these difficulties (Li et al., 2002) and this issue is revisited in this study. Means are given with their SDs.

Recordings were made using stage 37/38 *Xenopus* tadpoles immobilised with 10 μM α-bungarotoxin. Paired recordings were made with whole-cell recording pipettes and neuron anatomy was revealed after neurobiotin filling. Results showed that dINs with descending ipsilateral axons directly excited more caudal CPG neurons of all types. These included: motorneurons (n = 24/28 pairs); commissural reciprocal inhibitory neurons (n = 4/4); ascending neurons, providing negative feedback to the sensory pathway and CPG (n=3/4); and other dINs (n=34/40). The unitary EPSPs produced by dIN impulses were often large (mean max amplitude 13.1 ± 1.7 mV; n = 10 pairs). Their latencies were short and consistent (1.32 ± 0.10 ms; n = 10 pairs) and neurobiotin staining also revealed close contacts between descending dIN axons and the postsynaptic neuronal dendrites or somata, suggesting these interactions are mono-synaptic (Li et al., 2002). During swimming dINs fired reliably and their spikes appeared earlier during each cycle than other CPG neurons at similar longitudinal locations (t-test, 16 dINs, 12 CPG neurons, $p<0.05$), suggesting that dINs are the source of the excitation that normally drives swimming. These new results confirm an excitatory role for dINs in tadpole swimming.

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

C111

Strychnine-sensitive mechanisms regulating primary afferent depolarization in the rat spinal cord

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Primary afferent depolarization (PAD) is a mechanism that accompanies some forms of pre-synaptic inhibition of the effects of primary afferents on their central targets. PAD is primarily mediated via GABAA-receptors and gives rise to a prolonged depolarization that may be monitored as an electrotonus with a pair of electrodes placed beneath a cut dorsal root (the dorsal root potential, DRP; see Rudomin and Schmidt 1999 for review). The pre-synaptic inhibition that accompanies PAD was shown to be enhanced by low-doses of strychnine in the cat (Eccles et al., 1963). Facilitation of stimulus-evoked DRPs in the presence of strychnine has also been reported (e.g. Jimenez et al., 1987). These results suggest that the DRP-generating spinal circuits are subject to glycinergic control.

Experiments were conducted in male Sprague-Dawley rats anaesthetized with urethane (1.25 g/kg i.p.). The trachea, carotid artery and jugular vein were cannulated and the spinal cord was exposed from the low thoracic level to the cauda equina and bathed in warm mineral oil. The cord was transected at mid-thoracic level (T8). Rats were observed to be deeply and stably anaesthetized for at least 1h and were then immobilised with gallamine triethiodide (20mg i.v.) and mechanically ventilated. Level of anaesthesia was monitored and maintained as described in Lidierth & Wall (1996). Heart rate and end-tidal CO2 were monitored and temperature was maintained at 37°C.

Systemic administration of strychnine was observed to have two effects. At low doses (0.2-2.0 μmol/kg i.v.), the DRPs evoked by electrical stimulation of neighbouring roots were dose-dependently enhanced (Fig. 1). In addition, the spontaneous DRPs that occur at approximately 10Hz in the spinally transected rat (Lidierth and Wall, 1996) were increased in amplitude. At high doses of strychnine (2.0-5.0 μmol/kg i.v.), these spontaneous DRPs dominated and stimulus-evoked DRPs were reduced in amplitude.

These data suggest that strychnine may block a tonic glycinergic inhibition that is intrinsic to the spinal cord and that serves to dampen both spontaneous and evoked DRPs. Evidence for tonic glycinergic inhibition of deep dorsal horn neurones has recently been presented (Cronin et al., 2004).The present data suggest that these neurones include those responsible for generating PAD.

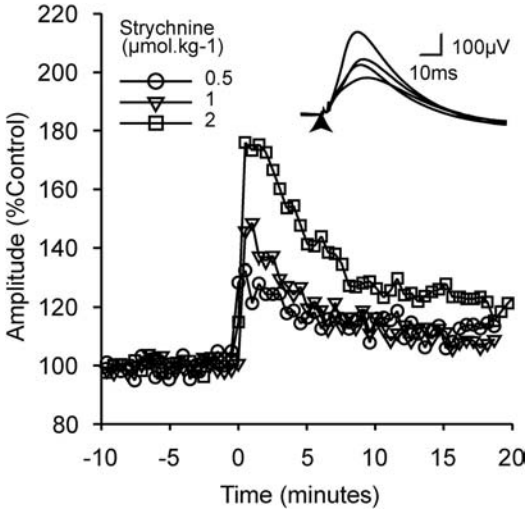

Fig. 1. Dose-dependent effects of strychnine on the amplitude of the DRP evoked on the L5 dorsal root by a submaximal stimulus to the L6 root. The inset shows averaged DRPs from the control period and for each of the doses of strychnine

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

C112

The role of persistent inward currents in transducing the respiratory drive in feline motoneurones

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Persistent inward currents (PICs), carried by non-inactivating voltage-sensitive Ca^{++} channels, are considered by many as necessary to produce a strong discharge in motoneurones, by amplification of synaptic excitation or by evoking plateau potentials (Powers and Binder, 2001). We have used intracellular recording and the application of graded depolarizing currents (Hultborn et al., 2003) to investigate the roles of PICs in producing respiratory drive potentials and respiratory discharges in motoneurones innervating a variety of muscles.
In cats decerebrated under isofluorane anaesthesia (Hultborn et al. 2003), recordings were made under neuromuscular blockade (pancuronium bromide 0.6 $mg.hr^{-1}$) and artificial ventilation, including added CO_2. A strong respiratory drive was evoked, monitored as inspiratory discharges (phrenic or T6 external intercostal nerve). Intracellular recordings were made from inspiratory motoneurones (C5 phrenic or T8 external or internal intercostal nerve), expiratory motoneurones (T8 internal intercostal nerve), or from hindlimb motoneurones (L7/S1). Intracellular electrodes contained K^+ acetate or, for studying drive potentials without firing, K^+ acetate with QX314. Discontinuous current clamp (typical sampling 3kHz) was used to monitor membrane potential during the application of currents through the recording electrode.
In some inspiratory motoneurones, graded depolarization produced amplification of the excitatory phase of the drive potentials by a factor of about 2, but with only a minimal appearance of any plateau-like behaviour. In expiratory motoneurones graded amplification was not detected, but some of these motoneurones demonstrated clear plateau potentials evoked by the expiratory depolarization.
Depending on the state of the preparation, hindlimb muscle nerves showed various patterns of discharge, most often firing in early expiration (post-inspiration, pI) or inspiration. In such a state, pI drive potentials were seen, often with a negligible amplitude until depolarizing currents were applied (cf. Kirkwood et al. 2002). The amplification was therefore large ($\geqq 20\times$) in such cells, which also showed prominent plateaux.
We conclude that the expression of PICs in spinal motoneurones varies widely, according to the muscle innervated and the state of the animal.

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

C113

In vivo analysis of monosynaptic reflex transmission in adult SOD1-G93A ALS mice

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While *in vitro* studies on the transgenic SOD1-G93A mouse model of human amyotrophic lateral sclerosis (ALS) provided valuable insights into the molecular and cellular disruptions underlying familial ALS, little is known about the significance of those observations for the *in vivo* situation of the intact animal. To elucidate underlying mechanisms we investigated lumbar spinal reflexes in SOD1-G93A mice *in vivo* and compared the results to those from wild type (WT) littermates.
Adult (>90 days) WT mice of strain B6SJL and transgenic SOD1-G93A mice (~90 days) were used: initial anaesthesia with pentobarbital sodium 70 mg kg-1 i.p., continuation with i.v. infusion of methohexital sodium 40-60 mg kg-1 h-1 as required. Body core temperature, ECG and breathing volume and rate were controlled throughout the experiment. A laminectomy was performed from L1 to L5. Nerves of the left hind limb (posterior biceps, common peroneal, tibial and sural nerve) were prepared, distally cut, and mounted for recording of monosynaptic reflexes. The left dorsal root L4 (main input from the hind leg) was cut and mounted for central stimulation (stimuli: rectangular pulses of 0.1 ms duration, strength 2-10 times threshold). The recurrence interval of the stimulus started with 1.84 s, and was gradually reduced to 50 ms. The reduction of monosynaptic reflexes with increasing stimulus frequency and the required recovery time of the reflex after repetitive stimulation were determined.
Before the experiments there were different degrees of motor dysfunctions in the SOD1-G93A mice. WT mice showed a stable reflex amplitude with 1.84 s stimulus interval, and a decreasing reflex amplitude during higher stimulus frequencies. In all animals, recovery of reflexes was complete within a few minutes. SOD1-G93A mice with *light* motor deficits showed a distinct reduction of the monosynaptic reflex amplitude during repetitive stimulation, even with long stimulus intervals. In mice with *severe* motor deficits, a monosynaptic response could hardly be elicited. In general, recovery of monosynaptic reflexes after repetitive stimulation required a longer time in SOD1-G93A mice and was mostly incomplete. In SOD1-G93A mice *without* obvious motor deficits, the monosynaptic reflex amplitude was normal

with 1.84 s stimulus interval. Although recovery times were longer compared to WT, recovery was in most cases complete. The experiments demonstrate a clear deficit in the monosynaptic reflex of SOD1-G93A mice, reflex profiles correlating with apparent motor deficits. The results on reflex recovery i) are in agreement with models suggesting a contribution of impaired cellular energy supply to the pathogenesis of ALS and ii) provide a basis for future *in vivo* studies on ALS-related spinal disturbances.

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

C114

Retinal photoreceptor arrangements in South American rodents (Octodontidae: *Octodon degus, lunatus and bridgesi*).

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The distribution of retinal rod and cone photoreceptors shows considerable variation across mammalian species. We were interested to see how these differences relate to different adaptive pressures in closely related species. The Octodontidae are endemic rodents from South America and show a marked diversity at the species level in their lifestyle (diurnal vs nocturnal) and habitat use. We have studied the population densities and distributions of the rods and the different spectral cone types in the retinae of three Octodon species: *Octodon degus*, crepuscular-diurnal; *O.lunatus* and *O. bridgesi*, both nocturnal. Animals were killed by a lethal intraperitoneal dose of ketamine and xylazine. In isolated formalin-fixed retinae, rods were labelled with the rod opsin-specific mouse antibody rho4D2. The middle-to-long-wave-sensitive (L-)cones were labelled with the L-opsin-specific rabbit antiserum JH492, and the short-wave-sensitive (S-)cones with the S-opsin-specific rabbit antiserum JH455 or goat antiserum sc-14363.

In all three species, rods are the most abundant photoreceptors. All three species possess L-cones and S-cones (cone dichromacy). The cone proportion among the photoreceptors is about 30% in the diurnal O. degus and only about 2% in the nocturnal *O.bridgesi* and *O.lunatus*. In all three species, the S-cones form a minority of 5% or less of the cones, with the exception of ventral *O.bridgesi* retina where they attain up to 17%. The range of rod densities across the retina is 200,000-320,000/mm^2 in *O.bridgesi* and 200,000-430,000/mm^2 in *O.lunatus*. The cones show a centro-peripheral density gradient with central maxima: 7000-2700 L-cones/mm^2 and 700-40 S-cones/mm^2 in *O.bridgesi*, 8500 L-cones/mm^2 and 525-20 S-cones/mm^2 in *O.lunatus*. Double-labelling for L-cone and S-cone opsin was performed by incubating the tissue in a mixture of antisera JH 492 and sc-14363. In *O.degus* and *O.bridgesi*, each and every cone exclusively expresses either the L- or the S-opsin. In *O.lunatus*, a marked proportion of the S-cones co-express the L-opsin (dual pigment cones).

In conclusion, our observation of highly different cone proportions in diurnal vs nocturnal species from the same genus Octodon suggest that their photoreceptor arrangements were shaped by species-specific visual demands, rather than representing phylogenetic relationships. The functional role of dual pigment cones is still enigmatic.

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

C115

Traveling waves of visually induced very fast oscillations in the optic tectum of the pigeon (*Columba livia*)

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Brief periods of fast oscillatory potentials (100-600 Hz) have been described in the neocortex and hippocampus of mammals, spontaneously (Chrobak & Buzsaki, 1996; Grenier et al. 2001) or during electrical (Kandel & Buzsaki, 1997) and natural sensory stimulation (Jones & Barth, 1999). In particular, fast oscillations are prominent components of the somatic evoked potential recorded in the somatosensory cortex of anesthetized and awake animals, including humans (sigma bursts) (Curio et al. 1997). Although fast oscillatory activity is becoming a subject attracting increasing interest, its mechanisms and functional, or pathological role remain to be determined. Here we report that visual stimulation elicits robust traveling waves of fast oscillatory activity in the optic tectum (OT) of awake and anaesthetized pigeons. The avian OT is a complex multilayered sensorial structure that is considered operationally analogous to the mammalian sensory cortex. Adult pigeons were anaesthetized with a mixture of Ketamine (40 mg/kg) and Xylazine (12 mg/kg) injected intramusculary in the pectoral muscles and supplemented by 13 mg/kg ketamine and 4 mg/kg every 2 hours. The body temperature was maintained at 39-41°C by a DC-powered blanket. The heart rate was continously monitored and local wounds and pressure points were treated with anaesthesic ointment. A craneotomy exposing the dorso-lateral tectum was performed and tungsten microelectrodes were then lowered into the tectum under visual guidance. Visually triggered tectal fast oscillations consist of trains of 3-12 short (2-6 msec), very large (400 microvolts), high frequency (600 Hz) bursts, separated by 25 ms, corresponding to a 42 Hz gamma frequency. Due to their high amplitude and fast dynamics, these oscillations may have been confused previously with regular multi-unitary potentials. Tectal fast oscillations have three main characteristics also found in stimulus evoked fast oscillations in mammalian somatosensory cortex. First, they are always superimposed on the rising phase of a slower evoked field negative potential. Second, they are generated locally by radially oriented dipoles. And third, focal visual stimulation elicites traveling waves of fast oscillations that propagated laterally for distances up to 1 mm from the stimulated site. These results demonstrate that sensory evoked waves of fast oscillations are not particular to the mammalian cortex. Notwithstanding the differences in neural architecture, the remarkable similarities we found between tectal and cortical phenomenology suggest that these waves of fast oscillations may represent the action of a neural mechanism that plays a key role in

the operation of sensorial laminar structures. We suggest this role is related to a local attentional mechanism that modulates surrounding neural circuits prior to the arrival of the sensory stimulus.

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Supported by Fondecyt 1030761

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

C116

A Detailed Study of the Organization of the Rotundo-Entopallial Projections in the Pigeon (Columba livia)

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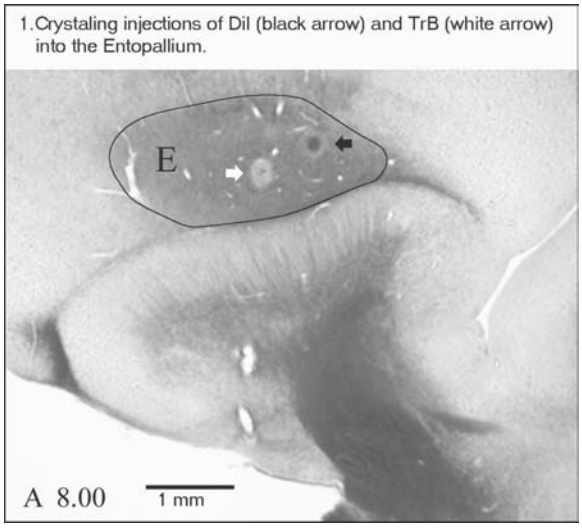
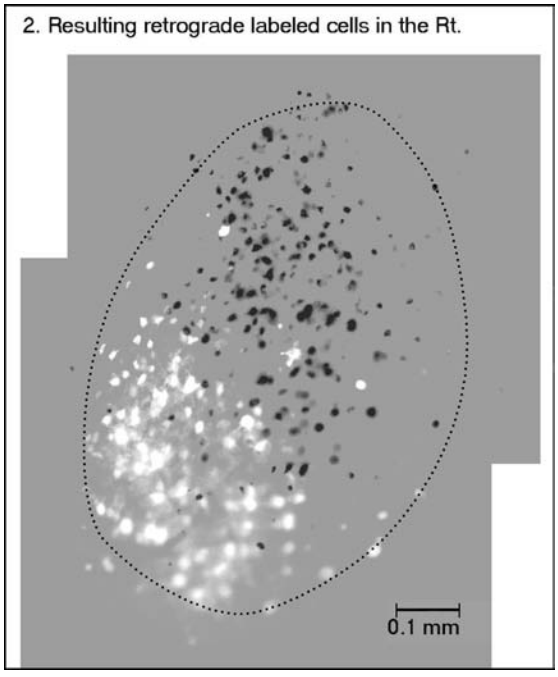
The tecto-fugal pathway is the major ascending visual pathway in non-mammalian vertebrates. In pigeons, most of the retinal axons target the optic tectum in a strictly topographical manner. The tecto-fugal projections arise exclusively from several different kinds of layer 13 tectal ganglion cells, which in turn target the different subdivisions of the thalamic nucleus rotundus (Rt) in an interdigitating non-retinotopic fashion (Marin et al. 2003). The cells in the Rt innervate the entopallium (E), a nuclear area that lies in the pallial telencephalon. Previous studies in zebra finch have shown that the rotundus-entopallial projections have a zonal topography, so that the rostro-caudal order of the rotundal subdivisions is preserved through the projection (Lavergheta & Shimizu, 2003; Krutszfeldt & Wild, 2004). However, the detailed organization of the projection from each rotundal subdivision to the E has yet to be described. We have investigated this organization performing pin-point single and double injections of small crystals of the retrograde tracers DiI and True Blue into a given zone of the E of adult pigeons, using a specially designed solid microinjector apparatus (Marin et al. 2001).

Pigeons were anesthetized with a mixture of Ketamine (40 mg/kg) and Xilazine (12 mg/kg) injected intramusculary in the pectoral muscles. Anaesthesia was maintained with supplementary doses every 2 h. The E was sterotaxically reached through a small craneotomy exposing the dorsal telencephalon. Local incisions and pressure points were treated with anaesthesic ointment. After recuperative surgery and a survival period of 3-5 days animals were deeply anaesthetized and perfused via the aorta with 4% paraformadehyde. The brains were excised and processed according to standard procedures.

Single injections into restricted loci of the E resulted in the labeling of a well-defined cluster of cells in the Rt. These clusters are mainly confined within the limits of a given rotundal subdivision. Double injections of DiI and True Blue located near one another in the same zone of E (figure 1) resulted in two close but clearly discernible clusters of retrograde labeled cells in the Rt (figure 2, dark cells labeled with DiI and white cells labeled with True Blue). These clusters appear to be confined to a single subdivision (in the figure 2, posterior subdivision), and the distance between them is proportional to the distance between the crystals in the E. Double-labeled cells were very scant, and located only in the overlapping periphery of the clusters. These results strongly suggest there is a broad, but consistent point-to-point topographical arrangement in the efferent projection of each rotundal subdivision to its corresponding entopallial zone. Thus, the activity of a given ectostriatal region could be modulated by the activity of a specific distributed ensemble of tectal ganglion cells via an intermediate cluster of rotundal neurons.





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Supported by Fondecyt 1030522

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

C117

Insulin-induced membrane currents in capsaicin-sensitive primary sensory neurones

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The non-selective cation channel vanilloid receptor 1 (TRPV1) is expressed by a sub-population of primary sensory neurones. It is activated either by direct interactions with activators, such as capsaicin or heat <43°C, or indirect actions by ligands acting on other receptors, such as nerve growth factor on the tyrosine kinase A (trkA) receptor. This latter type of activation is mediated by post-translational changes of TRPV1. Recent data shows that TRPV1 is often co-expressed with the insulin receptor in sensory ganglion neurones and that trkA and the insulin receptor have common components in their signalling pathways, suggesting that insulin may activate TRPV1 indirectly. Therefore, we studied the effects of insulin on membrane currents in cultured primary sensory neurones.

Neuronal cultures were prepared from dorsal root ganglia of terminally anaesthetized (isoflurane) Wistar rats (n=7), and kept in F-12 culture medium supplemented with 50ng/ml NGF. Capsaicin (500nM)- and insulin (10μM)-induced whole cell currents were recorded at E_m=-60mV at room temperature from neurones of 3-6 day-old cultures. The composition of the bath and pipette solutions were (mM): NaCl 130, KCl 5, $CaCl_2$ 2, $MgCl_2$ 2, Hepes 10, glucose 10 (pH 7.4), and KCl 130, NaCl 5, $MgCl_2$ 2, Hepes 10 (pH 7.4), respectively. Data are expressed as mean±S.E.M., statistical analysis was performed by Student's t test, $p<0.05$.

Thirty of the 94 capsaicin-sensitve, but non of the capsaicin-insensitve cells responded to insulin. The peak amplitude of the insulin-evoked current was 227±52pA. The activation kinetics of the insulin-induced currents was slower than that of the capsaicin-evoked responses (time to peak: 12.58±0.78s vs. 18.76±1.74s). The capsaicin-induced current was significantly greater in insulin-responsive (614±21pA) than in the insulin non-responsive cells (278±5pA, p=0.022). While capsaicin-evoked currents desensitised following repeated administration of capsaicin, there was no apparent desensitisation in the insulin-induced currents. The competitive TRPV1 antagonist, capsazepine (5μM) strongly reduced the capsaicin-induced but not the insulin-induced currents.

These findings show that insulin induces membrane currents in a proportion of capsaicin-sensitive cultured primary sensory neurones, though, at present it is not clear whether the insulin-induced responses are mediated by TRPV1. Nevertheless, our data suggest that insulin might significantly influence the functions of a proportion of primary nociceptors.

This study was supported by the VIth Framework Program of the European Union, Marie Curie Action, MEIF-CT-2003-500960.

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

C118

Responses of C and A fibres to small stretches of locally inflamed nerve trunks in anaesthetised rats

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Patients with neuropathic pain often have local tenderness over nerve trunks. In addition, pain may occur with limb movements, possibly because nerves stretch during such movements (Hall & Elvey, 1999). Inflammation of a nerve trunk can produce mechanosensitivity to pressure (Eliav, Benoliel, & Tal 2001; Bove *et al.* 2003). The present study examines the mechanosensitivity of nerve fibres in regions of local inflammation, and specifically looks for responses to nerve stretch.

In a preliminary procedure, local neuritis was induced by wrapping oxidised cellulose (Surgicel) saturated in complete Freund's adjuvant (CFA) around the sciatic or peroneal nerves of Sprague Dawley rats anaesthetised with halothane/N2O. In some cases a small slit was made in the perineurium. In control rats, CFA was replaced with saline. 2-9 days post surgery rats were anaesthetised with urethane (1.5 g/Kg i.p.) and recordings were made from sciatic nerve filaments dissected proximal to the lesion site. Units were isolated using electrical stimulation of either the sural nerve (for sciatic lesions) or the peroneal nerve. Conduction block was measured in peroneal lesioned animals using paired stimulating electrodes positioned proximal and distal to the lesion site. Mechanical sensitivity was assessed at the lesion site using a fine glass probe and by small (<5%) stretch. Experiments were in accord with UK Home Office regulations.

Pressure at the lesion site caused responses in 6% (33/545) of C and 9% (21/242) of A fibres. Responses to stretch were seen in 3.0% of C (15/501) (figure 1) and 4.0% of A fibres (8/211), all of which also responded to pressure. In 2 saline treated nerves there were no stretch sensitive units (0/97). A cutaneous or deep receptive field was found for only 1 of 54 units responding to pressure at the lesion site and overall relatively few receptive fields were found for C fibres from inflamed nerves. In 3 of 4 peroneal lesioned rats there was A fibre conduction block at the lesion site (range 22% to 89% blocked). There was no C fibre block.

In summary, nerve trunk inflammation can cause small numbers of A and C fibres to become locally sensitive to small nerve stretches. In patients with neuropathic nerve lesions, these stretch sensitive fibres may contribute to the pain felt during large limb movements.

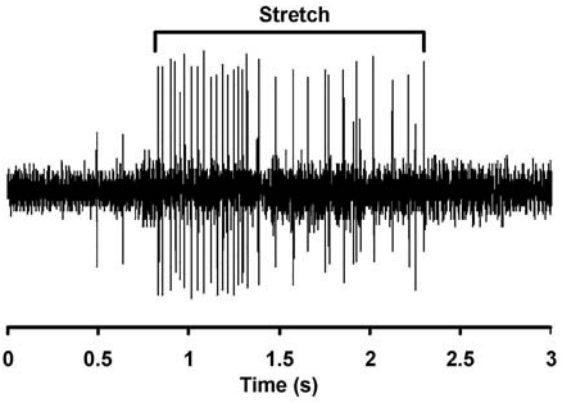

Figure 1. A typical C fibre response to stretch at the lesion site.

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This work was supported by The Wellcome Trust.

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

C119

Comparison of pattern and efficacy of neuronal transduction in the dorsal root ganglia by lentiviral pseudotypes in vivo

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Equine infectious anaemia virus (EIAV)-based lentiviral can transduce CNS neurones (Mazarakis et al., 2001). Our study compares the pattern and efficacy of transduction in the dorsal root ganglion (DRG) by EIAV vectors pseudotyped with either the rabies-G glycoprotein (rabies-G) or vesicular stomatitis virus glycoprotein (VSV-G), following different routes of administration.

Adult male Wistar rats and C57BL/6 mice were anaesthetized with Medetomidine (0.25 and 0.5 mg/kg, respectively, i.p.) and ketamine (60 and 75 mg/kg, respectively, i.p.). With sterile precautions an EIAV vector expressing β-galactosidase (titre 4-8 x 10^8 T.U. /ml) was infused slowly via a micropipette into the spinal cord, DRG, sciatic nerve or footpad. Animals were anaesthetized and perfused with 4 % paraformaldehyde at various times later. Expression of β-galactosidase was determined by incubating 20 μm cryostat tissue sections with either X-gal staining solution (1 mg/ml X-gal in 0.1 M phosphate buffer containing 1.3 mM $MgCl_2$, 3 mM $K_3Fe(CN)_6$, 3 mM $K_4Fe(CN)_6$) overnight at 37°C or rabbit anti-β-galactosidase (1:300, Europa Labs) followed by Alexa Fluor 488 (1:1000, Molecular Probes).

Intraspinal injections (2 μl/rat and 1 μl/mouse) of both VSV-G and rabies-G EIAV vectors produced abundant transduction within the cord at 3 and 5 weeks respectively (n=3) (Figure A). The rabies-G vector also transduced approximately 40 % of DRG neurons in the treated segment compared to < 5 % using the VSV-G vector (Figure B). Roughly equal numbers of NF200, CGRP and IB4 positive cells were transduced in DRG.

Direct DRG injection (2 x 0.5 μl) or peripheral sciatic nerve or footpad injections (6-10 μl) (n = 3 each, 4 week survival) transduced fewer DRG neurones but by all routes transduction was better using rabies-G pseudotyped EIAV vector.

This study demonstrates that the pattern and efficacy of neuronal transduction in the DRG is dependent on the pseudotype of the lentiviral vector as well as the route of injection. For retrograde expression of the EIAV vector in the DRG the rabies-G EIAV vector was more efficient while expression at the site of injection was more effective using the VSV-G pseudotyped EIAV vector. These pseudotyped lentiviral vectors can be used to manipulate experimentally gene expression of sensory neurones in the DRG.

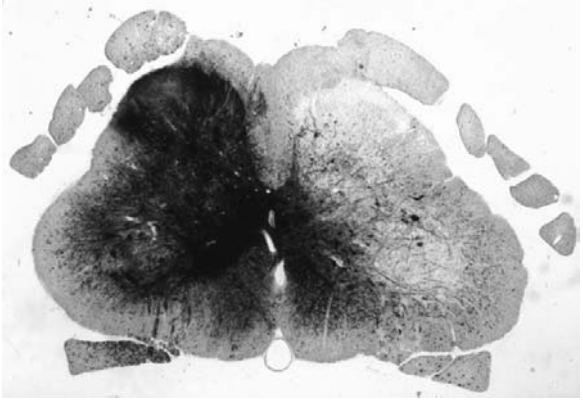

Figure A. Expression of beta-galactosidase using X-gal staining in adult rat spinal cord using VSV-G EIAV vector at 3 week post injection.

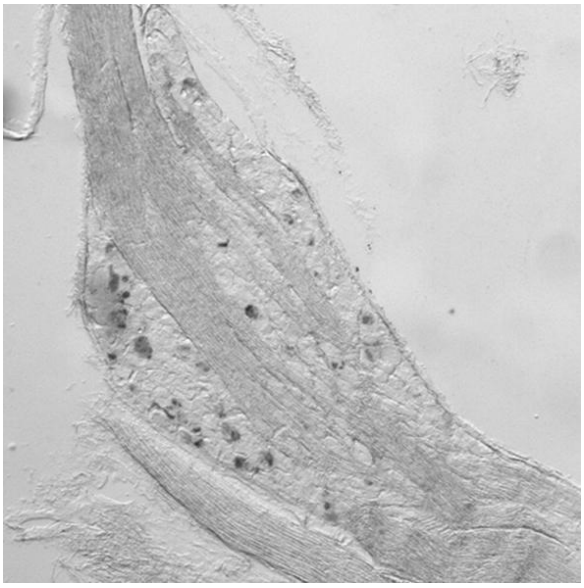

Figure B. Expression of beta-galactosidase using X-gal staining in adult dorsal root ganglion using rabies-G EIAV vector at 5 week post injection.

Mazarakis, N.D. et al. (2001). Human Mol. Genetics 10, 2109-2121.

This work was supported by a MRC Link grant with Oxford BioMedica Ltd.

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

C120

Use of a GDNF expressing lenti-viral vector to reverse neurochemical changes associated with a model of peripheral nerve injury

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Peripheral nerve injury leads to complex pathophysiological changes and often results in intractable neuropathic pain. Neu-

trotrophic factors can have neuroprotective roles and recently it has been shown that intrathecal administration of glial derived neurotophic factor (GDNF) to the spinal cord is highly effective in reversing pathological changes that occur following nerve injury (Boucher et al, 2000).

Targeted delivery of neurotrophic factors can be achieved using viral vectors which produce the desired protein in a defined anatomic location. We investigated the efficiency of a recombinant lentiviral vector overexpressing GDNF in a rat model of neuropathic pain. The viral vector was injected under isofluorane anaesthesia (5%) into the spinal cord of male Wistar rats at the L5 level. The rats received a 1.5ml injection of rLV vector encoding either GDNF (n=5) or the marker gene green fluorescent protein (GFP, n=5). 4 weeks later, the rats received a ligation of the fifth lumbar spinal nerve (5% isofluorane for induction, 2% for maintenance) (modified from Kim & Chung, 1992). 14 days post-surgery the rats were anaesthetised with pentobarbitone (60 mg/kg, i.p.) and perfused with 4% paraformaldehyde and the dorsal root ganglia (DRGs) and spinal cord were collected for immunocytochemical processing. DRGs and spinal cords were labelled for IB4 (10 μg/ml), a marker of GDNF sensitive neurones and DRGs were also stained for ATF3 (1:500), a marker of axotomy. All procedures were carried out under licence according to the UK Animals (Scientific Procedures) Act. All values are means ± SEM.

We followed the time-course of GFP expression in the spinal cord following the rLV-GFP. GFP was expressed from 2 weeks onwards, peaking at 4-5 weeks. GFP expression was restricted to one spinal segment and was detected in both neurons and glial cells.

Spinal nerve ligation induces ATF3 upregulation in axotomised primary sensory neurones and downregulation of IB4 binding in the small, nonpeptidergic DRG cell population. We observed that 89 ± 2% of ipsilateral L5 DRG cells in the GFP virus treated rats were ATF3 positive, compared to none in the contralateral side. rLV-GDNF delivery reduced the incidence of ATF3 labelling by 35%. rLV-GFP treated rats also showed a complete loss of IB4 binding in ipsilateral L5 DRGs and a marked reduction of IB4 binding in the spinal cord (to 15% ± 7 of control values). GDNF virus treatment has significantly reversed this downregulation in both the DRGs to 74% of control values and in the spinal cord to 140% of control values (one way ANOVA).

These findings suggest that spinal injection of recombinant virus may be a effective method of administration of GDNF to treat changes associated with nerve injury.

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

C121

EphB receptors modulate NR2B function in the adult spinal cord

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We have recently identified a novel role for Eph receptor tyrosine kinases and their ephrin ligands (well known guidance molecules during development) as modulators of synaptic strength in the spinal cord. Activation of EphB receptors in rat dorsal horn induced behavioural thermal hyperalgesia; blocking EphB receptor activation prevented or reduced pain-related behaviour in inflammatory pain and in the formalin test. We have now tested the hypothesis that EphB receptor activation induces hyperalgesia through the modulation of NMDA receptor activity, via phosphorylation of the NR2B subunit of the receptor by the non-receptor tyrosine kinase Src. Intrathecal cannulae were implanted in anaesthetized (Medetomidine, 250μg/kg and ketamine, 60mg/kg, i.p.) adult male Wistar rats. After 1 week, rats received 2 10 μl intrathecal injections (each followed by a 10 μl saline flush) at a 10 min interval according to the following protocol: saline (vehicle solution) + saline (Group 1) or + ephrin-B2-Fc solution (to activate EphB receptors; 2μg/rat) (Group2); the Src family kinases inhibitor PP2 (73 nmoles/rat) + saline (Group 3) or + ephrin-B2-Fc (Group 4); PP2 (7.3 nmoles/rat) + ephrin-B2-Fc (Group 5: only for behavioural analysis). For biochemical analysis, the rats were killed by decapitation. Immunoprecipitation of the NMDA receptor was performed on homogenates of the lumbar region of the spinal cord (dorsal horns only), and followed by Western blotting. Membranes were probed with mouse anti-phosphotyrosine (PY490) or rabbit anti-phosph-Src antibodies. The membranes were then stripped, and probed with antibodies to total NR2B. Densitometric analysis was made using Scion Image software. Behavioural testing was performed using a plantar test (Ugo Basile) 30, 60, 90 and 120 mins after injection.

In agreement with our hypothesis, the state of tyrosine phosphorylation of NR2B was significantly increased following ephrin-B2 treatment compared to control levels, as was the phosphorylation level of Src bound to NR2B, while levels of total NR2B remained unchanged: these increases were prevented by prior administration of PP2. We correlated these biochemical findings with behavioural analysis. As expected, intrathecal ephrin-B2 induced thermal hyperalgesia ($P<0.001$, n=5; ANOVA). Prior administration of PP2 reversed the effect of ephrin-B2 in a dose-dependant manner: animals treated with 7.3nmoles PP2 displayed latencies which were not significantly different from those of either control (n=10) or ephrin-B2 treated rats (n=6), while a dose of 73nmoles totally reversed ephrin-B2-induced hyperalgesia ($P<0.001$, n=8).

PHOSPHORYLATION LEVEL (arbitrary units)	CONTROL	EPHRIN-B2-FC	PP2
for NR2B (pY-NR2B/NR2B)	100±5.6 n=4	214.8±17.7* n=4	134.4±22** n=4
for Src (pY-Src/NR2B)	100±2.7 n=4	139±9.1* n=4	97.9±5.7** n=3

Table 1. *Significantly different from control, $p<0.05$ ANOVA: **difference from control, n.s.

Supported by the Wellcome Trust

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

PC117

Effects of flecainide on rat saphenous nerve compound action potentials

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Voltage-gated sodium channel (VGSC) blockers have clinical applications for chronic pain states and parasthesiae that arise from aberrant action potential firing in sensory neurons (Lai *et al.* 2003). Flecainide (FLEC), a VGSC blocker, is an anti-arrhythmic drug that has been found to have beneficial effects in animal models of neuropathic pain and inflammatory demyelinating disease (Ichimata *et al.* 2001; Bechtold *et al.* 2004). Here, we describe effects of FLEC on compound action potential (CAP) conduction in sensory nerve that may contribute to these actions.
Rat saphenous nerves (n = 25) were isolated following humane killing and CAPs were recorded *in vitro* using a grease-gap method. FLEC (10 μM-1 mM) reduced CAP amplitudes dose-dependently with IC_{50} values of 484.6 ± 78.0 μM (mean ± SEM,, n = 4 for 10 μM, n = 6 for 30 μM - 1 mM) in A fibres and 109.7 ± 27.2 μM (n = 7) in C fibres. Increasing extracellular potassium from 3 mM to either 8 mM (A fibres) or 5 mM (C fibres) reduced the IC_{50} values to 138.5 ± 19.5 μM (n = 5 for 10 and 300 μM, n = 7 for 30 and 100 μM) for A fibres and 67.1 ± 17.4 μM (n = 5) for C fibres. We have previously observed similar results with the anticonvulsants carbamazepine (CBZ) and lamotrigine (LTG) in the same preparation; these results are consistent with a voltage-dependent block of VGSCs.
During impulse trains (100 CAPs for A fibre and 50 for C fibre) FLEC showed a frequency-dependent block of CAPs that was not seen previously with CBZ or LTG. At 100 μM FLEC in A fibres, the inhibition of CAP amplitude between the first and last CAP in the train increased by 5 ± 1 % (paired t-test p = 0.003, n = 6) and 19 ± 2 % (p = 0.002, n = 6) at 1 and 10 Hz respectively in 3 mM potassium buffer and 5 ± 1 % (p = 0.0003, n = 7) and 16 ± 1 % (p < 0.0001, n = 7) when potassium was increased to 8 mM. With C fibres at 100 μM FLEC, increased inhibition at higher frequency was seen in 3 mM (1 Hz: 31 ± 5 %, p = 0.001, n = 6; 10 Hz: 39 ± 7 %, p = 0.005, n = 6) but not in 5 mM potassium buffer. Surprisingly, despite this frequency-dependent blocking effect FLEC, like CBZ and LTG, had no effect of the refractoriness of A fibres measured using a paired pulse protocol.
The frequency-dependent blocking effect that distinguishes FLEC from CBZ and LTG may be due to different mechanisms of VGSC block. CBZ and LTG bind predominantly to an inactivated state of the VGSC (Rogawski & Löscher 2004) whereas FLEC probably acts primarily via an open channel block (Ramos & O'Leary, 2004). If there is insufficient time for drug dissociation from channels between impulses then VGSCs blocked by FLEC may accumulate thereby increasing the inhibitory effect of the drug.

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Funded by an MRC PhD Studentship.

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

PC118

The contribution of descending pathways to the inhibitory effect of intravenous morphine on spinal reflexes in the decerebrated rabbit

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In the decerebrated rabbit intravenous fentanyl, a μ-opioid agonist, acts at spinal and supraspinal sites to inhibit reflex responses in medial gastrocnemius (MG) motoneurones evoked by electrical stimulation of the sural nerve. This inhibition is supported by descending adrenergic pathways thus spinal section or intrathecal (i.th.) administration of the selective α_2-adrenoceptor antagonist RX 821002 reduces the effect of fentanyl (Clarke et al., 1998). Studies with i.th. morphine however suggest that this opioid does not interact with endogenous noradrenaline in the same way (Clarke & Lo, 2004). The present study has examined this relationship further using i.v. morphine.
Experiments were performed on 26 rabbits decerebrated under isoflurane (3-5 %)/N_2O anaesthesia. Eight of these animals were also spinalized at L1. Electrical stimulation of the left sural nerve at C fibre intensity (144 times threshold) was used to evoke reflex responses in the ipsilateral MG muscle nerve. These were averaged, integrated by computer and analyzed in 3 post-stimulus time bands: 5-12 ms (phase 1); 12-100 ms (phase 2) and 100-250 ms (phase 3). After a control period of at least 30 min, i.v. morphine was injected at 30 min intervals in doses of 1, 2, 7 and 20 mg kg^{-1} to give a total cumulative dose of 30 mg kg^{-1}. In 9 non-spinalized animals RX 821002 (100 μg i.th.) was given prior to i.v. morphine. Experiments were terminated by i.v. injection of saturated KCl solution.
In decerebrated animals, i.v. morphine significantly (Friedman's ANOVA, p < 0.0001, n = 9) inhibited phase 1, 2 and 3 reflexes so that after 30 mg kg^{-1} cumulative, median responses were 7%, 15% and 11% of pre-drug levels respectively. In animals pretreated with RX 821002, i.v. morphine had no effect on phase 3 responses but it did significantly (Friedman's ANOVA, p < 0.002, n = 9) inhibit phase 1 and 2 reflexes to 29% and 39% of pre-morphine controls after 30 mg kg^{-1}: these values were not significantly different to those in the absence of RX 821002 (Mann-Whitney tests, p > 0.2). In the spinalized group, responses were significantly (Friedman's ANOVA, p < 0.004, n = 8) inhibited by morphine to 58%, 31% and 20% of control values for phases 1, 2 and 3 respectively. For phase 1 reflexes this inhibition was significantly reduced compared to spinally intact animals (Mann-Whitney test, p < 0.02).
These data corroborate our findings using i.th. morphine and show that inhibition of short-latency reflexes by i.v. morphine is partially mediated by descending pathways. However, adrenergic systems appear to support morphine only against long-latency reflexes.

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Supported by BBSRC

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

PC119

Locognostic acuity differs between the transverse and longitudinal axes of the human arm and leg

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It has been reported (Hamburger, 1980) that tactile point localization (locognosia) is more precise in the transverse than longitudinal axis of the arm. However, a potential motor-sensory confound was inherent to the technique employed which involved the subject manually marking the perceived stimulus site. We have re-examined the issue using a response mode that did not depend upon the subject's movement accuracy and have extended observations to the lower limb.

Twenty-eight (19 female, 9 male, mean ± S.D. age 20.6 ± 0.7 years) healthy, right-handed subjects participated with local ethics committee approval. A 7 x 7 cross of stimulus points (13 points, 5 mm separation), arrayed along intersecting lines, orientated in the transverse and longitudinal limb axes, was drawn on the shaved skin of the left forearm and lower leg. In each trial, a brief tactile stimulus was first applied, with a von Frey hair (rating 150 mN), to the central locus of the cross (reference), followed, after 1s, by a stimulus to one of the 13 test loci. The limb was obscured from the subject's view. The subject was required to state whether the test locus was "more distal"/ "more proximal" (longitudinal axis) or "more lateral"/"more medial" (transverse axis) to the reference locus. The four limb-axis combinations were tested separately in each subject. Each test locus received 10 stimuli (total 70 stimuli per axis). For each subject, at each test locus, the probability of the response "more distal" (longitudinal axis) or "more lateral" (transverse axis) was calculated. The interval of uncertainty (IU, a measure of discriminatory threshold) and point of subjective equality (PSE, the test locus perceived to correspond to the reference locus) were estimated from psychophysical functions (probability versus stimulus locus).

The group mean ± S.D. values (in mm) of IU for the different limb-axis combinations were: arm-transverse, 0.96 ± 0.11; arm-longitudinal, 1.72 ± 0.65; leg-transverse 1.17 ± 0.35; leg-longitudinal, 1.82 ± 0.51. Group mean PSE values were consistently small (< 1mm). Statistical analysis (2-way, repeated measures ANOVA) of IU values indicated significant main effects of axis ($p = 0.000$) and limb ($p = 0.045$), whilst the axis*limb interaction was non-significant ($p = 0.614$). Thus, our subjects showed (1) greater locognostic acuity (smaller IU) in the transverse than longitudinal axis of both the forearm, confirming Hamburger's (1980) findings, and lower leg and (2) more accurate tactile discrimination in the upper than lower limb.

Hamburger HI (1980). PhD Thesis, University of Amsterdam.

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

PC120

Protease-activated receptor 2 sensitizes TRPV4

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Transient receptor potential vanilloid 4 (TRPV4) is a cation channel activated by hypo–osmotic and mechanical stimuli, and the selective synthetic agonist 4α–phorbol didecanoate (4α–PDD). Hypo–osmotic stimulation produces 5',6'–epoxyeicosatrienoic acid (5',6'–EET), an endogenous TRPV4 agonist. TRPV4 is expressed on a subset of nociceptive neurons and may sense painful mechanical stimuli. Nociceptive neurons also express protease–activated receptor 2 (PAR2), and proteases that activate PAR2 induce thermal and mechanical hyperalgesia. PAR2–induced thermal hyperalgesia results from PKC–mediated TRPV1 sensitization. We examined the hypothesis that PAR2 activation also causes sensitization of the related TRPV4 channel.

We identified mRNA encoding TRPV4 and PAR2 in a human bronchial epithelial cell line (16HBE140–) and rat dorsal root ganglia neurons by RT–PCR. We investigated TRPV4 signalling by measuring $[Ca^{2+}]_i$ in HBE cells loaded with Fura–2 AM. 4α–PDD ($1x10^{-6}$M-$1x10^{-5}$M) produced a concentration-dependent increase in $[Ca^{2+}]_i$ in HBE cells ($p<0.05$; n=3; ANOVA + Dunnett's test vs. vehicle control). 5',6'-EET ($1x10^{-4}$M) also produced a significant increase in $[Ca^{2+}]_i$ in these cells ($p<0.01$; n=3; *t* test). The response to $1x10^{-6}$M 4α-PDD in HBE cells was potentiated by pre-treatment with $1x10^{-4}$M mouse PAR2 activating peptide (AP), but not PAR2 reverse peptide (RP; $p<0.05$; n=3; *t* test). Neither the PKC inhibitor GFX ($1x10^{-6}$M) nor the PKA inhibitor H-89 ($1x10^{-6}$M) affected this potentiation. To determine whether PAR2 sensitization affected release of the neuropeptides that mediate nociception, we measured release of calcitonin gene-related peptide (CGRP) from superfused segments of rat spinal cord. 4α-PDD ($1x10^{-5}$M and $1x10^{-4}$M) caused a concentration-dependent increase in CGRP release, indicative of activation of nociceptive sensory neurons. The CGRP release to $1x10^{-5}$M 4α-PDD was increased by $1x10^{-5}$M AP, compared to $1x10^{-5}$M RP ($p<0.05$; n=4; *t* test).

Thus, PAR2 activation sensitizes the response of TRPV4 channels to the selective agonist 4α–PDD, as indicated by increased Ca^{2+} signal in HBE cells and enhanced CGRP release from spinal nociceptive neurons. Further experiments are necessary to elucidate the mechanism by which PAR2 activation produces the sensitization of TRPV4, and to determine its importance to mechanical hyperalgesia *in vivo*.

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PC120A

Visual sensory-motor reaction latent periods signal intensity dependence and its relationship with indexes of psychophysical and autonomic functions

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Pavlov (1935) suggested that those likely to develop neuroses might show 'overloading inhibition' when exposed to long lasting or high intensity stimuli. The experiments reported here, done in 2003 and 2004, investigated the hypothesis that absolute sensory threshold was related to how the nervous system processes stimuli of different intensities and to patterns of cutaneous autonomic regulation.

With ethical committee permission and informed consent, healthy volunteers of both sexes (18-22 years) were studied. STATISTICA 5.5 was used. General sensitivity of the nervous system ('force gradient' (FG); Nesbylitsin, 1969) was assessed by latent period (LP) between the visual stimulus (white square of variable size) and button press. Stimulus size varied randomly (RM) or sequentially (HS); FG was estimated by least squares. Visual threshold following dark adaptation was measured (adaptometer) every 15 s after 1 min pre-adaptation to medium (250 cd m^{-2}; n=102) or high (1200 cd m^{-2}; n=96) intensity light. Cutaneous sensitivity was assessed by alternating current (0.1-50 mA; 30 Hz to 30 kHz) on skin of the left forearm, threshold and nature (nociceptive, tactile, anaesthetic) of sensations being reported. Electrodermal properties (static electrical potentials (STEP)), investigated in biologically active zones (BAZ; Podshibiakin, 1960) of facial skin were used to evaluate patterns of autonomic regulation. Potentials were recorded between different BAZ pairs via non-polarisable AgCl electrodes using a millivoltometer with high-input impedance.

LP was strongly dependent on the magnitude of stimuli but this differed significantly between RM and HS. With RM only, some subjects had increased LP with the largest stimulus, interpreted as indicating overloading inhibition. Information obtained by factor analysis was taken to indicate different visual sensitivities: early indexes reflecting cone receptors, late indexes reflecting rod properties. Skin threshold was related to the frequency of stimulating current. The higher the frequency, the higher the threshold (Table 1). The type of sensation showed some frequency specificity, but with overlap. At lower frequencies threshold feelings were nociceptive; at higher frequencies they were tactile or anaesthetising. Nociceptive thresholds were usually lower than tactile at the same frequency. Women had higher thresholds at high frequencies (Table 1); men had a more distinct separation of sensations, the border being at a higher frequency (1-3 kHz; n=35) than in women (0.3-1 kHz; n=50), as revealed by factor analysis. STEP was most negative in nasal and most positive in zygomatic BAZ. LP tests with rm were more informative than with HS. Several relationships existed between parameters, but were weak and differed in 2003 and 2004. A linear relation of sensitivity to LP curve slope was supported only for cone sensitivity with medium light pre-adaptation (250 cd m^{-2}; n=101; 2003) but not with high light pre-adaptation (1200 cd m^{-2}; n=96; 2004). In other cases we found inverse results, the higher the sensitivity, the steeper the LP curve and the lower the rate of overloading inhibition with large stimuli. Nociceptive threshold (30 Hz) was unrelated to the slope, but was related to STEP in some zones. Conversely, tactile threshold (3 kHz) was unrelated to STEP, but was related to LP curve parameters. STEP was related, in different zones, to slope, levels of visual sensitivity or nociceptive thresholds. We are unclear why some results differed in 2003 and 2004.

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

PC123

Simultaneous recordings from auditory nerve and cortex in chinchilla: temporal properties analysis

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Periodic sounds are commonly found in natural sources, speech and music. Therefore it is important to learn how neural responses change when exposed to stimulus sequences. We have studied the temporal properties of responses to auditory stimuli in the auditory nerve and cortex of the chinchilla. We recorded from 12 anaesthetised chinchillas (Ketamine 20 mg/kg IM, Acepromazine 0.7 mg/kg IM). At the end of the experiments the animals were euthanized (Thiopental 120 mg/Kg IV). Responses were recorded either with a round-window electrode positioned in the right cochlea (n=9), with tetrodes placed on the left auditory cortex (n=8), or simultaneously from round window and auditory cortex (n=5). Clicks and tones were digitally generated (TDT III), delivered through a closed cavity coupled to the ear, and presented at different rates (1 to 300 Hz) and sound pressure levels. Electrical responses were filtered, amplified (10,000x) and digitised with a NI® board. Auditory cortex units were sorted as described in Gray et al (1995). Auditory nerve compound action potential discharges followed stimulus presentation rates up to 300 Hz. These responses showed amplitude reductions and greater latencies for stimulus presentation rates higher than 50 Hz. Local field potentials recorded in the auditory cortex followed stimulus rates up to 20 Hz, while single units were able to follow stimulus frequencies up to 10 Hz. In a stimulus train preceded by five seconds of silence, the auditory nerve responses to the first stimulus in the train had the same amplitude and latency as responses to later stimuli, while cortical responses to the first stimulus of each train were stronger than those to later stimuli, with larger evoked potentials followed by an inhibitory period of about 500 ms. The mean first-spike latency to the first stimulus of cortical neurons was shorter than the mean first-spike latency to later stimuli(16.1±1.3 ms; 20.0±1.7 ms; means±SD, unpaired t test, $p<0.01$). The spike responses to the first stimuli showed an onset response followed by an inhibitory period of about 200 ms and a rebound that lasted to

about 800 ms. These results support the claim that auditory cortex neurons have a lower "cut-off" frequency than auditory nerve neurons, and also show auditory cortical properties to infrequent stimuli that had not been previously reported in the chinchilla.

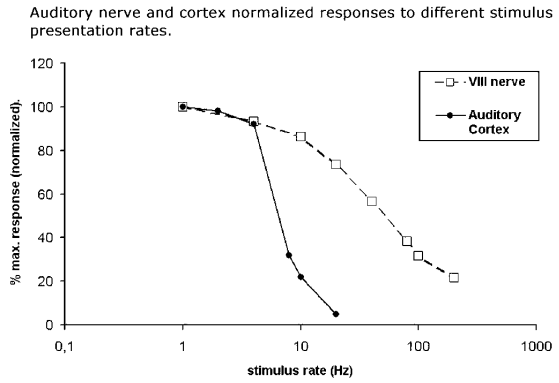

Fig 1. Auditory cortex responses can not follow stimulus rates higher than 10 Hz and decrease with a steep slope. Auditory nerve responses follow stimulus rates higher than 100 Hz.

Gray, C.M., Maldonado, P.E., Wilson M. & McNaughton B. (1995). J. Neurosci. Methods 63, 43-54.

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