Translating long-term potentiation from animals to humans: A novel method for non-invasive assessment of cortical plasticity

Supplemental Information

Behavioral Consequences

It is possible that the high frequency visual stimulation we used to induce long-term potentiation (LTP) could be accompanied by a change in visual performance. However, the role of LTP as a neural mechanism underlying behavior has been controversial. Although it is generally accepted that LTP alters synaptic strength in memory networks, the use of electrical stimulation to induce LTP in animal studies has been criticized as being unnatural and thus unlikely to underlie subsequent behavioral changes. However, others have shown LTP induction to more natural stimulation (1, 2). Here we examined whether a behavioral change can be induced by natural sensory stimulation that we found to induce LTP in human sensory cortex.

Supplemental Methods and Materials

Subjects

16 male subjects (aged 21-35, vision normal or corrected-to-normal) took part in this experiment.

Stimuli

The experiment was programmed on a Pentium II/200 computer. The stimuli were centered on a 15" flat-screen LCD monitor with a resolution of 640 x 480 pixels with 256 levels of gray.

Stimuli for threshold setting and reaction time experiments were 6 x 6 checkerboards subtending 1.25 degrees of visual angle presented on a white background with RGB setting of 255 (pure white, where all three channels, Red, Green and Blue are set at 255). Settings for the checkerboards refer to the RGB settings of the "black" squares, the "white" sections were identical to the background setting. They were presented at 1.75 degrees left or right of fixation (see Figure 5). Stimuli were presented for 33.4 milliseconds (2 refresh cycles at 60 Hz). Subjects responded on a standard computer keyboard. All

stimulus control and response timing was performed using well established methods for the DOS operating system (3).

Setting Threshold

A session began with subjects setting their detection thresholds, using the descending method of limits. The stimulus was randomly presented either to the left or right of fixation. If the stimulus was visible, subjects were to adjust the RGB value of the stimulus towards the background setting, until they could no longer detect it. Their threshold was then determined to be the RGB setting 1 step darker (more visible) than this setting. Subjects had the option to re-present stimuli at a given setting without adjustment. Other studies have found reliable threshold settings using the method of limits in the setting of visual (4, 5) and other perception thresholds (6).

Reaction Time Trials

Nine stimulus settings were tested: threshold, four consecutive steps above threshold, and four consecutive steps below threshold. For example, if a subject's threshold was determined to be an RGB setting of 245, the stimuli were presented with RGB settings of 241 through 249 inclusively. Stimuli were presented for 33 ms either to the left or right of fixation and subjects were given a forced choice as to which side the stimulus was on. The stimuli were randomized in terms of not only location (left/right) but also stimulus intensity. Subjects were instructed to respond as quickly as possible. If no response was issued within 600 ms, a 50 ms tone was presented to prompt responding. Twenty trials were presented at each of the stimulus intensities, with an equal number to the left and right of fixation. There was a delay of 800-1800 ms, in 5 bins of 200 ms, between the response and the presentation of the next stimulus (mean delay: 1300 ms).

After completion of reaction time trials (Block A = 1^{st} set), subjects were either presented with the photic tetanus (photic tetanus (PT) condition) or sat for a period of 2 min looking at the white computer screen (control condition).

Subjects were then asked to re-set their threshold and perform a block of reaction time trials identical to that described above (Block B = 2^{nd} set). This was done to determine if the high frequency stimulation

had changed their detection thresholds and/or had altered their reaction times. Note, although thresholds were re-measured at this point, the original stimulus settings were employed during the Block B reaction time trials.

Photic Tetanus

The stimulus for the photic tetanus was a checkerboard subtending 8.80 horizontal and 5.80 vertical, centered at fixation (see Figure 5), thus tetanizing the area where the threshold setting stimuli are presented. The tetanus stimulus was presented at a frequency of 9 Hz for 120 seconds (1000 presentations). The RGB value for the tetanus was zero, giving maximum contrast as to increase the efficacy of the potentiation as much as possible.

Experimental Procedure

All 16 subjects performed two testing sessions that were identical in procedure in all aspects apart from the presentation of the tetanus (Block A followed by Block B). Order of the sessions was counter-balanced across subjects with a minimum of 8 days between sessions.

Supplemental Results

Threshold Settings

A two-way within subjects analysis of variance (ANOVA) was performed on the threshold data with condition (photic tetanus vs. control) and block (pre vs. post) as variables. There was no main effect of condition ($F(1,15) < 1$). There was a significant effect of block ($F(1,15) = 24.20$, $p < .001$) and there was a significant condition by block interaction ($F(1,15) = 20.99$, $p < .001$).

During the control condition, subjects' setting of their threshold did not change ($t(15) = 0$, $p = 1$). However, in the photic tetanus condition, threshold settings were set significantly lower after the tetanus ($t(15) = 6.85$, $p < .001$), see Figure 5. Using a one-way ANOVA, it was shown that subjects' threshold settings before the tetanus was not significantly different from the threshold settings (pre or post) on the day they did not receive a tetanus ($F(2,30) = 1.80$, $p > .05$). However, subjects' post-tetanus threshold

settings were significantly lower than the threshold settings on the day they did not receive a tetanus ($F(2,30) = 8.21$, $p < .05$) suggesting some carryover effect of the potentiation.

Reaction Times

The median reaction time for correct responses was analyzed in a three-way ANOVA with condition (photic tetanus vs. control), block (pre vs. post) and stimulus intensity (9 luminance settings) as factors; leaving out the super-threshold condition. Greenhouse-Geisser correction was used for analyses that violated the assumption of sphericity. The reaction times for blocks A and B are shown for both conditions in Figure 5.

There was a main effect of stimulus intensity ($F(2.17,32.48) = 61.03$, $p < .001$). Neither the main effect of block (Block A vs. Block B: $F(1,15) = 1.11$, $p > .05$) nor the main effect of condition (PT vs. control: $F(1,15) = 3.67$, $p > .05$) reached significance.

There was a significant interaction between block and condition ($F(1,15) = 7.50$, $p < .05$). Stimulus intensity did not interact with condition ($F(2.52,37.78) = 2.07$, $p > .05$) nor did it interact with block ($F(3.94,59.07) < 1$). Finally, the overall three-way interaction between block, condition, and stimulus intensity was significant ($F(3.18,57.92) = 3.14$, $p > .05$).

To explore the three-way interaction, each condition was analyzed separately. A two-way ANOVA of the control data revealed that there was no main effect of block (pre vs. post tetanus) ($F(1,15) < 1$). There was a significant effect of threshold setting ($F(2.43,36.42) = 33.93$, $p < .001$), but not a significant interaction between block and threshold settings ($F(3.46,51.85) = 1.94$, $p > .05$).

A two-way ANOVA of the tetanus data revealed that there was a main effect of block (pre vs. post tetanus) ($F(1,15) = 11.78$, $p < .01$). Additionally, there was a significant effect of threshold setting ($F(2.11,31.67) = 36.03$, $p < .001$), but not a significant interaction between block and threshold settings ($F(3.54,53.10) = 1.91$, $p > .05$).

These results confirm that when subjects were in the photic tetanus condition, they tended to improve during Block B, while when in the control condition they did not change. Response times to super-threshold stimuli were analyzed in a two-way ANOVA and there was no main effect of condition ($F(1,15) <$

1) or block ($F(1,15) = 3.83$, $p > .05$) and the interaction between block and condition was also non-significant ($F(1,15) = 3.31$, $p > .05$).

Percentage Correct

Response accuracy was analyzed in the same manner as the response times.

There was a main effect of condition in the accuracy comparisons ($F(1,15) = 5.98$, $p > .05$). However, there was no main effect of block ($F(1,15) = 2.06$, $p > .05$). There was a significant effect of stimulus intensity ($F(8,88) = 59.19$, $p < .05$).

There was a significant interaction between block and condition ($F(1,15) = 8.47$, $p < .05$) which reflected the larger decrease in performance for Block B of the control condition relative to the photic tetanus condition.

The interaction between condition and stimulus intensity did not reach significance, ($F(3.55,53.24) = 2.31$, $p > .05$). Similarly, the interaction between block and stimulus intensity did not reach significance, ($F(4.5,67.6) = 2.09$, $p > .05$). The three-way interaction between condition, stimulus intensity and block was significant ($F(8,120) = 2.03$, $p < .05$).

A two-way ANOVA of the control data revealed that there was a main effect of block (pre vs. post tetanus) ($F(1,15) = 5.87$, $p < .05$). There was also a significant effect of threshold setting ($F(3.43,51.51) = 37.53$, $p < .001$), and also a significant interaction between block and threshold settings ($F(8,120) = 3.28$, $p < .01$) indicating that in the control condition there was a slight decrease in accuracy after the break.

A two-way ANOVA of the tetanus data revealed that there was no main effect of block (pre vs. post tetanus) ($F(1,15) < 1$). There was a significant effect of threshold setting ($F(3.39,50.79) = 40.19$, $p < .001$), but not a significant interaction between block and threshold settings ($F(4.08,61.26) = 1.23$, $p > .05$).

Response accuracy to super-threshold stimuli were analyzed in a two-way ANOVA and there was no main effect of condition ($F(1,15) = 1.77$, $p > .05$) or block ($F(1,15) < 1$) and the interaction between block and condition was also non-significant ($F(1,15) < 1$). The results did not suggest any possibility of a speed-accuracy trade-off (see Figure 5).

Supplemental Discussion

The results showed that detection thresholds improved (i.e., less intense stimuli could be detected) following the high-frequency stimulation (Figure 5). When the high-frequency stimulation was omitted, detection thresholds remained stable. Response times to near threshold stimuli improved after high-frequency stimulation (Figure 5). Detection accuracy measures indicated that the response time improvement was not due to a speed-accuracy trade-off, and therefore suggests that the stimuli were more readily detected. Thus it seems likely that presenting visual checkerboards at a rapid rate activates synapses within the visual system in a manner similar to electrical stimulation, inducing changes within the visual network akin to those seen in cellular studies of LTP. Once potentiated, these neuronal assemblies are more likely to respond to previously subthreshold stimuli, allowing subjects to see what they previously could not.

Supplemental References

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