1   **Effect of fluoxetine on adult amblyopia: a placebo-controlled study combining neuroplasticity-**

2   **enhancing pharmacological intervention and perceptual training**

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1 **ABSTRACT**

2 Amblyopia is a common visual disorder that is treatable in childhood. However, therapies have limited

3 efficacy in adult patients with amblyopia. Fluoxetine can reinstate early-life critical period-like neuronal

4 plasticity and has been used to recover functional vision in adult rats with amblyopia. This phase 2,

5 randomized, double-blind (fluoxetine vs. placebo), multicenter clinical trial examined whether or not

6 fluoxetine can improve visual acuity in amblyopic adults. This interventional trial included 42 participants

7 diagnosed with moderate to severe amblyopia. Subjects were randomized to receive either 20 mg

8 fluoxetine (n=22) or placebo (n=20). During the 10-week treatment period, all subjects performed daily

9 computerized perceptual training and eye patching. There was no significant difference in treatment

10 efficacy between the groups. Visual acuity at the primary endpoint had significantly improved over

11 baseline in both the fluoxetine (-0.167 logMAR) and placebo (-0.194 logMAR) groups (both $p < 0.001$).

12 Because patching alone is not effective in adults, the visual acuity improvement likely resulted from

13 perceptual training. There was a positive correlation between visual acuity improvement and the

14 perceptual training time. While this study failed to provide evidence that fluoxetine enhances

15 neuroplasticity, our data support the usefulness of perceptual training for vision improvement in adults

16 with amblyopia.

17

INTRODUCTION

Amblyopia is a condition in which the best-corrected visual acuity (BCVA) is impaired in one eye or, less frequently, both eyes, even though no ocular abnormalities are generally present. Amblyopia develops when one or both eyes have abnormal visual input (either physical or physiological) during the sensitive period in childhood (from birth to 6 years).[1,2] Amblyopia is most commonly caused by strabismus in very young children (<3 years of age, 82%)[3] and by refractive errors (anisometropia, isoametropia) in older children.[4,5] In the clinical setting, moderate amblyopia is defined as a logarithm of the minimum angle of resolution (logMAR) BCVA of ≥ 0.30 (Snellen equivalent: $\leq 20/40$) and/or an interocular BCVA difference of 0.2 logMAR or more (in cases of unilateral amblyopia). The prevalence of amblyopia in the general population varies from 1.3% to 3.6%, and it is one of the most common causes of monocular visual impairment in adults.[4,6,7] Patients with monocular amblyopia have a significantly increased risk of visual impairment if vision in their "good" eye is lost as a result of trauma or disease.[8,9]

Amblyopia can be treated in early life,[10–12] but visual gains diminish in school-aged children because of a decline in visual system neuroplasticity and, possibly, treatment compliance.[1,13–15] Amblyopia is very difficult, if not impossible, to treat in adults.[16]

An improved understanding of the neuronal mechanisms underlying amblyopia and adult brain neuroplasticity[17–19] has led to the development of visual rehabilitation methods that can be used after the critical period.[20–24] Experimental models of amblyopia are based on the effects of monocular deprivation on the structure and function of the visual cortex.[25,26] Using an animal model, Espinosa and Stryker[26] showed that the effects of amblyopia can be reversed during the critical period in early postnatal development, but not later in life. However, recent evidence indicates that environmental enrichment[27,28] and pharmacological treatment[29] can reactivate critical period-like plasticity in the visual cortex of adult rodents. In particular, fluoxetine, a selective serotonin reuptake inhibitor (SSRI), promotes neuroplasticity and neurogenesis[30] and reactivates critical period-like plasticity in the rat visual cortex.[18]

An emerging body of literature suggests that vision improvements can be achieved with conventional therapies (e.g., occlusion therapy) in adolescents (i.e., older children and teenagers)[31–33] and adults[20,21,34,35] with amblyopia even though post-childhood amblyopia is generally considered untreatable.

3 (25)

1   In addition, catecholamine-based medical treatments can temporarily improve vision in human amblyopic

2   patients, including adults.[1, 36, 37] Perceptual training[38], dichoptic non-action and action videogame use[39],

3   and videogame use during patching[40] can improve vision in the amblyopic eye and binocular vision in

4   adults. This placebo-controlled study examined whether fluoxetine can enhance neuroplasticity and

5   improve vision in adults with amblyopia. The treatment period included eye patching and computerized

6   perceptual training on a web-based system for all subjects.

7

8   **RESULTS**

9   **Study subjects**

10   A total of 42 subjects were enrolled in the study, with 22 and 20 subjects randomly assigned to the

11   fluoxetine and control group, respectively. Table 1 presents a complete list of the eligibility and exclusion

12   criteria used for patient selection. Forty-one of 42 subjects (97.6%) required new spectacles before

13   randomization. Four subjects were non-compliant, 3 subjects withdrew their consent, and 1 subject was

14   lost to follow-up. Therefore, a total of 37 subjects ultimately completed the 10-week treatment period,

15   including primary endpoint assessments, and 34 completed the 3-month post-treatment follow-up period

16   (20 in the fluoxetine group and 14 in the control group; Figure 1). Data from all 42 randomized subjects

17   were subjected to an intention-to-treat analysis and were included in analyses. Subjects that completed the

18   study showed good medication compliance (>85%) and completed >85% of the computerized perceptual

19   training sessions.

20   Baseline demographic and ocular parameters are summarized in Table 2. Briefly, best-corrected

21   logMAR visual acuity at baseline was between 0.30 and 1.08 in the amblyopic eye and not worse than

22   0.10 in the dominant eye. The majority of subjects had anisometropic amblyopia [19 of 20 control subjects

23   (95.0%), 18 of 22 fluoxetine subjects (81.8%)]. Four subjects (1 in the control group and 3 in the

24   fluoxetine group) exhibited combined strabismic-anisometropic amblyopia, while 1 subject in fluoxetine

25   group exhibited strabismic amblyopia. These 5 subjects had undergone strabismus surgery during

26   childhood. Twenty subjects (8 in the control group and 12 in the fluoxetine group) had moderate

1   amblyopia and 22 (12 in the control group and 10 in the fluoxetine group) had severe amblyopia (Figure

2   1A).

3   Mean subject age was 36.4 ± 11.5 years in the control group and 38.5 ± 12.5 years in the fluoxetine

4   group. Mean baseline logMAR visual acuity in the amblyopic eye was 0.620 ± 0.190 (Snellen equivalent:

5   20/83) in the control group and 0.649 ± 0.252 (20/89) in the fluoxetine group. Overall, the average

6   difference in the logMAR visual acuity between eyes was 0.728 in both groups. For hyperopic subjects

7   (n=33), the mean refractive error in the amblyopic eye was +3.13 ± 1.49 and +3.07 ± 1.79 D in the control

8   and fluoxetine groups, respectively, and for myopic subjects (n=9), the mean refractive error in the

9   amblyopic eye was -1.23 ± 0.66 and -2.81 ± 1.07 D, respectively. Mean anisometropia was 2.19 ± 1.86

10   and 1.95 ± 1.44 D in the control and fluoxetine groups, respectively. Baseline binocular vision testing

11   revealed that 9 of 20 control group subjects (45.0%) and 13 of 22 fluoxetine group subjects (59.1%) had

12   suppression or anomalous retinal correspondence (ARC) at the 4-m distance. At the 33-cm distance, 7 of

13   20 subjects in the control group (35.0%) and 8 of 22 subjects in the fluoxetine group (36.4%) showed

14   suppression or ARC at baseline. All subjects had impaired near visual acuity (with crowding effect; near

15   logMAR visual acuity < 0.7) at baseline, but only 2 control group subjects (10.0%) and 6 fluoxetine group

16   subjects (27.3%) had abnormal contrast sensitivity at the intermediate spatial frequency measured using

17   the Pelli-Robson chart. Mean baseline visual parameters are summarized in Table 2.

18   The 10-week treatment regimen included a combination of medication, eye patching, and perceptual

19   training. A game-based perceptual training software was specifically developed for enhancing the use of

20   the amblyopic eye during patching. The game tasks are illustrated in Figure 2 and Supplementary video 1.

21   The study was designed to include a placebo control group for the medication only, and all participating

22   subjects followed the same daily patching and training instructions.

23

24   **Visual acuity**

25   Twenty-two of the 42 subjects (52.4%) showed a clinically relevant improvement in visual acuity [≥2

26   lines of vision (0.2 improvement in logMAR visual acuity)], and 7 of 42 subjects (16.6%) had improved to

27   normal visual acuity (VA of logMAR 0 or better) during the study period (Table 3). Visual acuity

1   significantly improved in the amblyopic eye in both treatment groups (Figure 3A). At the primary efficacy

2   endpoint (10 weeks), the change in logMAR visual acuity from baseline was -0.167 [95% confidence

3   interval (CI): -0.226 to -0.108] in the fluoxetine group and -0.194 (95% CI: -0.254 to -0.133) in the control

4   group (both p < 0.001, Figure 3B). The marginal difference in visual acuity improvement between

5   treatment groups was not statistically significant (p = 0.524). The visual acuity improvements observed in

6   both groups were maintained 10 to 22 weeks after discontinuing all treatments (medication, eye patching,

7   and perceptual training). In addition, visual gains of at least 0.2 logMAR units persisted in many subjects

8   in both groups [9 of 20 fluoxetine subjects (45.0%), 6 of 14 control subjects (42.9%); Figure 3C].

9

10   **Binocularity, contrast sensitivity and crowded near visual acuity**

11   Both treatment groups exhibited a positive response to treatment, with 30 of the 42 subjects (71.4%)

12   showing an improvement in at least one visual function test (i.e., visual acuity, contrast sensitivity,

13   binocularity, crowded near visual acuity) at any visit after the randomization visit. Binocularity, contrast

14   sensitivity and crowded near visual acuity improved in both the control and fluoxetine groups. Binocular

15   vision was assessed for both near (33 cm) and distant (4 m) vision. Twenty-two subjects (9 control group

16   subjects, 13 fluoxetine group subjects) had suppression or ARC in the 4-m test at baseline (Figure 4A). At

17   10 weeks, the number of patients with suppression or ARC had decreased to 16 subjects (5 control group

18   subjects, 11 fluoxetine group subjects). This change persisted through 22 weeks in 11 subjects (3 control

19   group subjects, 8 fluoxetine group subjects). The results in the 33-cm test were similar for both groups

20   (data not shown).

21   Contrast sensitivity was normal (1.70 log) at baseline in all but 2 fluoxetine subjects (4.8%) with

22   severe amblyopia (both had 0 log values at baseline). Following fluoxetine treatment and perceptual

23   training, both subjects had almost normal contrast sensitivity (10 weeks: 1.20 and 1.95 log, 22 weeks:

24   1.95 and 1.65 log; Figure 4B).

25   Crowded near visual acuity was assessed using Landolt C ring charts and was considered to be normal

26   when 0.7 or smaller optotypes were detected. The baseline mean crowded near visual acuity in the

27   amblyopic eye was 0.171 ± 0.116 in the control group and 0.165 ± 0.141 in the fluoxetine group.

1   Improvements in these values were observed after treatment in both study groups (Figure 4C). At 10

2   weeks, the control group had improved by 0.181 ± 0.027 (95% CI: 0.126 to 0.236) and the fluoxetine

3   group had improved by 0.148 ± 0.026 (95% CI: 0.095 to 0.201, both $p < 0.001$). At 22 weeks, the control

4   group had improved by 0.221 ± 0.036 (95% CI: 0.150 to 0.293) and the fluoxetine group had improved by

5   0.197 ± 0.033 (95% CI: 0.131 to 0.262, both $p < 0.001$). Figure 4D shows the distribution of patients with

6   normal (≥ 0.7) and impaired (< 0.7) crowded near visual acuity.

7   The proportion of patients with an improvement in at least one of the visual function parameters was

8   60% in both treatment groups after 10 weeks of treatment. Three months after treatment completion (22

9   weeks), this proportion had increased to 70% in the fluoxetine group and 64% in the control group.

10   Moreover, 73% of the fluoxetine group subjects and 70% of the control group subjects showed a clinically

11   relevant improvement in at least one visual function parameter at some point during the study.

12   Improvements in visual function parameters are summarized in Table 3.

13   Our clinical study protocol also included prespecified subgroup analyses, which assessed the effect of

14   the severity of amblyopia (moderate/severe), binocularity (suppression/normal fusion), sex (female/male),

15   compliance with software-based training ($\geq 85\%/<85\%$), and age ($<40/\geq 40$ years) on changes in visual

16   acuity (logMAR). The subgroup analysis data are summarized in Table 4. Although the differences were

17   not statistically highly significant ($p = 0.048$), there were more responders with normal fusion in the

18   control group (6/11; 54.5%) than in the fluoxetine group (1/9; 11.1%), whereas there were more

19   responders with suppression in the fluoxetine group (7/11; 63.6%) than in the control group (3/7; 42.9%).

20   Finally, the visual acuity gains were associated with training compliance ($p = 0.012$) in both treatment

21   groups (Table 4).

22

23   **Safety**

24   A total of 66 adverse events (AEs) were reported after initiating study treatments. Fifty-eight (87.9%)

25   AEs occurred during treatment and 8 AEs occurred (12.1%) after treatment. Only 16 AEs (24.2%) were

26   related or possibly related to the study treatment and all were reported during the treatment period. Eleven

27   (16.7%) of these AEs occurred in the fluoxetine group and 5 (7.6%) occurred in the placebo group. None

1   of the treatment-related AEs were reported following the 10-week treatment period and no AEs led to

2   study withdrawal. One subject in the fluoxetine group exhibited transient mild diplopia that resolved

3   spontaneously. Other reported AEs were not related to visual function. One serious adverse event (benign

4   ovarian cyst of moderate severity) occurred during the study, but it was not related to study treatment.

5

6   **DISCUSSION**

7   Amblyopia is a complex brain disorder that can restrict everyday life because of the visual limitations

8   it imposes. Despite good screening programs and effective childhood treatments, amblyopia remains a

9   common cause of lifelong visual impairment independent of location or ethnic origin.[4, 7, 9, 41] The

10   combination of adequate refractive correction and occlusion therapy (patching of non-amblyopic eye) has

11   been the mainstay therapy for amblyopia of all etiologies. However, the benefits of various forms of

12   occlusion therapy are greatest when therapy is started at an early age (<8 years). Therefore, early

13   amblyopia detection and treatment is the most important factor for obtaining successful visual outcomes.

14   Physiologically, the brain has the greatest plasticity during the critical period in early postnatal life.

15   However, recent evidence strongly indicates that the primary sensory cortex may remain plastic into

16   adulthood.[17–24, 27–30] This finding suggests that there is a physiological basis for treating amblyopia in

17   adulthood, which provides an opportunity to potentially alleviate this world-wide public health problem.

18   The current study examined whether fluoxetine, an SSRI known to modulate adult rat visual cortex

19   plasticity[18], can enhance the effects of patching/computer-based perceptual training combination therapy

20   in adults with amblyopia. The treatment response was good in both the fluoxetine and placebo study

21   groups, with an overall visual rehabilitation success rate of 52%. In addition, 17% of patients achieved

22   normal visual acuity in the amblyopic eye (Table 3). Our results are in agreement with those of Li et al.[40],

23   who found that 33% of adult amblyopic subjects had a substantial improvement in visual acuity following

24   video game-based perceptual training. In addition, our subjects had an average gain of approximately 2

25   lines of vision (0.2 logMAR), which was similar to improvements observed with other published training

26   protocols.[38, 39]

1   The change in visual acuity after 10 weeks of study medication/perceptual training therapy was not

2   significantly different between subjects taking fluoxetine and subjects taking a matching placebo. It may

3   be that 20 mg of fluoxetine, the dose typically used to begin treatment of depression, was too small a dose

4   to modulate neuroplasticity. It may also be that the training paradigm (new spectacles, patching, and

5   computerized perceptual training) was so effective that the 20-mg dose of fluoxetine did not provide any

6   additional benefit. Larger fluoxetine doses (up to 80 mg/day) are often used in depressed patients when the

7   initial dose does not have the desired therapeutic effect. In addition, the 10-week treatment period may

8   have been too short to maximize fluoxetine benefits and it is possible that a difference between treatment

9   groups could have emerged after a longer treatment period.

10   Although there is evidence that some amblyopia treatments may be additive (optical correction in

11   combination with patching or atropine)[32], some reports have documented that all amblyopia treatment

12   effects may not be additive. The effects of multiple amblyopia treatment paradigms were not synergistic in

13   rodent models of amblyopia.[42] Furthermore, environmental enrichment and fluoxetine treatment have been

14   shown to induce similar levels of amblyopia recovery in rodents.[28] Therefore, it is possible that perceptual

15   training alone (with refractive correction) promotes the maximum amount visual cortex plasticity and that

16   further treatments do not have additive benefits. The current study was not designed to determine this and

17   future studies should include a group of subjects only treated with fluoxetine (no perceptual training).

18   However, a rodent study found that fluoxetine alone had no effect on vision.[18] Furthermore, there are no

19   prior reports of visual benefits from fluoxetine monotherapy in amblyopic patients, even though millions

20   of patients, and presumably thousands of amblyopic patients, have used the medication over the past three

21   decades. In addition, a recent placebo-controlled, double-blinded, clinical study showed that 20 mg/day of

22   fluoxetine for 19 days did not significantly affect visual perceptual learning in humans.[43] These prior

23   studies support the theory that 20 mg/day of fluoxetine may not be a large enough dose to effectively

24   modulate visual cortex plasticity in adult humans.

25   Subjects in the current study received new spectacles with the proper refractive correction at baseline.

26   Initiating the use of appropriate prescription glasses can improve amblyopia in children[44] and adults[45].

27   Therefore, the use of new corrective lenses may have added to the visual gains observed in the current

1   study. However, adults have lower plasticity than children so the effects of glasses may have been less

2   prominent in our adult population. Furthermore, test-retest variability should be taken into account; this

3   was low in the present study because visual acuity was measured under the same conditions and in the

4   same locations by the same observers. Moreover, regression to the mean must be taken into account;

5   because our study was placebo-controlled, the regression to the mean was reduced because both groups

6   most likely exhibited an equal tendency.

7   We found that training compliance was well correlated with improved visual acuity. Therefore, the

8   training software used in the current study could potentially be used in the clinical setting to personalize

9   training and remotely monitor patient compliance. This would allow clinicians to adjust follow-up

10   intervals based on treatment response rates. Understanding early treatment responses would also aid in

11   determining which patients are likely to benefit from treatment and would allow for adjustment or

12   discontinuation of treatment on the basis of individual responses. If no improvement is detected despite

13   good compliance, treatment may be discontinued and the diagnosis of amblyopia may need to be

14   reconsidered. Follow-up visit schedules can also be determined based on perceptual training performance.

15   Our study had several limitations. First, we did not have a true no-treatment control group. However, in

16   both the placebo and fluoxetine groups, perceptual training compliance was well correlated with the

17   magnitude of vision improvement. This finding strongly suggests that perceptual training resulted in

18   neuroplasticity and subsequent visual benefits. Future clinical trials should include several control groups

19   to examine the effects of individual interventions and their combined effects. Furthermore, the dose of

20   fluoxetine and duration of its use should be varied in these studies. Second, the response to treatment was

21   remarkably variable in both treatment groups. This may have resulted from the large amount of variation

22   in amblyopia severity and etiology in our study population. Twenty-two subjects (52.4%) had abnormal

23   binocularity at baseline, 10 of which had improvements in binocularity with study treatment. In addition,

24   only 2 subjects with severe amblyopia had low contrast sensitivity at baseline. Contrast sensitivity deficits

25   are sometimes found to correlate with the visual acuity in the amblyopic eye.[46–48]. Both subjects exhibited

26   remarkable improvements in visual test results. Our results are in agreement with those of Zhou et al.,[49]

27   who showed that perceptual learning can improve visual acuity and contrast sensitivity in adult amblyopia

1   patients. Nine of our subjects (21.4%) had improved crowded near visual acuity (Table 3). Hussain et al.[50]

2   found a significant association between reduction in crowding and visual acuity improvement in

3   amblyopic adults. However, we did not find a significant correlation between visual acuity improvement

4   and crowding, contrast sensitivity, or binocularity. It should be noted that the number of subjects in these

5   subgroups was relatively small and that larger subgroups may have revealed additional statistically

6   significant findings.

7   In conclusion, both fluoxetine and the software-based perceptual training were safe and well-tolerated,

8   with fluoxetine treatment not offering further benefits over perceptual training. The training software used

9   in the study simultaneously determined training compliance and improvements in visual function and

10   showed that good training compliance is essential for treatment benefit. Therefore, software-based

11   training, combined with eye patching, may improve visual function in adult patients with amblyopia.

12   Furthermore, the computer program used here has the potential to become a robust tool for the treatment

13   of amblyopia.

14

15   **MATERIALS AND METHODS**

16   All study conduct adhered to the tenets of the Declaration of Helsinki and followed Good Clinical

17   Practices. This study was reviewed and approved by the Regional Ethics Committee of Tampere

18   University Hospital (centralized process for all centers in Finland) and the Research Ethics Committee of

19   the University of Tartu (Estonia). Written informed consent was obtained from all subjects prior to

20   performing any study examination or procedure. The study was registered in the European Clinical Trials

21   Database (EudraCT) on October 1st, 2010 under the number 2010-023216-14.

22   This phase 2, multi-center, clinical study was a randomized, double-blind, placebo-controlled (for drug

23   treatment), parallel-group trial performed to assess visual acuity improvement in the amblyopic eye, as

24   measured by the Early Treatment of Diabetic Retinopathy Study (ETDRS) chart, following 10 weeks of

25   medication (20 mg fluoxetine or placebo) and computer-based training (with the dominant eye patched).

26   The following assumptions were made to calculate sample size: comparison of two equally sized groups,

27   an intergroup difference (fluoxetine vs. placebo) in the change in logMAR visual acuity of at least 0.15, a

1 standard deviation (SD) of 0.15, and a subject drop-out rate of 10%. Thirty-four subjects needed to be

2 randomized to power the study to 80%, assuming a two-sided type I error rate of 5%.

3

4 **Study subjects**

5 Four eye clinics in Finland and Estonia enrolled 42 subjects between June 2011 and April 2013. Study

6 inclusion and exclusion criteria are fully described in Table 1. Briefly, adult patients with monocular

7 amblyopia with no other ocular or neurological abnormalities were considered for enrollment. Included

8 subjects were 19 to 57 years of age and had moderate (0.3−0.6 logMAR difference) to severe (>0.6

9 logMAR difference) amblyopia due to myopic or hyperopic anisometropia (≤4.25 D) or congenital

10 esotropia. The lower limit for anisometropia was not set in the study protocol. The investigators

11 considered the amblyopia to be of the anisometropic type if no strabismus had been diagnosed in

12 childhood and the refractive error was at least 1 D of anisometropia, determined as the spherical

13 equivalent, in childhood. Patients with other primary forms of strabismus, extrafoveal (eccentric) fixation,

14 or who used antidepressant drugs in the past 6 months were excluded.

15

16 **Study examinations**

17 Eligibility, demographic data, medical history, relevant medication, vital signs, physical examination,

18 blood and urine samples (including urine pregnancy test for fertile women), and amblyopia were assessed

19 at screening. Amblyopia was confirmed at screening and was defined as an interocular ETDRS best-

20 corrected visual acuity difference of at least two lines and/or a logMAR visual acuity between 0.30 and

21 1.10 in the amblyopic eye and 0.10 or better in the dominant eye. Prior to randomization, patients received

22 new spectacles based on non-cycloplegic refraction to ensure best-corrected vision during the study.

23 A thorough ophthalmic examination was conducted at each of the seven scheduled visits (at

24 weeks -2 (screening), 0 (randomization), 2, 6, 10, 14 and 22; Figure 1) during the 26-week study period.

25 Vision tests included the assessment of binocularity, visual acuity, crowded near visual acuity, and

26 contrast sensitivity and were performed with the refractive error corrected. In addition, presbyopic

27 correction was used for crowded near visual acuity testing in presbyopic subjects.

1   Binocularity was examined using the Bagolini striated glass test[51] before monocular testing. Lens

2   striations were placed at 135° before the right eye and 45° before the left eye using lorgnette frames. This

3   testing setup allows each eye to receive the same fusible image with each fixation streak oriented

4   perpendicular to the striations and 90° away from the other eye. The test enables the evaluation of

5   simultaneously perceived images with a minimal dissociative effect and it was performed at near (33 cm)

6   and distance (4 m) under normal lighting conditions. Binocularity was categorized as suppression (1 light

7   and only 1 line were seen), normal fusion (binocular single vision, BSV; 2 lines were seen as X and 1 light

8   at the center), anomalous retinal correspondence (ARC; harmonious if 1 light and 2 lines were seen, but

9   one of the lines was broken due to foveal suppression, or inharmonious if 1 light and 2 lines were seen,

10   but the lines did not cross at the center where the light was located) or diplopia (2 lights and 2 lines were

11   seen). However, none of the subjects had diplopia in the current study.

12   Visual acuity was assessed under standardized lighting conditions (self-calibrated test lighting with a

13   constant light level of 85 cd/m^2) using a large-format standardized ETDRS light box (ESV3000 with LED

14   lights, VectorVision, Greenville, OH) placed 4 meters from the subject. Three different ETDRS charts

15   (charts R, 1 and 2) were used to prevent subjects from memorizing eye charts. Visual acuity was assessed

16   in the amblyopic eye first and was measured as the number of correctly identified letters. A clinically

17   relevant visual acuity improvement was defined as a 0.2 or greater decrease in the logarithm of the

18   minimum angle of resolution (logMAR) visual acuity (i.e., 2 lines or 10 characters on the ETDRS chart).

19   Contrast sensitivity was determined under standardized lighting conditions using a Pelli-Robson chart

20   at a distance of 1 m (charts A and B), using previously established age-dependent normative values.[52]

21   Crowded near visual acuity was assessed using a specific crowded Landolt C ring chart booklet at a

22   distance of 40 cm.[53] Crowded near visual acuity was defined by the smallest line in which the subject

23   correctly identified at least 8 of 12 letters (≥66.7%). The right eye was tested first in all tests requiring

24   charts. The contralateral eye was occluded during testing and charts were switched between eyes. All eye

25   examination test charts and Bagolini striated glasses tests were standardized and validated for trial

26   endpoint measurement. All staff that evaluated vision were masked to subject group assignment.

1   Treatment safety was assessed using ophthalmoscopy, biomicroscopy, intraocular pressure (IOP)

2   measurement, laboratory safety tests [hematology (hemoglobin, hematocrit, erythrocyte count, leukocyte

3   count, platelet count), clinical chemistry (alanine aminotransferase, alkaline phosphatase, aspartate

4   aminotransferase, creatinine, gamma-glutamyl transferase, potassium, sodium, urea), and urine analysis

5   (blood, glucose, ketones, protein, pH)], vital signs, and physical examination performed at screening and

6   at each study visit. Adverse events and changes in concomitant medications were recorded at each study

7   visit.

8

9   **Study medication**

10   Fluoxetine capsules were manufactured by Orion Corporation (Espoo, Finland) and the matching placebo

11   capsules were manufactured by Corden Pharma GmbH (Plankstadt, Germany). Study subjects were

12   randomly assigned to receive either 20 mg fluoxetine each day (hard capsule) or a matching placebo.

13   Randomization was done in a 1:1 fashion in blocks of 4 and was stratified by site. Randomization was also

14   stratified by amblyopia severity, determined using interocular visual acuity difference (moderate: 0.3−0.6

15   logMAR difference, severe: >0.6 logMAR difference). The randomization structure was designed by a

16   biostatistician and the final randomization list was generated by an independent person who had no

17   contact with study subjects or study data. Medication was pre-packed and serially numbered so that

18   subjects were assigned to a study group by giving them the next available medication number in the

19   sequence. A drug accountability log was maintained by study-authorized personnel. The receipt, dispense

20   and return of study medication was recorded in this log. Patients were instructed to return dispensed

21   medication bottles at the next visit, even if the bottles were empty. The number of capsules dispensed and

22   returned was reconciled against the number of days between the visits and any discrepancies were

23   accounted for. After 10 weeks of receiving study medication, subjects were weaned off the daily

24   medication (1 capsule every other day for the next 2 weeks, Figure 1B).

25

26   **Perceptual training**

1   All subjects completed daily computerized training with eye patching during the 10-week period of

2   receiving study medication (Figure 1A). The principle underlying the perceptual training software

3   developed for this study is fully described in the Electronic Supplementary Materials and is illustrated in

4   Figure 2 and Supplementary video 1. All subjects received new spectacles before randomization and were

5   instructed to wear an eye patch over their dominant eye while performing daily computerized perceptual

6   training. The training software was used to track training compliance, which was calculated by dividing

7   the total accomplished training time with the total prescribed training time. Training compliance was

8   automatically reported to the study site prior to each scheduled visit.

9   All subjects were given an eye patch and were instructed to wear it over the non-amblyopic eye for

10   1 hour each day. Subjects were also instructed to complete approximately 30 minutes of the computer-

11   based training each day while they were wearing the patch and their spectacles.

12   The training period was divided into ten 1-week segments and each subject played an identical

13   composition of games each week. The maximum total training time over the 10-week training period was

14   35 hours. The training program was made up of seven different games wherein the performance was

15   primarily determined by visual acuity and contrast sensitivity and secondarily by attention and mental

16   effort. Thus, the training was primarily focused on visual acuity and contrast sensitivity and was aimed at

17   their improvement. A schematic illustration of the training game task design and composition is shown in

18   Figure 2. In addition, the computerized training setup, training protocol structure, and individual training

19   game design are described in detail in the Electronic Supplementary Materials. Data on behavioral

20   performance were collected on a per-game basis. For each game type, the corresponding weekly test

21   outcome measures were obtained by pooling the data from all individual games of that type played in that

22   week (see Electronic Supplementary Materials).

23

24   **Study outcomes**

25   The primary outcome of the study was an improvement in visual acuity in the amblyopic eye, as measured

26   by the ETDRS chart, from baseline (week 0, randomization) to the 10-week visit (end of treatment).

27   Secondary outcomes included the change from baseline in binocularity, contrast sensitivity and crowded

near visual acuity to week 10 week. The persistence of changes observed at 10 weeks was also evaluated at the end of the follow-up period (week 22). Treatment safety was assessed using adverse event incidence and ophthalmological examination findings throughout the study. Exploratory outcomes included changes from baseline in training measures at each study visit.

Data analyses

The primary analysis population was the full analysis dataset (FAS), which included all randomized patients who had received at least one dose of study medication (intention-to-treat principle). A last observation carried forward (LOCF) imputation was applied up to week 10 for subjects who did not complete the study and those who were non-compliant with the treatment.

Differences in visual acuity and crowded near visual acuity between the two treatment groups were evaluated using the repeated measurements of analysis of covariance (RM ANCOVA) method with baseline values as a covariate. The model included the study center, treatment and time point (visit) as main effects, and treatment by time point (visit) as an interaction effect. With regard to the primary endpoint, differences between the treatment groups with regards to change in the logMAR visual acuity at 10 weeks (and a 95% CI for the difference) were estimated using RM ANCOVA models with a contrast. A secondary RM ANCOVA analysis was used to compare the least square means between the treatment groups at the end of the follow-up (22 weeks) period to determine if treatment effects were maintained. Contrast sensitivity was not analyzed with RM models because of low variability in the dataset. Pearson's chi-square test was used to compare categorical variables. Some binocularity categories contained a low number of subjects. Therefore, differences between treatment groups in binocularity were evaluated using Fisher's exact test.

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19   **ACKNOWLEDGEMENTS**

21

22   **ADDITIONAL INFORMATION**

1   **FIGURE LEGENDS**

2

3   **Figure 1. Study design and flow.** (A) Disposition and patients. (B) Visit and assessment schedule and

4   duration of medication and active training.

5

6   **Figure 2. Schematic illustration of the training game task design and composition.** For details, please

7   see Materials and Methods section: *Training paradigm*. In short, the training program comprised seven

8   different games, Games 1-7, that tapped primarily on visual acuity and contrast sensitivity in multiple

9   attentional and working memory tasks. Subjects were presented with a pre-determined selection of games

10   for each training day. The total training duration per week was ~3.5 h, excluding the time spent on game

11   parameter adaptation. In all tasks, the subject responded with a single keyboard-button press or withheld

12   the response. **Games 1 and 2** were single- or multi-object visual tracking tasks where complex shaped

13   objects moved along curved paths on screen and the subjects' task was to respond whenever they observed

14   a feature-change in any of the objects. Different game segments exhibited different numbers of to-be

15   attended objects (attentional loads 1, 2, 3, and 4). Prior to each game, there was a calibration period with

16   one (Game 1) or two (Game 2) objects during which the magnitude of the feature change (C) was adjusted

17   to yield a detection rate (HR) of 64−73%. **Games 3 and 4** were visual-tracking games like Games 1 and 2

18   and had an identical calibration procedure and object mobility, but involved only attentional loads of 1 and

19   2, and exhibited in two out of four conditions six feature-wise distinct distractor objects to impose visual

20   crowding. **Game 5** was a continuous single-object tracking task where the subjects reported the feature

21   changes of a single object (as in Games 1−4). Game 5 had no calibration but rather started with very

22   salient feature changes that in each of the 12 game segments decreased by a factor of 1.6 so that the

23   subjects on average were able to reach segments 7−8 at a detection rate of >25%. **Game 6** was a Go/No-

24   Go 1-back working memory task where the subject was presented stimuli with an object in one quadrant

25   lasting ~1 s at a rate of one stimulus in ~2.5 s. The subjects task was to indicate whether the object in the

26   current stimulus was different from the one in the previous stimulus regardless of quadrant and object

27   rotation. **Game 7** was a threshold-stimulus-detection task where semi-transparent complex visual objects

1   were presented randomly for 0.1 s and the subjects' task was to report perceived stimuli. The object

2   transparency was calibrated so that for an alpha-level A, detection rate of 0.5 was obtained at 0.5A.

3   During the games, objects were at five equiprobable levels of A so that A were 0, 0.25, 0.5, 0.75, and 1.0.

4

5   **Figure 3. Improvement of visual acuity.** (A) Scatter plots showing each individual patient's visual

6   acuity (amblyopic eye) at baseline, at week 10 (end of treatment/training) and at week 22 (end of follow-

7   up), as measured by ETDRS chart (logMAR). Control group is shown on the left and fluoxetine group on

8   the right. The limit of normal visual acuity (logMAR 0) is shown with a hatched line. (B) Average change

9   in visual acuity from baseline as measured by ETDRS chart (logMAR) at baseline and after 2, 6, 10, 14

10   and 22 weeks. Average +/- 95% CI in each timepoint when visual acuity was determined by ETDRS chart

11   is shown. (C) Number of patients per group who showed improved visual acuity by ≥0.2 or <0.2 logMAR

12   units at week 10 and 22 as compared to baseline.

13

14   **Figure 4. Change in binocularity, contrast sensitivity, crowded near visual acuity.** (A) Number of

15   patients per group who showed change in binocular vision (suppression, anomalous retinal

16   correspondence (ARC) or normal fusion) at week 10 and 22 as compared to baseline. Bagolini striated

17   glass test results at 4 meter distance are shown. (B) Mean contrast sensitivity (log value) as measured by

18   Pelli-Robson chart. Normal contrast sensitivity (1.70) is indicated by a hatched line. Only two patients in

19   the whole patient population (n=42) had significant contrast sensitivity impairment (i.e. 0 log) at baseline.

20   Both patients received fluoxetine and improved to almost normal contrast sensitivity. (C) Crowded near

21   visual acuity as measured by Landolt C ring charts. Normal crowded near visual acuity was considered as

22   ≥0.7 (hatched line). In panels B and C, the direction of improvement is indicated by an arrow. (D) Number

23   of patients per group who improved in crowded near visual acuity test at week 10 and 22 as compared to

24   baseline.

25

26

27

1

2 **TABLES**

3 **Table 1. Eligibility and exclusion criteria.**

Eligibility criteria
Age 18-60 years, male or female
Diagnosed with amblyopia due to myopic or hyperopic anisometropia, or, congenital esotropia
Visual acuity in the amblyopic eye ≥0.30 and <1.10 logMAR
Visual acuity in the dominant eye ≤0.10 logMAR
Anisometropia ≤4.25 (spherical equivalent in diopters)
Judged to be otherwise healthy by the Investigator, based on medical history, brief physical examination, eye examination and clinical laboratory assessments
Females of childbearing potential were eligible for the study provided (i) they have a negative urine pregnancy test at the screening visit and (ii) they agreed to use adequate contraception (e.g. oral, depot or implanted hormonal contraception, intrauterine device, surgical sterilization or partner vasectomy) from the screening visit until at least 4 weeks after the last dose of study medication

Exclusion criteria
Diagnosed with other reasons of strabismus (than infantile esotropia) as the primary reason for amblyopia
History of any amblyopia therapy in the 2 years before the screening visit
Any eye surgery less than 6 months before the screening visit
Observed off-fixation by ophthalmological examination (extra-foveal/eccentric fixation)
Other ophthalmological pathologies that may affect the patient's rehabilitation
Pregnant, planning to become pregnant during the study, or breast feeding
History of depressive illness or treatment with antidepressant medication within 6 months before the screening visit
Use of psychiatric medication within 6 months before the screening visit
Receipt of an experimental treatment for any disease within 4 weeks before the screening visit
History or presence of illicit drug use or alcohol abuse
History or presence of any medical or psychiatric condition or disease, or laboratory abnormality that, in the opinion of the Investigator, may place the patient at unacceptable risk or that could prevent the patient from completing the study

4

1 **Table 2. Demographic data and mean visual parameters at baseline.**
2

Demographic data and mean visual parameters at baseline		
	Control (n = 20)	**Fluoxetine (n = 22)**
Age (y)	36.4 ± 11.5 (19-57)	38.5 ± 12.5 (20-57)
Gender	11 male (55%)	11 male (50%)
	9 female (45%)	11 female (50%)
Cause of amblyopia		
Anisometropia	19 (95%)	18 (82%)
Anisometropia and strabismus	1 (5%)	3 (14%)
Strabismus only (congenital esotropia)	0 (0%)	1 (4%)
Mean visual acuity (logMAR, amblyopic eye)	0.620 (0.190)	0.649 (0.252)
Interocular visual acuity difference (logMAR)	0.728 (0.257)	0.728 (0.245)
>0.2 to <0.5	4 (20%)	6 (27%)
>0.5 to <0.8	7 (35%)	9 (41%)
>0.8	9 (45%)	7 (32%)
Refractive error in amblyopic eye (D)[1]		
< 0	1 (5%)	3 (14%)
≥ 0 to ≤ +1.00	4 (20%)	3 (14%)
> +1.00 to < +3.00	4 (20%)	7 (32%)
≥ +3.00	11 (55%)	9 (40%)
Mean (SD), hyperopes, n=33	+3.13 (1.49)	+3.07 (1.79)
Mean (SD), myopes, n=9	-1.23 (0.66)	-2.81 (1.07)
Refractive error in non-amblyopic eye (D)[1]		
< 0	4 (20%)	3 (14%)
≥ 0 to ≤ +1.00	10 (50%)	10 (45%)
> +1.00 to < +3.00	4 (20%)	7 (32%)
≥ + 3.00	2 (10%)	2 (9%)
Mean (SD), hyperopes, n=33	+1.43 (1.89)	+1.14 (1.04)
Mean (SD), myopes, n=9	-0.95 (0.70)	-2.28 (1.20)
Mean anisometropia (D)[1]	2.19 (1.86)	1.95 (1.44)
Mean contrast sensitivity (log, amblyopic eye)	1.913 (0.096)	1.732 (0.568)
Crowded near visual acuity (decimal, amblyopic eye)	0.171 (0.116)	0.165 (0.141)
Binocularity (normal fusion in Bagolini striated glasses test, 33 cm)	13 (65%)	14 (64%)
Binocularity (normal fusion in Bagolini striated glasses test, 4 m)	11 (55%)	9 (41%)

3   [1]Spherical equivalent in diopters

23 (25)

1 **Table 3. Summary of vision improvement in all 42 patients enrolled in the study, observed at any**

2 **visit after the randomization visit.**

Summary of vision improvement (at any visit after randomization visit)			
Visual parameter	**Test**	**Number of patients with improvement**	**Notes**
Visual acuity	ETDRS	22 / 42 (52%)	7 / 42 patients (17%) achieved normal visual acuity (<0.10 logMAR).
Crowded near visual acuity	Landolt C ring	9 / 42 (21%)	9 patients improved to normal, defined as ≥0.7
Contrast sensitivity	Pelli-Robson	2 / 2 (100%)	Two patients had abnormal contrast sensitivity at baseline. Both improved to normal contrast sensitivity, defined as ≥1.65 log.
Binocularity	Bagolini striated glasses	10 / 22 (45%)	Twenty-two patients had suppression or anomalous retinal correspondence at baseline. Ten patients changed to normal fusion.
Improve in at least one visual function test (during the whole course of the study)		30 / 42 (71%)	Different patients improve on different visual parameters. No relation between visual acuity improvement and crowded near visual acuity, contrast sensitivity or binocularity. 6 / 42 patients improved in at least two visual parameters.

3

1 **Table 4. Pre-specified subgroup analyses of visual acuity.**

Pre-specified subgroup analyses of visual acuity (logMAR)			
Subgroup variable	**Interaction p-value[1]**	**Subgroup p-value[2]**	**Treatment p-value[2]**
Severity of amblyopia[3]	p=0.774	p=0.711	p=0.633
Binocularity (4 m)[4]	**p=0.048**	p=0.976	p=0.544
Gender[5]	p=0.161	p=0.252	p=0.493
Training compliance[6]	p=0.873	**p=0.012**	p=0.846
Age[7]	p=0.678	p=0.864	p=0.525

2
3 The subgroup analysis results were obtained by incorporating the subgroup main effect and treatment-by-
4 subgroup interaction effect into the primary RM ANCOVA model.
5 [1]Statistical significance of treatment-by-subgroup interaction in the FAS (LOCF) dataset
6 [2]Statistical significance of the subgroup and 10-week treatment effects in the FAS (LOCF) dataset from
7   the model without the interaction effect (amblyopia severity, age, gender, compliance) or with the
8   interaction effect (binocularity)
9 [3]Moderate/Severe
10 [4]Suppression/Normal fusion
11 [5]Female/Male
12 [6]$\geq$85% / <85%
13 [7]<40 / $\geq$40 years
14 Note: Severe amblyopia was more pronounced with younger, compliant, female and, especially,
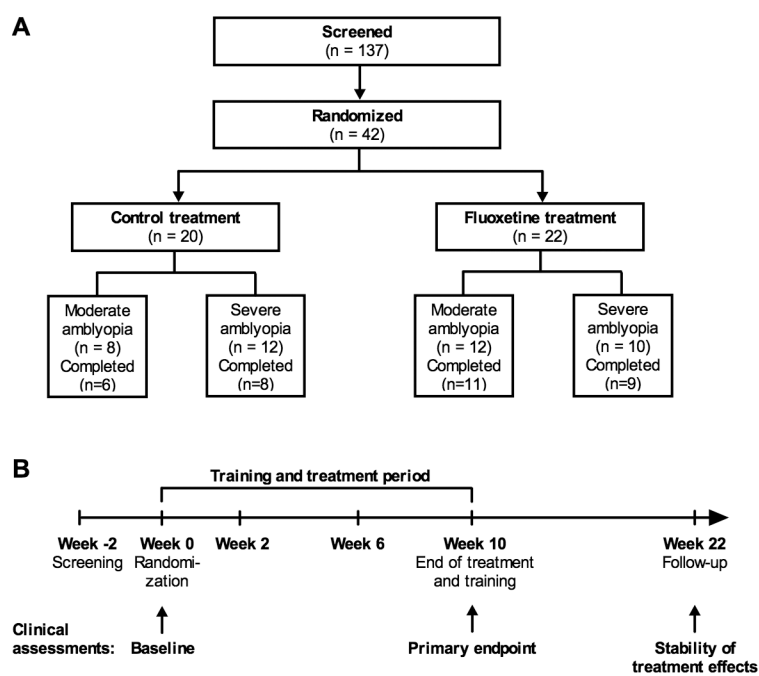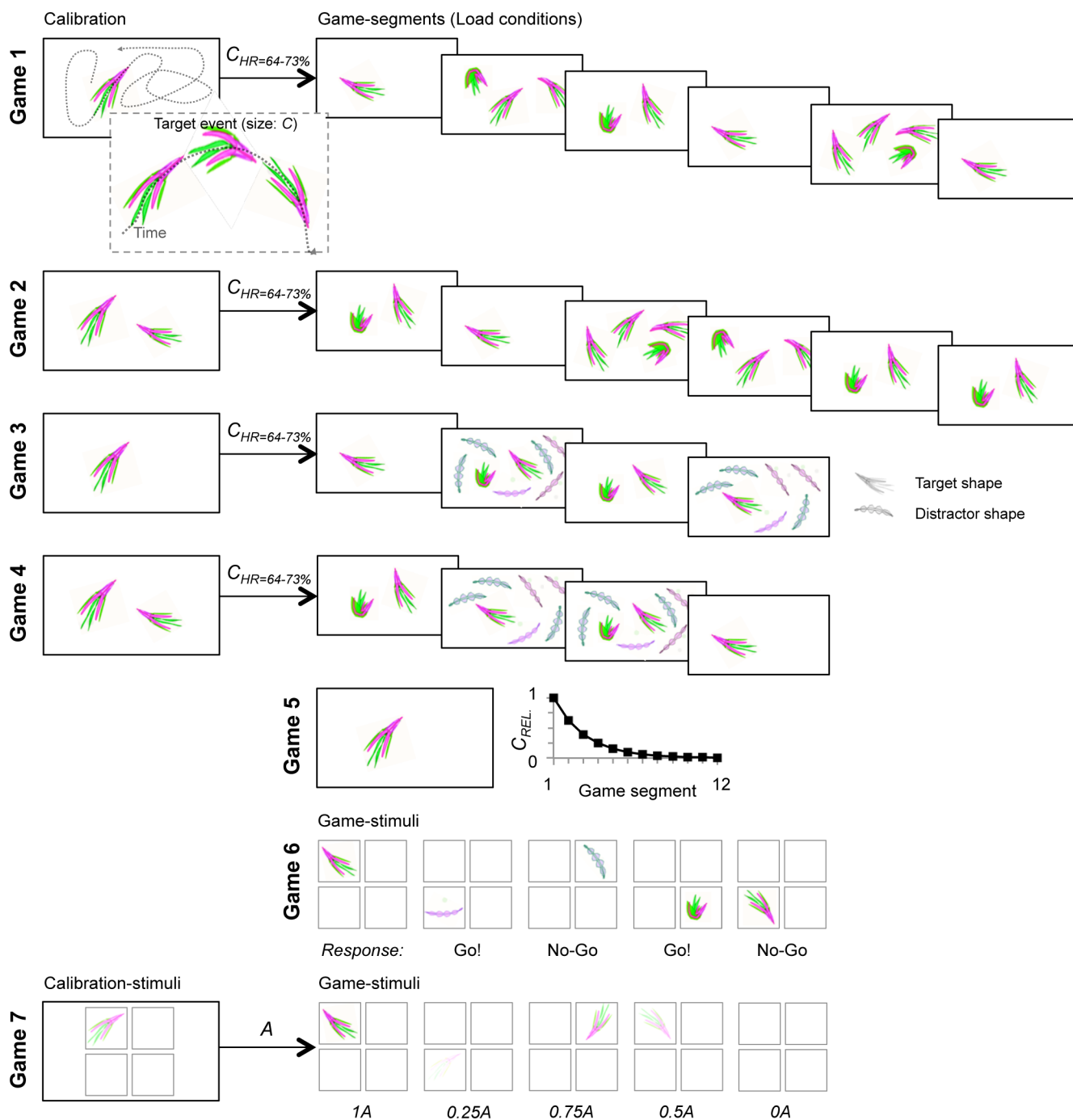15   suppression patients.
16

Figure 1

Figure 2
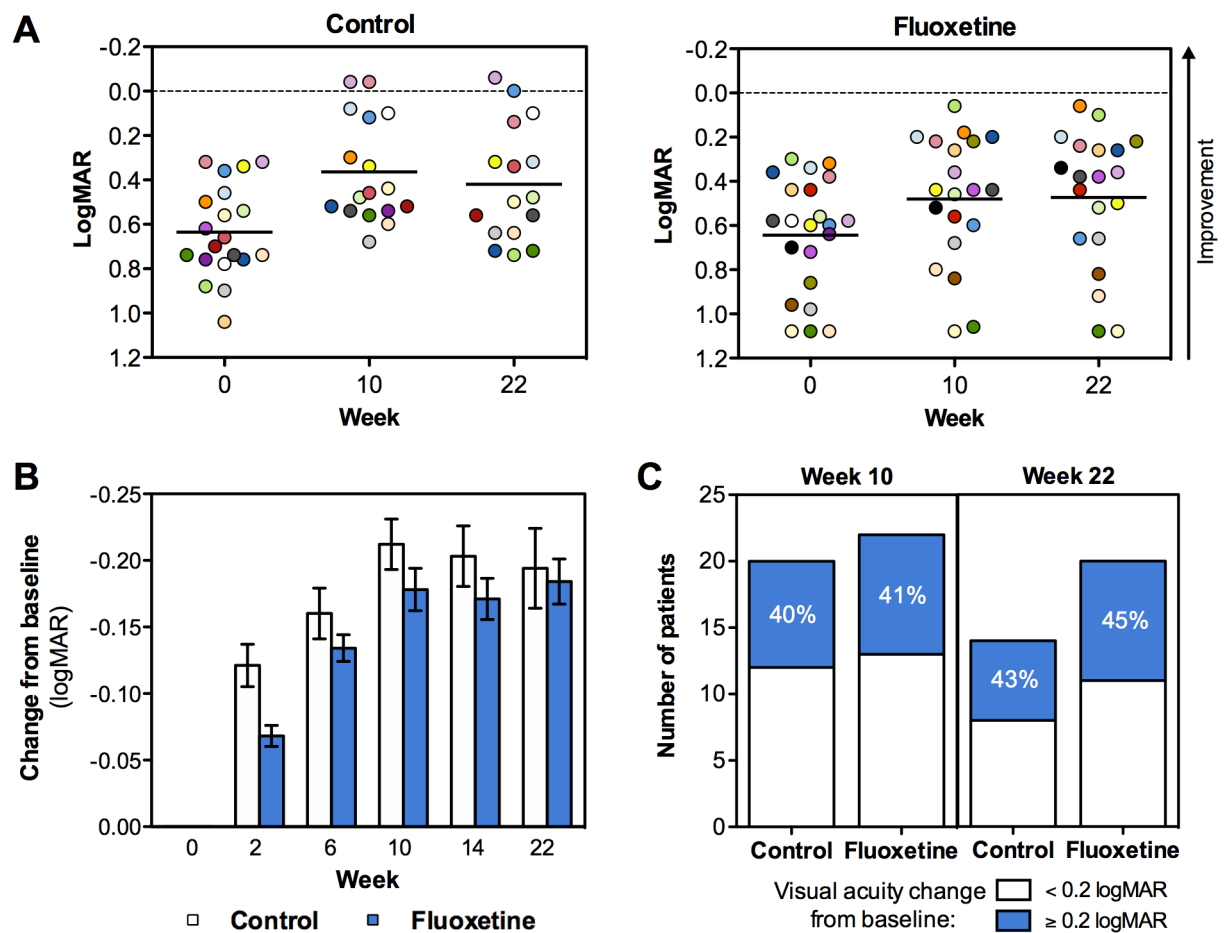
Figure 3

Figure 4