Report

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

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Summary

Learning through experience underlies the ability to adapt to novel tasks and unfamiliar environments. However, learning must be regulated so that relevant aspects of the environment are selectively encoded. Acetylcholine (ACh) has been suggested to regulate learning by enhancing the responses of sensory cortical neurons to behaviorally relevant stimuli [1]. In this study, we increased synaptic levels of ACh in the brains of healthy human subjects with the cholinesterase inhibitor donepezil (trade name: Aricept) and measured the effects of this cholinergic enhancement on visual perceptual learning. Each subject completed two 5 day courses of training on a motion direction discrimination task [2], once while ingesting 5 mg of donepezil before every training session and once while placebo was administered. We found that cholinergic enhancement augmented perceptual learning for stimuli having the same direction of motion and visual field location used during training. In addition, perceptual learning with donepezil was more selective to the trained direction of motion and visual field location. These results, combined with previous studies demonstrating an increase in neuronal selectivity following cholinergic enhancement [3–5], suggest a possible mechanism by which ACh augments neural plasticity by directing activity to populations of neurons that encode behaviorally relevant stimulus features.

Results

The neurotransmitter acetylcholine (ACh) has been proposed to regulate neural plasticity by selectively increasing the responses of neurons to behaviorally relevant stimuli [1]. Cholinergic neurons in the basal forebrain project widely to cortex, where they release more ACh when animals are performing a task requiring sustained attention [6]. In addition, application of ACh to sensory cortex induces persistent modifications of neuronal tuning [7], and pairing of basal forebrain electrical stimulation with presentation of a sensory stimulus causes changes in cortical tuning that are similar to those observed when the animal performs a task on the presented stimulus [8]. In humans, pharmacological reduction of cholinergic transmission has been shown to prevent learning-dependent changes in fMRI responses [9]. Cholinesterase inhibitors such as donepezil (trade name: Aricept) reduce the activity of the enzyme that breaks down ACh in the synaptic cleft, thereby prolonging the effects of endogenously released ACh. Previous results suggest that cholinesterase inhibitors may benefit cognitive functions such as attention and memory [10, 11].

We examined the effects of cholinergic enhancement with donepezil on perceptual learning of a motion direction discrimination task [2]. Perceptual learning is a persistent and stimulus-specific improvement in performance of a perceptual task with training [12]. The specificity of perceptual learning suggests possible changes in coding in neurons selectively tuned to the characteristics of the training stimuli. Indeed, physiological studies in humans [13] and other primates [14–16] have described changes in coding in visual cortical areas containing neurons exhibiting selectivity for the stimulus characteristics employed during training.

Twelve participants (seven female; mean age: 23 ± 6; tobacco smokers were excluded from participation; all subjects had normal or corrected-to-normal vision) performed a task in which they reported whether two fields of moving dots, presented sequentially, were moving in the same direction [2] (Figure 1A; see also Supplemental Experimental Procedures available online). The angular difference between the stimuli was adjusted according to a psychophysical staircase, converging on 70% correct performance, and a threshold was estimated from all of the trials in each staircase [17]. Two quadrants of the stimulus, located on opposite sides of the fixation point, contained 100% coherent motion, and the remaining quadrants contained 0% coherent motion (Figure 1B). Stimuli were created using the Psychophysics Toolbox [18, 19].

Each subject completed two courses of training (Figure 1C), once while ingesting a pill containing 5 mg of donepezil before every training session and once while an inactive placebo was administered. Drug administration was double blind, and the order of drug and placebo administration was counterbalanced between subjects. Each course of training was preceded by 3 days of donepezil or placebo administration, bringing drug plasma levels to within the steady-state range (the half-life of donepezil in the human body is approximately 80 hr [20]), and drug or placebo administration continued daily throughout training and the posttraining assessment. Before and after training, thresholds were measured for both pairs of visual field locations and for eight different directions of motion.

For each course of training, subjects performed the task for a particular stimulus with coherent motion in one direction and in one of the two possible pairs of locations (Figure 1B). Because training in this task is specific for visual field location and motion direction [21], the effects of the two courses of training were separately assessed in each subject by training under donepezil with a stimulus presented in the other visual field location and in the opposite motion direction than the stimulus used for the placebo training course. Human subjects exhibit differences in performance of this task for oblique and cardinal directions of motion [21, 22]. We therefore used only oblique directions for training. During training, participants performed 1000 trials every day. Subjects underwent 5 days of training, except for one subject who trained for 6 days in both the placebo and donepezil conditions. At least 2 weeks passed between the two courses of training, allowing for donepezil, if present, to be eliminated.

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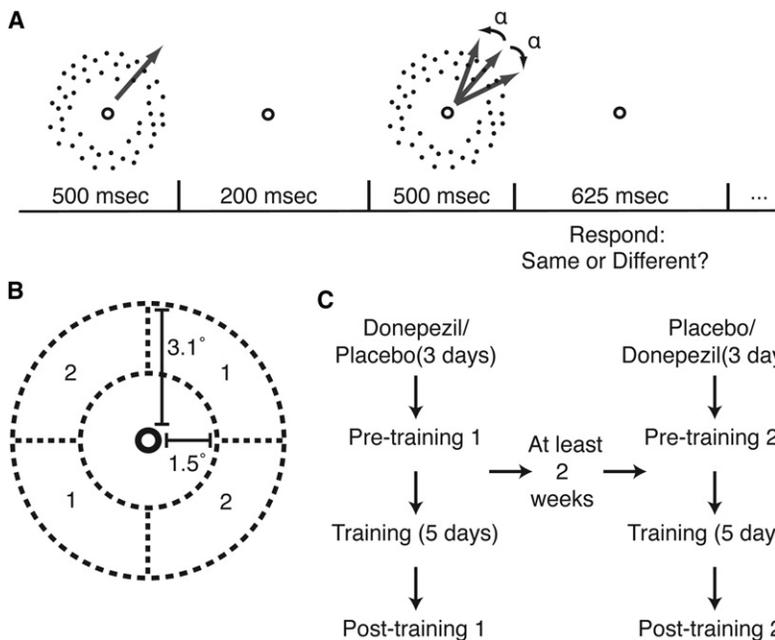
Figure 1. Experimental Procedure

(A) Task description. In each trial, two fields of dots with 100% coherent motion were sequentially presented. The two fields contained either the same or slightly different directions of motion.

(B) Stimulus configuration. Coherent motion was presented in one of two pairs of spatial locations (1 or 2) and in the same direction of motion throughout the course of training.

(C) Training procedure. Subjects participated in two courses of training. Donepezil or placebo was administered beginning 3 days before the pretraining measurement and daily throughout training and the posttraining measurement.

Perceptual learning resulted in an improvement in performance for the trained condition, defined as the direction of motion and visual field location used for training (Figure 2). The average decrease in angular difference threshold (Figure 1A) for the trained condition, combining placebo and donepezil training courses, was $4.2° \pm 1.2°$. However, the main effect of training (pre- versus posttraining thresholds, across all directions of motion, both locations, and both drug conditions [placebo and donepezil], as assessed by the significance of the training factor in the analysis of variance [ANOVA]) was not significant ($F_{1,9} = 0.53$, $p = 0.49$), demonstrating the specificity of learning for the training stimulus.

To further characterize the specificity of learning, we compared the improvement (change in threshold) that occurred in the trained condition (combining drug and placebo training courses), relative to the improvement that occurred in performance of the task on other stimuli. Direction specificity of perceptual learning was assessed by subtracting the improvement in performance in the untrained directions of motion (in the trained location) from the improvement in the trained direction (in the trained location). This difference in improvement was $2.7° \pm 0.9°$ (planned comparison, $t_{36} = 3.26$, $p < 0.05$), indicating that learning was specific to the trained direction of motion.

A similar measure of location selectivity was also calculated by subtracting the improvement in the untrained visual field locations (in the trained direction) from the improvement in the trained locations (in the trained direction). This difference in improvement was $1.3° \pm 0.7°$ and was not significant (planned comparison, $t_{36} = 1.53$, $p = 0.13$). The lack of location specificity of learning for the combined donepezil and placebo training courses replicates previous results in which this paradigm showed substantial transfer of learning to visual field locations immediately adjacent to the training location [21]. In addition, the spatial layout of our training stimulus, which requires simultaneous discrimination of motion direction in both visual hemifields, may have led to more spatial generalization of learning than in previous studies.

Comparison of the drug and placebo conditions revealed that administration of donepezil had an overall effect on learning, as evidenced by a significant interaction of drug and training factors in the ANOVA ($F_{1,9} = 5.89$, $p < 0.05$; see Supplemental Experimental Procedures). There were significant reductions in threshold in the trained condition following perceptual learning, both when subjects were taking donepezil, with an average improvement of $6.2° \pm 2.1°$ (planned comparison, $t_{36} = 6.81$, $p < 0.05$), and under placebo, $2.2° \pm 0.8°$ (planned comparison, $t_{36} = 2.42$, $p < 0.05$). However, the improvement in performance in the trained condition during donepezil administration was significantly larger than the improvement under placebo (planned comparison, $t_{36} = 3.1$, $p < 0.05$).

In addition to enhancing the amount of learning in the trained condition, donepezil also increased its selectivity to this condition. Direction and location selectivity were computed separately for the placebo and donepezil training courses using the same procedure described above for combined drug and placebo data. Direction selectivity was $4.0° \pm 1.2°$ under donepezil (planned comparison, $t_{36} = 3.94$, $p < 0.05$) and $1.4° \pm 1.1°$ under placebo (planned comparison, $t_{36} = 1.34$, $p = 0.19$). The difference between these two values was statistically significant, indicating that, compared to placebo, donepezil increased the direction selectivity of learning (planned comparison, $t_{36} = 2.82$, $p < 0.05$). Similarly, location selectivity was $3.0° \pm 1.2°$ under donepezil (planned comparison, $t_{36} = 2.36$, $p < 0.05$) and $-1.2° \pm 1.0°$ under placebo (planned comparison, $t_{36} = 0.92$, $p = 0.36$). The increase in location specificity under donepezil (relative to placebo) was also statistically significant (planned comparison, $t_{36} = 2.68$, $p < 0.05$).

Comparisons of raw threshold values are sensitive to between-subject performance differences and to effects of the drug on overall task performance. In addition to donepezil's enhancement of the magnitude of perceptual learning, there was an overall deleterious effect of donepezil on discrimination thresholds ($F_{1,9} = 12.76$, $p < 0.05$, combining all directions, both locations, and both pre- and posttraining measurements, as assessed by the significance of the drug factor in the ANOVA), which may stem from nonspecific effects of cholinergic enhancement on visual perception and/or task performance (see Discussion).

In particular, pretraining thresholds for the direction of motion and visual field location used for training were numerically higher under donepezil ($13.3° \pm 2.4°$) than under placebo ($10.7° \pm 0.8°$), raising the possibility that the drug effect on the magnitude of learning was due to this difference between donepezil and placebo in pretraining thresholds. However, this

Pre-training
Post-training

Donepezil
trained location

Donepezil
untrained location

Placebo
trained location

Placebo
untrained location
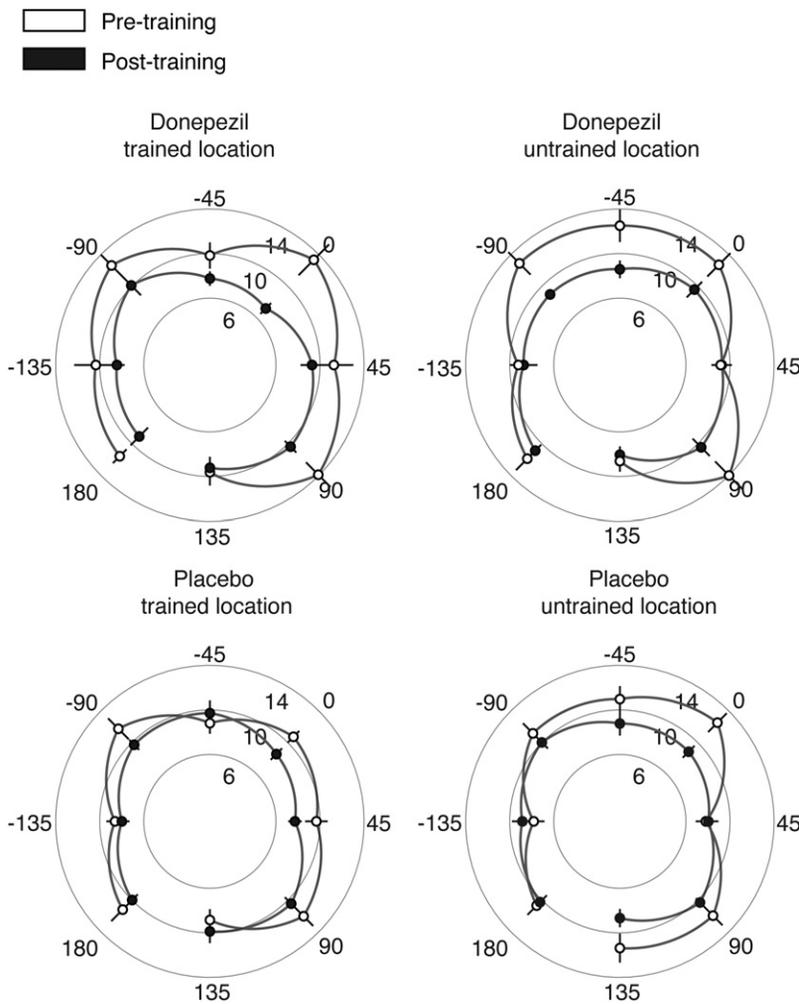
Figure 2. Effects of Training and Cholinergic Enhancement with Donepezil on Motion Direction Discrimination Thresholds

Each plot displays angular difference thresholds in degrees (Figure 1A) for different directions of motion, where 0° corresponds to the direction used for training. There was a significant improvement in performance for the trained condition (trained direction and visual field location), and this improvement was substantially larger under donepezil than under placebo. Error bars denote standard error of the mean (SEM). Single-subject data are presented in Figure S1.

condition for both donepezil and placebo (Figure 4). A single-parameter model of learning was fit to the data (see Supplemental Experimental Procedures). Average learning rates (in units of percent change/session) were greater for donepezil (4.9% per session ± 0.8% per session) than for placebo (2.0% per session ± 0.5% per session). Statistical significance of the effect of cholinergic enhancement on learning rate was calculated using a nonparametric permutation test (see Supplemental Experimental Procedures). This test demonstrated that learning was significantly more rapid under donepezil ($p < 0.05$).

Discussion

Cholinesterase inhibitors such as donepezil are commonly prescribed drug treatments for Alzheimer's disease. It would therefore be beneficial to understand the specific aspects of cognition and behavioral performance that are enhanced by increases in synaptic ACh. A previous study has shown that administration of the cholinesterase inhibitor physostigmine to healthy humans enhanced the behavioral effects of visual spatial attention [11], but another study reported no effects of this drug on performance in tasks requiring visual spatial attention [23]. Also, administration of physostigmine [10], as well as donepezil [24], can improve long-term retention of memorized items, but this effect has also not always been found [25]. Our findings demonstrate the possibility of enhancing the beneficial cognitive effects of the cholinergic system, even in a young healthy population, and suggest that the cognitive improvement associated with cholinergic enhancement in Alzheimer's disease may stem from an augmented capacity to learn new information.

Studies in animals have shown that ACh increases transmission at feedforward thalamocortical synapses relative to lateral intracortical connections [26]. ACh reduces the spatial spread of excitatory activity following electrical stimulation of rat visual cortical slices [27] and decreases the preferred stimulus length of cells in marmoset area V1 [4]. In addition, electrical stimulation of the basal forebrain results in a more reliable representation of the stimulus in visual cortical neurons [28]. In humans, donepezil reduces the spatial spread of excitatory fMRI visual responses in early visual cortex [5], consistent with a reduction in excitatory receptive field size of visual cortical neurons, and physostigmine increases the selectivity of responses in visual association

difference in pretraining thresholds for the condition that was then used for training was not statistically significant (post hoc t test: $t_{11} = 1.17$, $p = 0.27$). On the other hand, posttraining thresholds for the trained condition were significantly lower under donepezil (7.2° ± 0.6°) than under placebo (8.5° ± 0.5°, post hoc t test: $t_{11} = 2.81$, $p < 0.05$). There were no significant effects of the drug on thresholds for any other combination of visual field location and motion direction, either before or after training. This finding provides additional evidence that donepezil enhances perceptual learning in a stimulus-specific manner, because any general effect of the drug on task performance would presumably have affected both pre- and post-training thresholds (see Supplemental Data for additional analysis of donepezil's effect on overall thresholds and Figure S1 for individual subject data).

To isolate donepezil's effects on perceptual learning from its effects on overall task performance, we calculated the percent learning for each subject relative to that subject's pretraining performance (Figure 3). Percent learning in the trained condition was greater for donepezil than for placebo (planned comparison, $t_{36} = 2.5$, $p < 0.05$), further demonstrating that the beneficial effects of donepezil on learning were not due to the drug's effects on overall performance.

In order to determine whether the increase in the magnitude of learning under donepezil was a consequence of more rapid learning, we examined the progression of learning in the trained
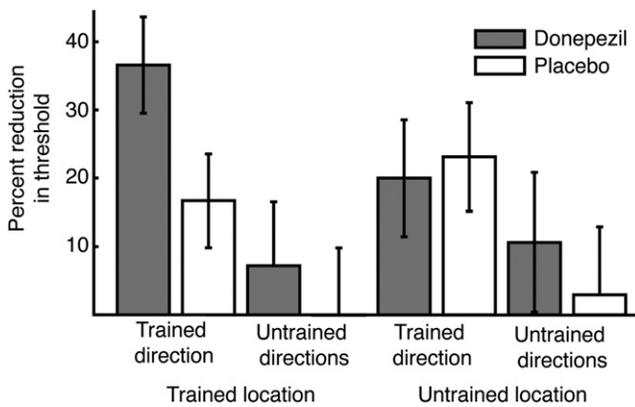
Figure 3. Donepezil Increases Magnitude and Specificity of Perceptual Learning

Percent reduction in threshold following training was significantly larger under donepezil in the trained condition, and learning under donepezil was more specific to the direction of motion and visual field location used for training. Error bars denote SEM. These data are presented without normalization (i.e., raw threshold values) in Figure S2.

cortex [3]. Our findings suggest that during perceptual learning, these increases in neural selectivity by ACh may enhance learning-dependent changes in tuning of the neurons that encode task-relevant stimuli. This is consistent with previous models of the role of the cholinergic system in learning and memory [29].

One factor that could be mediating the effects of cholinergic transmission on learning is visual attention. Attention has been found to play an important facilitatory role in some types of perceptual learning [30], and ACh modulates allocation of attention [1, 31]. In particular, when an animal is attending to a particular visual field location, visual cortical neurons with receptive fields at that location exhibit larger responses to visual stimulation. This increase in firing rate due to visual
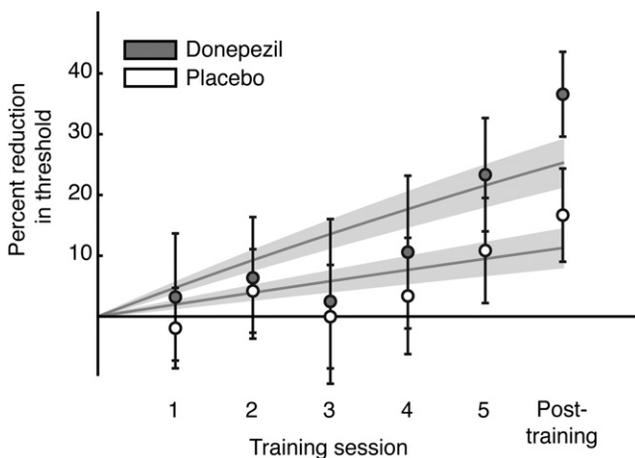
Figure 4. Donepezil Increases Rate of Perceptual Learning

Percent learning in the trained condition is presented as a function of training session. Training under donepezil (●) proceeded at a more rapid rate than training under placebo (○). Learning rates were computed by fitting a single-parameter model of learning to the data (gray continuous lines, where the shaded area is the standard deviation derived from a jack-knife estimate; see Supplemental Experimental Procedures). Error bars denote SEM.

spatial attention is augmented by local administration of ACh to cortical area V1 and is attenuated following local administration of the muscarinic ACh receptor antagonist scopolamine [32]. Previous studies in humans have shown increases in the effects of sustained visual attention following pharmacological enhancement of the cholinergic system [11, 31]. In the present study, donepezil may have facilitated processing of the training stimulus through enhanced allocation of attention to this stimulus, thereby augmenting perceptual learning. Nevertheless, we cannot rule out the possibility that donepezil's enhancement of perceptual learning is due to a direct modulation of plasticity rather than to an effect mediated by attention.

It is important to note that perceptual learning does not always require attention to be directed to the stimulus and that learning can occur even in the absence of conscious perception of the stimulus. Watanabe et al. [33] instructed participants to perform a difficult sensory judgment in the center of the visual field while a task-irrelevant motion stimulus was presented in the peripheral visual field. Although the amount of coherent motion in the peripheral stimulus was undetectable, subjects improved in performance of a motion discrimination task for the direction of motion contained in the peripheral stimulus, and the learning was specific to that direction of motion. However, even for this kind of task-irrelevant perceptual learning, training was still not entirely subliminal. Specifically, for the peripheral subthreshold stimuli, learning occurred only when the peripheral stimulus was presented at the same time that the target appeared in central vision [34]. That is, simultaneous presentation of task-relevant information was required in order for plasticity of the neural representations of the task-irrelevant stimulus to take place.

Furthermore, another study [35] demonstrated that task-irrelevant perceptual learning depends on the relative visual field locations of the task-irrelevant and task-relevant stimuli. Task-irrelevant perceptual learning was demonstrated for stimuli that were near the task-relevant stimulus but was not observed for stimuli that were farther away (6.6° of visual angle) from the attended stimulus. Acetylcholine is released in cortex when animals are performing a task requiring sustained attention [6], and a recent study showed that ACh can be released in frontal cortex in a transient and spatially specific manner and that this transient release of ACh increases the probability of stimulus detection [36]. We hypothesize that ACh release may facilitate task-irrelevant perceptual learning when the task-irrelevant stimuli appear in temporal and spatial proximity to the allocation of spatial attention. Further research is needed to determine the role of ACh in task-irrelevant perceptual learning (see [37] for a review of perceptual learning, attention, and neuromodulatory signals).

In the present study, subjects' overall task performance (across both trained and untrained conditions) was impaired by administration of donepezil, indicating that the presumed increase in selectivity of the neural response by ACh did not translate into an overall improvement in motion direction discrimination. However, the decrease in performance could also be the result of other effects of the drug. Donepezil was administered systemically in our study, and although this drug is relatively selective for the form of cholinesterase expressed in the central nervous system [38, 39], it may have affected nonspecific task-related cognitive functions and cholinergic synapses regulating processes such as lens accommodation and pupil dilation [40]. These nonspecific

effects of the drug would have affected performance in all conditions (including both pre- and posttraining measurements) and therefore would have been independent of the effect of donepezil on the magnitude and specificity of perceptual learning. Importantly, increased learning in the trained condition under donepezil was observed even when performance was normalized to each subject's pretraining threshold (Figure 3). Thus, overall differences in performance do not account for the beneficial effects of the drug on perceptual learning (see Supplemental Data).

In conclusion, we have shown that the magnitude, direction and location specificity, and rate of perceptual learning of a visual motion direction discrimination task are greater when donepezil is administered during the training procedure. These results demonstrate the possibility of enhancing the beneficial cognitive effects of the cholinergic system, even in a young, healthy population. Our finding that donepezil increases the specificity of perceptual learning suggests that ACh may augment plasticity and tuning in populations of neurons that encode task-relevant stimulus features.

Supplemental Information

Supplemental Information includes Supplemental Data, Supplemental Experimental Procedures, and two figures and can be found with this article online at doi:10.1016/j.cub.2010.08.027.

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