**Current Biology, Volume *20***

# Supplemental Information

# Cholinergic Enhancement Augments Magnitude and Specificity of Visual Perceptual Learning in Healthy Humans

**Ariel Rokem and Michael A. Silver**

## Supplemental Data

### General Drug Effects on Task Performance Do Not Account for the Effects of Donepezil on Perceptual Learning

In addition to donepezil's beneficial effects on perceptual learning, there was an overall deleterious effect of the drug on task performance. However, as shown in the Results section, this effect on overall task performance does not account for the effects of the drug on the magnitude of perceptual learning.

Further evidence for this comes from excluding one subject whose pre-training threshold for the training stimulus while taking donepezil was 34 degrees (see Figure S1A for single-subject thresholds). This value was 2.7 standard deviations above the group mean pre-training donepezil threshold for the condition that was then used for training. This outlier subject accounts for most of the drug effect on mean pre-training threshold but is not responsible for the pattern of drug effects on learning. When this subject's data were removed from the sample, the donepezil/placebo difference in pre-training thresholds was reduced (donepezil: 11.5 +/- 1.5 deg; placebo: 10.5 +/- 0.8 deg, $t_{10}=0.6$, $p>0.5$), while the difference in post-training thresholds was still statistically significant (donepezil: 7.1 +/- 0.7 deg; placebo: 8.6 +/- 0.6 deg, $t_{10}=2.7$, $p<0.05$).

We also conducted the statistical analysis of donepezil's effects on learning after excluding this subject's data from the sample. The enhancing effect of donepezil on the magnitude of perceptual learning was still present (donepezil: 33 +/- 6%, placebo: 15 +/- 8%; $t_{32}=2.05$, $p<0.05$) and donepezil still increased the location specificity ($t_{32}=2.21$, $p<0.05$) and direction specificity ($t_{32}=2.34$, $p<0.05$) of learning. Finally, although the outlier subject had a very large donepezil pre-training threshold for the stimulus that was then used for training, three subjects exhibited greater effects of donepezil on learning (difference between percent learning under donepezil and percent learning under placebo for the trained condition; see Figure S1B for single-subject values). We conclude that the worse pre-training performance under donepezil does not account for the increases in magnitude and specificity of perceptual learning under donepezil.

## Individual Differences

Perceptual learning is often found to be variable between subjects [1]. In our study, subjects also differed in the magnitude of the effect of donepezil on perceptual learning. We examined a number of factors to determine whether they predicted either the magnitude of donepezil's effect on learning or the amount of learning under donepezil: 1) the pre-training thresholds, which correspond to baseline motion direction discrimination performance, 2) percent learning under placebo, which represents the amount of learning in the absence of pharmacological manipulation, and 3) average percent learning (average of placebo and donepezil conditions), which serves as an unbiased estimate of the amount of learning for a given subject.

Figure S1A relates pre- and post-training thresholds for each participant separately for placebo and donepezil. Pre-training performance did not predict the magnitude of the effect of donepezil on learning: the correlation between pre-training thresholds (averaged between drug and placebo conditions) and the drug effect on percent learning (in the trained condition, defined as the difference between percent learning under donepezil and percent learning under placebo) was not significant ($r^2$=0.09, p=0.35). We also correlated percent learning under placebo with percent learning under donepezil and found no significant relationship between these two measures ($r^2$=0.001, p=0.96) (Figure S1B). This indicates that the amount of learning for a given subject under placebo does not predict how much learning occurs for that subject during cholinergic enhancement. In addition, there was no correlation between the overall amount of learning for a given subject (average of percent learning in donepezil and placebo conditions) and the magnitude of the effect of donepezil on learning (difference between percent learning in donepezil and placebo conditions) ($r^2$=0.01, p=0.77).

## The Effect of Drug Administration Order

Overall, the magnitude of perceptual learning was greater during the first course of learning than during the second course. Because half of the subjects trained first under placebo and then under donepezil and the other half trained in the opposite order, the order in which drug and placebo were administered affects the size of the measured drug effect on learning for a given subject. The four subjects who learned less under donepezil than under placebo were all randomly assigned to the group that received placebo during the first course of learning. In the analysis of variance we conducted, the effect of order was discounted by including order as a between-subjects covariate. We also directly measured the effect of order by comparing percent learning in the first course of training with percent learning in the second course of training, regardless of whether subjects were administered drug or placebo. The difference due to order was 47%. To correct for this order effect, half of the average order effect (23.5%) was subtracted from the magnitude of donepezil's effect on percent learning for each of the subjects who trained with the drug first, and half of this was added to the drug effect for

each of the subjects who trained with placebo first. Following this procedure to eliminate the order effect, 10 of the 12 subjects showed a facilitatory effect of donepezil on learning.

**Changes in Response Bias Do Not Account for the Drug Effect**

Discrimination thresholds are influenced by both sensitivity (the subject's ability to discriminate two different directions of motion) and response bias (a measure of how certain a subject needs to be before reporting that the two stimuli contained different directions of motion). In the motion direction discrimination task employed here, response bias can be measured by computing the proportions of "same direction of motion" and "different directions of motion" responses, independent of whether those responses were correct or incorrect.

We measured the effects of donepezil and perceptual learning on response bias. For each condition, response bias was calculated as the absolute percentage deviation from an equal number of "same" and "different" responses. The same analysis of variance that was conducted on the discrimination threshold values was also conducted on the response bias values. There was no effect of either drug ($F_{1,9}$=0.35, p=0.56) or learning ($F_{1,9}$=0.02, p=0.89) on response bias. There was also no significant drug by learning interaction effect on response bias ($F_{1,9}$=0.005, p=0.95).

In addition, none of the planned comparisons conducted for the discrimination thresholds were significant when applied to the response bias data. Specifically, there was no effect of the drug on response bias in the trained condition and no location or direction specificity of response bias to the trained condition (p>0.05 for all planned comparisons). Furthermore, between-subject variance in changes in response bias did not account for the between-subject variance in the effects of the drug on changes in discrimination threshold due to learning. There was an extremely low correlation between the drug effect on changes in response bias in the trained condition and the drug effect on changes in discrimination threshold in that condition ($r^2$=0.0001, p=0.99). There were similarly low correlations between the drug effect on response bias and the drug effect on threshold for direction specificity ($r^2$=0.004, p=0.99) and location specificity ($r^2$=0.01, p=0.97). We conclude that changes in response bias do not account for the effects of donepezil on the magnitude and specificity of perceptual learning.

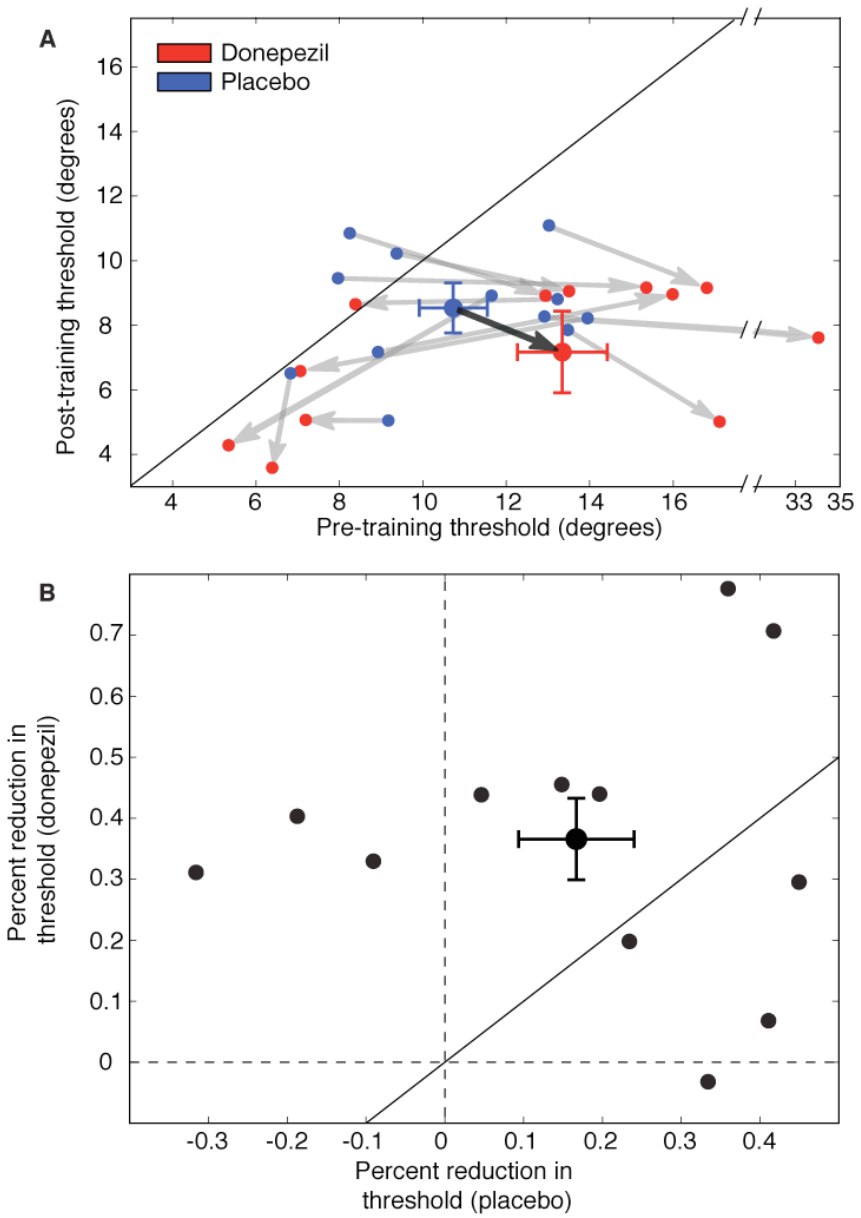


**Figure S1: Individual differences in perceptual learning and their modulation by donepezil, related to Figure 2.** A, pre- and post-training thresholds for placebo and donepezil. Each subject's behavioral performance is displayed as a pair of points (blue = placebo, red = donepezil) connected by an arrow. The distance of each point from the equality (solid black) line indicates how much learning (change from pre- to post-learning threshold) occurred in that condition. The direction of the arrow connecting the placebo and donepezil points for a given subject indicates the effect of the drug on learning. Arrows pointing away from the equality line (rightwards and downwards) indicate that donepezil increased the magnitude of learning. Large points are the group averages with SEM error bars. B, comparison of percent learning under donepezil and placebo. The distance of each point from the equality (solid black) line indexes differences in the amount of learning under placebo and donepezil. Points above the line represent subjects who exhibited more learning under donepezil than under placebo. The large point represents the group average with SEM error bars.

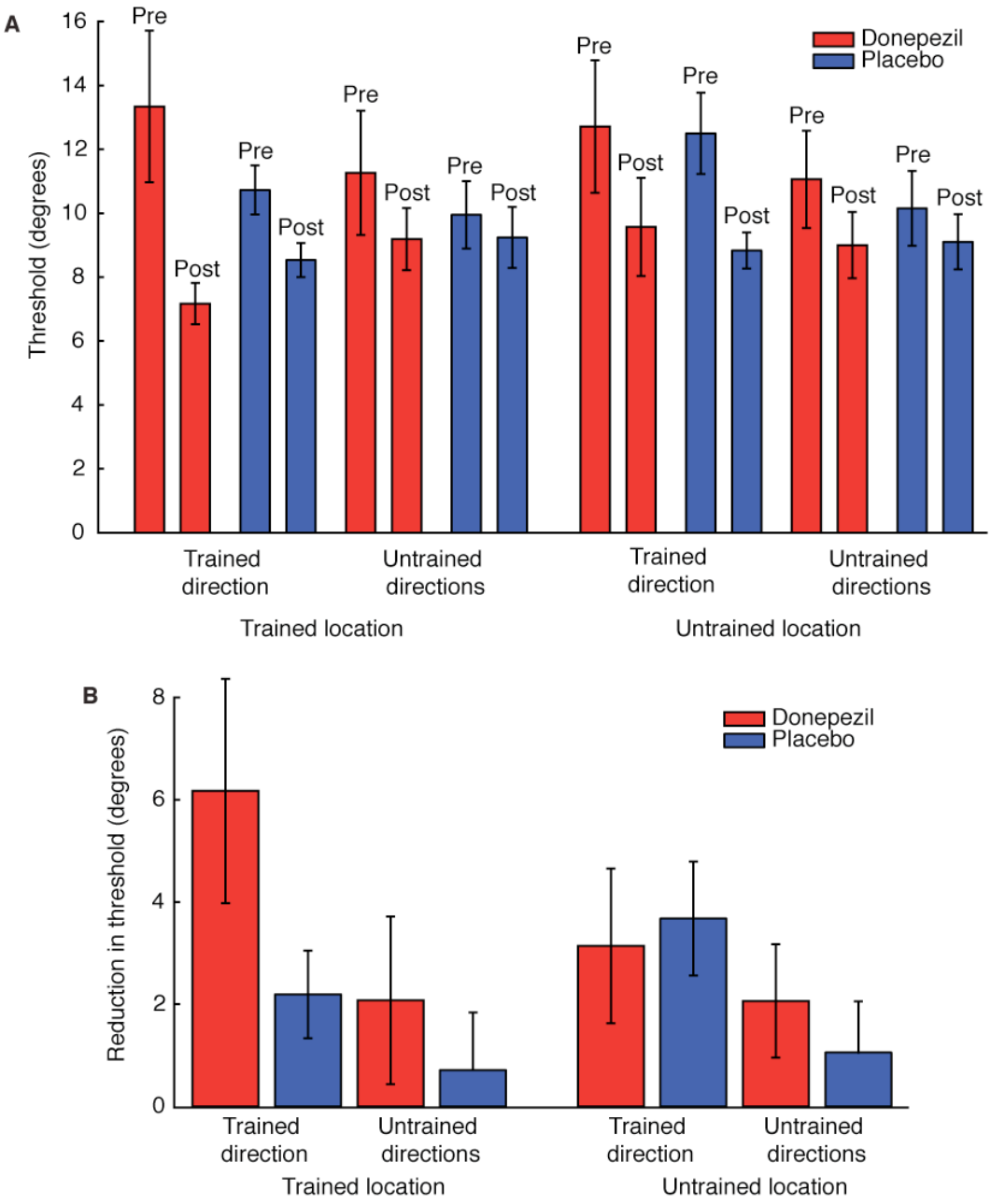


**Figure S2: Reduction in threshold and its modulation by donepezil, related to Figure 3.** A, pre- and post-training thresholds for placebo (blue) and donepezil (red), divided according to trained and untrained location and trained and untrained directions of motion. B, Change in threshold (difference between pre- and post-training). Error bars are SEM.

## Supplemental Experimental Procedures

### Stimuli

The stimuli covered an annulus subtending 1.5-3.1 deg of visual angle, centered at the fixation point (Figure 1B). The radius of each dot was 0.03 deg, and the dot density was 17 dots/deg$^2$. The dots were moving at a speed of 8 deg/sec, and each dot moved continuously for two monitor frames (approximately 24 msec at the 85 Hz monitor refresh rate used) before being reassigned to another random location within the annulus.

### Analysis

Differences in task performance were evaluated using a mixed-model ANOVA, with drug condition (donepezil vs. placebo), training (pre- vs. post-), visual field location (trained vs. untrained), and direction of motion (five levels: 0, 45, 90, 135, and 180 degrees offset from trained direction) as within-subject factors. In order to discount the effect of order of donepezil/placebo administration on thresholds (which was orthogonal to the effect of the drug, due to the counterbalance), statistical testing was performed with order of drug administration as a between-subjects covariate. In addition, planned comparisons between conditions were conducted in order to investigate specific hypotheses. These planned comparisons are based on the error term in the full ANOVA model and have the degrees of freedom associated with that error term, while correcting for the number of conditions compared [2]. Because the planned comparisons were based on specific *a priori* hypotheses, we did not correct for multiple comparisons. One subject did not perform a post-training assessment under placebo in the untrained locations, and these values were entered into the analysis as missing values.

For each subject and each condition, percent learning was calculated using the following formula:

$$\%\ \text{learning} = 100 \cdot \left(1 - \frac{threshold(post)}{threshold(pre)}\right)$$

In order to test whether learning was significantly faster under donepezil than under placebo, the average percent learning in each daily session was calculated for each subject and then averaged across subjects. A single-parameter model was fit to the progression of learning:

$$\%\ \text{learning}\ (\text{session}) = 100 \cdot \left(1 - e^{\tau \cdot session}\right)$$

where $\tau$ is the parameter that quantifies the rate of learning. Since the data did not allow for a reliable model fit on the single-subject level, a jackknife procedure was employed [3]. The model was fit to twelve resamples from the data. For each resample, the data from one subject were omitted, and the learning curves from the remaining eleven subjects were averaged. The model was then fit to this average learning curve. This

produced twelve different values of learning rate ($\tau$) for each condition. The values of the learning rates were then compared across the jackknife samples. In order to estimate the statistical significance of the difference between learning rate under donepezil and under placebo, a non-parametric permutation test was used: 10,000 surrogate samples were created by randomly recoding the condition from which each value of the learning rate was taken (donepezil or placebo). This was done independently for each jackknife sample. Thus, the distribution of the differences between the means of these recoded distributions corresponds to that expected for the null hypothesis (no effect of donepezil on learning rate). The mean difference between the actual jackknife distributions (donepezil and placebo) was then compared to the $95^{th}$ percentile of the randomly recoded samples created to test whether the probability of the measured differences between donepezil and placebo learning rates occurring by chance is smaller than 0.05.

**Supplemental References**


1. Mukai, I., Kim, D., Fukunaga, M., Japee, S., Marrett, S., and Ungerleider, L.G. (2007). Activations in visual and attention-related areas predict and correlate with the degree of perceptual learning. J. Neurosci. 27, 11401-11411.
2. Keppel, G., and Wickens, T.D. (2004). Design and Analysis: A Researcher's Handbook, (Upper Saddle River, NJ: Pearson Education).
3. Efron, B., and Tibshirani, R.J. (1993). An Introduction to the Bootstrap, (New York, NY: Chapman and Hall).