Predhodno priopćenje
Preliminary study

MAGNETIC BRAIN STIMULATION MODULATES NEURONAL PLASTICITY IN SPINAL CORD INJURY PATIENTS

MAGNETSKA STIMULACIJA MOZGA MODULIRA NEURONALNI PLASTICITET U BOLESNIKA SA OZLJEDOM LEĐNE MOŽDINE

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Summary: Patients can often recover good motor function in muscles below an incomplete spinal cord injury. Within a few days of injury natural processes lead to down-regulation of inhibitory pathways within the motor cortex than can be demonstrated electrophysiologically. We believe that this natural change might encourage motor recovery by allowing an increased excitability of surviving descending corticospinal neurones. Repetitive transcranial magnetic stimulation (rTMS) can produce similar changes in corticospinal inhibition in normal uninjured individuals, albeit rather short-lasting. In this preliminary study we have delivered a sham rTMS over one week followed by another week of real treatment to four stable incomplete spinal cord injury patients who already showed reduced inhibition compared with controls. Intracortical inhibition was further reduced during the week of treatment but recovered basal levels within the three-week follow-up period. Longer-term improvements were seen in the clinical scores for both motor and sensory function, perceptual threshold to electrical stimulation of the skin and the time taken to complete a standard peg-board test. Although the measurable electrophysiological effects of rTMS are short-lived it would appear that functional recovery persists for at least three weeks after the treatment. Spinal cord injury patients might be more susceptible to the plastic cortical changes evoked by rTMS than non-injured individuals. This preliminary study provides promising data on which to base a larger investigation with the aim of substantiating the use of rTMS as a tool for routine use in rehabilitation.

The central nervous system has a remarkable natural ability to reorganise itself and restore function after it is damaged (see review[1]). Patients who make a good recovery of motor function following incomplete spinal cord injury (iSCI) exhibit: (i) reduced corticospinal inhibition,[2,3] similar to that seen following stroke[4] and (ii) altered patterns of corticospinal facilitation.[5] These changes occur within a month of injury[6] and have been identified in patients with iSCI in the cervical spinal cord by recording electromyographic (EMG) responses in hand muscles to transcranial magnetic stimulation (TMS) of the motor cortex. Imaging studies using magnetic resonance spectroscopy (MRS)[7] and positron emission tomography (PET)[8] have shown an elevated level of the neuronal metabolite *N-acetylaspartate* (NAA) and a changed distribution of the inhibitory neurotransmitter *gamma aminobutyric acid* (GABA) in the sensorimotor cortex. These biochemical and pharmacological changes are consistent with a reduced inhibitory drive within the motor cortex. We propose that such natural down-regulation of cortical inhibition is associated with, and may promote recovery of motor function.

The increased excitability to TMS in normal subjects following an ischaemic nerve block[9] is also thought to result from a reduction in intracortical inhibition. This short-term cortical modulation has been manipulated with drugs[10] providing evidence that the inhibition is GABA-mediated; indeed, this has also been suggested by our own ligand-binding work in iSCI.[8] Application of repetitive transcranial magnetic stimulation (rTMS) produces modulation of both intracortical inhibition and facilitation. Furthermore, rTMS over the contralateral cortex during the period of deafferentation (nerve block) results in reduced inhibition and enhanced facilitation whereas rTMS over the ipsilateral cortex results in enhanced inhibition and reduced facilitation.[9] This work suggests that disordered ascending afferent feedback to the cortex, nearly always seen in iSCI, may influence the processes of plasticity that modulates output from the motor cortex. Other studies have also shown that rTMS can modulate inhibitory and excitatory neuronal connections within the motor cortex[11,12] making it a potentially important tool that might improve motor recovery in iSCI, although, to date no conclusive study has been conducted.[13] A more functional

sign of change reflected by improvement of voluntary power in quadriceps muscles following delivery of rTMS, while the muscle was contracting, has also been reported.[14]

We have now studied four patients with stable iSCI before, during and following a week of daily treatment using a combination of low and high frequency rTMS (see Methods).

Corticospinal inhibition: All patients showed corticospinal inhibition in response to single-pulse TMS in each of the assessments made during the baseline and sham periods before the week of rTMS treatment. The index of inhibition (see Methods) was plotted in bar charts for each testing session in each patient (figure 1A). Examination of data from each patient reveals that the index of inhibition dropped in the four testing sessions conducted during the week of treatment for all four patients. Patient number 3 showed a negative index of inhibition in the second testing session during the treatment week; this indicates that the inhibition had disappeared completely and that a small increase in EMG above pre-stimulus levels was evident at the time of expected inhibition. The first follow-up assessment shows that the inhibition had returned towards pre-treatment levels in all four patients. In subsequent follow-up assessments there was little further change. The lower histogram in figure 1A shows the mean levels of inhibition in all four patients seen before (baseline and sham assessments combined), during the treatment week and during the follow-up period. The mean index of inhibition is reduced by 66 % during the treatment week (**P** < 0.05; ANOVA on ranks with Dunn's correction) compared with the assessments made before treatment and by 60 % compared with assessments made during the follow-up period. Figure 1B shows four example traces recorded from patient number 2 in the assessment sessions before treatment, in the middle and at the end of the treatment week and two weeks into the follow-up period. The inhibition is clearly evident before treatment, during there is a vestige present in the mid-treatment record but this has disappeared completely by the end of the treatment week. Two weeks into the follow-up period there is evidence of the inhibition returning. From this evidence it would appear that the naturally-reduced corticospinal inhibition seen in incomplete spinal cord injury can be accentuated by rTMS treatment. Although the index of inhibition remains depressed from day to day during the week of treatment it has substantially recovered pre-treatment levels one week after the end of the treatment.

Perceptual threshold, ASIA clinical assessments and timed peg-board: All the patients showed a small drop in perceptual threshold to an electrical stimulus applied to the skin of the hand during the week of treatment. Perceptual threshold values for each assessment session in each patient are plotted in the bar charts in figure 2A. This indicates an increase in sensitivity to sensory stimulation of the skin. Data from the four patients show that the effect is not immediate and takes time to become established during the week of treatment. However, the perceptual thresholds remain lowered during the follow-up period. The lower histogram in figure 2A shows the mean levels of perceptual threshold in all four patients seen before (baseline and sham assessments combined), during the treatment week and during the follow-up period. Mean perceptual threshold were decreased by an average of 0.25 mA (15 %) during the treatment week and follow-up period compared with pre-treatment levels ($P < 0.05$; repeated measures ANOVA with Tukey correction). From this data it would appear that the rTMS has altered the circuitry, perhaps within the sensory cortex, involved in perceiving stimulation of the skin and increased the sensitivity of the skin to electrical stimulation.

Conventional ASIA clinical assessments of sensory and motor function were also made as part of each patient assessment session. The same clinician performed all the tests in all the patients. Sensory measurements were made over each of the 28 dermatomes on both sides of the body using light touch to assess posterior column function and pin-prick to assess spinothalamic pathways. Each dermatome was scored as follows: no sensation = 0; abnormal sensation = 1; normal sensation = 2. The scores were added up to give a maximum (normal) score of 112. The mean pin-prick score (figure 2B) increased by 9/112 during the week of treatment compared with pre-treatment levels and remained elevated during the follow-up period ($P < 0.05$; ANOVA with Tukey correction). The mean light touch score (figure 2C) was elevated during the week of treatment by 6/112 and remained elevated by 5/112 into the follow-up period but this change was not statistically significant ($P > 0.05$; ANOVA on ranks with Dunn's correction). Clinical scores of motor function were also made according to ASIA criteria. Five key muscles innervated by the cervical cord and five by the lumbar cord were measured for power and scored out of 5, with no perceived voluntary movement scoring 0 and normal power scoring 5. The scores for each key muscle on both sides of the body were added to give a maximum (normal) score of 100. The motor score (figure 2D) was elevated during the week of treatment by 4/100 and remained elevated into the follow-up period ($P < 0.05$; ANOVA on ranks with Dunn's correction). This clinical data suggests a small improvement in motor function that persisted up to three weeks after the end of treatment.

Each patient completed a ten-peg peg-board test against the clock as part of each assessment session. They were instructed to remove all ten pegs from the holes in the board and then replace them as quickly as possible using only their right hand. The time for this task in seconds was recorded. There was an improvement of 4 seconds in the mean time to complete this test in the treatment week compared with the pre-treatment times; however this difference was not statistically significant ($P > 0.05$; ANOVA with Tukey correction). There was a further improvement (averaging another 2 seconds) into the follow-up week; the mean times in the follow-up week were significantly different from the pre-treatment values ($P < 0.05$; ANOVA with Tukey correction). The results of this test suggest that the physiological and clinical

changes that we observed might have some functional relevance in the motor performance of the patients.

It would appear that the naturally occurring down-regulated corticospinal inhibition commonly seen in patients with incomplete spinal cord injury[3] was reduced further during and immediately following rTMS treatment. This result is consistent with evidence from normal individuals where rTMS has been shown to modulate corticospinal excitability by altering cortical inhibition. Two studies have used test-conditioning paired pulse TMS to assess intracortical inhibition following rTMS; Wu *et al.*[12] demonstrated that 30 pulses of 15 Hz rTMS could reduce intracortical inhibition for up to 3.2 minutes while Peinemann *et al.*[15] showed a reduction in inhibition for 10 minutes following 1250 pulses of 5Hz rTMS. Other work[16] showed that only 20 pulses of rTMS at frequencies of 5-20 Hz could increase the amplitude of motor evoked potentials (MEPs), possibly reflecting reduced inhibition, but for only for a period of 1 second. However, lower intensity rTMS produced a reduction in MEP amplitudes such at those reported by Touge *et al.*[17] Another study reported that 15 minutes of 1 Hz rTMS produced increases in MEP thresholds whether delivered at 85% or 115% of pre-treatment motor threshold[18] and that there was no effect on cortical inhibition. These studies show that a variety of acute effects of rTMS can be evoked in the motor cortices of normal man and that stimulus frequency, intensity and duration are all important factors in determining the precise resultant effect. The spinal cord injury patients in this study had already shown the kind of reduction in corticospinal inhibition following injury that is commonplace following this sort of trauma.[3] Although they had reached a stable state clinically their brains may be more receptive to further change as a result of the intervention with rTMS. The rTMS was applied for much longer periods of time than the control studies outlined above and it contained both high (10 Hz) and low (0.1 Hz) frequency components.

The clinical changes observed are consistent with the idea that reduced corticospinal inhibition can facilitate a more functional recovery and this is reflected by the increase in ASIA motor scores and possibly the improved dexterity indicated by faster peg-board completion times. The changes we observed in sensory function illustrated by reduced perceptual threshold to electrical stimulation and by increases ASIA sensory scores are less easy to explain. Clearly the rTMS coil, because of its large size, lay over sensory as well as motor cortex. There is little evidence that TMS can activate sensory cortex but there is one report of body sensations evoked by TMS in spinal cord injury patients;[19] possibly sensory gating mechanisms are altered following the deafferentation that occurs as a result of spinal injury making the sensory cortex more amenable to stimulation by TMS. It is possible that rTMS can also affect plasticity within the sensory cortex and produce changed sensory thresholds in the patients in this study.

Clearly a more extensive continuation of this preliminary study must be conducted before rTMS can be advocated as a routine therapy in spinal cord injury; nevertheless we feel that the present results provide very positive evidence of its future potential.

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Methods

Patients: With local ethical committee approval we studied four patients with stable incomplete spinal cord injury recruited from the National Spinal Injuries Centre at Stoke Mandeville Hospital. Three of the patients (all male, ages 41, 54 and 54 years) suffered their spinal cord trauma between seven and eight years prior to the study and the fourth (female, age 26 years) fifteen months prior to study. All patients had their clinical level of lesion (the last segment of normal function) diagnosed at the fifth cervical segment and all had D-gradings when assessed according to American Spinal Injury Association (ASIA) criteria.[20] A previous study from our laboratory indicated that the natural recovery processes in incomplete spinal cord injury become stable at a time 6 months to one year after trauma.[6] Out-patient clinical assessments of all the patients showed them to have been clinically stable for at least nine months and the three males for several years.

Experimental protocol and rTMS application: The patients were assessed twice, using the tests outlined below, before any intervention. They then received daily *sham* treatment for five days with the magnetic stimulating coil held tangentially over the occipital cortex. Patients were assessed on a further two occasions during the *sham* treatment week. Patients then received the putative *therapeutic* treatment with rTMS over one motor cortex. A circular (9 cm diameter) stimulating coil was positioned over the vertex with the current flowing in a clockwise direction so as to activate the left motor cortex.[21] The stimulus intensity was set to 90 % of motor threshold. Motor threshold was defined as the lowest stimulus intensity that produced identifiable motor evoked potentials in *thenar* muscle EMG recordings to at least 50 % of presentations. Each rTMS treatment took place over five consecutive days with each session lasting for one hour (a total of 360 doublet pulses). Double pulses of rTMS were applied separated by 100 ms at a frequency of 0.1 Hz, a frequency shown to modulate cortical plasticity (Ziemann *et al.*, 1998a). Application of 10 Hz rTMS over the prefrontal cortex has been reported to be efficacious in drug-resistant depression[22] and obsessive compulsive disorder.[23] The safety of rTMS has been tested and guidelines for use and exclusion criteria have been derived (see[24,25]). During the week of treament, patients were assessed on four occasions. Patients were assessed on three further occasions at approximately weekly intervals.

Continuous assessment of patients: Clinical, functional and electrophysiological patient assessments were made on four occasions before the week of treatment, four times during the week of treatment and three time after the week of treatment. *Clinical* assessments of sensory (light-touch and pin-prick) and motor function were made, always by the same clinician, to American Spinal Injury Association (ASIA) standards.[20] Perceptual threshold to electrical stimulation of the skin[26] over the right hand was used to identify more accurately deficits in sensory function. A timed peg-board test was used to assess changes in *functional* ability (see[27]) as it measures speed and dexterity in a task with known reliance on corticospinal function. *Electrophysiological* testing assessed intracortical inhibition from surface EMG to single-pulse TMS of the motor cortex (see[3]). The thenar muscles were chosen as target muscles as all the patients had their neurological lesion level above cervical spinal segment 7/8 from where these muscles are innervated. Experiments were conducted during weak voluntary contraction (5-10% maximum voluntary contraction) to allow investigation of corticospinal inhibition. TMS was applied to the left motor cortex to produce motor evoked potentials (MEPs) and periods of inhibition (silent periods) in the right *thenar* muscles. In the first baseline testing session, the stimulus intensity for each patient was adjusted so that a cortical silent period could be identified in the on-going EMG in the absence of a MEP. This intensity was used for that patient in all subsequent testing sessions. A full session of the assessments described above lasted approximately 60-90 minutes and each patient was assessed on eleven different occasions.

Data analysis: No patients showed a difference between the baseline and sham week assessments so these were combined into one data set before the week of treatment. Expressing the area of inhibition as a proportion of a fixed area (50 ms) of pre-stimulus voluntary activity allowed us to derive an index of corticospinal inhibition. This index, perceptual threshold, ASIA scores, and time to complete the peg-board test were compared before during and after the week of treatment. For the perceptual threshold data we used a repeated measures ANOVA analysis with Tukey correction to compare the means for each patient in each phase of the trial. Perceptual thresholds before treatment were very different between subjects and pair-wise analysis highlighted the consistent reductions in perceptual threshold during and after the treatment. All other data was analysed using unpaired ANOVAs on the raw data where data was normally distributed (ASIA pin-prick scores and timed peg-board) or ANOVA on ranks (index of inhibition, ASIA light-touch and ASIA motor scores). Level of significance was taken as $P < 0.05$ throughout.

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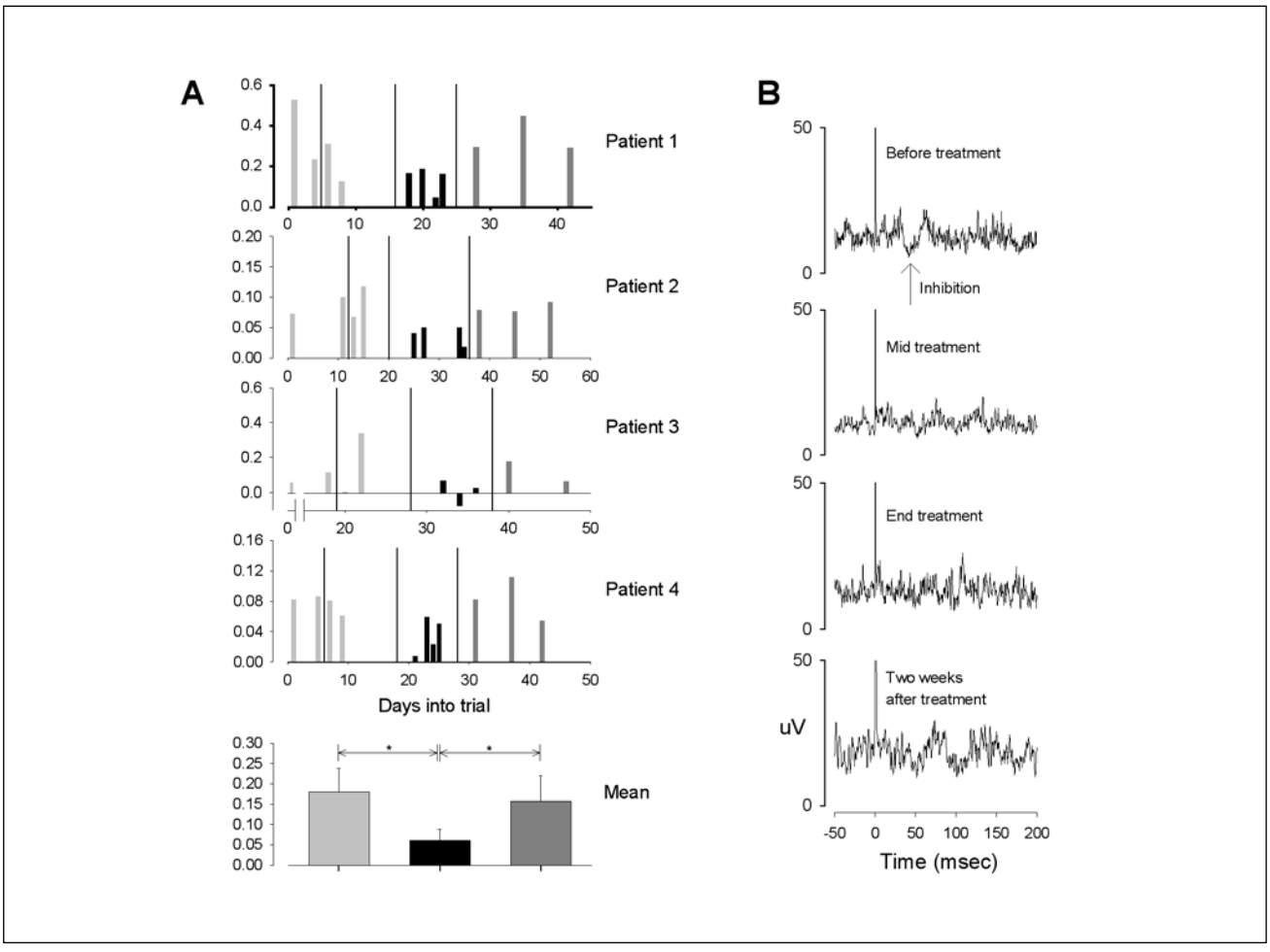

Figure 1
A: Bar charts showing the index of corticospinal inhibition (see Methods) during the baseline assessment, the week of sham treatment, the week of rTMS treatment and during the month following the week of treatment. Data is shown for each patient. Averaged data from all four patients are shown in the lower histogram in which the baseline and sham data have been combined. The inhibition became weaker during the week of treatment and recovered during the follow-up period. Error bars indicate one standard error of the mean. Asterisks indicates a statistically significant difference between bars where $P < 0.05$ (ANOVA on ranks with Dunn's correction).

B: Example EMG responses to TMS from patient 2. A small period of inhibition is evident before treatment which became small or absent during the week of treatment. The assessment two weeks following treatment showed signs of a weak inhibition. Each record represents an average of 30 full-wave rectified responses to TMS delivered at 35 % of the maximum stimulator output.

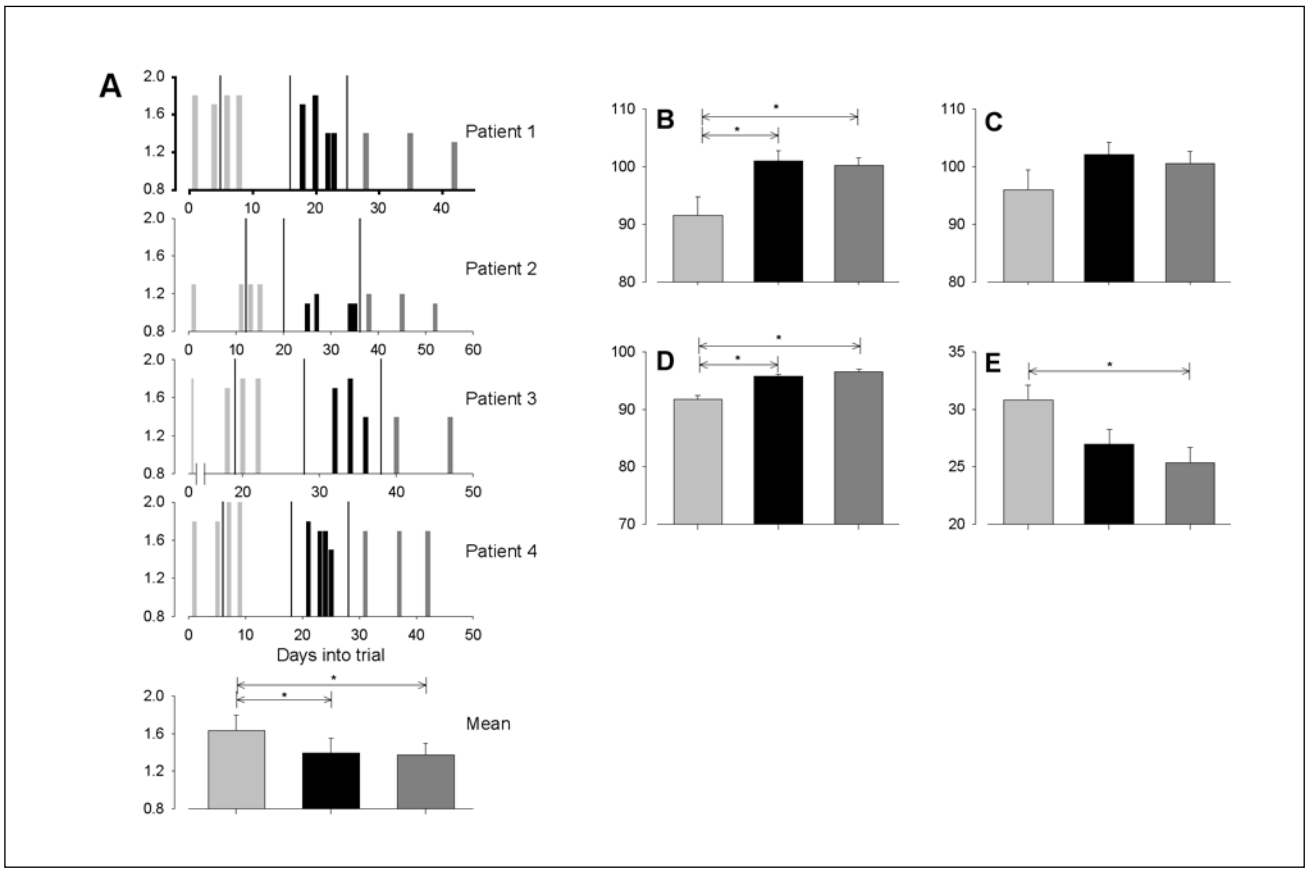

Figure 2

A: Bar charts showing perceptual threshold to an electrical stimulus to the hand (see Methods) during the baseline assessment, the week of sham treatment, the week of rTMS treatment and during the month following the week of treatment. Data is shown for each patient. Averaged data from all four patients are shown in the lower histogram in which the baseline and sham data have been combined. Perceptual threshold was lower during the week of treatment and remained low during the follow-up period ($P < 05$, repeated measures ANOVA with Tukey correction).

Mean ASIA clinical scores for pin-prick sensation **(B)**, light touch sensation **(C)** and motor function **(D)** over the whole body (see Methods). The clinical scores indicate an improvement during the week of treatment that is sustained into the follow-up period.

Mean time to complete a peg-board test is shown in **(E)**. Error bars indicate one standard error of the mean. Asterisks indicates a statistically significant difference between bars where $P < 0.05$ (**B,D:** ANOVA with Tukey correction; **C,E:** ANOVA on ranks with Dunn's correction).

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