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Hebbian LTP in inhibitory hippocampal interneurons of αCaMKII-T286A mutant mice

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NMDA receptor- (NMDAR) dependent long-term potentiation (LTP) has been extensively documented in hippocampal pyramidal neurons, where it requires autophosphorylation of the alpha isoform of Ca^{2+}-calmodulin-dependent kinase type IIα (αCaMKII). We have recently shown that excitatory synapses onto a subset of inhibitory interneurons in stratum radiatum of the rat hippocampus also show Hebbian NMDAR-dependent LTP. αCaMKII has been reported to be absent from interneurons. We have therefore explored the role of CaMKII in Hebbian LTP in interneurons. We have used knock-in mutants to study the requirement of αCaMKII autophosphorylation. These mutants have the T286A point mutation in the endogenous αCaMKII gene preventing autophosphorylation. We performed gramicidin perforated patch recordings from stratum radiatum interneurons in hippocampal slices obtained either from αCaMKII-T286A mutant mice or from wild type mice. In mutant mice high-frequency stimulation (100 Hz, 1 s, x 2) of Schaffer collaterals resulted in LTP (90 ± 33% pathway-specific potentiation of EPSP initial slope; mean ± SD) lasting > 30 min in 7 interneurons, and failed to evoke LTP in 5 other cells. These results were not significantly different from either wild-type mice (LTP in 4 out of 7 cells; chi square test) or rats (LTP in 5 out of 9 cells). We verified that strong hyperpolarisation during the high-frequency stimulus prevented LTP induction in mutant mice (n = 4). These results argue against an obligatory role for αCaMKII. However, KN-62 (10 μM), a selective inhibitor of several CaMK isoforms, blocked LTP in the interneurons of αCaMKII-T286A mutant mice (n = 3). Hebbian LTP in stratum radiatum interneurons thus requires another KN-62-sensitive kinase such as βCaMKII. αCaMKII-independent Hebbian plasticity in interneurons may contribute to some residual memory formation observed in the αCaMKII-T286A mutant mice.

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

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Maturation of motor control pathways involved in coordination of speech and posture

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The integration of speech production within ongoing postural tasks requires children to learn to coordinate multiple motor programs. In early development speech is achieved at the cost of maintained posture. Later in normal development, speech and posture can occur simultaneously without noticeable impact to either skill. In this study we examined the integration of the motor control pathways involved in this dual task in normally developing children.

Surface EMG was recorded on the right side of the body from the intercostals, rectus abdominis, obliques, lower erector spinae, and latissimus dorsi muscle groups. Three adults and 18 individual children from 4-10 years of age were studied. The trials included sitting with upright posture-no speech, upright posture-normal speech, and upright posture-loud speech. Temporal and spectral analyses of the EMG data revealed differences in muscle activation patterns across all three conditions and with development.

Younger children showed a less refined amplitude modulation to produce loud speech than older children and adults. Temporal examination of muscle firing patterns revealed a lack of synchronization in motor unit firing (a broad peak in the cross-correlogram; Fig. 1B & Table 1), presumably required for efficient speech production, in the younger children compared to the adults. Interestingly, these same young subjects also showed a higher level of common motor cortical drive (β-band inter-muscular coherence; Gerloff et al. 2006; Halliday & Rosenberg, 1999) to the muscles during the loud speech task. Adults did not show this common motor cortical drive during this task (see Fig.1 for examples and Table 1 for quantification).

Changes in amplitude modulation and coherence are indicative of an evolution of control processes for the integration of posture, respiration, and speech. The changes in firing patterns suggest a refinement of the implementation of these control processes. We hypothesize that as children mature they learn a more efficient method of producing loud speech involving an increased utilization of distributed motor control pathways and an enhanced spatio-temporal relationship between synergistic muscle groups. Children with neurodevelopmental disorders, such as cerebral palsy, may not exhibit this pattern of maturation.

Table 1. Changes in neurophysiological parameters of speech and posture with age

Age	Mean amplitude of B-band coherence (above 95% confidence level)	Width of peak in cumulant density
5	0.0839	15 ms
6	0.0651	10 ms
7	0.0301	8 ms
Adult	0	4 ms

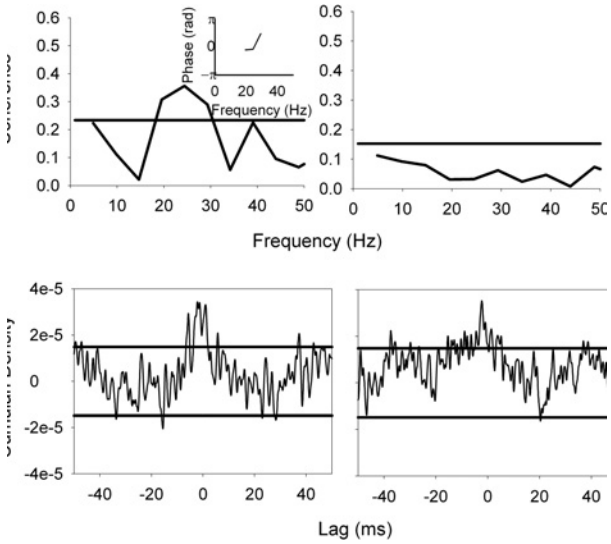

Figure 1

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Dose-dependent effects of rTMS on human pharyngeal motor cortical excitability

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Transcranial magnetic stimulation (TMS) has been used to demonstrate that recovery from stroke is associated with increased size and excitability of the swallowing motor cortex [1]. In healthy volunteers the optimal frequency of repetitive TMS (rTMS) to induce excitation in swallowing motor cortex is 5Hz [2]. However, little is known about the dose effects of intensity or duration of rTMS in this region. The aim of our study was to assess differing doses of rTMS prior to studies on dysphagic stroke patients.

Twelve healthy subjects took part. EMG responses were recorded using a pharyngeal catheter with ring electrodes. Thenar responses were recorded to determine the thenar motor threshold (TT). Twenty EMG responses to single pulse TMS over one hemisphere were recorded and averaged before and up to 90 min following a range of doses of rTMS. Subjects underwent 6 interventions on separate days, given in a randomised order and double blinded: A (100 pulses at 90% TT); B (250 pulses at 90% TT); C (1000 pulses at 90% TT); D (100 pulses at 120% TT); E (250 pulses at 120% TT); and sham stimulation(250 pulses at 120%TT, with coil tilt). Thenar threshold was chosen to comply with rTMS safety guidelines. EMG amplitude was normalised to baseline for each individual. Statistical comparisons were performed using repeated measures ANOVA.

All interventions showed an increase in cortical excitability compared to sham, which varied no more than 14% from baseline. The ANOVA revealed a significant effect of time ($F(3.7,110)=5.3$, $p<0.001$), indicating a strong effect of rTMS, and a significant treatment vs time interaction ($F(3.7,110)=3.3$;$p=0.016$), showing excitability differences between the interventions (Fig. 1, Table 1). To probe this further, an area under the curve analysis using paired t tests comparing each intervention with sham showed a significant difference with B ($p=0.006$), C ($p=0.004$), D ($p=0.001$) and E ($p=0.007$).

Differing doses of rTMS seem able to induce significant increases in pharyngeal cortical excitability. Our data suggest that while increasing the intensity and duration of stimulation may lead to larger increases in excitability, there is no additional benefit gained by giving more than 250 pulses of stimulation. We propose that 250 pulses given at 120% of thenar threshold may be the optimal regimen for inducing excitability in swallowing motor cortex.

	Baseline	Immediate	30 min	60 min	90 min
A	64.07 (9.5)	74.05 (10.4)	81.35 (11.7)	74.53 (9.5)	78.87 (12.9)
B	60.35 (9.6)	67.34 (8.6)	80.67 (11.7)	85.95 (13.3)	83.90 (10.2)
C	54.32 (8.5)	61.05 (11.9)	61.40 (8.2)	77.92 (11.1)	72.07 (11.9)
D	53.62 (4.0)	66.23 (7.2)	78.28 (8.0)	78.59 (10.5)	62.91 (6.6)
E	48.77 (5.7)	67.83 (8.2)	76.03 (8.2)	66.51 (5.8)	79.21 (9.2)
Sham	67.89 (8.5)	79.11 (11.8)	61.18 (11)	74.63 (11.7)	67.42 (10.8)

Table 1. Pharyngeal EMG amplitude μV (standard error).

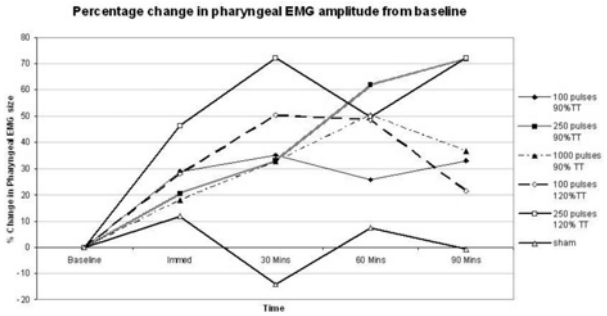

Figure 1. Change in pharyngeal EMG amplitude over time in response to each of the active interventions and sham.

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Visualisation of neuromuscular synaptic protection in *Wld*^{S} mutant mice by confocal microendoscopy

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Wallerian degeneration describes the characteristic degeneration of the severed distal segments of axons and their terminals after they have been separated from their cell bodies. In the slow Wallerian degeneration (*Wld*^{S}) mutant mouse, this active process is delayed by expression of a chimeric *Nmnat/Ube4b* gene: disconnected axons are preserved for up to 3 weeks, rather than 24-48 h characteristic of wild-type mice (Coleman, 2005). However, synaptic terminals are less well protected by expression of the chimeric protein. For instance, motor nerve terminals appear to withdraw from motor endplates over a 3-10 day period (Gillingwater et al. 2002). This suggests that neurodegenerative mechanisms may be compartmentalised in neurones and that presynaptic terminals represent an independent neurodegenerative compartment (Gillingwater & Ribchester, 2001). To gain further insights into the pattern and mechanism of neuromuscular synaptic degeneration, we have developed a minimally invasive *in vivo* imaging protocol based on confocal microendoscopy. This methodology has potential application to analysis covert neuromuscular phenotypes, for example, following random germ-line mutations induced by ethylnitrosourea (ENU) mouse mutants (Nolan et al. 2002).

Wld^S mice were first crossbred with two transgenic lines expressing Yellow Fluorescent Protein in neurones: *thy1-YFP16* which expresses the fluorochrome in all motor neurones, and *thy1-YFPH* in which only about 5% of motor neurone expresses fluorescent protein (Feng et al. 2000). The offspring were backcrossed to *Wld*^{S} mice to generate fluorescent lines that were homozygous for *Wld*^{S}. Next, we transected the sciatic nerve in these mice under 3% halothane/40-50% $N_2$0/40-50% O_2 anaesthesia. Mice were inspected daily. All of them compensated within 24 h for their unilateral lower hind-limb paralysis. Following recovery 1-7 days later, we reanaesthetised the mice (ketamine/xylazine, 100/10 mg/kg, I.P.). We visualised the severed distal axons in the tibial nerve and superficial neuromuscular junctions in the gastrocnemius muscle via small incisions in the skin, using Proflex S-1500, S-650 and prototype Z-1000 fibre-optic probes connected to a Cell Vizio confocal microendoscope (Mauna Kea Technologies, Paris). In two mice from control transgenic YFP lines, axons and neuromuscular junctions had visibly undergone fragmentation and degeneration within 48 h. In the *YFP-Wld*^{S} variants (n=4), however, intact axons and neuromuscular synapses were visible for at least 4 days after sciatic nerve section, proving that the Cell Vizio system can resolve intact and degenerating synapses *in vivo*. We are currently adapting this methodology to carry out a) repeated, semi-continuous visualisation of axonal and synaptic degeneration *in vivo* with minimal surgical invasion; b) high-throughput screening of ENU mutants (Nolan et al. 2002) for novel phenotypes showing slow synaptic degeneration.

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