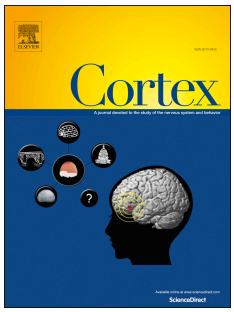
Accepted Manuscript



Stimulus uncertainty enhances long-term potentiation-like plasticity in human motor cortex

Dr. Martin V. Sale, Abbey S. Nydam, Jason B. Mattingley

PII: S0010-9452(16)30352-5

DOI: 10.1016/j.cortex.2016.12.008

Reference: CORTEX 1897

To appear in: *Cortex*

Received Date: 26 July 2016

Revised Date: 6 October 2016

Accepted Date: 9 December 2016

Please cite this article as: Sale MV, Nydam AS, Mattingley JB, Stimulus uncertainty enhances long-term potentiation-like plasticity in human motor cortex, *CORTEX* (2017), doi: 10.1016/j.cortex.2016.12.008.

Title: Stimulus uncertainty enhances long-term potentiation-like plasticity in human motor cortex

Authors: Martin V. Sale[a,b], Abbey S. Nydam[c], Jason B. Mattingley[a,c]

Affiliations: The University of Queensland, QLD 4072, Australia: [a]Queensland Brain Institute; [b]School of Health and Rehabilitation Sciences; [c]School of Psychology

Correspondence: Dr. Martin Sale, School of Health and Rehabilitation Sciences, Building 84A, The University of Queensland, St Lucia, QLD 4072, Australia.

Email: m.sale@uq.edu.au or martinvsale@gmail.com

Tel: +617 33653008

Abstract

Plasticity can be induced in human cortex using paired associative stimulation (PAS), which repeatedly and predictably pairs a peripheral electrical stimulus with transcranial magnetic stimulation (TMS) to the contralateral motor region. Many studies have reported small or inconsistent effects of PAS. Given that uncertain stimuli can promote learning, the predictable nature of the stimulation in conventional PAS paradigms might serve to attenuate plasticity induction. Here, we introduced stimulus uncertainty into the PAS paradigm to investigate if it can boost plasticity induction. Across two experimental sessions, participants (n = 28) received a modified PAS paradigm consisting of a random combination of 90 paired stimuli and 90 unpaired (TMS-only) stimuli. Prior to each of these stimuli, participants also received an auditory cue which either reliably predicted whether the upcoming stimulus was paired or unpaired (no uncertainty condition) or did not predict the upcoming stimulus (maximum uncertainty condition). Motor evoked potentials (MEPs) evoked from abductor pollicis brevis muscle quantified cortical excitability before and after PAS. MEP amplitude increased significantly 15 minutes following PAS in the maximum uncertainty condition. There was no reliable change in MEP amplitude in the no uncertainty condition, nor between post-PAS MEP amplitudes across the two conditions. These results suggest that stimulus uncertainty ~~boost~~ may provide a novel means to enhance plasticity induction with the PAS paradigm in human motor cortex. To provide further support to the notion that stimulus uncertainty and prediction error promote plasticity, future studies should further explore the time course of these changes, and investigate what aspects of stimulus uncertainty are critical in boosting plasticity.

Highlights

- Plasticity can be induced in human cortex by repeated pairing of stimuli.
- This form of induced plasticity mimics behavioral associative learning.

- We manipulated stimulus uncertainty, which influences learning and plasticity.
- When stimulus uncertainty was high, plasticity was enhanced in human motor cortex.

Keywords: entropy, neuroplasticity, prediction error, paired associative stimulation, Bayesian

1. Introduction

The ability to learn relationships between sensory events (cues) and their expected consequences is critical for human function (Esber & Haselgrove, 2011). Yet the relationship between cues and learning is not linear; more cues do not necessarily equate to more effective learning. Animals and humans quickly learn predictive relationships between sensory inputs and their expected outcomes (Gallistel & Matzel, 2013), and if the relationship between sensory inputs and outcomes becomes predictable, neural activity (Alink, Schwiedrzik, Kohler, Singer, & Muckli, 2010) and learning are significantly reduced (Hogarth, Dickinson, Austin, Brown, & Duka, 2008; Kording & Wolpert, 2004; Orban & Wolpert, 2011; Pearce & Hall, 1980; Vanni-Mercier, Mauguiere, Isnard, & Dreher, 2009). This suggests that although the contiguity of events is important (Wheeler & Miller, 2008), the associative relationship between these events is crucial to learning. More specifically, when the relationship between a cue and an outcome is not predictable, but instead is uncertain, learning is enhanced. Here, we report on the effect of stimulus uncertainty in an associative-stimulation paradigm in which learning-like plastic changes were induced in human motor cortex using non-invasive brain stimulation.

One of the candidate mechanisms contributing to learning is a change in synaptic efficacy. An increase in synaptic efficacy is referred to as long-term potentiation (LTP). LTP-like changes can be induced in humans using non-invasive brain stimulation. Paired associative stimulation (PAS) repeatedly pairs a peripheral electrical nerve stimulus targeting an intrinsic hand muscle with transcranial magnetic stimulation (TMS) over the motor cortical region representing that muscle (Stefan, Kunesch, Cohen, Benecke, & Classen, 2000). When the timing of these two stimuli is adjusted such that the afferent volley arising from the electrical nerve stimulus arrives in the motor cortex just before a TMS pulse depolarizes the

output neurons, LTP-like changes in cortical excitability are induced. The plastic changes arising from PAS are quantified indirectly by comparing the size of the motor evoked potential (MEP) evoked with TMS before and after PAS (Stefan et al., 2000). The duration of the PAS-induced change in MEP amplitude persists for up to 30-90 minutes after stimulation (Stefan et al., 2000; Wischnewski & Schutter, 2015). Although several variants of PAS have been developed, the repeated pairing of the stimuli is invariably predictable and rhythmic. For example, in the seminal study that first described PAS, Stefan et al. (2000) delivered ninety pairs of stimuli at a fixed interval of 0.05 Hz over 30 minutes. Such an approach has been used by many other subsequent studies employing PAS (e.g., Cirillo, Lavender, Ridding, & Semmler, 2009; Di Lazzaro et al., 2009; Fratello et al., 2006; Player, Taylor, Alonzo, & Loo, 2012). Critically, however, in all variants of PAS, the pairing of the peripheral and cortical stimulation occurs in a regular and entirely predictable manner, which would appear to make it non-optimal for inducing learning-related changes.

We developed a novel PAS paradigm in which the arrival of the plasticity-inducing paired stimuli was uncertain. By pseudo-randomly introducing non-plasticity inducing single-pulses of TMS throughout the procedure, the participant was never certain whether the upcoming stimulus would be paired (plasticity-inducing) or unpaired (non-plasticity inducing). Further, we incorporated an auditory cue which either predicted with no uncertainty (100% certainty) whether the upcoming stimulus was paired or unpaired (no uncertainty condition), or predicted with 50% certainty, at the level of chance (maximum uncertainty condition) whether the upcoming stimulus was paired or unpaired. Given the role of stimulus uncertainty in boosting learning (Hogarth et al., 2008; Kording & Wolpert, 2004; Orban & Wolpert, 2011; Pearce & Hall, 1980; Vanni-Mercier et al., 2009), we investigated whether plasticity induced with PAS could be altered by manipulating stimulus uncertainty. We hypothesized that PAS-

induced plasticity would be increased when auditory cues did not reliably predict whether the forthcoming stimulus was paired or unpaired.

2. Materials and Methods

2.1. Participants

Data from 28 healthy volunteers were included (16 male; mean ± SEM = 23.3 ± 0.5; range, 20-32 years). All were right-handed (mean LQ = 0.9, range 0.6-1.0) as assessed by the Oldfield handedness questionnaire (Oldfield, 1971). Participants attended two experimental sessions, each approximately one week apart. All participants were naïve to the experimental paradigm. No participants were taking neuroactive medication. All participants provided written informed consent, and the study was approved by The University of Queensland Medical Research Ethics Committee.

2.2. Experimental arrangement

Participants were seated comfortably in a chair. Surface electromyography (EMG) recordings from left *abductor pollicis brevis* (APB) muscle were obtained using bipolar Ag-AgCl electrodes in a belly-tendon montage. EMG signals were amplified 1000 times, filtered (20-2000 Hz; NeuroLog, Digitimer), digitized (2 kHz) via a CED 1401 interface (Cambridge Electronic Design), and stored on computer for offline analysis. EMG signals were displayed on an oscilloscope to assist (via verbal feedback) the participant in maintaining EMG silence when required.

2.2.1. TMS and peripheral nerve stimulation

Monophasic TMS was applied through a 70 mm figure-of-eight coil and a Magstim 200^2 stimulator (Magstim). The site for TMS was defined as that which consistently elicited the largest MEPs from left APB at a suprathreshold stimulus intensity. The coil was held tangentially to the skull with the handle pointing backwards and laterally at ~45º to the sagittal plane, inducing a posterior-to-anterior current in the cortex. This location was targeted throughout the session using an infrared stereotaxic navigation system (Visor, ANT). Electrical stimuli were applied to the median nerve of the left wrist using a constant current stimulator (DS7 stimulator; Digitimer) with bipolar surface electrodes (30 mm spacing), and with the cathode proximal. Stimuli were square waves with a pulse width of 200 µs.

2.2.2. Paired associative stimulation (PAS)

The PAS protocol involves a series of paired peripheral and cortical stimuli (Stefan et al., 2000). The peripheral electrical stimulus was delivered to the left median nerve at the wrist. The stimulus intensity was set as the minimum intensity required to elicit a motor response (M-wave) > 200 µV in amplitude. This stimulus was followed 25 ms later by a TMS pulse to the cortical representation targeted by the peripheral stimulation in the right motor cortex. The left hand/right motor cortex was chosen because it allowed us to directly compare the results of the present study with previous PAS experiments conducted in our laboratory (Kamke, Hall, et al., 2012; Kamke, Nydam, Sale, & Mattingley, 2016; Kamke et al., 2014), and because it has been shown previously that there are no hemispheric differences in PAS-effectiveness (Ridding & Flavel, 2006). The TMS intensity was adjusted to evoke an MEP of ≃0.5-1mV in peak-to-peak amplitude (test intensity). There were 90 paired stimuli delivered in 15 minutes (Kamke et al., 2014; Sale & Mattingley, 2013). The conventional PAS paradigm repeatedly and predictably delivers these paired stimuli at regular interstimulus intervals. We introduced non-plasticity

inducing, single pulses of TMS (n = 90) pseudo-randomly throughout the PAS paradigm. These 'unpaired' TMS (TMS-only) pulses thus allowed us to manipulate the level of uncertainty as to whether the upcoming stimulus was 'paired' (i.e., contributing to plasticity induction with PAS) or 'unpaired' (i.e., non-plasticity inducing single pulse TMS) (Figure 1). Critically, across the two experimental sessions, the order of the paired and unpaired stimuli was equivalent.

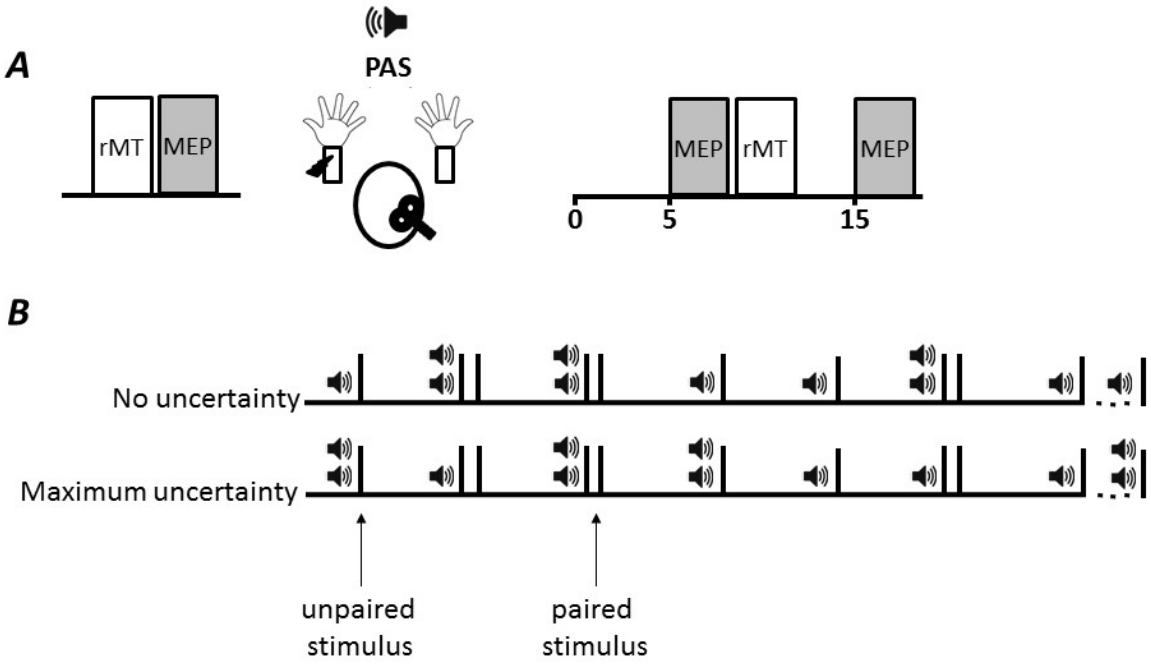


Figure 1: Experimental overview and auditory cueing design. A, Plasticity was induced in right motor cortex using the paired associative stimulation (PAS) procedure. Plasticity was probed by measuring the peak-to-peak amplitude of motor evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS) before and at 5 minutes and 15 minutes after PAS. rMT, resting motor threshold. B, In separate sessions participants heard either a single auditory tone (🔊) or a double auditory tone (🔊🔊) prior to receiving either the TMS pulse alone (i.e., non-plasticity inducing) or the TMS pulse paired with a peripheral electrical stimulus (i.e., plasticity inducing). In the no uncertainty condition, the

single auditory tone always preceded a single TMS pulse, and a double auditory tone always preceded the paired stimuli (i.e., the auditory tone was 100% predictive of the subsequent stimulus). In the maximum uncertainty condition, the single and double auditory tones predicted with 50% accuracy the subsequent stimulus (i.e., maximally uncertain). Note that the sequence of paired and unpaired stimuli was exactly the same in both the no uncertainty and maximum uncertainty conditions – the only difference was the relationship with the preceding auditory cue.

Uncertainty was manipulated by the introduction of auditory cues, as described in detail below. There were a total of 180 trials in the PAS paradigm, consisting of 90 paired pulses, and 90 unpaired pulses. Pulse types (paired, unpaired) were randomised in blocks of 20 to ensure no runs of either paired or unpaired pulses exceeded three successive trials. Pulses were delivered at 0.2 Hz, so that the paired stimuli were delivered at an average frequency of 0.1 Hz, which has been shown previously to induce reliable effects on cortical excitability (Kamke, Hall, et al., 2012; Kamke et al., 2014; Player et al., 2012; Sale & Mattingley, 2013). Each experimental session was conducted at approximately the same time of day to minimize the known influence of circadian factors on PAS-induced plasticity (Sale, Ridding, & Nordstrom, 2007a, 2008).

Auditory stimuli served as predictors of the subsequent paired or unpaired pulses. Tones (frequency 1000 Hz) were delivered as either a single tone (duration 100 ms) or double tone (duration 100 ms with 200 ms spacing) using Creative® speakers set at a constant, suprathreshold intensity. Each series of auditory stimuli preceded the paired or unpaired pulses by 2 seconds, and there was a further 3 second break after the paired or unpaired pulses before the next auditory stimuli were presented. In the no uncertainty condition, a single auditory tone predicted with 100% certainty that the upcoming stimulus was unpaired, and a double auditory tone predicted with 100% certainty that the upcoming stimulus was a paired pulse. Thus, the tone was 100% predictive of the subsequent type of stimulation pulse (either paired or

unpaired). In the maximum uncertainty condition, the relationship between auditory stimuli and pulses was at the level of chance, and thus entirely uncertain (Figure 1).

The relevance of the auditory tones and their relationship with the subsequent stimuli was not explicitly explained to participants before the experiments commenced. However, in order to maximize the effectiveness of PAS-induced plasticity, and to control for attention across sessions (Kamke, Hall, et al., 2012; Kamke et al., 2014; Stefan, Wycislo, & Classen, 2004), participants were asked to attend to the auditory stimuli and respond to an 'oddball' tone that occurred periodically throughout the protocol. The oddball was a single auditory tone delivered at a lower pitch (800 Hz) on 18 randomly occurring trials. Participants were asked to verbally respond 'yes' when they heard the oddball tone. Trials in which the responses were absent or delayed (occurring after the trial had ended) were tallied as errors. This served as a simple means of maintaining participants' attention during the procedure. The 2 s break between tones and pulses allowed participants enough time to respond without their verbal response interfering with the stimulation pulse. Furthermore, oddball tones were only ever presented in place of a single auditory tone occurring before an unpaired pulse so as to not contaminate paired PAS pulses with possible activity caused by the verbal response. The distribution of oddball tones was equivalent across blocks of 20 trials for both conditions. We also investigated whether participants' awareness of the contingency between auditory cues and subsequent paired or unpaired stimuli influenced plasticity induction. Participants were questioned afterwards whether they were aware of the relationship between auditory cues and subsequent stimuli. Participants who correctly reported that the double auditory tones occurred before the paired pulses and the single auditory tones before the unpaired pulses in one of their sessions, and that this association was not present on their other session, were categorized as being *aware* of the contingency. The other participants were categorized as being *unaware* of the contingency.

The effectiveness of PAS ~~-induced plasticity~~ was probed indirectly in two ways: by quantifying motor cortical excitability, and by quantifying the variability of MEPs. This involved measuring MEP amplitude at various stages during the experiment. Specifically, average (n = 20) peak-to-peak APB MEP amplitude evoked with single pulse TMS at test intensity was calculated at three time points: pre-PAS, 5 minutes post-PAS and 15 minutes post-PAS across the two sessions (Figure 1). The variability of MEP amplitude fluctuations was quantified by determining the coefficient of variation (cv) of MEPs at each of the three time points for the two conditions. Trials containing voluntary muscle activity in the 500 ms prior to TMS were discarded from the analysis (<1.5% of trials). There were a similar number of excluded trials in the "no uncertainty" condition (24 trials) and the "maximum uncertainty" condition (26 trials).

2.3. Data analysis

The APB MEP amplitude data were initially inspected for violations of normality, and were transformed as required. Preliminary analysis of mean MEP data revealed positively skewed distributions and significant Shapiro-Wilk tests, indicating the assumptions of normality were violated. After performing a natural log transform on the data, the Shapiro-Wilk test was no longer significant for any variable ($p > .05$ for all). The data were then analyzed with repeated-measures analyses of variance (ANOVA) with within-subject factors of time (3 levels: pre-PAS, 5 min post-PAS, 15 min post-PAS) and contingency (2 levels: no uncertainty, maximum uncertainty). A separate repeated-measures ANOVA was also conducted on the coefficient of variation (cv) of MEP amplitudes with within-subject factors of time and contingency. To investigate whether there were any carry-over effects of PAS, a separate repeated-measures ANOVA with within-subject factors of time and session (2 levels: first session, second session) was conducted. Further, the data were split according to whether

participants were aware or unaware of the stimulus contingency. *Post-hoc* investigation of whether results differed for the two awareness groups was conducted using a three way mixed ANOVA with factors of Time, Contingency and Awareness (Aware *vs.* Unaware).

The modified PAS protocol provided an opportunity to investigate changes in cortical excitability *during* plasticity induction. MEPs taken from the 90 single TMS pulses were divided into six epochs consisting of roughly 15 pulses and representing 2.5 minutes of stimulation. Trials in which EMG activity was present prior to stimulus onset were excluded for each participant. To investigate intra-PAS activity, MEPs were compared across the six epochs for the two conditions. A 2 x 6 repeated measures ANOVA was conducted with factors of epoch (6 levels) and contingency (no uncertainty, maximum uncertainty). Data were analysed using SPSS 19 (IBM) and are expressed as mean ± within-subjects error. Statistical significance was assumed at an α–level of $P < 0.05$, with corrections made for multiple comparisons.

3. Results

All participants completed both experimental sessions, and no adverse effects were noted.

3.1. Behavioral data

Across all participants and experimental sessions, a total of eight errors were made in the no uncertainty contingency and five errors were made in the maximum uncertainty contingency. A chi-squared test of independence indicated this difference was not significant, χ^2 (3, N = 56) = .72, *ns*. These results suggest participants were attending to the auditory stimuli equally across the two contingencies.

3.2. Baseline physiological measures

The stimulus intensities used for median nerve stimulation, resting motor threshold and baseline (pre-PAS) MEPs are shown in Table 1. As expected, there were no differences across the contingency conditions for median nerve stimulation, $t(27) = 1.203$, $p = .24$, or in baseline MEPs, $t(27) = .809$, $p = .43$. Thus, any differences in MEPs found between contingency conditions following the PAS protocol could not be accounted for by differences in stimulation characteristics. Further, there was no change in rMT following PAS in either the no uncertainty, $t(27) = .563$, $p = .58$, or maximum uncertainty, $t(27) = 1.156$, $p = .26$, condition, indicating that resting membrane potential was unaltered following PAS.

3.3. PAS-induced effects

Consistent with our *a priori* hypothesis, MEP amplitudes following PAS were greater in the maximum uncertainty condition than in the no uncertainty condition. The change in MEP amplitude for the two conditions, relative to baseline, is shown in Figure 2. ANOVA revealed a significant main effect of time, such that MEPs increased from pre-PAS ($M = .78$, $SD = .04$) to post-PAS 5 minutes ($M = .82$, $SD = .07$) and post-PAS 15 minutes ($M = .95$, $SD = .09$), $F(2, 26) = 4.48$, $p = .016$, $\eta_p^2 = .14$. There was no significant main effect of contingency, $F(1, 27) = 0.61$, $p = .441$, $\eta_p^2 = .02$, but there was a significant two-way interaction between time and contingency, $F(2, 26) = 3.42$, $p = .040$, $\eta_p^2 = .11$. The significant interaction was followed up with simple effect comparisons for time, conducted separately for each level of stimulus contingency.

The simple effects of time were significant for the maximum uncertainty contingency, $F(2, 26) = 6.52$, $p = .003$, $\eta_p^2 = .19$. Follow up pairwise comparisons revealed a significant increase in MEP amplitude at post-PAS 15 minutes ($M = .98$, $SD = .10$) relative to the pre-PAS baseline ($M = .75$, $SD = .05$), $t(27) = -2.62$, $p = .014$, and at the 15 minute post-PAS relative to the 5 minute post-PAS ($M = .75$, $SD = .07$), $t(27) = -3.65$, $p = .001$. These data show that,

relative to baseline, MEP amplitude in the maximum uncertainty condition increased by 32% 15-minutes following PAS. There was no significant change in MEP amplitude from pre-PAS to the 5 minute post-PAS, $t(27) = .348$, $p = .731$.

In contrast, the simple effects of time were not significant for the no uncertainty contingency, $F(2, 26) = 1.36$, $p = .264$, $\eta_p^2 = .05$ (see Figure 2), suggesting that changes in MEPs over time were not reliable for this condition. The mean MEP amplitudes for the three time points in the no uncertainty contingency were: baseline ($M = .79$, $SD = .05$), post-PAS 5 minutes ($M = .88$, $SD = .08$), and post-PAS 15 minutes ($M = .92$, $SD = .10$). In the no uncertainty condition, MEP amplitude increased by only 17% 15-minutes after PAS, approximately half the increase observed in the maximum uncertainty condition at the same time point post-stimulation. There was no significant difference in MEP amplitudes at the 15 minute post-PAS time point between contingencies, $t(27) = .754$, $p = .457$. There were no detectable carry-over effects of PAS, evidenced by a non-significant main effect of session, $F(1, 27) = 0.85$, $p = .365$, $\eta_p^2 = .03$.

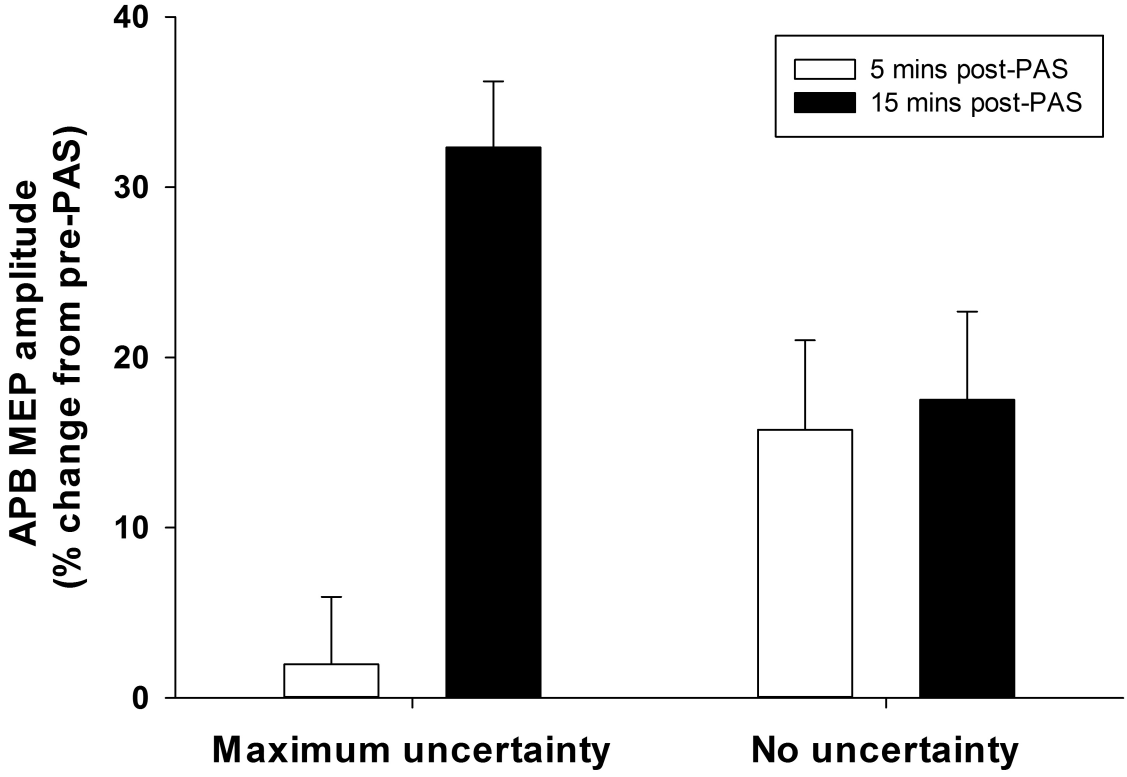


Figure 2: PAS-induced effects under maximum uncertainty and no uncertainty conditions. Mean MEP amplitudes at 5 minutes (white bars) and 15 minutes (black bars) following PAS are shown relative to baseline (pre-PAS) levels. Following PAS, MEPs were significantly larger 15 minutes post-PAS relative to baseline, but only in the maximum uncertainty condition (left; $p < 0.05$). There was no reliable increase in MEPs in the no uncertainty condition (right). Error bars indicate within-subjects errors.

MEP amplitude variability was unaffected by time or contingency. There was no significant main effect of time, $F(2, 27) = 0.47$, $p = .622$, $\eta_p^2 = .12$, nor contingency, $F(1, 27) = 1.03$, $p = .318$, $\eta_p^2 = .16$, on the coefficient of variation of MEP amplitudes. There was also no significant interaction between time and contingency, $F(2, 26) = 0.11$, $p = .893$, $\eta_p^2 = .07$.

3.4. Cortical excitability during PAS

There was a significant main effect of epoch, indicating a general increase in MEPs from the first epoch to the sixth epoch, $F(5, 135) = 5.22$, $p < .001$, $\eta_p^2 = 162$. There was no significant main effect of contingency, $F(1, 27) = .89$, $p = .353$, $\eta_p^2 = .03$, however, and no significant interaction, $F(5, 135) = 1.51$, $p = .191$, $\eta_p^2 = .05$, indicating that cortical excitability increased during the PAS procedure, but this was not influenced by contingency (Figure 3).

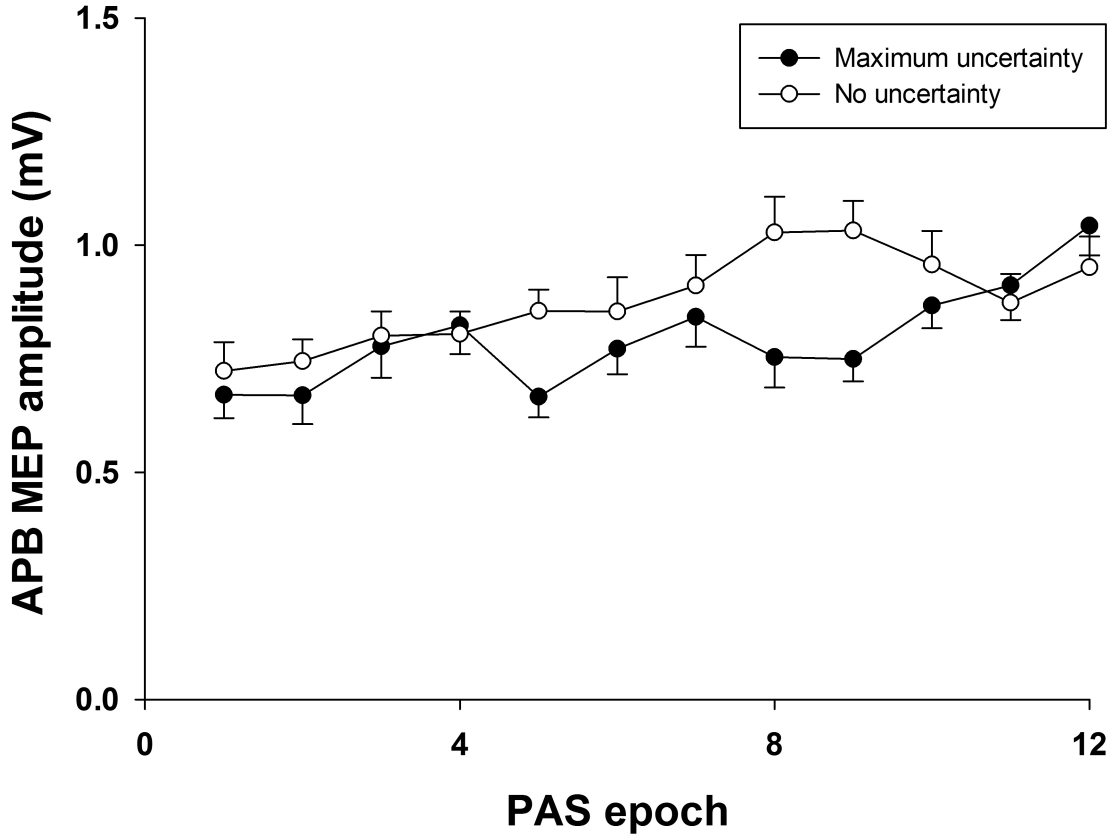


Figure 3: Cortical excitability during paired associative stimulation (PAS) in the maximum uncertainty and no uncertainty conditions. Motor evoked potential (MEP) amplitudes for the transcranial magnetic stimulation (TMS)-only stimuli across the 12 epochs spanning the 15 minutes of PAS for the maximum uncertainty (black circles) and no uncertainty (white circles) conditions. There was no significant difference in MEP amplitudes between the two conditions. Error bars indicate within-subjects errors.

3.5. Contingency awareness

A total of 18 participants could not report any relationship between the auditory tones and the paired or unpaired pulses (Unaware group). Ten participants were able to report that single auditory tones were related to the unpaired pulses, and double auditory tones were related to the paired pulses (Aware group). Of the 10 aware participants, five underwent the no uncertainty session first and five underwent the maximum uncertainty session first. Therefore, the order in which participants experienced the conditions appeared not to affect their propensity to detect the association between auditory tones and subsequent stimuli. Results for the predictive contingency data indicated no main effect of awareness, $F(2, 26) = 3.68$, $p = .066$ and no interaction , $F(2, 52) = .10$, $p = .906$ (Figure 4). Similarly, the random condition showed no significant effect of awareness, $F(2, 26) = 1.63$, $p = .213$ and no interaction, $F(2, 52) = .771$, $p = .468$. These results confirmed there was no reliable difference in the pattern of results depending on participants' awareness of the stimulus contingency.

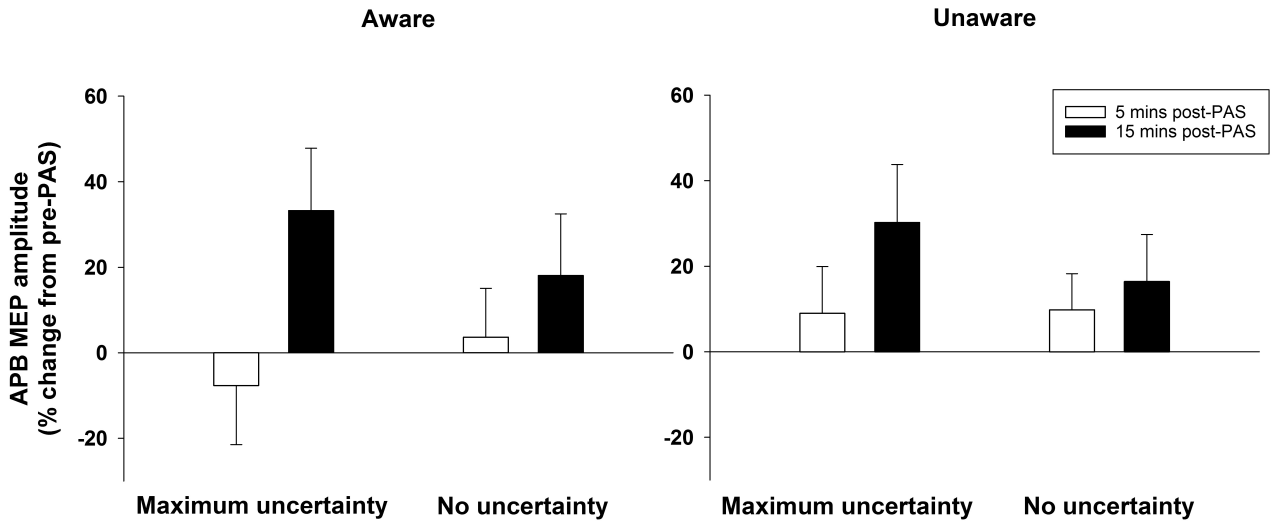


Figure 4: PAS-induced effects under maximum uncertainty and no uncertainty conditions for participants who were aware of the contingency manipulation (Aware, n = 10, left panel), and for those who were unaware of the contingency manipulation (Unaware, n = 18, right panel). Mean MEP amplitudes at 5 minutes (white bars) and 15 minutes (black bars) following PAS are shown relative to

baseline (pre-PAS) levels. There was no reliable difference in effects induced by PAS irrespective of whether participants were aware of the contingency manipulation or not. Error bars indicate within-subjects errors.

4. Discussion

Plasticity can be induced in humans using non-invasive brain stimulation protocols. These techniques are seen as potentially useful in the clinical sphere, as they may normalize aberrant plasticity and promote functional recovery (Lefaucheur et al., 2014; Ridding & Rothwell, 2007; Sale, Mattingley, Zalesky, & Cocchi, 2015). To be of greatest benefit, these protocols should induce robust and reliable plastic changes in the targeted brain region(s). Unfortunately, the induction of plastic changes with non-invasive brain stimulation is currently variable and unreliable (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2013; Ridding & Ziemann, 2010; Sale, Ridding, & Nordstrom, 2007b). There are several factors that have already been identified that seem to contribute to the variability of induced effects (for review see Ridding & Ziemann, 2010), including genetics (Witte et al., 2012), age (Todd, Kimber, Ridding, & Semmler, 2010), history of previous cortical activity (Sale & Mattingley, 2013), time of day of stimulation (Sale et al., 2007b, 2008), and cognitive factors such as attention (Kamke, Hall, et al., 2012; Kamke et al., 2014). Here we investigated whether stimulus uncertainty, a factor known to boost learning (Hogarth et al., 2008; Kording & Wolpert, 2004; Orban & Wolpert, 2011; Pearce & Hall, 1980; Vanni-Mercier et al., 2009), can increase the effectiveness of plasticity induced in the human motor cortex.

In separate sessions, auditory cues either reliably predicted whether an upcoming stimulus was paired or unpaired (no uncertainty condition), or provided no reliable information about the nature of the upcoming stimulus (maximum uncertainty condition). This

manipulation in stimulus uncertainty influenced the time course of changes in corticospinal excitability following PAS. When the auditory cues did not predict the upcoming stimulus type, plasticity induced with PAS was enhanced. However, when the auditory cues reliably predicted the upcoming stimulus (i.e., the stimuli were *not* uncertain), no plasticity was induced with PAS. This finding shows for the first time that stimulus uncertainty can boost plasticity induced in human cortex using non-invasive brain stimulation. It is important to point out that there was no difference between the two conditions in MEP amplitude at either of the two time points following PAS. Although this limits our interpretation and conclusions, we believe that our results are nevertheless informative in terms of the influence of stimulus uncertainty on changes in corticospinal excitability following PAS.

We only probed changes in cortical excitability up to 15 minutes following PAS, and so any longer-evolving temporal effects of stimulus uncertainty might not be captured in the current design. Indeed, some studies have shown that MEP changes following PAS reach their maximum 30-60 minutes following PAS (Frantseva et al., 2008; Morgante, Espay, Gunraj, Lang, & Chen, 2006). Our choice of measuring MEPs up to 15 minutes following PAS was motivated by the earlier work of Stefan et al. (2000), which suggested that the largest changes in cortical excitability occur at approximately this time point, before gradually returning to baseline at 60 minutes post-PAS. The MEP changes we report in the maximum uncertainty condition possibly suggest that the changes continue to manifest 15 minutes following PAS. We therefore suggest that future studies should probe cortical excitability changes for longer periods following PAS. This would allow for a better understanding of the temporal manifestation of the PAS effect when stimulus uncertainty is increased. Further, it appears that participants' awareness of the contingency between auditory cues and subsequent stimuli did not affect the magnitude of PAS-induced plasticity. However, the small participant numbers

used in this component of the analysis prevent us from unequivocally commenting on the influence of conscious awareness on plasticity induction.

We manipulated stimulus uncertainty by altering the contingency of auditory cues across the two experimental sessions. In every other aspect, the two sessions were identical. Therefore, any changes in MEP amplitudes arising from PAS over time ~~difference in MEP amplitudes~~ between the two conditions necessarily arose from an interaction between these cues and the paired stimuli of the PAS protocol. The findings of the present study suggest that when uncertainty is introduced into a plasticity-inducing paradigm, the effects are enhanced. Stimulus uncertainty can be considered to act as a sensory signal that feeds forward in a hierarchical model of perceptual processing (Friston, 2010). Bayesian models suggest the brain relies on both prior and current sensory information to create the best estimate of the current state of the world (Vilares & Kording, 2011). According to this framework, perceptual learning is described as a process of updating the prior distribution based on the current inferred posterior distribution (Knill & Pouget, 2004). The effect of sensory input on the current internal model depends on the degree to which the prior distribution differs from the posterior. For a predictable event, the prior and the posterior are equivalent, and there is little or no updating of the internal model. For uncertain stimuli, however, there is a divergence, and this leads to a greater effect of the sensory information on the model as evidenced by enhanced processing of that sensory input. In the current study, when the auditory cue did not reliably predict whether the upcoming stimuli were paired or unpaired, the PAS-induced increase in MEP amplitude was reliable.

There are several potential mechanistic explanations that could account for the increase in plasticity induction in the maximum uncertainty condition. First, animal (Perrett, Xiao, Barraclough, Keysers, & Oram, 2009) and human research (Alink et al., 2010; Garrido, Sahani, & Dolan, 2013) has shown that unpredictable stimuli evoke a larger cortical response

compared with predictable stimuli. Thus, there may have been a generalized increase in motor cortical excitability in the maximum uncertainty condition throughout the PAS protocol, reflecting an increase in arousal. Cortical excitability during PAS was probed in the present study by quantifying the amplitude of single-pulse TMS-evoked MEPs (see Figure 3). Although MEP amplitude increased throughout the PAS protocol, there was no difference between the maximum uncertainty and no uncertainty conditions. Therefore, a generalized increase in cortical excitability or arousal seems unlikely as an explanation for our results. It is important to point out that we cannot discount the possibility that the single pulses of TMS, added to allow the manipulation of uncertainty, may have interacted with the paired stimuli to alter the effects of PAS. Given the large time that separated these pulses (5 seconds), however, this seems an unlikely possibility. The interstimulus interval between pulses was an order of magnitude longer than any previous studies that investigated the influence of a preceding stimulus on the response to a subsequent TMS pulse (Schabrun, Weise, Ridding, & Classen, 2013).

Similarly, it is also possible that the auditory cues may have interacted with the PAS stimuli to affect plasticity induction. When sensory stimuli are presented simultaneously, or within a tight temporal window (< 100 ms), the perception of the stimuli can be dramatically affected (Kamke, Vieth, Cottrell, & Mattingley, 2012; Shams, Kamitani, & Shimojo, 2000; Violentyev, Shimojo, & Shams, 2005). Given that there were differences in the number of auditory cues preceding the paired stimuli of PAS in the two conditions, any interaction between the auditory cues and the sensory stimulation associated with PAS may have influenced PAS-effectiveness. Again, we consider this mechanistic explanation unlikely, as the delay between the auditory cues and the PAS stimuli was 2000 milliseconds, far greater than the <100 millisecond temporal window required for the multi-sensory interaction to occur (Shams et al., 2000).

A second possible explanation for the increase in PAS effects in the maximum uncertainty condition relates to the mode of action of PAS. The plastic changes arising from PAS are thought to reflect long-term potentiation-like changes in synaptic efficacy (Stefan, Kunesch, Benecke, Cohen, & Classen, 2002; Stefan et al., 2000). These LTP-like changes are N-methyl-D-aspartate (NMDA) receptor dependant (Stefan et al., 2002). The NMDA receptor is often referred to as a coincidence detector (Hasan et al., 2013), as it requires the coincident binding of glutamate and depolarization of the post-synaptic cell to expel Mg^{2+} ions from the channel to permit the influx of Na^{+} ions, thereby initiating the cascade of cellular effects that underpin LTP. This associativity of inputs formed the rationale for the development of the PAS protocol (Stefan et al., 2000; Wolters et al., 2003): the afferent volley from the peripheral electrical stimulus is assumed to release glutamate in the targeted cortical neurons, while the TMS pulse over motor cortex depolarizes cortical output cells. If the afferent volley releases more glutamate in the maximum uncertainty condition, due to an increase in excitability induced by uncertainty, activation of the NMDA receptor (and therefore LTP) is more likely to occur.

Our findings suggest that uncertainty increases the effectiveness of PAS, but that the variability of motor cortical excitability following PAS is unaffected by manipulating stimulus uncertainty, as the coefficient of variation of MEPs was not altered. Involvement of the NMDA receptor in uncertainty processing is also supported by research on the mismatch negativity (MMN), which is an electrophysiological marker of stimulus uncertainty processing. In a typical paradigm used to elicit the MMN, an oddball auditory tone is embedded within a series of regular, standard tones (Garrido, Kilner, Stephan, & Friston, 2009). The largest MMN response is evoked for maximally uncertain stimuli (Garrido et al., 2013). Interestingly, the MMN is NMDA-receptor dependent (Korostenskaja, Nikulin, Kicic, Nikulina, & Kahkonen, 2007; Umbricht, Koller, Vollenweider, & Schmid, 2002),

providing further support to the notion that the NMDA receptor may be involved in the processing of the predictive error between the auditory cue and subsequent stimuli. This account of the possible link between the NMDA receptor, uncertainty, and PAS is speculative, however, and would benefit from further research to establish a causal interaction between these factors, with the use, for example of NMDA receptor antagonists.

5. Conclusion

We have shown that introducing stimulus uncertainty can boost the effects of plasticity induced in the human motor cortex using PAS. That is, corticospinal excitability increases following PAS when stimulus uncertainty is high. Conversely, when stimuli are predictable, the temporal changes in MEP amplitude following PAS are not significant. This result adds to a large body of literature that indicates stimulus uncertainty and prediction error are beneficial to learning. Interestingly, in the paradigm used here, the learning-related changes were induced with non-invasive brain stimulation, rather than a cognitive task. Although we are unable to unequivocally identify the mechanisms leading to this effect, it appears likely that the NMDA receptor is involved. Plasticity inducing protocols such as PAS are often touted as potentially important treatment tools in clinical neuroscience, but currently remain unreliable and variable. The present study adds to growing research that has identified ways to boost plasticity induction in human cortex, suggesting that the simple yet effective manipulation of PAS by introducing stimulus uncertainty can increase its effectiveness, but does not alter its variability.

Table 1.

Stimulation characteristics for the peripheral nerve stimulation (M-wave intensity), the amplitude of baseline MEPs, and resting motor threshold (rMT), expressed as a percentage of maximum stimulator output (%MSO) before and after PAS.

	M-wave intensity	Baseline MEP	rMT (%MSO)	
Contingency	(mA)	(mV)	pre-PAS	post-PAS
No uncertainty	11.10 (1.52)	.79 (.05)	40.43 (1.45)	40.68 (1.48)
Max uncertainty	9.79 (1.33)	.75 (.05)	40.14 (1.36)	39.54 (1.44)

Note: Standard errors are given in parenthesis.

Acknowledgements

This work was supported by a Training Fellowship from the National Health and Medical Research Council of Australia (APP1012153) awarded to MVS. JBM was supported by the Australian Research Council (ARC) SRI Science of Learning Research Centre (SR120300015), the ARC Centre of Excellence for Integrative Brain Function (ARC Centre Grant CE140100007), and an ARC Australian Laureate Fellowship (FL110100103). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding bodies. The authors declare no competing financial interests. We would like to thank Dr. David Lloyd for technical assistance.

References

Alink, A., Schwiedrzik, C. M., Kohler, A., Singer, W., & Muckli, L. (2010). Stimulus predictability reduces responses in primary visual cortex. *J Neurosci, 30*(8), 2960-2966. doi: 10.1523/JNEUROSCI.3730-10.2010

Cirillo, J., Lavender, A. P., Ridding, M. C., & Semmler, J. G. (2009). Motor cortex plasticity induced by paired associative stimulation is enhanced in physically active individuals. *J Physiol, 587*(Pt 24), 5831-5842. doi: 10.1113/jphysiol.2009.181834

Di Lazzaro, V., Dileone, M., Pilato, F., Profice, P., Oliviero, A., Mazzone, P., . . . Tonali, P. A. (2009). Associative motor cortex plasticity: direct evidence in humans. *Cereb Cortex, 19*(10), 2326-2330. doi: 10.1093/cercor/bhn255

Esber, G. R., & Haselgrove, M. (2011). Reconciling the influence of predictiveness and uncertainty on stimulus salience: a model of attention in associative learning. *Proc Biol Sci, 278*(1718), 2553-2561. doi: 10.1098/rspb.2011.0836

Frantseva, M. V., Fitzgerald, P. B., Chen, R., Moller, B., Daigle, M., & Daskalakis, Z. J. (2008). Evidence for impaired long-term potentiation in schizophrenia and its relationship to motor skill learning. *Cereb Cortex, 18*(5), 990-996. doi: 10.1093/cercor/bhm151

Fratello, F., Veniero, D., Curcio, G., Ferrara, M., Marzano, C., Moroni, F., . . . De Gennaro, L. (2006). Modulation of corticospinal excitability by paired associative stimulation: reproducibility of effects and intraindividual reliability. *Clin Neurophysiol, 117*(12), 2667-2674. doi: 10.1016/j.clinph.2006.07.315

Friston, K. (2010). The free-energy principle: a unified brain theory? *Nat Rev Neurosci, 11*(2), 127-138. doi: 10.1038/nrn2787

Gallistel, C. R., & Matzel, L. D. (2013). The neuroscience of learning: beyond the Hebbian synapse. *Annu Rev Psychol, 64*, 169-200. doi: 10.1146/annurev-psych-113011-143807

Garrido, M. I., Kilner, J. M., Stephan, K. E., & Friston, K. J. (2009). The mismatch negativity: a review of underlying mechanisms. *Clin Neurophysiol, 120*(3), 453-463. doi: 10.1016/j.clinph.2008.11.029

Garrido, M. I., Sahani, M., & Dolan, R. J. (2013). Outlier responses reflect sensitivity to statistical structure in the human brain. *PLoS Comput Biol, 9*(3), e1002999. doi: 10.1371/journal.pcbi.1002999

Hamada, M., Murase, N., Hasan, A., Balaratnam, M., & Rothwell, J. C. (2013). The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex, 23*(7), 1593-1605. doi: 10.1093/cercor/bhs147

Hasan, M. T., Hernandez-Gonzalez, S., Dogbevia, G., Trevino, M., Bertocchi, I., Gruart, A., & Delgado-Garcia, J. M. (2013). Role of motor cortex NMDA receptors in learning-dependent synaptic plasticity of behaving mice. *Nat Commun, 4*, 2258. doi: 10.1038/ncomms3258

Hogarth, L., Dickinson, A., Austin, A., Brown, C., & Duka, T. (2008). Attention and expectation in human predictive learning: the role of uncertainty. *Q J Exp Psychol (Hove), 61*(11), 1658-1668. doi: 10.1080/17470210701643439

Kamke, M. R., Hall, M. G., Lye, H. F., Sale, M. V., Fenlon, L. R., Carroll, T. J., . . . Mattingley, J. B. (2012). Visual attentional load influences plasticity in the human motor cortex. *J Neurosci, 32*(20), 7001-7008. doi: 10.1523/JNEUROSCI.1028-12.2012

Kamke, M. R., Nydam, A. S., Sale, M. V., & Mattingley, J. B. (2016). Associative plasticity in the human motor cortex is enhanced by concurrently targeting separate muscle representations with excitatory and inhibitory protocols. *J Neurophysiol, 115*(4), 2191-2198. doi: 10.1152/jn.00794.2015

Kamke, M. R., Ryan, A. E., Sale, M. V., Campbell, M. E., Riek, S., Carroll, T. J., & Mattingley, J. B. (2014). Visual spatial attention has opposite effects on bidirectional plasticity in the human motor cortex. *J Neurosci, 34*(4), 1475-1480. doi: 10.1523/JNEUROSCI.1595-13.2014

Kamke, M. R., Vieth, H. E., Cottrell, D., & Mattingley, J. B. (2012). Parietal disruption alters audiovisual binding in the sound-induced flash illusion. *Neuroimage, 62*(3), 1334-1341. doi: 10.1016/j.neuroimage.2012.05.063

Knill, D. C., & Pouget, A. (2004). The Bayesian brain: the role of uncertainty in neural coding and computation. *Trends Neurosci, 27*(12), 712-719. doi: 10.1016/j.tins.2004.10.007

Kording, K. P., & Wolpert, D. M. (2004). Bayesian integration in sensorimotor learning. *Nature, 427*(6971), 244-247. doi: 10.1038/nature02169

Korostenskaja, M., Nikulin, V. V., Kicic, D., Nikulina, A. V., & Kahkonen, S. (2007). Effects of NMDA receptor antagonist memantine on mismatch negativity. *Brain Res Bull, 72*(4-6), 275-283. doi: 10.1016/j.brainresbull.2007.01.007

Lefaucheur, J. P., Andre-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., . . . Garcia-Larrea, L. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin Neurophysiol, 125*(11), 2150-2206. doi: 10.1016/j.clinph.2014.05.021

Morgante, F., Espay, A. J., Gunraj, C., Lang, A. E., & Chen, R. (2006). Motor cortex plasticity in Parkinson's disease and levodopa-induced dyskinesias. *Brain, 129*(Pt 4), 1059-1069. doi: 10.1093/brain/awl031

Oldfield, R. C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia, 9*(1), 97-113.

Orban, G., & Wolpert, D. M. (2011). Representations of uncertainty in sensorimotor control. *Curr Opin Neurobiol, 21*(4), 629-635. doi: 10.1016/j.conb.2011.05.026

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychol Rev, 87*(6), 532-552.

Perrett, D. I., Xiao, D., Barraclough, N. E., Keysers, C., & Oram, M. W. (2009). Seeing the future: Natural image sequences produce "anticipatory" neuronal activity and bias perceptual report. *Q J Exp Psychol (Hove), 62*(11), 2081-2104. doi: 10.1080/17470210902959279

Player, M. J., Taylor, J. L., Alonzo, A., & Loo, C. K. (2012). Paired associative stimulation increases motor cortex excitability more effectively than theta-burst stimulation. *Clin Neurophysiol, 123*(11), 2220-2226. doi: 10.1016/j.clinph.2012.03.081

Ridding, M. C., & Flavel, S. C. (2006). Induction of plasticity in the dominant and non-dominant motor cortices of humans. *Exp Brain Res, 171*(4), 551-557. doi: 10.1007/s00221-005-0309-2

Ridding, M. C., & Rothwell, J. C. (2007). Is there a future for therapeutic use of transcranial magnetic stimulation? *Nat Rev Neurosci, 8*(7), 559-567. doi: 10.1038/nrn2169

Ridding, M. C., & Ziemann, U. (2010). Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J Physiol, 588*(Pt 13), 2291-2304. doi: 10.1113/jphysiol.2010.190314

Sale, M. V., & Mattingley, J. B. (2013). Selective enhancement of motor cortical plasticity by observed mirror-matched actions. *Neuroimage, 74*, 30-36. doi: 10.1016/j.neuroimage.2013.02.009

Sale, M. V., Mattingley, J. B., Zalesky, A., & Cocchi, L. (2015). Imaging human brain networks to improve the clinical efficacy of non-invasive brain stimulation. *Neurosci Biobehav Rev*. doi: 10.1016/j.neubiorev.2015.09.010

Sale, M. V., Ridding, M. C., & Nordstrom, M. A. (2007a). Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Experimental Brain Research, 181*(4), 615-626. doi: 10.1007/s00221-007-0960-x

Sale, M. V., Ridding, M. C., & Nordstrom, M. A. (2007b). Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp Brain Res, 181*(4), 615-626. doi: 10.1007/s00221-007-0960-x

Sale, M. V., Ridding, M. C., & Nordstrom, M. A. (2008). Cortisol inhibits neuroplasticity induction in human motor cortex. *Journal of Neuroscience, 28*(33), 8285-8293. doi: 10.1523/Jneurosci.1963-08.2008

Schabrun, S. M., Weise, D., Ridding, M. C., & Classen, J. (2013). A new temporal window for inducing depressant associative plasticity in human primary motor cortex. *Clin Neurophysiol, 124*(6), 1196-1203. doi: 10.1016/j.clinph.2013.01.004

Shams, L., Kamitani, Y., & Shimojo, S. (2000). Illusions. What you see is what you hear. *Nature, 408*(6814), 788. doi: 10.1038/35048669

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., & Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J Physiol, 543*(Pt 2), 699-708.

Stefan, K., Kunesch, E., Cohen, L. G., Benecke, R., & Classen, J. (2000). Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain, 123 Pt 3*, 572-584.

Stefan, K., Wycislo, M., & Classen, J. (2004). Modulation of associative human motor cortical plasticity by attention. *J Neurophysiol, 92*(1), 66-72. doi: 10.1152/jn.00383.2003

Todd, G., Kimber, T. E., Ridding, M. C., & Semmler, J. G. (2010). Reduced motor cortex plasticity following inhibitory rTMS in older adults. *Clin Neurophysiol, 121*(3), 441-447. doi: 10.1016/j.clinph.2009.11.089

Umbricht, D., Koller, R., Vollenweider, F. X., & Schmid, L. (2002). Mismatch negativity predicts psychotic experiences induced by NMDA receptor antagonist in healthy volunteers. *Biol Psychiatry, 51*(5), 400-406.

Vanni-Mercier, G., Mauguiere, F., Isnard, J., & Dreher, J. C. (2009). The hippocampus codes the uncertainty of cue-outcome associations: an intracranial electrophysiological study in humans. *J Neurosci, 29*(16), 5287-5294. doi: 10.1523/JNEUROSCI.5298-08.2009

Vilares, I., & Kording, K. (2011). Bayesian models: the structure of the world, uncertainty, behavior, and the brain. *Ann N Y Acad Sci, 1224*, 22-39. doi: 10.1111/j.1749-6632.2011.05965.x

Violentyev, A., Shimojo, S., & Shams, L. (2005). Touch-induced visual illusion. *Neuroreport, 16*(10), 1107-1110.

Wheeler, D. S., & Miller, R. R. (2008). Determinants of cue interactions. *Behav Processes, 78*(2), 191-203. doi: 10.1016/j.beproc.2008.02.002

Wischnewski, M., & Schutter, D. J. (2015). Efficacy and time course of paired associative stimulation in cortical plasticity: Implications for neuropsychiatry. *Clin Neurophysiol*. doi: 10.1016/j.clinph.2015.04.072

Witte, A. V., Kurten, J., Jansen, S., Schirmacher, A., Brand, E., Sommer, J., & Floel, A. (2012). Interaction of BDNF and COMT polymorphisms on paired-associative stimulation-induced cortical plasticity. *J Neurosci, 32*(13), 4553-4561. doi: 10.1523/JNEUROSCI.6010-11.2012

Wolters, A., Sandbrink, F., Schlottmann, A., Kunesch, E., Stefan, K., Cohen, L. G., . . . Classen, J. (2003). A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol, 89*(5), 2339-2345. doi: 10.1152/jn.00900.2002