O71

Enhancement of changes in dim flash response kinetics during light adaptation by 9-demethylretinal in isolated salamander rods

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During light adaptation Ca^{2+} acts on a step early in phototransduction that takes place with a time constant of ~0.5 s (Matthews, 1997). In rods that have been bleached and regenerated with 11-*cis*-9-demethylretinal, which forms a photopigment with a prolonged photoactivated lifetime (Corson *et al*. 1994), the time course of this Ca^{2+}-dependent step is greatly prolonged so that it dominates the recovery of the bright flash response (Matthews *et al*. 2001). In order to investigate the shaping of the dim flash response, we have studied dim flash response kinetics during light adaptation in rods containing the 9-demethylretinal pigment.

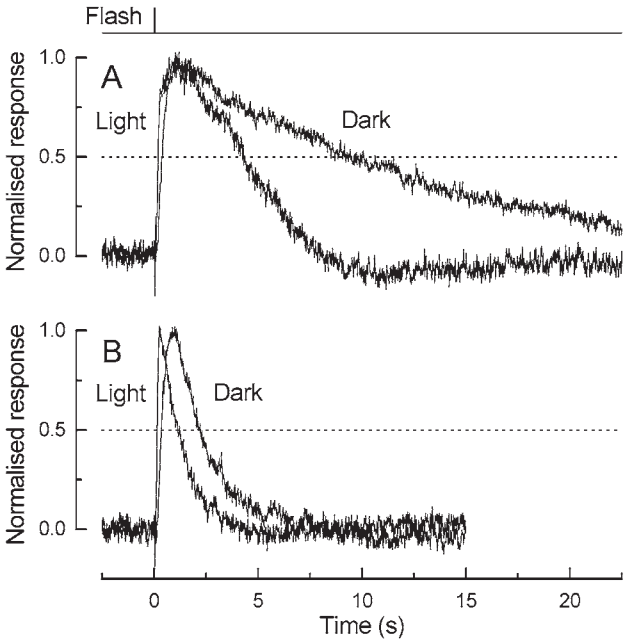

Figure 1. Normalised responses to dim flashes at *A*, 440 nm, and *B*, 650 nm of a rod regenerated with 9-demethylretinal in darkness (Dark) and during steady illumination delivering 3.59×10^4 photons μm^{-2} s^{-1} at 610 nm (Light).

Aquatic tiger salamanders were killed by stunning, decapitation and pithing. Isolated rods were exposed to intense 500 nm light calculated to bleach >99 % of the photopigment, and regenerated by exposure to phospholipid vesicles containing 9-demethlyretinal. In a bleached rod regenerated with 9-demethlyretinal, the response in darkness to a 440 nm dim flash (Fig. 1*A*, Dark), which preferentially excites the analogue photopigment, was substantially prolonged in comparison with the response to a 650 nm flash (Fig. 1*B*, Dark), which preferentially excites remaining native pigment. Steady near-saturating 610 nm light had a much more profound effect on the recovery kinetics of the response to the 440 nm flash (Fig. 1*A*, Light) than that to the 650 nm flash (Fig. 1*B*, Light). This steady light accelerated the time for 50 % recovery of the response to the 440 nm flash from 8.3 ± 0.5 to 3.5 ± 0.3 s (means ± S.E.M., 9 cells), and the response to the 650 nm flash from 2.1 ± 0.1 to 0.89 ± 0.09 s. The mean decrease in response duration at 440 nm (4.9 ± 0.6 s) was significantly larger than that at 650 nm (1.2 ± 0.1 s) (Student's unpaired t test, $t = 6.35$; better than 0.1 % level). This observation suggests that the recovery phase of the response to a dim 440 nm flash is dominated by the greatly slowed Ca^{2+}-sensitive quenching of the analogue photopigment, thereby enhancing the modulation of response recovery as $[Ca^{2+}]_i$ falls during background light.

Corson D *et al.* (1994). *Vis Neurosci* **11**, 91–98.

Matthews HR (1997). *J Gen Physiol* **109**, 141–146.

Matthews HR *et al.* (2001). *J Gen Physiol* **118**, 377–390.

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All procedures accord with current UK legislation.

O72

Broadly oriented motion detectors in marmoset V1 respond to local features in 2-D patterns

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The visual system analyses the motion of 2-D patterns in two stages (Adelson & Movshon, 1982). First, orientation-selective neurons in primary visual cortex (V1) extract the motion of oriented components. Second, oriented motion signals from V1 are synthesized by neurons in visual area MT to compute speed and direction of motion (Movshon *et al.* 1985).

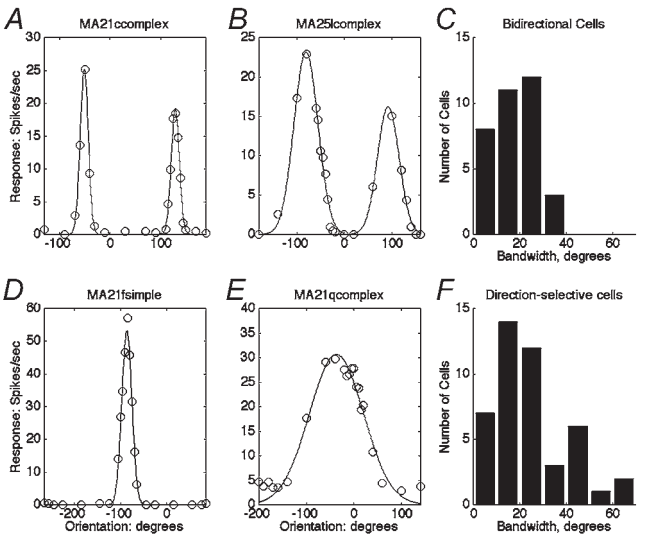

Figure 1. Orientation-tuning curves and distributions of bandwidths. Sample broad and narrow tuning curves from bidirectional (*A* and *B*) and direction-selective (*D* and *E*) neurons. Smooth curves are best-fitting Gaussian functions. Histograms show the distribution of bandwidths of bidirectional (*C*) and direction-selective (*F*) neurons.

Here we examine the orientation selectivity of direction-selective and bidirectional neurons recorded in V1 of marmosets anaesthetized during preparation with saffan (I.V.) and anaesthetised and neuromuscularly blocked during recording with a respiratory mixture of 2:1 nitrous oxide and oxygen with

supplementary fluothane (0–0.5 %) as required to maintain a stable ECG and synchronized EEG and intravenous delivery of 20 μg kg^{-1} h^{-1} fentanyl citrate and 100 μg kg^{-1} h^{-1} I.V. pancuronium bromide in glucose saline I.V. Procedures were licensed under the UK Animals (Scientific Procedures Act) 1986 (Derrington *et al.* 2002).

Responses to moving sinusoidal gratings depend strongly on orientation. The range of orientation bandwidths is similar to that found in macaque (Ringach *et al.* 2002) but the best marmoset neurons have narrower bandwidths than macaque neurons. About 25 % of direction-selective neurons have bandwidths greater than 45 deg. When tested with 2-D patterns made by adding differently oriented component gratings, broadly oriented neurons signal the motion of local features rather than the motion of component gratings.

Adelson EH & Movshon JA (1982). *Nature* **300**, 523–525.

Derrington AM *et al.* (2002). *Phil Trans Roy Soc B* **357**, 975–985.

Movshon JA *et al.* (1985). *Pattern Recognition Mechanisms,* ed. Chagas *et al.*, pp. 117–151. Springer Verlag, New York.

Ringach DL *et al.* (2002). *J Neurosci* **22**, 5639–5651.

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All procedures accord with current UK legislation.

O73

Input from beyond the classical receptive field modulates response gain in marmoset primary visual cortex

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Recent research suggests that neurons in primary visual cortex (V1) are capable of integrating information from regions beyond their classical receptive field (e.g. Levitt & Lund, 1997). Here we investigate, in anaesthetized and neurosmuscularly blocked marmosets, how input from regions extending beyond the classical receptive field modulates the gain of V1 neurons.

Anaesthesia was induced with Saffan (Alphadalone/Alphaxalone acetate; 1.5 ml kg^{-1} I.M.) and was maintained with a respiration mixture of 70 % N_2O and 30 % O_2 and fentanyl citrate (20 μg kg^{-1} h^{-1} I.V.). Skeletal muscles were neurosmuscularly blocked with vecuronium bromide (0.1 mg kg^{-1} h^{-1} I.V.). Appropriate levels of anaesthesia were maintained by ensuring electroencephalogram activity was synchronised and heart rate was stable. Supplementary fluothane was given if necessary (Webb *et al.* 2002).

We examined how drifting sinusoidal gratings that flanked or surrounded the classical receptive field modulated single neuron responses in V1 to an optimal drifting grating on the classical receptive field. The grating on the classical receptive field could have several different contrasts, in the range 0–100 %. The grating was presented alone or with a grating of the same or orthogonal orientation that either (1) surrounded the receptive field to fill a square that was 10 deg × 10 deg in size, (2) extended laterally to fill flanks that were the length of the receptive field and 10 deg wide, or (3) extended at each end of the receptive field to fill a region 10 deg in length. To characterise how gain was modulated by surround stimuli, the Michaelis-Menten equation was fitted to response *versus* contrast functions obtained under each stimulus condition. Surround modulation of gain was modelled best as a reduction in the maximum obtainable firing rate (response gain control). Response gain varied with the orientation of surround stimuli and was most severely reduced when the orientation of surround stimuli matched the preferred orientation of the classical receptive field. There was no laminar organization to neurons whose response gain was modulated by surround stimuli, suggesting that gain control from beyond the classical receptive field is a general property of V1.

Levitt JB & Lund JS, (1997). *Nature* **387**, 73–76.

Webb BS *et al.* (2002). *Visual Neurosci* **19**, 583–592.

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All procedures accord with current UK legislation.

O74

Object motion, with or without retinal motion, activates human cortical area MT+

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Moving objects viewed through stationary eyes appear to move, but during smooth-pursuit eye movements, stationary objects in the field are not perceived as moving. Moreover a moving object tracked with the eyes seems to move, although its retinal image is virtually stationary (Leigh & Zee, 1991). This implies that the brain compares retinal motion and eye position to compute the perceived movement of objects (Lisberger & Movshon, 1999). We measured Blood Oxygenation Level-Dependent signals by means of functional MRI (Siemens-Varian 3T scanner) to investigate brain regions underlying this computation.

Seven subjects, who gave informed consent, viewed a display consisting of high-contrast vertical white stripes, with a central cross that they fixated constantly, on a black background. To maintain attention constant, they had to look for and report occasional small changes in the shape of the cross. Stimulation conditions, each exposed 8 times for 27 s, included: (1) fixation cross stationary, stripes oscillating horizontally back and forth with sinusoidal time-course (2.4 s period; 10 deg amplitude); (2) stripes stationary, cross oscillating, producing the same retinal motion as in (1) (Leigh & Zee, 1991); (3) stripes and cross both stationary; and (4) stripes and cross moving together, producing perceived motion of the entire display with only small residual slip of the retinal image.

Our results suggest that the temporo-occipital cortical area MT+, thought to be homologous to monkey MT & MST (Zeki *et al.* 1991; Watson *et al.* 1993), is involved in computing external object motion. MT+ was strongly activated by motion of the stripes when the eye was stationary but showed no obvious activation when identical retinal motion was generated by smooth pursuit eye movements. MT+ was also activated when the eyes moved with the stripes, i.e. without significant retinal motion.

These results, which imply that MT+ receives an extraretinal signal about eye position as well as information about retinal motion, are compatible with a recent report that neurons in monkey MT respond differently to retinal motion caused by stimulus movement and eye movement (Thiele *et al.* 2002).

Leigh RJ & Zee DS (1991). *The Neurobiology of Eye Movements.*

Lisberger SG & Movshon JA (1999). *J Neurosci* **19**, 2224–2246.

Thiele A *et al.* (2002). *Science* **295**, 2460–2462.

Watson JD *et al.* (1993). *Cereb Cortex* **3**, 79–94.

Zeki S *et al.* (1991). *J Neurosci* **11**, 641–649.

This work was supported by the University of Pisa, the Medical Research Council and the Oxford McDonnell Centre for Cognitive Neuroscience.

All procedures accord with current local guidelines and the Declaration of Helsinki.

O75

Visual discrimination of natural-scene stimuli by amblyopic people

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In amblyopes, one of the eyes has poorer vision than the other, usually because the eye was neglected (e.g. due to squint) in early childhood. It is unclear whether the poor vision in complex visual tasks can be explained by lack of sensitivity to simple stimuli, or whether information from the amblyopic eye is processed differently in the brain (Levi *et al.* 2002).

We performed experiments on five amblyopic women (aged 18–30) with Ethical Committee approval. We measured thresholds in both eyes of each observer for two or four sets of natural scene stimuli: series of slightly different pictures made by morphing one picture into another (Parraga *et al.* 2000). For instance, pictures might show slight changes in facial expression. A total of 14 such experiments was performed. For comparison, we also measured the observer's contrast detection thresholds for sinusoidal gratings of different spatial frequencies. Thresholds were measured by presenting pictures or gratings (2 deg square) on a CRT monitor, following a modified two-alternative forced-choice protocol (Parraga & Tolhurst, 2000).

In all experiments with complex natural-scene stimuli, the thresholds in the amblyopic eye were 1.83 times those in the fellow eye (mean = 1.83, $n = 14$, S.D. = 0.44). Often, the contrast thresholds for the gratings were also higher in the amblyopic eye, raising the question whether poor performance in complex discriminations results just from a failure to see the Fourier components of the complex scenes as well.

We have previously developed a simplistic model of discrimination for normal observers (Parraga *et al.* 2000), in which we presume that the visual system breaks complex pictures down into separate spatial frequency bands; discrimination depends on detecting differences in contrast in those bands. The parameters of the model include the oberver's thresholds for sinusoidal gratings. When we ran such models on the amblyopic eyes, we found that the poor sensitivity to gratings *did* predict poor performance on the complex discrimination task. However, the actual performance in the complex task was worse even than predicted by the model; the actual thresholds were on average 1.4 times the predicted thresholds (S.D. = 0.41).

There are many assumptions in such a simplistic model, so it is interesting to note that our two best-studied observers (4 experiments each) actually had similar grating thresholds in their two eyes but performed the complex discrimination worse in their amblyopic eye than their fellow eye; the amblyopic threshold was 1.57 times the threshold in the good eye ($n = 8$; S.D. = 0.27).

Levi DM *et al.* (2002). *Vision Research* **42**, 1379–1394.

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CAP was a Fight for Sight Fellow.

All procedures accord with current local guidelines and the Declaration of Helsinki.

O76

Functional magnetic resonance imaging of spatial auditory perception in blind and sighted human subjects

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*Departments of *Physiology and ‖Psychobiology, University of La Laguna, Tenerife, †Magnetic Resonance Center at Esplugues de Llobregat, CETIR Grup Medic, Barcelona, ‡Magnetic Resonance Centre IMETISA, University Hospital, Tenerife and § Institute for Astrophysics, Tenerife, Spain*

Virtual acoustic space (VAS) is an R+D multidisciplinary project on sensory substitution designed to study the human auditory capability of perceiving complex spatial patterns using a sound code. The aim is to improve blind people's mobility and orientation. A device has been developed that transduces on-line visual scene information captured by a pair of microcameras into a specially processed sound delivered through headphones. It is perceived by the subject as multiple irregularly repetitive clicks coming from the coordinates occupied by the surfaces of objects in that scene, generating a unitary perception of every object's whole surface extending over the relevant coordinates in the field of view (González Mora *et al.* 1999).

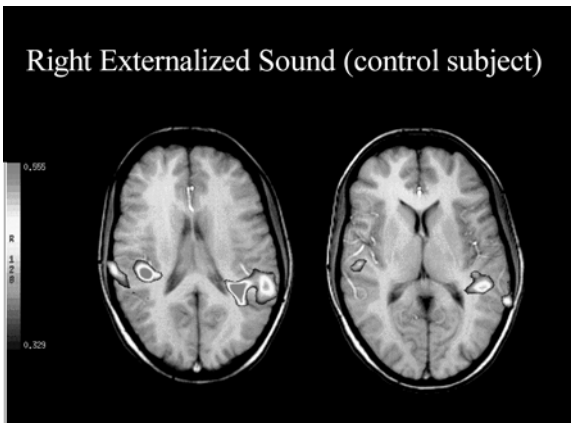

Figure 1. fMRI horizontal sections of the brain of a sighted subject showing temporal areas involvement in auditory localization of a virtual single sound source.

A fMRI research line has been initiated to study the cerebral substrate for this kind of perceptual experience and the possible plastic changes derived from its maintained utilization by blind users. We present preliminary results from exploring cerebral areas involved in the auditory perception of a single sound, virtually externalized 1 m, at 45 degrees to the right, in the horizontal plane. So far, six sighted female subjects and four blind females (2 congenital and 2 late onset) were scanned while presented with a rest–stimulus protocol consisting of repetitive trains of clicks perceived as a single point inside or outside the head at a virtual position. It was found that in the blind subjects there was a relative shift of the areas activated by the sound stimulus towards more posterior areas (temporal–occipital) that in sighted people are involved in visual processing. In addition to Heschl-planum temporal bilateral activation with apparent left predominance, activation appeared at the bilateral temporal-occipital union, areas 37–19. The figures show an example from sighted (Fig. 1) and a blind (Fig. 2) subject.

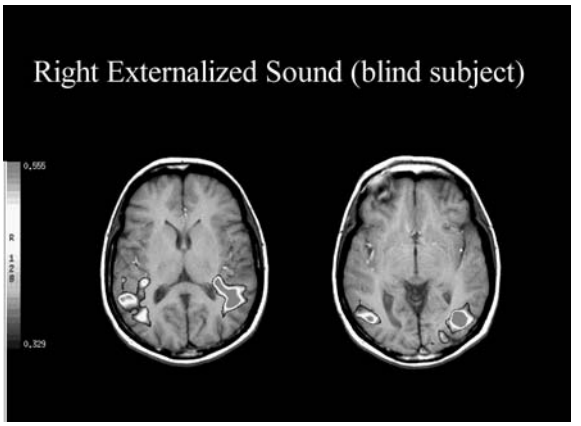

Figure 2. fMRI pictures of a blind subject performing the same task mentioned in Fig 1. Notice the posterior shift of the activated areas towards regions which are involved in sighted people on visual processing.

Our results support the previous findings, particularly in congenital blindness (Weeks *et al.* 2000), of an involvement of occipital areas in cross-modal auditory processing, and suggest that a similar response can be found in late onset blindness, strengthening the hypothesis that the visual cortex remains functional in peripheral blindness, possibly as a result of plastic reorganization as well as by functional recruitment.

Current studies are directed to further verification of the involvement of parieto-occipital areas in perceiving the surrounding acoustic stimulus both in early and late onset blindness.

González Mora JL *et al.* (1999). *Proc Int Work Conf Artificial & Natural Networks* **2**, 321–330.

Weeks R *et al.* (2000). *J Physiol* **7**, 2664–2672.

We thank all the participating volunteers. This work is supported by grants from the Spanish Science and Technology Ministry and from Europeans Founds (FEDER), ref. 1FD1997-1237.

All procedures accord with current local guidelines and the Declaration of Helsinki.

O77

Comparative study of the acoustic response of the human ears, head and torso and its sculptural reproduction in silicone, oriented to the development of a sensory substitution device

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In order to localize an external sound source, the human brain processes several acoustic cues which vary depending on its position relative to the subject. They are primarily, the interaural arrival time and intensity differences and the monaural spectral modifications by the pinnae, head and torso. For every position these cues can be gathered as a mathematical function, the so-called head-related transfer function (HRTF), that applied to any crude sound makes it perceived as coming from such position, in spite of being presented through headphones. A R+D project on sensory substitution for blind people's orientation and mobility using sounds aim to present the subject with an auditory coded image of the frontal scene (Carlile & Pralongn, 1994). For every coordinate in the scene where a piece of object surface exists, image after image is sent on-line as a spatialized and very locatable 'click' that make the subject perceive the scene as if it were virtually covered by sound sources. HRTFs obtaining for every coordinate imply an individual long-lasting recording session with a miniature microphone located at the entrance of each blocked ear channel. The need for higher resolution has led us to study the possibility of substituting the human subject by a mannequin replica of him with a comparable acoustic spatial response.

An accurate reproduction in silicone of the pinnae and head of an adult male was made. Binaural pairs of HRTFs were obtained for 27 positions at different horizontal and elevation coordinates, at distances of 0.5 and 1.34 m in the frontal arc, for the human original (O), the mannequin (M) and a second human subject as control (C). The spectrum range of interest went from 0.3 to 18 kHz.

We analysed the graphical similarities between these subjects for a random selection of the HRTFs, concerning the spectral features usually considered to be implied in the localization process (primarily spectral peak and dip amplitude and position, and the low order spatial frequency of the curve) (González Mora *et al.* 1999).

A quantitative measurement of the similarity and a perceptual validation in a localization task using virtual sound sources obtained with individual and non-individual HRTFs were carried out.

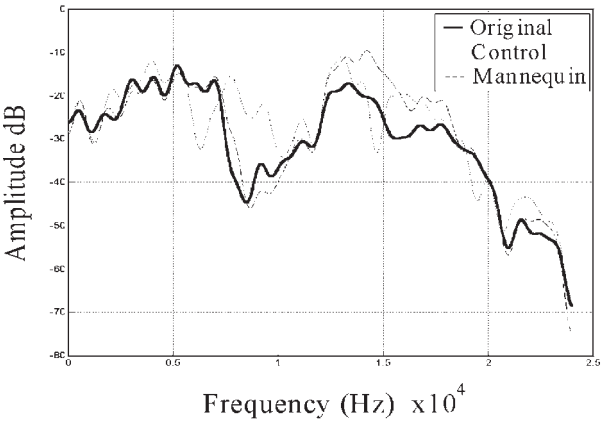

Figure 1. Right ear recorded head related transfer functions for one position. Notice the similarity between the mannequin and the original *versus* control.

We have observed two HRTF spectral feature behaviours in the spectral ranges below and above the 10 kHz point. The similarity of M-O in the first range for the whole graphics studied, and their clear distinction from the control for a majority of them (see figure) are remarkable. In the second range, there was a conservation of the general structure of the curve for M-O with the possible appearance of differences whose perceptual relevance remains unknown, but that could be due to the methods measurement variability.

The results support the possibility that a mannequin reproduction of a human subject has a comparable acoustic behaviour to this one, in such a way that the person can localize with a similar precision virtual sound sources generated using

both its own and the replica obtained HRTFs. Quantitative comparison and perceptual validation will allow a more precise conclusion on the value of these preliminary results.

Carlile S & Pralongn D (1994). *J Acoust Soc Am* **95**, 3445–3459.

González Mora JL *et al.* (1999). *Proc Int Work Conf Artificial & Natural Networks* **2**, 321–330.

This work is supported by grants from the Spanish Science and Technology Ministry and from Europeans Founds (FEDER), ref. 1FD1997-1237.

All procedures accord with current National and local guidelines and the Declaration of Helsinki.

O78

A simple procedure for auditory stimuli to ameliorate Parkinsonian deficits of movement

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Parkinson's disease (PD) is the most common disorder of the basal ganglia. Patients characteristically exhibit tremor at rest, muscle rigidity, limited initiation of movements and slowness in performing complex voluntary movements. In addition to the typical neurochemical deficits, abnormal motor performance in PD results from impaired motor programming, with functional alterations of the supplementary motor area and pre-motor cortex, resulting in a failure of the internal rhythm formation process. It has been suggested that sequential movements in Parkinsonian patients might be improved by the effects of external rhythmic cues, either visual or acoustic, acting as a sort of timekeeper.

In line with that idea, we have developed a portable system which allows the patient suffering from bradykinesia and rigidity to initiate appropriate auditory stimulation when he/she is unable to move. Here we present data from six PD patients studied by means of surface electromyography while walking . They walked along an 8.5 m walkway, turned 180 deg at the walkway end and returned to the starting position. The electromyographic activities (EMG) of two leg muscles (tibialis anterior and gastrocnemius) were recorded by mean of surface electrodes. We studied the following temporal parameters: interval between burst of EMG activity, slope of each burst and its duration. We have used the coefficient of variability as an indicator for temporal stability (CV = standard deviation/mean × 100). In control trials, no external auditory cues were available. In test trials, the patients were asked to proceed in the presence of an external rhythm (click tone) at a fixed frequency of 100 clicks min^{-1}, a standard value for a normal elderly human walk cadence. The tone was delivered by a device constructed in-house (comprising a battery-operated metronome and small headphones), which was controlled by the patient. We compared the performance during the task with and without stimulation using the Mann-Whitney *U* and Wilcoxon tests for a $P \leqslant 0.05$. Informed consent for participation was obtained from all individuals and protocol procedures were reviewed and approved by the University of Coruña Ethical Board and were in accordance with The Declaration of Helsinki.

All patients showed remarkable improvement in the EMG parameters studied while using the device. With acoustic stimulation, the coefficient of variability improved for each of the parameters (see Fig. 1), which reached values very close to those recorded for control subjects (for which stimulation and control situations were not statistically different). The results are consistent with prior reports on rhythmic auditory facilitation in Parkinson's disease gait, and we suggest that this represents a novel and inexpensive tool to help PD sufferers in daily motor performance.

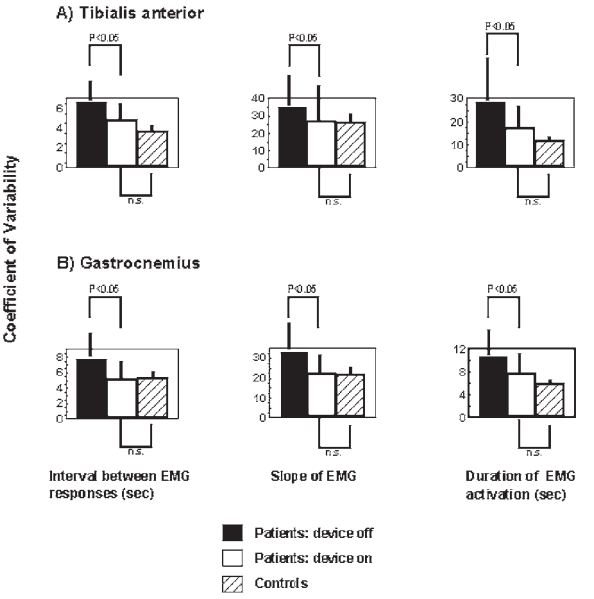

Figure 1.

This work was supported by XUGA (PGDIT), Spain.

All procedures accord with current local guidelines and the Declaration of Helsinki.

O80

The sense of the movement elicited by transcranial magnetic stimulation is due to sensory input, not efference copy

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Subjects have reported awareness of moving 80–90 ms before voluntary muscle activation (Libet *et al.* 1983). When transcranial magnetic stimulation (TMS) of motor cortex was used to elicit forearm muscle contractions, some subjects reported a sense of movement even when movement was abolished by ischaemic block (Amassian *et al.* 1989; Brasil-Neto *et al.* 1993). These findings support the idea of a corollary discharge or efference copy. Our experiments were devised to resolve whether TMS evokes a sensation of movement directly in the brain or as a result of sensory feedback.

Local ethical approval and informed consent was obtained for the following procedures carried out on six normal subjects (aged 23 to 56). Twitch contractions evoking left and right finger extensions were elicited by electrical stimulation of muscles. In another series, finger extension of one hand was elicited by TMS (MagStim 200, 9 cm round coil) of the contralateral motor cortex and on the other side by muscle stimulation. Left and right

finger movements were matched in amplitude using accelerometer recordings. The time interval between left and right stimuli was varied randomly (range ± 90 ms in 15 ms steps) from trial to trial. Allowance was made for the extra conduction time with TMS. Subjects were asked to report whether they sensed that the left or the right movement occurred first, or if they could detect no difference.

Sequence discrimination plots (percentage correct responses against time interval) indicated that left and right movements evoked by dual electrical stimulation were sensed as near simultaneous when there was zero delay between them. The sense of movement for the six subjects ranged from right preceding left by 10 ms to left preceding right by 5.5 ms, for simultaneous movements. When TMS was combined with muscle stimulation the cortically evoked movement was felt on average 20 ms after the movement evoked by muscle stimulation. The sense of movement in response to TMS ranged from 7 ms preceding to 53 ms following the electrically induced movement.

Since the TMS response was felt late rather than early, the results do not support the notion of corollary discharge or efference copy being elicited by TMS but favour sensory feedback as the source of the sense of movement. The delayed perception of the TMS-induced movement may reflect cortical suppression of the sense of movement.

Amassian VE *et al.* (1989). *Brain Res* **479**, 355–360.

Brasil-Neto JP *et al.* (1993). *Brain* **116**, 511–525.

Libet CA *et al.* (1983). *Brain* **106**, 623–642.

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All procedures accord with current local guidelines and the Declaration of Helsinki.

P115

Computer simulation of the role of priors in the visual cortex (V1) contrast code

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The problem faced by an animal using sensory information to find out about its environment may be suited to Bayesian analysis. The animal will be most accurate if it guesses that the event occurring is the event for which the probability of event given neural responses, $p(e|r)$, is maximum. In order to estimate $p(e|r)$, an organism must know the probabilities of occurrence of events $p(e)$ (the priors), and the probabilities of neural responses given events $p(r|e)$. Given these, one can apply Bayes' formula:

$$p(e|r) = p(r|e).p(e)/p(r).$$

Yet it is difficult to see how the statistical formalism could be implemented in the brain. We examined the problem, developing a computer model of contrast identification in which 16 simplified V1 neurons must infer, from their noisy responses (variance = 2 × mean, Tolhurst *et al.* 1983), the value of stimulus contrast presented. The contrast response function is modelled by the Naka-Rushton equation (Albrecht & Hamilton, 1982). An important parameter is the level of contrast at which the neuron attains half of its maximum response (σ). We ran simulations in which the σ distribution was taken from cat (D.J. Tolhurst, unpublished data) and monkey (D. Ringach, personal communication) neurophysiology and found performance in contrast identification to be different from that of a control population with a uniform σ distribution. For the animal σ simulations performance peaked at the mid contrast range, corresponding to the peak in the distribution of contrasts in natural scenes, as estimated by an equivalent contrast method (Peli, 1990). This would give the animal the advantage of being best at coding the contrasts most frequently occurring in the natural world.

This observation was supported by calculations of mutual information between a set of natural contrasts (c) and model contrast estimates ($\hat{c}$):

$$I(c;\hat{c}) = \sum\sum p(c;\hat{c}).\log_2[p(c;\hat{c})/p(c).p(\hat{c})],$$

which found I to be greater for cat and monkey populations (2.4 and 2.2 bits, respectively) than the control set (2.1 bits), when measured with a flat $p(e)$ distribution.

Measurement with a natural scenes $p(e)$ did not increase I for the control population, but did shift peak accuracy closer to the peak of the natural contrast distribution – an effect analogous to making the σ set more like that of the cat or monkey populations. The finding that the effect of a probability distribution can be mimicked by adjusting a neuronal parameter is relevant to the issue of biological implementation of Bayesian analysis. It suggests that by setting σ values, V1 is able to encode prior information.

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P116

Commissural interneurons in cat spinal motor pathways: identification of excitatory and inhibitory cells

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Detailed investigations have been performed on only a small number of spinal interneurons. Earlier studies focused on analysis of input to interneurons in various pathways (see Jankowska, 1992) and, with the exception a limited number of types of interneuron, very little is known about their postsynaptic actions. We have combined electrophysiological methods and immunocytochemistry to investigate the organization of commissural interneurons in midlumbar spinal segments and the transmitter content of their axons.

Experiments were performed on three adult cats which were deeply anaesthetised (with chloralose, up to 5 mg kg^{-1} h^{-1} I.V., after induction with 40 mg kg^{-1} I.P. of sodium pentobarbital). If any increase occurred in the heart rate or blood pressure or if the pupils dilated, an additional dose of anaesthetic was given. Neuromuscular transmission was blocked (Pavalon 0.2 mg kg^{-1}). Cells in laminae VII–VIII were activated monosynaptically by stimulation of muscle nerves at intensities consistent with activation of group II muscle afferent fibres or by descending fibres from the reticular formation. Stimuli were then applied within contralateral motor nuclei in lower lumbar segments to select neurons which were activated antidromically and thus to identify them as commissural interneurons. Cells were labelled intracellularly with a mixture of rhodamine dextran and Neurobiotin. At the end of experiments, animals were killed

humanely and fixed by perfusion. Four neurons had well labelled axons that could be followed into the contralateral grey matter where they formed clusters of boutons in laminae VII, VIII and IX. Labelled structures were scanned with a confocal laser scanning microscope and reconstructed. Sections containing axonal processes were incubated with antibodies raised against the vesicular glutamate transporters 1 and 2 (VGLUT1 and 2), which are markers for glutamatergic terminals (Varoqui *et al.* 2002). Further sections were reacted with antibodies raised against molecules associated with inhibitory transmitters: glycine transporter molecule 2 (GlyT2) to reveal glycine-containing axons and glutamic acid decarboxylase (GAD) to reveal GABAergic axons.

Analysis of individual boutons indicated that two neurons reacted positively for GlyT2 and that one was positive for VGLUT2. The fourth cell was negative for all four markers. These findings provide the first firm evidence that feline commissural interneurones include both excitatory and inhibitory neurons. Since all of the neurons investigated were antidromically activated from motor nuclei, these findings also support the hypothesis that the earliest crossed inhibitory actions of group II afferents may be evoked disynaptically via commissural neurones (Arya *et al.* 1991).

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All procedures accord with current National guidelines.

P117

Enhancement of the potency and duration of fentanyl antinociception by subeffective doses of the NSAID nitroparacetamol (NCX-701)

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The μ-opioid agonist fentanyl is a very effective analgesic drug, though its effect is very short. Subeffective doses of the NSAID dexketoprofen trometamol enhanced the effect of fentanyl in responses to noxious mechanical stimulation (Gaitan & Herrero, 2002). We have now studied the possible interaction between NCX-701 (NOP, nitroparacetamol), the NO-releasing derivative of paracetamol (acetaminophen), in comparison to that of dexketoprofen trometamol (DKT) in single motor units (SMU) activated by noxious mechanical stimulation and high intensity electrical stimulation (wind-up).

Preparatory surgery, in male Wistar rats under halothane anaesthesia included the cannulation of the trachea, two superficial jugular veins and one carotid artery. The experiments were performed under chloralose anaesthesia and the animals were humanly killed by an overdose of pentobarbitone. The I.V. administration of fentanyl induced a dose-dependent inhibition of pinch-evoked responses to noxious mechanical stimulation (ID_{50}: 18 μg kg^{-1}, $n = 10$) that fully recovered in 30 min. In the presence of 60 μmol kg^{-1} of NOP, fentanyl caused an inhibition of responses with an ID_{50} of 5.2 μg kg^{-1} ($n = 7$). This enhancement of the potency of fentanyl was similar to that observed when studied in the presence of 0.1 μmol kg^{-1} of DKT (ID50: 4.7 μg kg^{-1}, $n = 7$). In the presence of either NOP or DKT, no recovery of the effect of fentanyl was observed for at least 45 min, and was not reversed by the I.V. administration of the opioid antagonist naloxone (200 μg kg^{-1}) or the α_2-adrenoceptor antagonist atipamezol (100 μg kg^{-1}). SMU wind-up was reduced by fentanyl with a minimal effective dose (MED) of 32 μg kg^{-1} and fully recovered in 15 min. In the presence of NOP, the fentanyl MED was of 16 μg kg^{-1}, and the effect was still significant 15 min later. In the presence of DKT, however, although the MED was lower (16 μg kg^{-1}) to that observed in the absence of DKT, the effect fully recovered 30 min after the administration of the highest dose of fentanyl.

In conclusion, the presence of subeffective doses of NCX-701 induces a potent enhancement of the potency and duration of the antinociceptive effect of the μ-opioid receptor agonist fentanyl, in responses to noxious mechanical stimulation. This effect seems to be independent on the presence of NO since a similar action was observed in the presence of DKT. In wind-up, however, the enhancement of the effect observed after the administration of fentanyl seems to be more dependent on the release of NO since the enhancement observed in the presence of DKT was not so evident.

Gaitan G & Herrero JF (2002). *Br J Pharmacol* **135**, 393–398.

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P118

The effects of sham and full spinalization on the systemic potency of the NO-releasing NSAID NCX-701 (nitroparacetamol) in rat spinal nociceptive reflexes and wind-up

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NCX-701 (nitroparacetamol) is a NO-releasing derivative of paracetamol (acetaminophen) that has shown potent antinociceptive and anti-inflammatory properties (Romero-Sandoval *et al.* 2002). We have compared the antinociceptive activity of I.V. NCX-701 *vs.* paracetamol in single motor unit recordings (SMU) from monoarthritic male Wistar rats. NCX-701 was also tested in sham and full-spinalized animals to assess whether or not the effect was located at spinal cord sites. Monoarthritis was induced by the administration of 50 μl of carrageenan in the knee cavity under brief halothane anaesthesia 16 h previous to the experiment. Preparatory surgery was also made under halothane anaesthesia and included the cannulation of the trachea, two superficial branches of the jugular veins, and one carotid artery. Spinalization or sham operation was performed at the vertebral T9–10 segment. SMU recordings were performed in hind limb muscles by means of bipolar tungsten electrodes under systemic chloralose anaesthesia. The animals were humanly killed at the end of the experiment by an overdose of pentobarbitone.

In normal animals, the administration of cumulative doses of NCX-701 induced a dose-dependent reduction of responses to noxious mechanical stimulation, similar to that seen with paracetamol (ID_{50} values of 320 and 305 μmol kg^{-1}, respectively, $n = 9$). The maximum effect observed was of 30 % of control response for paracetamol and 22 % for NCX-701. SMU wind-up, however, was significantly ($P < 0.05$, ANOVA with Dunnett's *post hoc* test) reduced by NCX-701 but not modified by paracetamol, indicating a different mechanism of action. In spinalized animals, NCX-701 reduced responses to mechanical and electrical stimulation with a similar potency to that seen in

normal non-spinalized animals (ID_{50} of 327 μmol kg^{-1}, $n = 8$). In sham-spinalized animals, however, the effect of NCX-701 on responses to noxious mechanical stimulation was very week and not dose dependent, with a maximum effect of 58 % of control response. Also, wind-up responses remained unchanged in sham spinalized rats.

We conclude that NCX-701 induces a potent antinociceptive effect by a mechanism different from and complementary to that of paracetamol, and its action is mainly located within the spinal cord in monoarthritic animals.

Romero-Sandoval *et al.* (2002). *Br J Pharmacol* **135**, 1556–1562.

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P119

Modulation of spinal reflexes by M-current modulators: an electrophysiological study *in vitro*

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M-currents constitute a unique effector system to control neuronal excitability due to their voltage and ligand sensitivities. We have used M-channel openers and blockers to study the possible role of these currents in the modulation of nociceptive and non-nociceptive spinal reflexes using the *in vitro* hemisected cord.

Spinal cords were dissected from urethane-anaesthetised (2 g kg^{-1} I.P.) Wistar rat pups, hemisected and superfused with standard oxygenated ACSF. Animals were then killed with an overdose of urethane. Simultaneous AC and DC recordings of responses to electrical stimulation of the L5 dorsal root were obtained from the ventral root using suction electrodes. The effects of bath-applied retigabine, an M-current opener (Rundfelt, 1997), and XE-991, an M-current blocker (Zaczek *et al.* 1998), were tested on responses to single (and repetitive) stimulation at A- and C-fibre intensities. Quantification of signals was made according to previous reports (Hedo & Lopez-Garcia, 2001). Data are expressed as means ± S.E.M.

Retigabine applied at 1, 3 and 10 μM ($n = 5$) produced a concentration-dependent inhibition of the integrated area of responses to single and repetitive C-fibre intensity stimulation of the dorsal root as well as of the action potential counts elicited by repetitive stimulation (overall ANOVA, $P \leqslant 0.001$). The minimum effective concentration was 3 μM (Dunnet's *post hoc* test, $P \leqslant 0.05$). Retigabine applied at 10 μM did not change the monosynaptic reflex evoked by C-fibre intensity stimuli but increased the threshold intensity to obtain a ventral root response from 27 ± 5 to 38 ± 4 μA ($n = 6$; Student's paired t test, $P \leqslant 0.01$). Superfusion of XE-991 ($n = 8$) produced an enhancement of spinal reflexes elicited by single and repetitive C-fibre stimulation which was reflected in an increased integrated area and in a larger number of action potential counts (minimum effective concentration was 10 μM, Dunnet's *post hoc* test, $P \leqslant 0.05$). XE-991 applied at 10 μM reversed the inhibitory effects of retigabine 3 μM ($n = 3$).

These observations indicate the presence of functional M-currents within the mammalian spinal cord and suggest that their modulation can be relevant to controlling the flow of nociceptive signals through spinal circuits.

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P120

Spinomedullary cells in the rat dorsal horn receive synaptic contacts from axons that possess α2c-adrenergic receptors

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It is likely that the α2c subclass of adrenergic receptor (α2c-AR) mediates some of the antinociceptive actions of noradrenaline in the spinal cord (Fairbanks *et al.* 2002). Axon terminals, which possess this receptor, are concentrated in the superficial dorsal horn and originate from spinal interneurons (Stone *et al.* 1998). We performed a series of combined tract-tracing and immunocytochemical studies to determine if α2c-AR-immunoreactive axons target projection neurons that possess the neurokinin-1 (NK-1) receptor as such neurons are likely to transmit nociceptive information to the brain (Naim *et al.* 1997).

Spinomedullary neurons were labelled by stereotaxic injection of the B subunit of cholera toxin (CTb) in the caudal ventrolateral medulla of three anaesthetized adult rats (ketamine–xylazine mixture, 7.33 and 0.73 mg per 100 g I.P.). After 3 days, the animals were anaesthetized again with sodium pentobarbitone (1 ml I.P.), killed humanely and fixed by perfusion. Sections were cut from midlumbar segments and reacted with antibodies to reveal α2c-ARs, CTb and NK-1 receptors. Retrogradely labelled neurons possessing the NK-1 receptor ($n = 45$) were examined with confocal microscopy to investigate their relationship with α2c-AR-immunoreactive axons. Numerous α2c-AR axons were apposed to cell bodies and proximal dendrites of cells in lamina I and also with distal dendrites that originate from labelled cell bodies in lamina III. A combined confocal and electron microscopic method revealed that appositions were synaptic. Further experiments showed that most α2c-AR terminals in contact with labelled cells are immunoreactive for the vesicular glutamate transporter 2 and therefore are glutamatergic.

These data suggest that noradrenaline can modulate excitatory synaptic transmission from spinal interneurons to projection cells by acting at α2c-ARs. This could be one of the mechanisms that underlie the antinociceptive actions of noradrenaline.

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