Sequential perceptual learning of letter identification and "uncrowding" in normal peripheral vision: Effects of task, training order, and cholinergic enhancement

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Human adults with normal vision are capable of improving performance on visual tasks through repeated practice. Previous work has shown that enhancing synaptic levels of acetylcholine (ACh) in healthy human adults with donepezil (trade name: Aricept) can increase the magnitude and specificity of perceptual learning (PL) for motion direction discrimination in the perifovea. In the current study, we ask whether increasing the synaptic levels of ACh in healthy human adults with donepezil boosts learning of low-contrast isolated letter identification and high-contrast flanked letter identification in normal peripheral vision. Two groups of observers performed sequential training over multiple days while ingesting donepezil. One group trained on isolated low-contrast letters in Phase 1 and crowded high-contrast letters in Phase 2, and the other group performed the reverse sequence, thereby enabling us to differentiate possible effects of drug and training order on PL of letter identification. All testing and training were performed monocularly in peripheral vision, at an eccentricity of 10 degrees along the lower vertical meridian. Our experimental design allowed us to evaluate the effects of sequential training and to ask whether increasing cholinergic signaling boosted learning and/or transfer of low-contrast isolated letter identification and high-contrast flanked letter identification in normal peripheral vision. We found that both groups improved on each of the two tasks. However, our results revealed an effect of training task order on flanked letter identification: Observers who trained on isolated targets first showed rapid early improvement in flanked letter identification but little to no additional improvement after 30 training blocks, while observers who first trained with flanked letters improved gradually on flanked letter identification over the entire 100-block course of training. In addition, we found no effect of donepezil on PL of either isolated or flanked letter identification. In other words, donepezil neither boosted nor blocked learning to identify isolated low-contrast letters or learning to uncrowd in normal peripheral vision.

Introduction

Visual perceptual learning (PL) can be quite specific to the trained task, orientation, eye, and so on (for recent reviews, see Sagi, 2011; Watanabe & Sasaki, 2015; Dosher & Lu, 2017). Elucidating the sites and mechanisms of PL are fundamental goals for vision science and may be of practical importance for the treatment of visual disorders such as amblyopia (Levi & Li, 2009; Tsirlin et al., 2015).

The neurotransmitter acetylcholine (ACh) plays important roles in visual cortical plasticity and PL (reviewed in Kang et al., 2014). In a previous study,

Rokem and Silver administered the cholinesterase inhibitor donepezil (trade name: Aricept) to increase synaptic levels of ACh in healthy human adults during PL of motion direction discrimination in the perifovea. In that study, participants ingested donepezil once per day over several days during one phase of PL and were administered placebo in the other phase. Donepezil increased the magnitude and specificity of PL of motion direction discrimination (Rokem & Silver, 2010), and the beneficial effects of donepezil on PL were still evident when retested up to 15 months later (Rokem & Silver, 2013).

Donepezil blocks the metabolism of ACh in the synapse, thereby prolonging its effective lifetime and presumably augmenting the normal physiological effects of ACh. Cholinesterase inhibitors increase cholinergic signaling through both nicotinic and muscarinic ACh receptor subtypes. Donepezil is widely used in the treatment of Alzheimer's disease and is considered safe, with few side effects. This medication is therefore potentially useful for boosting brain plasticity in adults with visual disorders. However, our most recent study found that training under donepezil failed to boost PL of letter identification in adults with amblyopia and may even have halted learning and transfer in an uncrowding task (i.e., learning to reduce the deleterious effect of nearby flanking letters on visual discrimination; Chung, Li, Silver, & Levi, 2017).

This lack of effect of donepezil on learning to reduce crowding in amblyopic vision is unfortunate because amblyopia is one of the most frequent neurodevelopmental causes of vision loss (Ciuffreda, Levi, & Selenow, 1991) and because crowding is a major bottleneck for spatial vision and reading, both in normal peripheral vision (Bouma, 1970; Levi, 2008; Whitney & Levi, 2011) and in individuals with amblyopia (particularly amblyopia associated with strabismus) (Levi, Song, & Pelli, 2007; Song, Levi, & Pelli, 2014). The findings of Chung et al. (2017) are also surprising, since amblyopic observers can learn to reduce crowding in the absence of pharmacological treatment (Chung et al., 2012; Hussain et al., 2012). Chung et al. (2017) speculated that the reduction in learning to uncrowd under donepezil might be a consequence of increased intracortical suppression, as cholinergic signaling decreases visual responses outside layer 4c in macaque V1 (Disney, Aoki, & Hawken, 2012), while at the same time boosting feedforward inputs to V1 (Disney, Aoki, & Hawken, 2007).

The variability of effects of cholinergic enhancement by donepezil on PL (Rokem & Silver, 2010; Chung et al., 2017) therefore raises questions regarding the interactions between ACh and the specific task(s) used for PL and whether cholinergic effects on PL are different for amblyopia and normal vision. Recent work (Frangou, Correia, & Kourtzi, 2018) suggests that the role of GABA in learning can depend on the task: Reductions in GABA in human occipitotemporal cortex during training were associated with a higher rate of learning to detect a visual signal in clutter, while increases in GABA in this brain region predicted enhanced learning of sensitivity to fine feature differences. Similarly, enhancing cholinergic signaling with donepezil can have task-dependent effects on PL and performance in visually normal adults. For example, donepezil enhanced PL of motion direction discrimination (Rokem & Silver, 2010) but not PL of texture discrimination (Byrne et al., in press). Moreover, acute administration of donepezil improved performance on a surround suppression task but not a crowded letter identification task (Kosovicheva et al., 2012).

Nine of the 10 amblyopic participants in the Chung et al. (2017) study performed sequential training under donepezil. First, they trained on identification of low-contrast isolated letters, and they improved by about the same amount as participants in a previous study with no donepezil administration (Chung et al., 2012). Subsequently, six of the subjects returned for further training on the flanked letter identification task while taking donepezil, and they showed little or no learning on this task. This type of sequential double training has been shown to facilitate visual PL on a number of tasks in studies with no drug administration (Xiao et al., 2008; Zhang et al., 2010; Wang et al., 2012; Wang et al., 2014; Zhang et al., 2014). In Chung et al. (2017), a 10th amblyopic observer performed the reverse sequence, training first on the flanked letter identification task and then on the low-contrast isolated letter task, while ingesting donepezil during both courses of training. This observer also showed no learning for flanked letters but substantial learning for isolated letters.

The lack of beneficial effects of donepezil on PL of letter identification in individuals with amblyopia (Chung et al., 2017) is in contrast to the clear effects of donepezil on PL of motion direction discrimination in participants with normal vision (Rokem & Silver, 2010, 2013). The discrepancy in these findings could be due to differences between subjects with and without amblyopia and/or differences in training task. Therefore, in the current study, we ask whether (a) there are sequential training effects in learning to uncrowd in normal peripheral vision and (b) whether increasing the synaptic levels of ACh with donepezil boosts learning of low-contrast isolated letter identification and high-contrast flanked letter identification. In the present study, two groups of observers performed sequential training while ingesting donepezil. One group ("Isolated-first") trained on isolated low-contrast letters in Phase 1 and on crowded high-contrast letters in Phase 2, and the other group ("Flanked-first") performed the reverse sequence, thereby enabling us to examine possible effects of
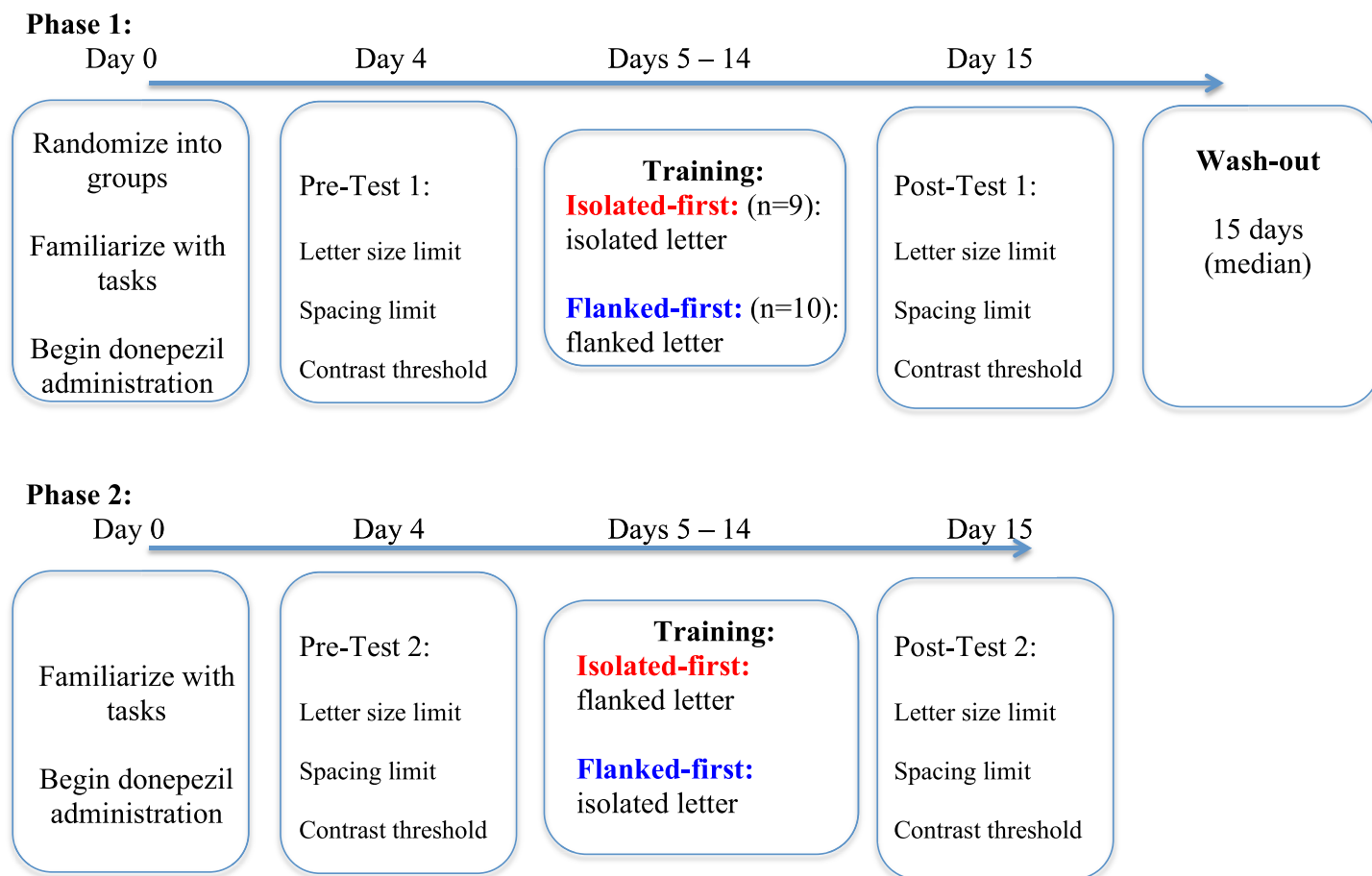
Phase 1:

Day 0	Day 4	Days 5 – 14	Day 15	
Randomize into groups Familiarize with tasks Begin donepezil administration	Pre-Test 1: Letter size limit Spacing limit Contrast threshold	**Training:** **Isolated-first:** (n=9): isolated letter **Flanked-first:** (n=10): flanked letter	Post-Test 1: Letter size limit Spacing limit Contrast threshold	**Wash-out** 15 days (median)

Phase 2:

Day 0	Day 4	Days 5 – 14	Day 15
Familiarize with tasks Begin donepezil administration	Pre-Test 2: Letter size limit Spacing limit Contrast threshold	**Training:** **Isolated-first:** flanked letter **Flanked-first:** isolated letter	Post-Test 2: Letter size limit Spacing limit Contrast threshold

Figure 1. Experimental timeline. Top row: Phase 1 training consisted of 10 blocks of 100 trials/block each day for 10 consecutive days, and 5 mg donepezil was administered on each of Days 0 to 15 for both phases. Bottom row: Phase 2 had the identical testing (Pre-test 2 and Post-test 2) and training protocol as Phase 1 but with the training task that was not used in Phase 1 (isolated letter or flanked letter).

both cholinergic enhancement and training task order on PL of letter identification in normal peripheral vision.

Our impetus for using sequential double training is based on the suggestion that there may be two stages of learning to uncrowd: a rapid early stage in which subjects learn to segment target and flankers, resulting in improved performance that generalizes to other stimuli, and a later stage in which performance gradually improves in a stimulus-specific manner as subjects learn to refine the target representation (Zhu et al., 2016). If true, this might predict different time courses and magnitudes of learning of flanked letter identification based on task training order: a more rapid time course for learning to uncrowd when target representation (isolated letter identification) is trained first and a greater magnitude of learning to uncrowd when flanked letter training is performed first.

Methods

Study design

Nineteen young healthy adults (mean age = 23.2 ± 3.4 (*SD*) years; 10 males, 9 females) with normal or corrected-to-normal vision (Snellen 20/20 or better) were randomly assigned to one of two groups: Isolated-first (isolated letter training followed by flanked letter training, *n* = 9) or Flanked-first (flanked letter training followed by isolated letter training, *n* = 10). The experimental timeline is illustrated in Figure 1.

On Day 0, participants were familiarized with each of the psychophysical tasks as detailed below and were then administered the first of the daily doses of donepezil in the laboratory (one 5-mg tablet per day, same dosage as in our previous studies

([Rokem & Silver, 2010](); [Chung et al., 2017]())). Participants self-administered the drug on Days 1 to 3 to attain steady-state plasma levels of donepezil (half-life, approximately 80 hours; [Rogers et al., 1998]()) before data collection. No testing was performed during these three days. On Day 4, another dose of donepezil was administered, and Pretest 1 was performed, followed by visual perceptual training and continuing daily donepezil administration for 10 consecutive days (Days 5–14). Posttest 1 (same as Pretest 1) was performed on Day 15, and this completed the first phase of training. There was then a washout period of at least 11 days (median = 15 days), with no testing or training during this period. For 16 observers, the duration of the washout period was between 11 and 22 days; the remaining 3 observers had a washout period longer than 30 days. Thus, the washout duration was at least 3.3 half-lives of donepezil for each participant. The actual length of the washout period for each observer was determined by his or her availability for testing and training on 12 consecutive days.

Observers then began Phase 2 while again ingesting daily donepezil. Phase 2 consisted of the same testing (Pretest 2 and Posttest 2), training, and drug administration as Phase 1 but employed the other training task (isolated letter identification for the Flanked-first group and flanked letter identification for the Isolated-first group). There were no reports of adverse side effects of taking donepezil for any participant.

Stimuli and tasks

The visual stimuli, consisting of isolated letters or three letter sequences (trigrams), and tasks were identical to those used by [Chung et al. (2012](), [2017]()). The stimuli are shown later in [Figure 3]() and will be briefly described here. One day before (Pretest 1 and Pretest 2) and one day after (Posttest 1 and Posttest 2) each training phase, observers completed three tasks that were used to assess transfer of PL: (a) letter size limit (the smallest high-contrast isolated letter size that observers could correctly identify 52% of the time), (b) spacing limit (the minimum center-to-center separation between adjacent high-contrast letters resulting in correct identification of the middle letter of the trigram 52% of the time), and (c) contrast threshold for identifying isolated letters (the contrast level at which observers could correctly identify the letter 52% of the time). For all three of these tasks, chance performance was 1/26 letters, or 3.8%. For the spacing and contrast tests, the letter size was set to $1.5 \times$ the letter size limit (*x*-height, defined as the height of the lowercase letter "x") for each observer. The method of constant stimuli was used to measure psychophysical thresholds, as described in [Chung et al. (2012)]().

There were two training tasks: isolated letter training and flanked letter training. For isolated letter training, observers were asked to identify a low-contrast letter that was $1.2 \times$ their pretest letter size limit. For flanked letter training, observers identified the middle letter of a high (90%) contrast trigram, with center-to-center distance between adjacent letters fixed at $0.8 \times$ the letter size for each individual. At this small letter separation, adjacent letters often touched but did not overlap, except for the wider letters (e.g., "w" and "m"). The letter size was $1.5 \times$ the pretest letter size limit for each participant. For both training tasks, observers completed 10 blocks of trials (100 trials per block) per day for 10 consecutive days, while taking 5 mg donepezil daily. Note that the pretest letter size limits for isolated letter training and flanked letter training were determined separately for each individual observer, based on data from the pretest that immediately preceded the respective training phase (i.e., Pretest 1 for Phase 1 and Pretest 2 for Phase 2). There were no significant differences between the Isolated-first and the Flanker-first groups in either phase: average letter size (Phase 1: mean pretest letter size: 0.50 ± 0.04 [*SEM*] vs. 0.53 ± 0.02, $t = -0.66$, $p = 0.52$; Phase 2: mean pretest letter size: 0.45 ± 0.03 [*SEM*] vs. 0.49 ± 0.02, $t = -1.27$, $p = 0.23$).

All testing and training were performed monocularly in the peripheral visual field of the right eye, at an eccentricity of 10 degrees along the lower vertical meridian, while observers fixated a small square. To minimize eye movements, visual stimuli were briefly displayed for 150 ms for all pretests, posttests, and training.

Results

Training

[Figure 2]() presents mean training curves for the two groups; individual results can be found in supplemental materials (Supplementary Figure S1). In all figures, Isolated-first (isolated letter training followed by flanked letter training) data are shown in red, and Flanked-first (flanked letter training followed by isolated letter training) data are shown in blue. To facilitate comparison of changes in performance with training, data in the lower panels were replotted after normalizing to individual initial performance (Training Day 1, Block 1). The group data from both tasks were fit with an exponential function, with the time constant corresponding to the training time needed to reach 63% of asymptotic performance.

A 2×2 crossover design (AB/BA) Type III analysis of variance (ANOVA) with Satterthwaite's method, based on the normalized data averaged across Blocks
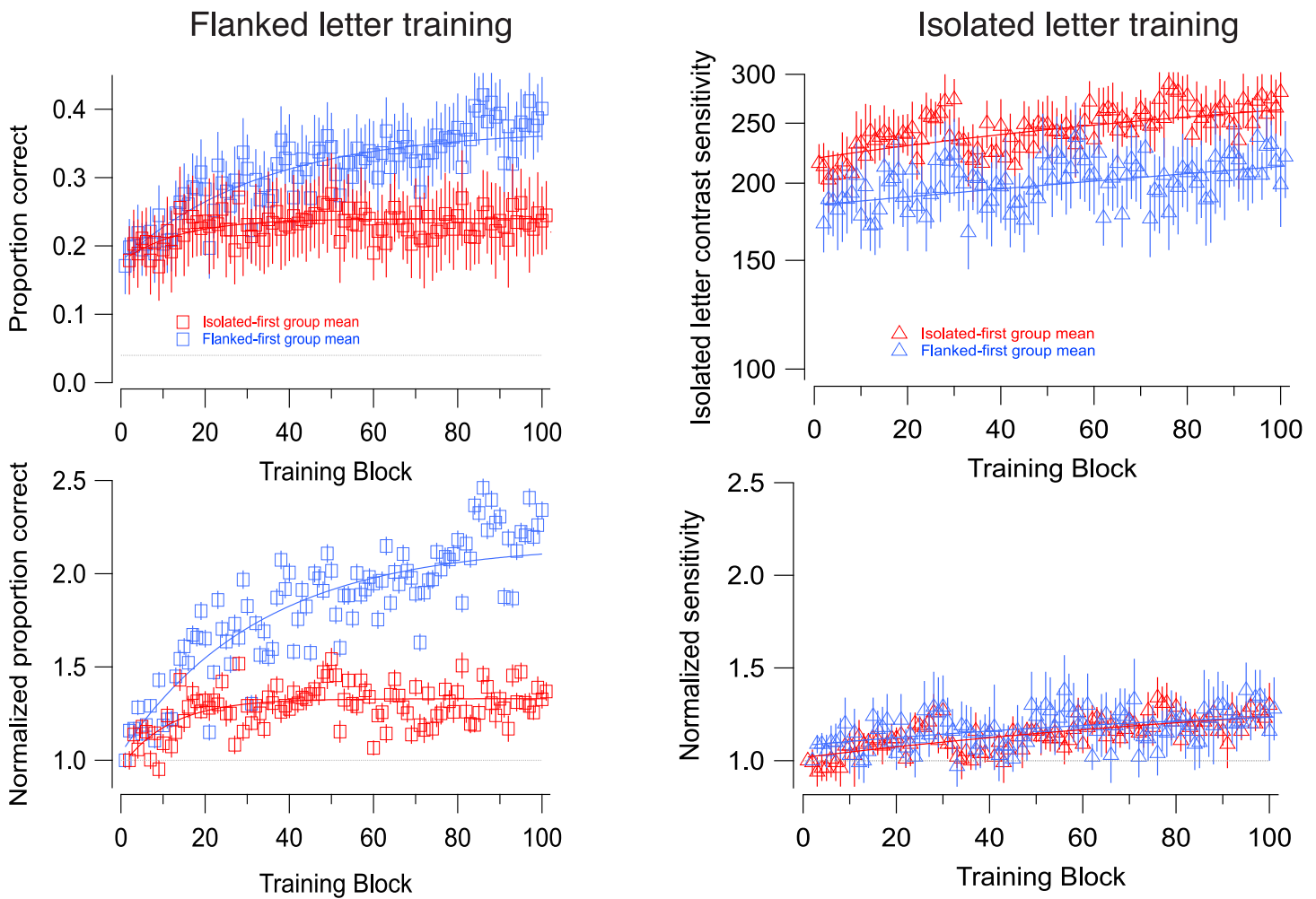
Figure 2. Sequential perceptual learning of letter identification under donepezil. Left panels show flanked letter training, and right panels show isolated letter training. Top panels are the geometric mean proportion correct (left) and contrast sensitivity (right) for both groups. Gray line in the top left panel indicates chance performance on the flanked letter identification task. Bottom panels replot the data from the top panels after normalizing each observer's performance by his or her own initial (Training Day 1, Block 1) performance. Colored lines show exponential function fits, and the gray dotted lines in the lower panels represent baseline performance (normalized value $= 1$). Error bars are ± 1 *SEM*.

	Mean Sq	Degrees of freedom	F	p
Task	3.76	(1, 34)	7.62	0.009
Phase	1.55	(1, 34)	3.15	0.085
Group	2.26	(1, 34)	4.58	0.040

Table 1. Type III analysis of variance table with Satterthwaite's method.

91 to 100 (i.e., data collected on the last training day), revealed significant effects of task (flanked vs. isolated) and group (Isolated-first vs. Flanked-first) but not of training phase (first vs. second) on PL (Table 1). The significant effect of group suggested a carryover effect, which we attribute to transfer of learning from the first to the second task in the Isolated-first group (discussed further below).

To further assess the effects of training task and group with more sensitivity, we conducted a two-way repeated-measures ANOVA on the normalized data. We again defined the amount of improvement as the average performance of training Blocks 91 to 100 (last day of training), normalized by that of Block 1. Table 2 summarizes the *t* test results characterizing the effects of task and group on PL. There was a significant overall main effect of task ($F = 7.15$, $p = 0.016$): The isolated letter task (Figure 2, lower right panel) showed less learning than the flanked letter task (Figure 2, lower left panel). For the flanked letter task, there was a significant effect of group on normalized improvement and time constant (Figure 2, lower left panel; Table 2). Specifically, compared to the Flanked-first group, the Isolated-first group showed much less learning of the flanked letter task, and this learning saturated more

	Isolated-letter task	Flanked-letter task	Task difference
Improvement in performance			
Isolated-first group	Phase 1: 1.24 ± 0.03 ($t = 4.58$; $p = 0.009$*)	Phase 2: 1.48 ± 0.13 ($t = 2.35$; $p = 0.023$*)	$t = 0.66$; $p = 0.520$
Flanked-first group	Phase 2: 1.32 ± 0.09 ($t = 2.25$; $p = 0.025$*)	Phase 1: 2.37 ± 0.22 ($t = 3.86$; $p = 0.002$*)	$t = 3.19$; $p = 0.005$
Group difference	$t = 0.26$; $p = 0.797$	$t = 2.77$; $p = 0.009$	
Time constants			
Isolated-first group	Phase 1: 136 ± 8.5 blocks	Phase 2: 10.1 ± 5.9 blocks	$t = 12.37$; $p = 0.0001$
Flanked-first group	Phase 2: 109 ± 13.4 blocks	Phase 1: 55.5 ± 14.1 blocks	$t = 2.75$; $p = 0.014$
Group difference	$t = 1.74$; $p = 0.1$	$t = 3.08$; $p = 0.007$	

Table 2. Training task improvement and time constants for task/group combinations. Asterisk indicates statistical test results for improvement in performance for which the mean was significantly greater than 1.

rapidly, at about a factor of 1.3 after approximately 30 blocks (Figure 2, lower left panel; Table 2). This group difference indicates that PL of flanked letter identification was reduced when it followed isolated letter training. In contrast, there was no significant effect of group on either normalized improvement in contrast sensitivity or the time constant of learning for isolated letter identification (Figure 2, lower right panel; Table 2). However, the Flanked-first group had lower absolute contrast sensitivity throughout training than the Isolated-first group (Figure 2, upper right panel) ($t = 17.27$, $p < 0.00001$).

Transfer

Figure 3 displays improvement relative to baseline due to PL for both training and transfer tasks. Here, as in our previous study (Chung et al., 2017), statistical significance was based on whether an improvement factor value of 1 was included in the 95% confidence interval (CI). The Isolated-first group (Figure 3, left panel) showed a small but significant transfer to size following isolated letter training in Phase 1 (improvement = 1.13 ± 0.03 [95% CI], $t = -3.157$, $p = 0.006$) but not after flanked letter training in Phase
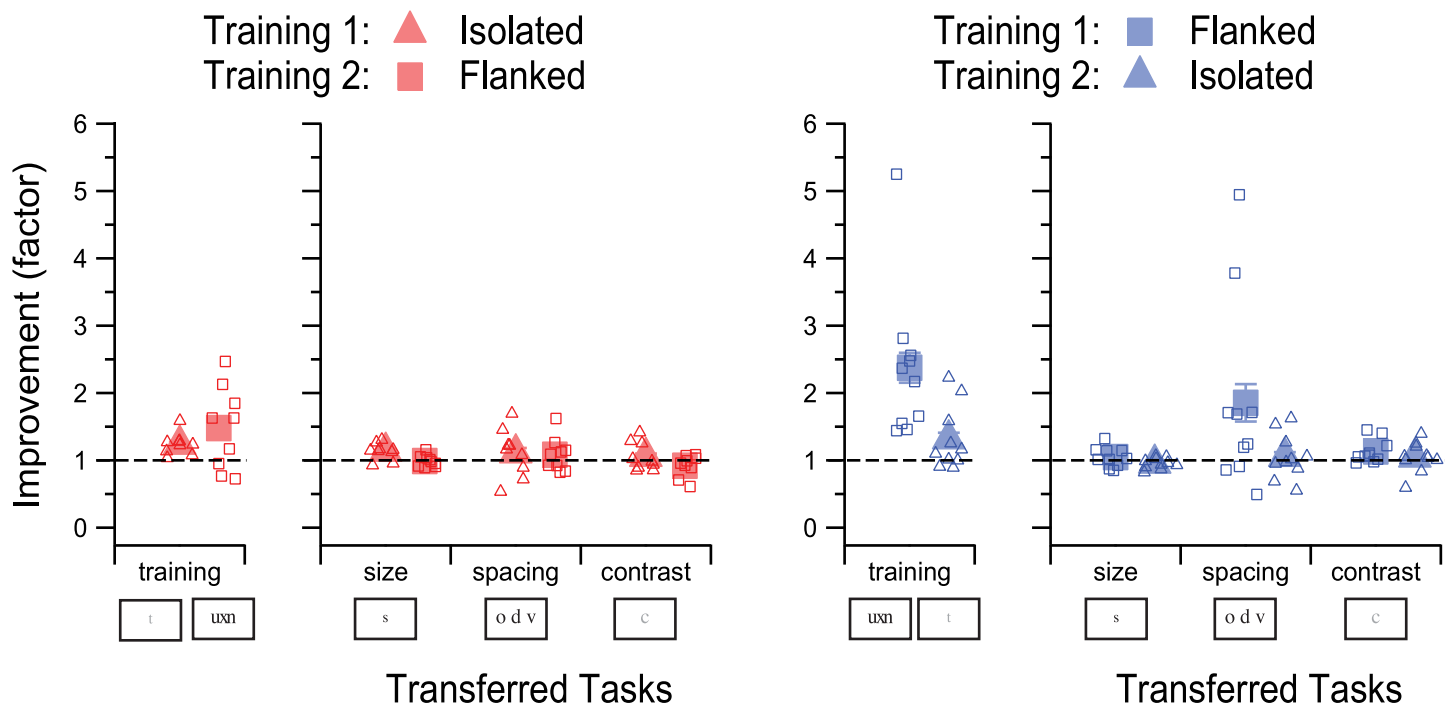

Figure 3. Training and transfer of learning. Improvement for both the training and transfer tasks for each phase is plotted, with a factor of 1 (dashed lines) representing no change relative to pretraining performance for that phase. Filled symbols are group averages, while small unfilled symbols are individual observers' data. Left panel: Isolated-first group. Right panel: Flanked-first group.
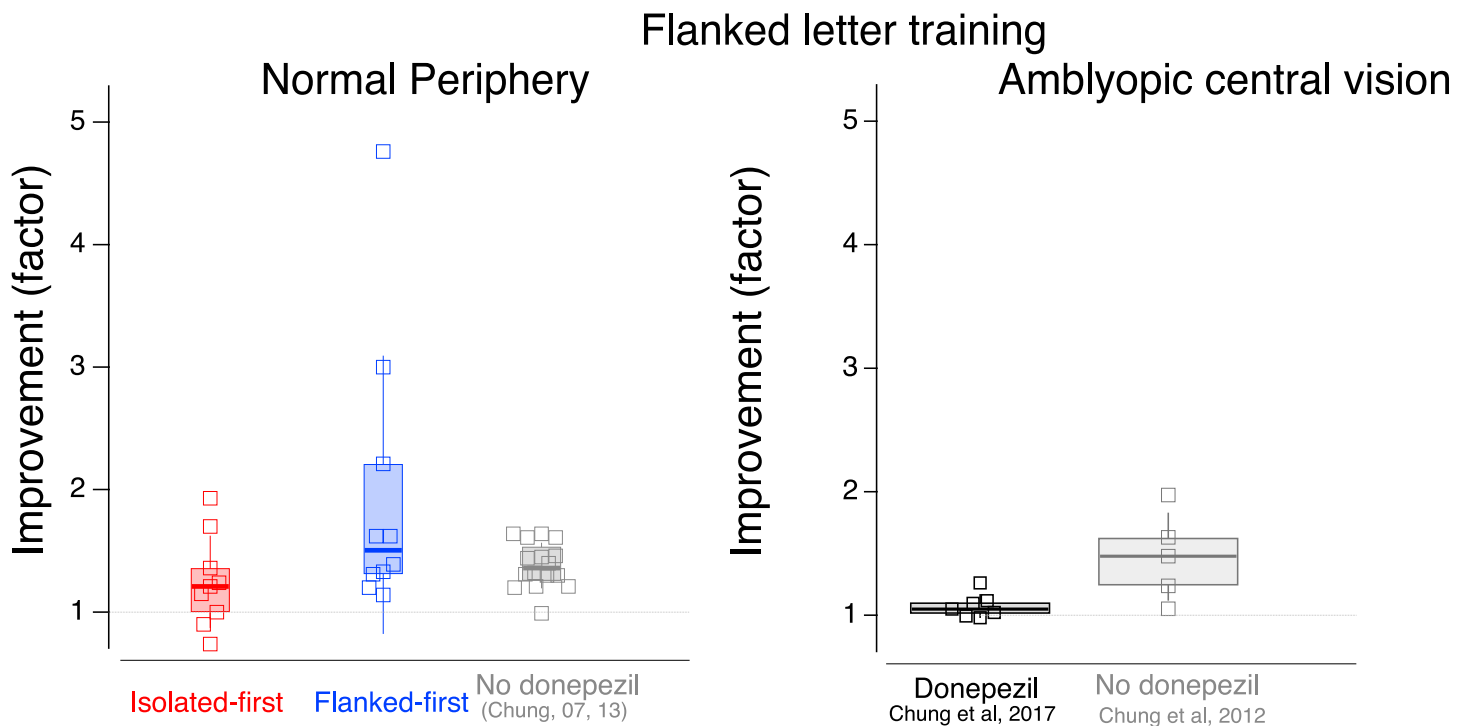
Figure 4. Comparison of PL of flanked letter identification in the current study with previous studies. Left panel: Normal periphery. Red and blue bars are from the current study; the gray bar shows the combined data from two previous studies using nonsequential flanked letter training without donepezil (Chung 2007; Chung & Truong 2013 [only data from the daily-training groups are shown here]). Right panel: PL of flanked letter identification in the fovea of amblyopic observers, replotted from previous studies (Chung et al., 2012; Chung et al., 2017). In this and the subsequent figure, the shaded boxes show the first and third quartiles, the thick horizontal lines are the median values, and the whiskers are ± 1 *SD*.

2 (0.99 ± 0.02 [95% CI], $t = 0.345$; $p = 0.73$) and little or no significant transfer after either phase to either spacing (Phase 1: 1.1 ± 0.08 [95% CI], $t = -0.09$; $p = 0.38$; Phase 2: 1.08 ± 0.05 [95% CI], $t = -1.01$; $p = 0.33$) or contrast (Phase 1: 1.05 ± 0.04 [95% CI], $t = -0.79$; $p = 0.44$; Phase 2: 0.92 ± 0.04 [95% CI], $t = 1.5$; $p = 0.15$). It is somewhat surprising that contrast training at 1.2 times the letter size limit for isolated letters did not transfer to another task that was identical except that it employed letters at 1.5 times the size limit.

For the Flanked-first group (Figure 3, right panel), flanked letter training in Phase 1 resulted in a substantial (although not statistically significant) transfer to the spacing limit (1.85 ± 0.28 [95% CI], $t = -1.91$; $p = 0.07$) and a significant transfer to contrast (1.13 ± 0.03 [95% CI], $t = -2.47$; $p = 0.024$) but little or no transfer to size (1.05 ± 0.03 [95% CI], $t = -1.02$; $p = 0.32$). It should be noted that the transfer of flanked letter PL to the spacing limit task was largely driven by two observers who showed a very substantial improvement in spacing limit, and the transfer was not significant if these two observers were removed from the analysis ($t = -1.4$, $p = 0.18$). For the Flanked-first group, the isolated letter training in Phase 2 produced little or no transfer to any of the tasks: size (0.94 ± 0.05

[95% CI], $t = 1.8$; $p = 0.09$); contrast (1.04 ± 0.15 [95% CI], $t = -0.65$; $p = 0.52$), or spacing (1.05 ± 0.07 [95% CI], $t = -0.51$; $p = 0.62$).

Assessing effects of donepezil and amblyopia on PL of letter identification

Figures 4 and 5 summarize the magnitude of PL for both tasks and groups, with all training occurring while ACh levels were elevated with donepezil. These data are compared to our previously reported findings in normal peripheral vision without donepezil or multiple phases of training (Chung, 2007; Chung & Truong, 2013; Figure 4, left panel) to effects of sequential training in the foveal visual field of observers with amblyopia during donepezil administration (Chung et al., 2017; Figures 4 and 5, right panels), and to foveal nonsequential training without drug administration (Chung et al., 2012; Figures 4 and 5, right panels). See Table 3 for a summary of these studies. To facilitate comparison with our previous studies, we fit the individual isolated letter data from the present study with an exponential function and the flanked letter
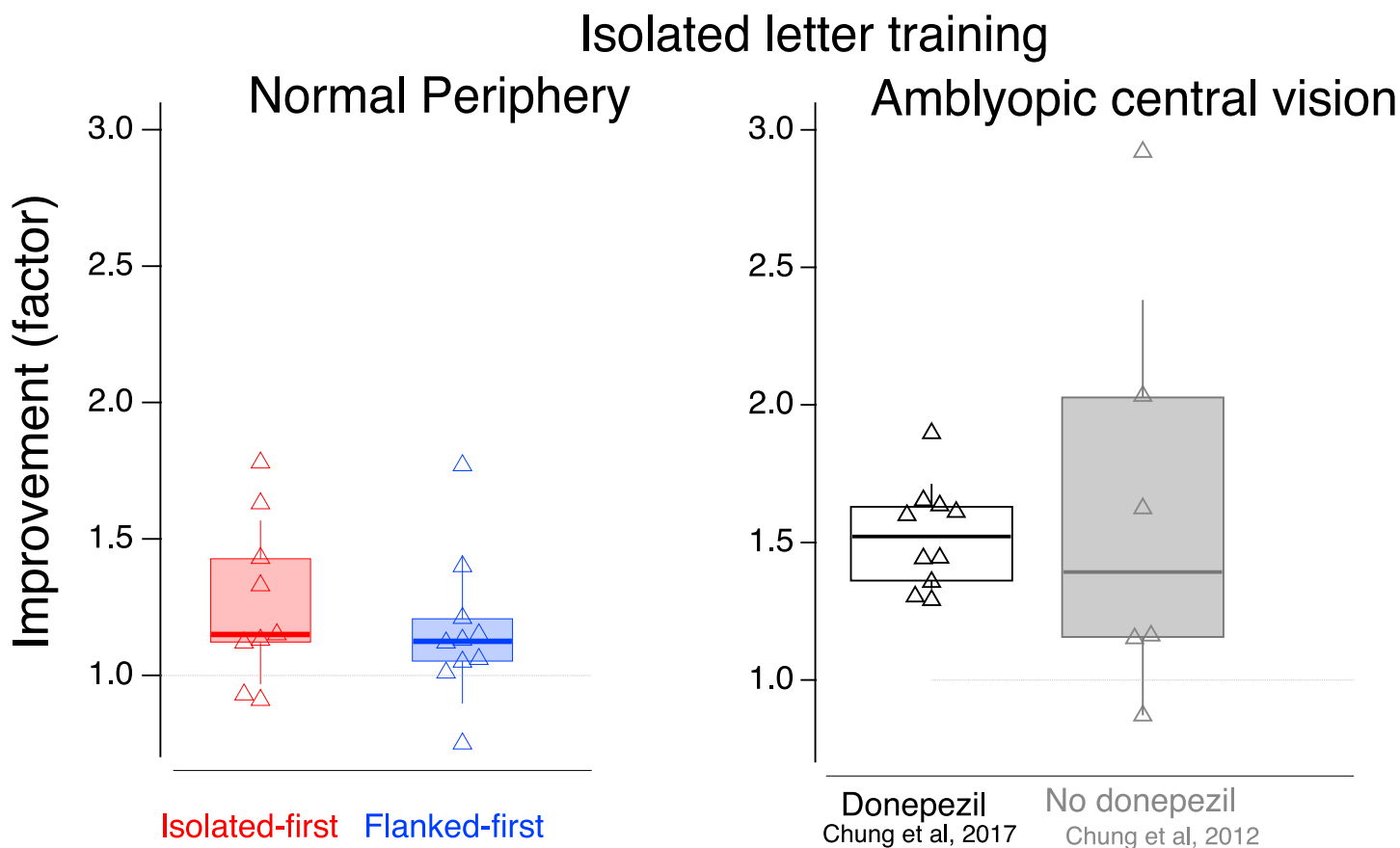
Figure 5. Comparison of PL of isolated letter identification in the current study with previous studies. Left panel: Normal periphery, training under donepezil. Blue and red bars are from the current study. Right panel: Foveal data from amblyopic observers, replotted from previous studies (Chung et al., 2012; Chung et al., 2017).

Study	Normal/Amblyopic	Central/Peripheral	Drug/no drug	Number of training phases	Improvement
Chung, 2007	Normal	Peripheral	No	One	Yes
Chung & Truong, 2013	Normal	Peripheral	No	One	Yes
Chung et al., 2012	Amblyopic	Central	No	One	Yes
Chung et al., 2017	Amblyopic	Central	Drug	Two	No

Table 3. Previous studies of PL of flanked letter identification.

data with a linear function (see supplementary figures) and defined improvement as the ratio of the regression parameters for Blocks 1 and 100. Note that this method resulted in slightly lower improvement factors than those shown in Figures 2 and 3.

For flanked letter training (Figure 4, left panel), effects of group (training task order) in the present study can be clearly seen (blue vs. red bars). In particular, improvement in flanked letter identification was greater when that task was trained first. However, there was no significant difference between the magnitude of PL of flanked letter identification under donepezil in the Flanked-first group (Figure 4, blue bar) in the present

study compared to the combined data from our two previous studies using identical flanked letter training in the periphery without the drug (Chung 2007; Chung & Truong, 2013) (gray bar in left panel of Figure 4) (Mann-Whitney $U = 45.5$, z score $= -1.178$, $p = 0.24$).

There was also no significant difference between PL of flanked letter identification under donepezil in the Isolated-first group (Figure 4, red bar) compared to the data combined from Chung (2007) and Chung and Truong (2013) (Mann-Whitney $U = 40$, z score $= 1.20$, $p = 0.23$). It should be noted that neither of the two previous studies employed sequential training. In conclusion, for PL of flanked letter identification in the

visual periphery of subjects with normal vision, there is no evidence for a benefit of cholinergic enhancement during training.

The foveal vision of patients with amblyopia has often been compared to normal peripheral vision (Levi, Klein, & Aitsebaomo, 1984; Katz, Levi, & Bedell, 1984; Levi & Klein, 1985). Like the normal periphery, the central visual field of the amblyopic eye is associated with reduced contrast sensitivity for small letters (Pelli, Levi, & Chung, 2004) and increased magnitude and spatial extent of crowding (Levi & Klein, 1985; Song, Levi, & Pelli, 2014). For flanked letter training without either donepezil or additional task training, the magnitude of PL in amblyopic participants (Chung et al., 2012) (Figure 4, right panel, gray bar) was similar to that in the normal periphery (Figure 4, left panel, gray bar; Mann-Whitney[1] $U = 27$, z score $= -0.49$, $p = 0.62$) and comparable to training in the normal periphery under donepezil for the Flanked-first group (Figure 4, left panel, blue bar; Mann-Whitney $U = 19$, z score $= 0.67$, $p = 0.50$).

However, when training with donepezil, observers with amblyopia showed no improvement in flanked letter identification (Chung et al., 2017) (Figure 4, right panel, white bar), and this was significantly less than the improvement in the Flanked-first group in the normal periphery with donepezil (Figure 4, blue bar; Mann-Whitney $U = 2$, z score $= 3.17$, $p = 0.0015$). This may be in part due to effects of training task order: Five of the six observers in our earlier study (Chung et al., 2017) first performed isolated letter training (like the Isolated-first group in the current study). Indeed, a direct comparison of the results from those five amblyopic participants (Chung et al., 2017) and the Isolated-first group from the present study (which used the same training sequence) (Figure 4, red bar) showed no significant difference (Mann-Whitney $U = 22$, z score $= 0.95$, $p = 0.34$).

For isolated letter training (Figure 5), the effect of training task order in peripheral vision was small and not significant (left panel, blue vs. red bars; Mann-Whitney $U = 35.5$, z score $= -0.73$, $p = 0.46$). There appears to be little, if any, benefit (or harm) from training with donepezil in either normal peripheral vision (left panel) or amblyopic foveal vision (right panel, white bar; Chung et al., 2017) compared to single-task training on isolated letters in amblyopic individuals without donepezil (right panel, gray bar; Chung et al., 2012). Interestingly, the difference in improvement in isolated letter identification between amblyopes who first performed sequential training with isolated letter training with donepezil (Chung et al., 2017) (Figure 5, white bar, right panel) and the corresponding normal periphery group (Figure 5, red bar) approached significance at the $p = 0.05$ level (Mann-Whitney $U = 21$, z score $= 1.92$, $p = 0.055$), perhaps because the amblyopic observers had more room for improvement.

Discussion

For literate adults, letters are among the most overlearned visual objects. Nonetheless, practice with near threshold letters (isolated or flanked) enhances letter identification in the periphery in individuals with normal vision (Chung, Levi, & Tjan, 2005; Chung, 2007; Chung & Truong, 2013; Yashar, Chen, & Carrasco, 2015) and in central amblyopic vision (Chung, Li, & Levi, 2008, 2012; Hussain et al., 2012; Chung et al., 2017). In the current study, two groups of observers with normal vision performed sequential training in the periphery while ingesting donepezil: The Isolated-first group trained on low-contrast isolated letters in Phase 1 and crowded letters in Phase 2, and the Flanked-first group performed the reverse sequence. This experimental design enabled us to evaluate sequential effects of successive training on the two tasks and to ask whether increasing synaptic levels of ACh with donepezil boosted learning and/or transfer of low-contrast isolated letter identification and high-contrast flanked letter identification in normal peripheral vision.

Sequential effects

Our experiments revealed a clear effect of training task order for learning to identify flanked letters. Practicing the flanked letter task first resulted in steady improvement, up to a factor of approximately 2.4, over the entire 10-day (10-kilo trials) course of training. In contrast, practicing flanked letter identification second (after 10-kilo trials of training with low-contrast isolated letters) resulted in a much smaller improvement (a factor of approximately 1.3) that almost saturated after just two days of training (86.5% of plateau performance after 2-kilo trials).

We note that observers in the Isolated-first group had a higher baseline sensitivity for identification of isolated letters than those in the Flanked-first group. Thus, one potential explanation for the group difference in PL of flanked letter identification is that the high sensitivity in the Isolated-first group might have resulted in rapidly saturating performance during the following flanked letter training. However, we think this is unlikely, as the magnitude and time course of isolated letter identification learning were similar in the two groups (Figure 2 and Table 2).

Rather, a more likely explanation is that the asymmetry in task training order effects is due to fundamental differences between learning to uncrowd and learning of the isolated letter training task (Law & Gold, 2008, 2010; Sun, Chung, & Tjan, 2010; Zhu, Fan, & Fang, 2016). Indeed, recent work by Zhu et al. (2016) suggests that there are two stages of learning to uncrowd: a rapid early stage in which subjects learn

to segment target and flankers, resulting in improved performance that generalizes to other stimuli, and a later stage in which performance gradually improves in a stimulus-specific manner as subjects learn to refine the target representation. Our results are broadly consistent with this framework. Observers in the Isolated-first group showed a rapid early improvement in flanked letter identification (red line in the lower left panel of Figure 2), with little improvement beyond 30 training blocks, while observers in the Flanked-first group (blue line in the lower left panel of Figure 2) continued to improve gradually on the flanked letter task over the entire 100-block course of training.

We speculate that in the early stage of flanked letter training, both groups learned to reduce crowding but that subjects in the Isolated-first group had already achieved the slow improvement in target representation during the first phase of training with isolated letters, so their subsequent PL for flanked letters was limited to learning to break crowding. This would be the opposite of the order described by Zhu et al. (2016) for orientation discrimination of crowded gratings (rapid general learning to uncrowd followed by slow refinement of target representation). An additional possibility is that the Isolated-first group may have overlearned the strategy of integrating all information presented across the perceptual window during Phase 1 training, a strategy that would not be ideal for learning to identify flanked letters in Phase 2.

Does donepezil boost perceptual learning of letter identification in subjects with normal vision?

Part of the motivation for this study was the observation that increasing synaptic levels of ACh of healthy human adults augmented the magnitude and specificity of PL of motion direction discrimination in the perifovea of subjects with normal vision (Rokem & Silver, 2010) and that these effects were long-lasting (Rokem & Silver, 2013). Crowding is a major bottleneck for visual processing in peripheral vision. Given that the effects of crowding in the periphery can be reduced through PL (Chung, 2007; Chung & Truong, 2013), we investigated whether donepezil would increase the effectiveness of PL of flanked letter identification in the periphery. However, compared with these previous nonpharmacological studies, our present findings suggest that cholinergic enhancement during training on flanked letters neither boosted nor interfered with learning to uncrowd. This negative result is consistent with our previous findings that donepezil did not significantly enhance PL of texture discrimination (Byrne et al., at press) and had no detectable acute effects on performance of a peripheral flanked letter identification task (Kosovicheva et al., 2012).

One limitation of our study is that we did not include a control group that performed sequential training without ingesting donepezil. However, neither the magnitude nor rate of PL of flanked letter identification for the Flanked-first group in the current study differed significantly from that of normal observers in previous studies (Chung, 2007; Chung & Truong, 2013) that used almost identical training methods without donepezil (Figure 4, left panel).

Donepezil, peripheral vision, and amblyopic vision

In a previous study, we found that donepezil failed to boost PL of isolated letter identification in adults with amblyopia and might even have halted learning and transfer of PL of flanked letter identification (Chung, Li, Silver, & Levi, 2017). Based on these results, we wondered whether this failure was specific to amblyopia or whether it might have been a consequence of the sequential training procedure employed in Chung et al. (2017). Nine of the amblyopic participants in Chung et al. (2017) practiced isolated letter identification in the first phase (in which they improved by about the same amount as participants with no donepezil), with six of these participants continuing with training on the flanked letter task (i.e., the same training sequence as the Isolated-first group in the present study). In fact, PL of flanked letter identification for the Isolated-first group from the present study was not significantly different from that of the amblyopic participants in Chung et al. (2017) (Figure 4). Although the Isolated-first group did show significant PL for flanked letter identification in Phase 2, the amblyopic subjects in Chung et al. (2017) did not. However, one of the amblyopic observers in Chung et al. (2017) performed the reverse training sequence (identical to the Flanked-first group in the present study) and also showed no significant improvement in flanked letter identification. While there are well-known individual variations in PL (Beard, Levi, & Reich, 1995), we note that this same amblyopic observer did show a significant performance improvement in subsequent training to identify isolated letters (Chung et al., 2017).

The effects of donepezil on PL are task specific

As noted above, Rokem and Silver (2010, 2013) showed that donepezil increased the magnitude and specificity of PL of motion direction discrimination in the perifovea. More recently, donepezil has been shown to enhance PL in an object-tracking task (Chamoun et al., 2017). Donepezil has also been shown to enhance contrast sensitivity in rats (Soma, Suematsu, & Shimegi,

2013) and humans (Boucart et al., 2015), with both studies suggesting the cholinergic benefit was greatest under more difficult perceptual conditions. In macaque monkeys, cholinergic signaling increases response gain in the primary thalamocortical recipient layer 4c (Disney, Aoki, & Hawken, 2007) and suppresses responses in V1 neurons outside this layer (Disney, Aoki, & Hawken, 2012). This increased intracortical suppression may be the reason that donepezil may have blocked the ability to learn to uncrowd in observers with amblyopia (Chung et al., 2017) who might already have elevated levels of intracortical inhibition (Sengpiel et al., 2006; Scholl, Tan, & Priebe, 2013).

Summary and Conclusions

Our results showed a clear effect of training task order on PL of flanked letter identification: Observers who first trained on isolated letters showed rapid early improvement in flanked letter identification but little to no improvement beyond ≈30 training blocks, while observers who first trained with flanked letters improved gradually over the entire 100 block course of training. These results are generally consistent with recent work suggesting that there may be two stages of learning to uncrowd (Zhu et al., 2016). In addition, compared with previous studies (Chung, 2007; Chung & Truong, 2013; Chung et al., 2012; Chung et al., 2017), we found no significant effect of donepezil on PL of flanked letter identification. In other words, donepezil neither boosts nor blocks learning to uncrowd.

Keywords: perceptual learning, cholinergic enhancement, donepezil, crowding, letter identification

Footnote

[1]The nonparametric Mann-Whitney *U* test was used for comparisons with data obtained from previous studies (Chung 2007; Chung & Truong, 2013; Chung et al., 2012; Chung et al., 2017).

References

Beard, B. L., Levi, D. M., & Reich, L. N. (1995). Perceptual learning in parafoveal vision. *Vision Research, 35*, 1679–1690.

Bouma, H. (1970). Interaction effects in parafoveal letter recognition. *Nature, 226*, 177–178.

Boucart, M., Bubbico, G., Szaffarczyk, S., Defoort, S., Ponchel, A., Waucquier, N., . . . Bordet, R. (2015). Donepezil increases contrast sensitivity for the detection of objects in scenes. *Behav Brain Res, 292*, 443–447, doi:10.1016/j.bbr.2015.06.037.

Byrne, K. N., McDevitt, E. A., Sheremata, S. L., Peters, M. W., Mednick, S. C., & Silver, M. A. (2020). Transient cholinergic enhancement does not significantly affect either the magnitude or selectivity of perceptual learning of visual texture discrimination. *Journal of Vision*, in press.

Chamoun, M., Huppé-Gourgues, F., Legault, I., Rosa-Neto, P., Dumbrava, D., Faubert, J., . . . Vaucher, E. (2017). Cholinergic potentiation improves perceptual-cognitive training of healthy young adults in three dimensional multiple object tracking. *Front Hum Neurosci, 11*, 128.

Chung, S. T. L., Levi, D. M., & Tjan, B. S. (2005). Learning letter identification in peripheral vision. *Vision Research, 45*, 1399–1412, doi:10.1016/j.visres.2004.11.021.

Chung, S. T. L., Li, R. W., & Levi, D. M. (2008). Learning to identify near-threshold luminance-defined and contrast-defined letters in observers with amblyopia. *Vision Research, 48*, 2739–2750, doi:10.1016/j.visres.2008.09.009.

Chung, S. T. L. (2007). Learning to identify crowded letters: does it improve reading speed? *Vision Research, 47*, 3150–3159.

Chung, S. T. L., Li, R. W., & Levi, D. M. (2012). Learning to identify near-acuity letters, either with or without flankers, results in improved letter size and spacing limits in adults with amblyopia. *PLoS One, 7*, e35829.

Chung, S. T. L., Li, R. W., Silver, M. A., & Levi, D. M. (2017). Donepezil does not enhance perceptual learning in adults with amblyopia: A pilot study. *Frontiers in Neuroscience, 11*, 448, doi:10.3389/fnins.2017.00448.

Chung, S. T. L., & Truong, S. R. (2013). Learning to identify crowded letters: does the learning depend on the frequency of training? *Vision Research, 77*, 41–50, doi:10.1016/j.visres.2012.11.009.

Ciuffreda, K. J., Levi, D. M., & Selenow, A. (1991). *Amblyopia: Basic and clinical aspects*. Stoneham, MA: Butterworth-Heinemann.

Disney, A. A., Aoki, C., & Hawken, M. J. (2007). Gain modulation by nicotine in macaque v1. *Neuron, 56*, 701–713, doi:10.1016/j.neuron.2007.09.034.

Disney, A. A., Aoki, C., & Hawken, M. J. (2012). Cholinergic suppression of visual responses in primate V1 is mediated by GABAergic inhibition. *J Neurophysiol, 108*, 1907–1923.

Dosher, B., & Lu, Z. L. (2017). Visual perceptual learning and models. *Annu Rev Vis Sci, 3*, 343–363, doi:10.1146/annurev-vision-102016-061249.

Frangou, P., Correia, M., & Kourtzi, Z. (2018). GABA, not BOLD, reveals dissociable learning-dependent plasticity mechanisms in the human brain. *Elife, 7*, doi:10.7554/eLife.35854.

Hussain, Z., Webb, B. S., Astle, A. T., & McGraw, P. V. (2012). Perceptual learning reduces crowding in amblyopia and in the normal periphery. *J Neurosci, 32*, 474–480.

Kang, J. I., Huppé-Gourgues, F., & Vaucher, E. (2014). Boosting visual cortex function and plasticity with acetylcholine to enhance visual perception. *Front Syst Neurosci, 8*, 172.

Katz, L. M., Levi, D. M., & Bedell, H. E. (1984). Central and peripheral contrast sensitivity in amblyopia with varying field size. *Doc Ophthalmol, 58*, 351–373.

Kosovicheva, A. A., Sheremata, S. L., Rokem, A., Landau, A. N., & Silver, M. A. (2012). Cholinergic enhancement reduces orientation-specific surround suppression but not visual crowding. *Front Behav Neurosci, 6*, 61.

Law, C. T., & Gold, J. I. (2010). Shared mechanisms of perceptual learning and decision making. *Top Cogn Sci, 2*, 226–238, doi:10.1111/j.1756-8765.2009.01044.x.

Law, C. T., & Gold, J. I. (2008). Neural correlates of perceptual learning in a sensory-motor, but not a sensory, cortical area. *Nat Neurosci, 11*, 505–513, doi:10.1038/nn2070

Levi, D. M. (2008). Crowding—an essential bottleneck for object recognition: a mini-review. *Vision Research, 48*, 635–654.

Levi, D. M., Klein, S. A., & Aitsebaomo, P. (1984). Detection and discrimination of the direction of motion in central and peripheral vision of normal and amblyopic observers. *Vision Research, 24*, 789–800.

Levi, D. M., & Klein, S. (1985). Vernier acuity, crowding and amblyopia. *Vision Research, 25*, 979–991.

Levi, D. M., & Li, R. W. (2009). Perceptual learning as a potential treatment for amblyopia: A mini-review. *Vision Research, 49*, 2535–2549.

Levi, D. M., Song, S., & Pelli, D. G. (2007). Amblyopic reading is crowded. *Journal of Vision, 7*(2):21, 1–17, doi:10.1167/7.2.21.

Pelli, D. G., Levi, D. M., & Chung, S. T. L. (2004). Using visual noise to characterize amblyopic letter identification. *Journal of Vision, 4*(10), 904–920, doi:10.1167/4.10.6.

Rogers, S. L., & Friedhoff, L. T. (1998). Pharmacokinetic and pharmacodynamic profile of donepezil HCl following single oral doses. *Br J Clin Pharmacol, 46*(Suppl 1), 1–6.

Rokem, A., & Silver, M. A. (2010). Cholinergic enhancement augments magnitude and specificity of visual perceptual learning in healthy humans. *Curr Biol, 20*, 1723–1728.

Rokem, A., & Silver, M. A. (2013). The benefits of cholinergic enhancement during perceptual learning are long-lasting. *Front Comput Neurosci, 7*, 66.

Sagi, D. (2011). Perceptual learning in Vision Research. *Vision Research, 51*, 1552–1566.

Scholl, B., Tan, A. Y., & Priebe, N. J. (2013). Strabismus disrupts binocular synaptic integration in primary visual cortex. *J Neurosci, 33*, 17108–17122, doi:10.1523/JNEUROSCI.1831-13.2013.

Sengpiel, F., Jirmann, K. U., Vorobyov, V., & Eysel, U. T. (2006). Strabismic suppression is mediated by inhibitory interactions in the primary visual cortex. *Cereb Cortex, 16*, 1750–1758, doi:10.1093/cercor/bhj110.

Soma, S., Suematsu, N., & Shimegi, S. (2013). Cholinesterase inhibitor, donepezil, improves visual contrast detectability in freely behaving rats. *Behav Brain Res, 256*, 362–367, doi:10.1016/j.bbr.2013.08.022.

Song, S., Levi, D. M., & Pelli, D. G. (2014). A double dissociation of the acuity and crowding limits to letter identification, and the promise of improved visual screening. *Journal of Vision, 14*(5), 3, doi:10.1167/14.5.3.

Sun, G. J., Chung, S. T. L., & Tjan, B. S. (2010). Ideal observer analysis of crowding and the reduction of crowding through learning. *Journal of Vision, 10*(5), 16, doi:10.1167/10.5.16.

Tsirlin, I., Colpa, L., Goltz, H. C., & Wong, A. M. (2015). Behavioral training as new treatment for adult amblyopia: A meta-analysis and systematic review. *Invest Ophthalmol Vis Sci, 56*, 4061–4075.

Wang, R., Zhang, J. Y., Klein, S. A., Levi, D. M., & Yu, C. (2012). Task relevancy and demand modulate double-training enabled transfer of perceptual learning. *Vision Research, 61*, 33–38, doi:10.1016/j.visres.2011.07.019.

Wang, R., Zhang, J. Y., Klein, S. A., Levi, D. M., & Yu, C. (2014). Vernier perceptual learning transfers to completely untrained retinal locations after double training: a "piggybacking" effect. *Journal of Vision, 14*(13), 12, doi:10.1167/14.13.12.

Watanabe, T., & Sasaki, Y. (2015). Perceptual learning: toward a comprehensive theory. *Annu Rev Psychol, 66*, 197–221.

Whitney, D., & Levi, D. M. (2011). Visual crowding: A fundamental limit on conscious perception and object recognition. *Trends Cogn Sci, 15*, 160–168, doi:10.1016/j.tics.2011.02.005.

Xiao, L. Q., Zhang, J. Y., Wang, R., Klein, S. A., Levi, D. M., & Yu, C. (2008). Complete transfer of perceptual learning across retinal locations enabled by double training. *Curr Biol, 18*, 1922–1926, doi:10.1016/j.cub.2008.10.030.

Yashar, A., Chen, J., & Carrasco, M. (2015). Rapid and long-lasting reduction of crowding through training. *Journal of Vision, 15*(10), 15, doi:10.1167/15.10.15.

Zhang, J. Y., Cong, L. J., Klein, S. A., Levi, D. M., & Yu, C. (2014). Perceptual learning improves adult amblyopic vision through rule-based cognitive compensation. *Invest Ophthalmol Vis Sci, 55*, 2020–2030.

Zhang, J. Y., Zhang, G. L., Xiao, L. Q., Klein, S. A., Levi, D. M., & Yu, C. (2010). Rule-based learning explains visual perceptual learning and its specificity and transfer. *J Neurosci, 30*, 12323–12328, doi:10.1523/JNEUROSCI.0704-10.2010.

Zhu, Z., Fan, Z., & Fang, F. (2016). Two-stage perceptual learning to break visual crowding. *Journal of Vision, 16*(6), 16, doi:10.1167/16.6.16.