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Olivo-cortico-nuclear localizations in crus I of the cat cerebellum

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It is widely accepted that the cerebellar cortex of the cat (and other mammals) includes a D1 and a D2 zone. Their climbing fibres originate from the principal subnucleus of the inferior olive (PO) and their Purkinje cells project to the lateral (dentate) cerebellar nucleus (NL). However, the precise topographical relationship between the two lamellae of PO and the two D zones remains unclear. Uncertainty also exists concerning the cortico-nuclear projections. In view of the conflicting evidence and because of our interest in the involvement of the cerebellar hemispheres in the control of volitional movements, we have carried out the following bi-directional axonal tract tracing experiments.

Cats were initially deeply sedated via subcutaneous injection of medetomidine hydrochloride (Domitor) and subsequently anaesthetised via continuous intravenous infusion of propofol at a rate sufficient to abolish flexion reflexes. Fluorescent-labelled axonal transport tracing agents were micro-injected into the folia of Crus I to investigate spatial localization in the olivo-cerebellar and cortico-nuclear projections. The folia were shown to be mainly occupied in rostro-caudal succession by three zones receiving their olivo-cerebellar afferents from territories respectively confined within the dorsal lamella of PO, the ventral lamella and the rostral half of the medial accessory olive. These zones are presumably parts of the D2, D1 and C2 cerebellar cortical zones, as earlier proposed by Rosina & Provini (1982); they were respectively c. 1.5, 2.5 and 4.5 mm wide and their respective nuclear targets were in the rostro-ventral quadrant of NL, the caudo-ventral quadrant of NL and the ventral half of nucleus interpositus posterior. In the most medial one or two folia a narrow C3 zone between the C2 and D1 zones was related to the rostral part of the dorsal accessory olive and the dorso-caudo-lateral part of nucleus interpositus anterior.

In the C2 and the D zones medial and lateral parts of the zone are innervated by different groups of olive cells and project respectively to medial and lateral parts of the nuclear territory for the zone, consistent with the existence of olivo-cortico-nuclear micro-complexes in Crus I (Ito, 1984). Within each zone a 'folial' localization is also present in both the olive and the cerebellar nuclei and intersects orthogonally with that for zone width.

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

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A putative site of synaptic relay for the motor cortical projection to the inferior olive in rats

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There is disagreement in the literature to whether or not there is a direct functional projection from the sensorimotor cerebral cortex to the inferior olive. A combination of electrophysiological and anatomical tract tracing techniques have been used in the present study to investigate the connectivity of this pathway. In pentobarbitone (50 mg/kg, i.p., Sagatal, Rhone Merieux, Harlow, UK) anaesthetised rats, single pulse electrical stimulation of the primary motor cortex evoked large climbing fibre potentials on the surface of the cerebellum. These inputs correspond with those defined by climbing fibre input from the periphery and were recorded in the C1 zone. Animals were killed humanely at the termination of the experiment. In anatomical studies, injections of anterograde tracer (Fluoro-Emerald and/or Fluoro-Ruby, Molecular Probes) were injected into the physiologically defined fore- and/or hindlimb areas of the motor cortex and retrograde tracerr (green beads and/or red beads, Lumafluor Inc.) into the C1 zone in the cerebellar cortex in the same animals. Overlaps between anterograde terminal and retrograde cell labelling were in the basal pontine nuclei. In contrast, only retrograde cell labelling was found in the inferior olive. Anterogradely labelled fibres continued past the inferior olive and after the decussation continued down to the spinal cord, although there was terminal labelling ventral to the gracile nucleus. In electrophysiological experiments, reversible inactivation of this anatomically identified area in the caudal brainstem with lignocaine resulted in a temporary abolition of climbing fibre field potentials evoked from cerebral cortical stimulation whilst responses evoked at the same C1 zone cerebellar cortical site by peripheral stimulation were unaffected. This suggests there is at least one relay prior to the inferior olive in the motor cortical cerebro-cerebellar pathway in rats.

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Spike trains prolong the post-spike after hyperpolarization in cat spinal motoneurones

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The interspike interval with the lowest firing frequencies during repetitive firing to injected current in cat spinal motoneurones corresponds to the duration of the post-spike afterhyperpolarization (AHP) (Kernell, 1965). When recording from motor units in humans during slowly increasing and followed by decreasing contraction strengths it has been repeatedly confirmed that the lowest firing frequencies are seen at the end of the spike

train (at de-recruitment with decreasing contraction) rather than just after recruitment (with increasing strength) (see Gorassini et al. 2002). Similar findings have been reported for motoneurones (intracellular recording) in the decerebrate cat when activated either with muscle stretch or intracellular current injections (Bennett et al. 1998). It has been suggested that the lower firing frequencies at de-recruitment may be explained by a sustained subthreshold depolarization with fluctuating synaptic noise occasionally driving the cell to fire (e.g. Matthews, 1996), rather than being determined by the AHP of the motoneurones as during a suprathreshold drive.

To address this question experimentally we have recorded the AHP trajectory following spikes evoked by short intracellular current pulses. These test AHPs were recorded either alone (control) or following spike trains evoked by triangular current pulses (conditioned AHPs). The duration and amplitude of the triangular current pulses could be varied. The interval after the termination of the conditioning triangular current injection and the test pulse was varied between 250 ms and 2 s. Results were obtained from 10 cats. Initial surgery was performed under general anesthesia (Isofluran) and the cats were then decerebrated and neuromuscular blockade established with pancuronium (0.6 mg/h) (see Crone et al. 1988) in order to study the both primary and secondary range firing (the latter dependent on anaesthesia-dependent persistent inward currents).

We confirmed that the firing frequency was lower at de-recruitment than at recruitment in all 51 neurones included in this report. We demonstrated that the AHPs were significantly prolonged in 30 motoneurones, when conditioned by a preceding spike train. This prolongation was often more pronounced at shorter than longer conditioning- test intervals (time between the end of the conditioning current injection to the test pulse) and with longer spike trains reaching high frequencies. We therefore conclude that the lower frequency at de-recruitment than at recruitment at least partly depends on a prolongation of the post-spike AHP.

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

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Modulation of motor intra-cortical inhibition and sensory perceptual threshold by repetitive transcranial magnetic stimulation in humans

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Following spinal cord injury (SCI), there is a natural reduction in motor intracortical inhibition (ICI) (Smith et al. 2000). Recently, we have shown that a specific pattern of repetitive transcranial magnetic stimulation (rTMS) leads to a reduction in ICI, a decrease in cutaneous perceptual threshold and to improvements in a functional test and clinical scores in incomplete SCI (Belci et al. 2004). Now, we evaluate the effect of this rTMS protocol in a group of healthy subjects.

With local ethics approval and consent, six healthy adult subjects were recruited. The rTMS pattern used was a doublet stimulus (100ms interval) applied every 10s for 30 min. Stimuli were delivered using a Magstim 200 stimulator connected to a 9cm circular coil centred over the vertex, A-side up, and at 90% of the resting motor threshold for the right thenar muscle. Before, during and after the rTMS, surface electromyography was recorded from right and left thenar muscles during 20% maximum voluntary contraction while single pulse TMS was employed as appropriate to elicit motor evoked potentials (MEP) and ICI in the right or left hand. Perceptual threshold (PT) to electrical stimulation of the right and left C6 dermatomes was also assessed using a Digitimer Stimulator-DS7. Measurements were made at intervals for two hours after rTMS. Statistical analysis was performed using repeated measures ANOVA with Holm-Sidak post hoc test.

In the right hand, there were significant decreases in ICI ($P=0.016$) and PT ($P<0.001$) following rTMS. Post-hoc test showed that ICI was decreased 10 minutes after the start of rTMS while PT was decreased at 60, 90 and 150 min. Mean values of ICI and PT had not recovered to baseline values after 2 hours. rTMS had no significant effect on MEP size ($P=0.90$). In the left hand, there were no significant changes in ICI ($P=0.16$) or MEP size ($P=0.25$) following rTMS. However, PT was lowered ($P=0.006$) at 30 and 60 min after start of rTMS.

Our findings suggest that the sub-threshold rTMS pattern that we have used has demonstrable motor and sensory effects on healthy subjects. The mechanism is thus not dependent on the plastic changes present in subjects with spinal cord injury.

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Mechanisms by which basement membrane regulates neutrophil recruitment in long-term endothelial cultures

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We reported previously that development of basement membrane (BM) during long-term culture of endothelial cells was associated with increased levels of adhesion and migration of neutrophils when the endothelial cells were stimulated with low concentrations of the inflammatory cytokine, tumour necrosis factor α (TNF). We therefore set out to investigate whether the basement membrane influenced neutrophil behaviour by directly interacting with migrating cells or indirectly modifying the responses of the endothelial cells growing on them.

Confluent monolayers of first passage human umbilical vein endothelial cells (HUVEC) were cultured for between 1 and 20 days, and then treated with 0, 1 or 100U/ml of TNF for 4h. The HUVEC were then stripped from the surface and the effects of the BM on cellular behaviour were studied as follows: (i) neutrophils were allowed to settle, adhere and migrate on the BM, with or without prior activation with interleukin-8 (10nM); (ii) fresh HUVEC were seeded and cultured on the stripped BM or on gelatin for 48h, TNF was added for 4h, and then neutrophils were allowed to settle, adhere and migrate on the HUVEC. After 20 days culture, stripped HUVEC left a distinct BM which formed a sheet judged by scanning electron microscopy, and which could be rolled up with a pipette tip. Unstimulated neutrophils did not adhere to BM under any conditions, while activated neutrophils bound in similar numbers regardless of the period of culture or whether the HUVEC had been stimulated or not before stripping. However, the neutrophils migrated more slowly on BM from 20 day cultures than on substrate laid down by day 3 cultures (velocity = $7.9 \pm 0.2\mu m$/min vs. $4.6 \pm 0.5\mu m$/min; mean ± SEM from 3 experiments; $p<0.05$ by paired t test). When primary HUVEC were seeded on day 20 BM and stimulated with low dose (1U/ml) TNF, they supported greater adhesion than HUVEC cultured on gelatin ($28 \pm 9\%$ of added cells adhered vs. $16 \pm 4\%$; mean ± SEM from 4 experiments). Nevertheless, when we measured the migration velocity of transmigrated neutrophils, they were slower under HUVEC grown on BM than under those grown on gelatin (velocity = $7.1 \pm 0.2\mu m$/min vs. $10.1 \pm 0.5\mu m$/min; mean ± SEM from 4 experiments; $p<0.05$ by paired t test)

These data indicate that substances laid down in the basement membrane directly influence the behaviour of migrating neutrophils. The BM also appears to modify the responses of the endothelial cells growing on it, but culture on day 20 BM does not fully recapitulate the effects of 20 days culture during which BM is deposited.

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