ABSTRACT

**Aims:** To evaluate the effectiveness of visual training aimed at reducing crowding in dyslexic children.

**Study Design:** Single-masked crossover pilot study.

**Place and Duration of Study:** University of Turin and the Gradenigo Hospital, Department of Ophthalmology, Turin, between January and November 2013.

**Methodology:** 15 dyslexic children underwent a visual training devised to reduce crowding. Patients were asked to recognize trigrams of letters with different spacing displayed at different eccentricities on both sides of the fixation point. As a placebo half of the sample was administered a contrast sensitivity test. Average reading rate for words and non-words with different interletter spacing was measured before and after the visual training and the placebo. The sample was divided into two subgroup: G1, who was first administered the training, and G2, who underwent first the placebo trial.

**Results:** After the training in G1 reading rate for words increased from 1.54 syl/sec (±0.60) to 1.74 syl/sec (± 0.64) (P=.001). Reading rate for non-words improved from 0.94 (0.68-1.55) syl/sec to 1.03 (0.85-1.63) syl/sec. No significant improvement was found after the administration of the placebo (T2) when testing words and non-words.
Analysis of variance showed a significant placebo x treatment effect for words ($P = .001$) and a barely significant effect for non-words ($P = .05$). In G2 no significant improvement was found after the placebo both at words and non-words (from $1.69 \text{ syl/sec} \pm 0.83$ to $2.01 \text{ syl/sec} \pm 0.94$ for words, from $1.07 \text{ syl/sec} \pm 0.51$ to $1.08 \text{ syl/sec} \pm 0.50$ for non-words). In this group the training increased the reading rate for words and non-words (from $2.01 \text{ syl/sec}$ to $2.12 \text{ syl/sec} \pm 1.13$; non-words: from $1.08 \text{ syl/sec}$ to $1.22 \text{ syl/sec} \pm 0.59$). However, analysis of variance did not show a significant effect of the treatment (words: $P = .70$; non-words: $P = .85$).

**Conclusion:** Factors other than visuoperceptive, in particular the phonological impairment, could help to account for the controversial results obtained in the small group of dyslexic children recruited in this study. In future investigations, performed on a larger sample, a classification aimed at ruling out patients mainly affected by phonological defects should be considered, in order to select the appropriate target suitable for such kind of approach.

**Keywords:** Dyslexia; visual training; crowding; reading rate; trigrams.

**1. INTRODUCTION**

Developmental dyslexia is a specific reading disability that affects approximately 4-10% of the population of school age [1,2]. It has been defined as a reading difficulty despite adequate instruction and education, normal intellective capacities and socio-cultural situation and not caused by reduced visual acuity or psychiatric pathologies [3]. A growing body of evidence is supporting the potential role of the visual function in the pathogenesis of this clinical condition. Apart from arguable evidence on abnormal motion perception [4-10], contrast sensibility deficits (see Skottun, for a critical review on this topic [11]), increased visual persistence time [5,12-17] or unstable binocular fixation [18-20], the reinforcement of crowding is believed to play a main role at least in a subtype of patients [21-27]. Crowding or lateral masking is the physiological phenomenon that makes neighboring letters unrecognizable if they are as close as to fall within a spatial interval, called critical spacing. According to the Bouma's law, the extent of the critical spacing (in degrees) is roughly half the eccentricity, so that the least spacing between letters required to make them recognizable is $0.5\phi$ (where $\phi$ is the eccentricity [28]). Even if increased crowding is typical of amblyopic subjects (see for example Levi et al. [29]), many studies found that critical spacing can be wider also in dyslexics [21-27]. In a part of dyslexics, in fact, the critical spacing at 4 deg of eccentricity along the horizontal axis is found to be increased compared to controls, so that the distance between letters in such subjects needs to be about one and a half wider compared to normal readers in order to make adjoining letters recognizable [24]. In a recent work [30], we have suggested wider critical spacing in dyslexics to depend on anisotropic spatial relationship perception: the perception of the visual space “shrunk” along the horizontal axis would lead to subjective reduction of the distance between characters so as to make them fall within the boundary of the critical spacing. As a matter of fact, increasing interletter distance tends to improve reading rate and accuracy in dyslexic patients but not in normal readers [30-31].

Larger critical spacing is claimed to hamper reading by reducing the number of characters recognizable at each fixation, that is to say the width of the visual span [32]. Since crowding increases as a function of eccentricity, the visual span narrows more, the further away the
fixation point: this is the reason why in normal subjects peripheral reading speed will remain always slower compared to central reading, even after the print size has been scaled.

Still, enlargement of the visual span is found to be achievable by training crowding in the peripheral visual field: intensive practice in recognizing the letters of trigrams displayed 10 deg above and below the fixation point is found to be effective in making the visual span wider, thereby the peripheral reading rate faster by 41% in a group of adult normal subjects [33].

If visual training focused on crowding is found to be effective in narrowing the critical spacing, therefore in enlarging the visual span and finally in increasing the peripheral reading rate in normal readers, it is worth wondering whether a similar practice can improve the reading rate of dyslexic children as well.

In this pilot study we have therefore devised a visual training aimed at improving parafoveal crowding of dyslexic children. The preliminary results of our protocol have been evaluated in a crossover case-control single masked investigation.

2. EXPERIMENTAL DETAILS

2.1 Participants

From a neuropsychiatry service, 15 dyslexic children (10 males, 5 females) aged 8-10 years were recruited. The diagnosis of dyslexia has been conducted according to the operational definition of the condition, i.e., lexical age reduced of at least 2.5 years with reading rate and accuracy below the second standard deviation compared to normal age-matched readers, normal intellectual ability and normal or above normal IQ, with visual acuity 60/60 and no behavioural problems or auditory impairment [3].

The parents of the recruited children were contacted by phone and their informed consent was obtained after explanation of the aim, nature and possible consequences of the study.

Selection criteria were: presence of developmental dyslexia as assessed by the neuropsychiatrist at the reading battery of Zoccolotti [34], average to above-average intellectual ability, normal IQ as measured by Wechsler Intelligence Scale for Children (WISC-R) scale, performance equal to normal readers in other academic subjects, best corrected visual acuity (BCVA) ≥ 6/6. Exclusion criteria were general or ophthalmological diseases, hyperopia/myopia >2D, astigmatism > 1.5D, eso/exotropy, poor convergence, auditory impairment, behavioral problems, poor collaboration.

Pupils were not affected from comorbid disorders. In particular no visuoattentional deficits were reported at the neuropsychiatric examination and QI in every case was normal or above-average.

All patients underwent a preliminary ophthalmological examination, then the sample was divided into two age- plus reading performance-matched groups, we will refer to as G1 (8 subjects) and G2 (7 subjects). Best corrected visual acuity was 60/60 in both groups. All but one participant in each group showed slight exophoria; convergence was good in all subjects of G1, fairly good in one subject and good in the remaining children of G2.
The members of each group belonged to the same socio-cultural contest.

2.2 Materials and Procedure for the Visual Training

Triplets of dark letters (luminance: 0.3 cd/m²) were displayed binocularly on a white background (luminance: 85 cd/m²) in tachistoscopic conditions (presentation time: 200 msec) along the horizontal meridian on the left and right side of the fixation point, and at different values of spacing. At the viewing distance of 40 cm, the stimulus size was arranged so as to be 30% greater than the resolution threshold at every tested value of eccentricity. The letters making the triplets were randomly sorted out from the Sloan characters with the characters of the next trigram differing from the characters of the previous triplet.

Trial after trial subjects were asked to look steadily at a central mark (an emoticon). At the same time they were required to recognize the three letters making up the trigram. The trigram was presented simultaneously with the central mark at different values of eccentricity, taking as the positional reference the middle letter. No feedback was provided. Fixation was constantly checked by a second operator and the subject was constantly reminded to look steadily at the emoticon. To help maintain the fixation stable, the emoticon changed randomly after a few numbers of presentations. Whenever a shift of fixation during a presentation was noticed by the physician, the trial was repeated. Within a session, all the eccentricities were trained according to the following order: right 2, 4, 6, 8, 10 deg, then left 2, 4, 6 deg, by administering a block of 45 presentations per locus. This testing order remained the same for all the sessions, whereas interletter spacing was progressively reduced. Each subject underwent 1 session per day (in the morning) for 5+5 consecutive days (two weeks from Monday to Friday). Therefore, in total each patient was administered 3600 trials.

At every session the distance between the three letters was computed as a multiplicative proportion of the critical spacing (center-to-center of the letters), according to the Bouma's law. The widest interletter spacing was 1.32 (φ/2) at the first session (day 1) and session after session it was reduced till to 1.05 (φ/2) at day 10.

2.3 Materials and Procedure for Reading Performance Assessment

A peculiar characteristic of this study was that, in order to better characterize the global reading performance of the subjects, before and after the treatment reading rate was estimated for different interletter distances, ranging from 0.2 to 0.51 deg (spacing computed center-to-center) and the obtained values for each observer were averaged.

On a high-resolution LCD screen, 22 presentations were displayed at a viewing distance of 70 cm in randomized order. Each presentation was made of 4 words placed side by side on the same line and made of, 3, 4, 3 and 2 syllables (font Free Monospace) or 5 non-words made of, 2, 2, 2, 3 and 3 syllables. Letter and background luminance was the same as in the training session. Mean character size was 0.4 deg at the viewing distance. At each trial the reading material was presented at a different value of interletter spacing, that is 0.2, 0.23, 0.25, 0.27, 0.28, 0.31, 0.36, 0.4, 0.44, 0.47 or 0.51 degrees, in random order. The subject was required, without being urged to the best performance, to read aloud each presentation in binocular conditions. Each presentation remained visible on the screen the time necessary to be read. In this way two reading rate measures (in syl/sec) and the correspondent Z-score (based upon previously collected normative data [35]) were obtained per each value of
spacing. The best estimate of each couple of trials was automatically selected by the instrument and averaged out as cumulative reading rate (CRR [30]; see Aleci [35] for a detailed description).

At the baseline, the two groups did not differ for reading performance for words and non words as assessed both at the conventional reading test of Zoccolotti and as CRR (CRR words: mean Z-score group G1= -2.14 ±0.44, group G2= -2.15±0.56, P= .95; CRR non-words: mean Z-score group G1= -1.55 ±0.47, group G2= -1.67±0.55, P=.66).

2.4 Design

Five consecutive days a week for two weeks, group G1 underwent the rehabilitative program (“trigram”) while group G2 was administered a placebo. As a placebo trial, sinusoidal gratings oriented upward or 45 deg to the left or to the right and with spatial frequency ranging from 0.5 to 12 c/deg, were presented sequentially. At each trial, the observer was required to discriminate the orientation of each grating. No feedback was provided. The duration of the training and of the placebo was roughly the same (about 15 minutes). At the end of the last session (T1), reading rate was compared in the two samples, then the rehabilitation/placebo groups were reversed (G1=placebo, G2= training), so that days after two additional weeks of training/placebo sessions were carried out. At the end of the last session (T2), reading rate was newly compared in the two samples Fig. 1.

![Fig. 1. The experimental design](image)

In each group results have been expressed as mean values (±SD) or median (+range) according to the parametric or non parametric distribution of the sample (normality verified by using the Kolmogorov Smirnov test).

All authors hereby declare that the experiment has been examined and approved by the ethics committee and has therefore been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki. All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed.
3. RESULTS

One child in the G1 group refused to undergo the non-word reading trial. In the G2 group one child dropped the rehabilitation protocol after the preliminary evaluation (T0). Results from G1 are shown in Table 1 and Fig. 2.

After two weeks (T1), in the sample who was administered the Trigram (G1) cumulative reading rate for words increased from 1.54 syl/sec (±0.60) to 1.74 syl/sec (±0.64). Cumulative reading rate for non-words improved from 0.94 (0.68-1.55) syl/sec to 1.03 (0.85-1.63) syl/sec. The pre-treated group G1 was then administered the placebo. At T2, cumulative reading rate changed from 1.74 syl/sec to 1.85 syl/sec (±0.70) when subjects were asked to read words, from 1.03 (0.85-1.63) syl/sec to 1.07 (0.83-1.78) syl/sec when they were required to read non-words.

Analysis of variance and Friedmann test performed respectively on CRR for words and non words showed an effect of the treatment compared to the placebo, even if for non-words the outcome was at the limits of significance (ANOVA: F [10.70], P=.001; Friedmann Fr [6.00], P=.05, respectively). Results are confirmed by computing the correspondent z-scores (words: ANOVA: F [8.43], P=.003; non words: Friedmann Fr [6.00], P=.05). The effect of the training on words reading was evident immediately after the practice (Tukey-Kramer [3.70]: q=3.96, P<.05) and remained significant after two weeks (Tukey-Kramer [3.70]: q=6.48, P<.01). The improvement with non-words was significant after two weeks from the end of the treatment (Dunn [8.96]: rank sum difference: -9.00, P<0.05). Contrary to the training sessions, no significant improvement was found after the administration of the placebo both at words and non words testing (Tukey-Kramer [q=2.52, P>.05], Dunn [8.96], rank sum difference:-3, P>.05).

Results from G2 are shown in Table 2 and Fig. 3. In the G2 sample no significant improvement was found both for words and non-words after the administration of the placebo (T1: average CRR for words changing from 1.69 syl/sec [±0.83] to 2.01 syl/sec [±0.94]; average CRR for non-words being unchanged: 1.07 syl/sec [±0.51] at T0 and 1.07 syl/sec [±0.50] at T1).

In this group even if the administration of the rehabilitation protocol increased the reading rate for words and non words (T2: words: average cumulative reading rate from 2.01 syl/sec to 2.12 syl/sec [±1.13]; non-words: from 1.07 syl/sec to 1.22 syl/sec [±0.59]). Table 2, Fig. 3. analysis of variance did not show a significant effect of the treatment (ANOVA, words: F [0.35], P=.70; non-words: F[0.15], P=.85). The lack of significance is confirmed by computing the correspondent z-scores. (ANOVA, words: F [0.45], P=.64; non words: F [0.30], P=.73).

Table 1. Mean/median reading rate (syl/sec) and Z-scores for words and non-words at baseline (T0), after the training (T1), and after the placebo treatment (T2). Group G1

<table>
<thead>
<tr>
<th></th>
<th>CRR words</th>
<th>CRR non-words</th>
<th>Z-score words</th>
<th>Z-score non-words</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>1.54 (±0.60)</td>
<td>0.94 (0.68-1.55)</td>
<td>-2.14 (±0.44)</td>
<td>-1.55 (±0.47)</td>
</tr>
<tr>
<td>T1</td>
<td>1.74 (±0.64)</td>
<td>1.03 (0.85-1.63)</td>
<td>-1.93 (±0.52)</td>
<td>-1.25 (±0.37)</td>
</tr>
<tr>
<td>T2</td>
<td>1.85 (±0.70)</td>
<td>1.07 (0.83-1.78)</td>
<td>-1.82 (±0.57)</td>
<td>-1.13 (±0.47)</td>
</tr>
<tr>
<td>P</td>
<td>.001</td>
<td>.031</td>
<td>.003</td>
<td>.05</td>
</tr>
</tbody>
</table>

*CRR non-words: median (range). T0: baseline, T1: after the training, T2: after the placebo treatment.
Fig. 2. Group G1. Reading rate (left panel) and Z-score (right panel) for words (continuous line) and non-words (dotted line) at baseline (T0), after the training (T1), and after the placebo treatment (T2).

Table 2. Mean reading rate (syl/sec) and Z-scores for words and non-words at baseline (T0), after the placebo treatment (T1), and after the training (T2). Group G2.

<table>
<thead>
<tr>
<th></th>
<th>CRR words</th>
<th>CRR non-words</th>
<th>Z-score words</th>
<th>Z-score non-words</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>1.69 (±0.83)</td>
<td>1.07 (±0.51)</td>
<td>-2.15 (±0.56)</td>
<td>-1.67(±0.55)</td>
</tr>
<tr>
<td>T1</td>
<td>2.01 (±0.94)</td>
<td>1.08 (±0.50)</td>
<td>-1.85 (±0.72)</td>
<td>-1.61(±0.57)</td>
</tr>
<tr>
<td>T2</td>
<td>2.12 (±1.13)</td>
<td>1.22 (±0.59)</td>
<td>-1.80 (±0.91)</td>
<td>-1.40 (±0.73)</td>
</tr>
<tr>
<td>P</td>
<td>.70</td>
<td>.85</td>
<td>.64</td>
<td>.73</td>
</tr>
</tbody>
</table>

*T0: baseline, T1: after the placebo treatment, T2: after the training.

Fig. 3. Group G2. Reading rate (left panel) and Z-score (right panel) for words (continuous line) and non-words (dotted line) at baseline (T0), after the placebo treatment (T1), and after the training (T2).

In Fig. 4 individual reading rates of G2 are shown for words and non-words.

With words, unexpected improvement of the performance after the administration of the placebo was found in one subject (subject 2). Yet, even after the aberrant trend has been ruled out, ANOVA remained not significant (reading rate: T0=1.55syl/sec [±0.82], T1=1.71syl/sec [±0.57], T2=1.72syl/sec [±0.78], F=0.43, \( P= .66 \)).

When reading non-words, in turn, one subject (subject 1) showed a dramatic worsening of the performance after the training. Once this aberrant trend has been removed, ANOVA turned out to be significant (F=6.79, \( P= .01 \)); the improvement was evident after the administration of the training (T2) (Tukey-Kramer [4.04]: 4.31, \( P< .05 \)) but not after the administration of the placebo (Tukey-Kramer [4.04]: 0.36, \( P> .05 \)).
One subject (subject 2) showed a strong improvement after the training at the non word-task. To further verify whether the significant effect obtained after the removal of subject 1 was biased by the performance of this patient, the data from subject 2 have been ruled out as well, and the remaining results have been analyzed once more. The effect of training remained significant (Friedman: p=0.004) with increased reading rate after the administration of the training compared to T0 (Dunn: p<0.05) but not after the placebo (Median reading rate: T0=0.71 syl/sec [0.49-1.27], T1=0.77 syl/sec [0.61-1.32], T2=1.09 syl/sec [1.00-1.33], Dunn: P> .05) Fig. 5.

In summary, average reading rate for non-words was increased after the training by 14-16.5%, in both groups, compared to 0-5.3% after the placebo. For words the improvement after the training was evident only in G1 (13% vs 6.3% after the placebo), but not in G2.
4. DISCUSSION

A growing deal of literature supports the effect of visual training in improving crowding both in the peripheral or paracentral visual field of normal subjects [33; 36-40] (see Huurneman et al. [41] for a review) and crowding-dependent functions in amblyopic patients, like positional and letter acuity [42], luminance- and contrast-defined letters identification [43] or crowding ratio [39] (see Levi & Li [44] for a review).

Although the effect of crowding and the outcome of the treatment is generally evaluated as crowding ratio or visual acuity [39,41], increased lateral masking is claimed to affect the capacity of reading fluently, so that reading rate is considered a main marker of the success of the remediation program in amblyopic children [45,46]. Practice focused on crowding in the peripheral visual field of adult normal readers is found to be effective in increasing their peripheral reading rate to a certain amount [33,36, 47]. Evidently, the abnormally strong crowding and its detrimental effect on the lexical performance via shrinking of the visual span, as found in many dyslexic children, is a hypothesis backed by this perspective. As a matter of fact, in many dyslexics crowding is found increased in the paracentral region; if training peripheral crowding is effective in improving peripheral reading speed of normal readers, training parafoveal crowding should be effective in improving the reading rate of dyslexic children as well, at least to a certain amount (the limit would be due to the phonological deficiency).

As a pilot study, the goal of the present investigation was to verify this assumption, that is to say to assess whether the visual training aimed at reducing the effect of crowding so as to enlarge the visual span could be effective in improving the lexical function of dyslexic children. The rationale, in particular, relies on a previous American investigation performed by Chung et al. [33], that found a visual training based on trigram recognition to be able to increase peripheral reading speed of normal young adult subjects.

In the aforementioned study, a visual training based on a letter-recognition task where characters were arranged into trigrams was administered to a group of adult normal readers. Trigrams were presented 10 deg in the upper and/or lower visual field. Peripheral reading speed at 10 deg above and below the fixation point as well as the visual span profile was measured by using a rapid serial visual presentation paradigm (RSVP, that is words making a sentence were presented in sequence one at a time) before and after the practice. Reading speed was found to be raised by 41% after the training. The improvement has been ascribed to visual span expansion and it is found to persist up to three months following the training.

The present investigation, that as far as we know is the first focused on this subject in dyslexics, made use of a similar rehabilitation paradigm but the effect was evaluated by testing the lexical performance with a sample of words and non-words using a model closer to normal reading compared to the RSVP paradigm.

Unlike the findings of the aforementioned studies, the obtained results are quite controversial and can be summarized as follows:

1- When tested with words, reading rate improved after the treatment but not after the placebo. The improvement lasted at least for two weeks. However, this finding has not been confirmed after the crossover. A possible explanation for the lack of correspondence between results obtained in G1 and G2 with words could rely on
differences in the two groups not detected at the preliminary clinical assessment. In G2, for example, visual attention could be worse and/or the visual span at baseline could be smaller compared to G1. These, indeed, are two possible explanations advanced by Yu et al. [47] to account for the weaker training effect based on a trigram paradigm similar to the one adopted in our study in their older subjects compared to the younger observers trained by Chung et al. Since nor visual attention neither visual span had been estimated at pre-training when matching the two groups, these solutions at the moment remain unsolved.

2- When tested with non-words, reading rate improved significantly two weeks after the treatment, but not after the placebo. Even if this finding has not been confirmed after the crossover in the small sample recruited, the lack of ameliorative effect of the training could depend on the performance of a subject that, being in sharp counter-tendence, may have biased the overall trend.

However the observed foveal reading improvement after the training is not greater than 13-16.5% for words and non-words, respectively: a small gain compared to 41% in the normal sample when reading peripherally found by Chung et al. Yet, it should be recalled that normal readers when reading peripherally can make use of the automatisms they have learned during their normal lexical development whereas for our patients, evidently, this was not the case. Second, as already pinpointed, the way the lexical function has been measured in this experiment is different: in the American study reading speed was estimated according to a RSVP paradigm and computed as a function of the words exposure time that yields 80% of words read correctly. This method allows ruling out the oculomotor factor (saccades number and fixation duration). However, in our investigation we were interested in assessing whether dyslexic children could benefit of the visual training in a real everyday reading scenario. For this reason we chose to adopt a simpler, more “natural” way to measure their reading rate. The detrimental effect of the well known abnormal saccadic movements of dyslexics during the lexical task could contribute to jeopardize the improvement provided by the treatment.

In addition, it has been ascertained that dyslexics, even in case of transparent languages, are affected by a prevalent phonological impairment [48,49]. Such a phonological influence would presumably make the results obtained from a selective visual approach less evident than expected. The recruited sample in the present survey has not been classified, so that the amount of the phonological component in the two sub-sample is unknown. A preponderance of more phonologically impaired children in the G2 group could help to explain the lack of correspondence in the outcome after the crossover. In effect, in G2 non-word performance, considered as a phonological marker [50,51], is (even if slightly) worse than in G1.

Therefore, our purpose for a future investigation is to classify the patients according to one of the methods available in literature (for example according to the paradigm of Castles & Coltheart [51] or Valdois [52]) in order to stay focused on those subjects whose visuoperceptive component looks to be prominent (and, in turn, the phonological factor is less relevant). We believe this can be an important cue to better judge the effectiveness of a purely visual remediation strategy.

Moreover, it is worth underlining that the reading rate is found to improve (even if not to a significant level) also after the administration of the placebo, especially in group G2. On closer inspection, this trend suggests that even a training based on contrast sensitivity could be effective (to a certain extent) in improving the lexical performance, all the more
considering that defective contrast sensitivity at low spatial frequencies has been reported in a subtype of dyslexic children [11]. If it were the case, the study may have been underpowered to detect a significant level after the placebo trials. In order to rule out the potential effect of a (presumed) placebo trial, whatever it be, future investigations should make use of different strategies (for example simple reading practice) to control the outcome of the training.

The lack of fixation monitoring via eye-tracker is an additional flaw, even if for a paradigm very similar to the one adopted in the present study "little detectable differences between observers with and without eye-movement monitoring" was reported [33].

Finally, no estimate of the change of the visual span has been carried out. We intend to take into account also this factor in future studies.

5. CONCLUSION

In conclusion, in light of the conflicting preliminary data reported in this paper, we believe the visual training focused on crowding is worth to be further investigated in a wider sample of disabled readers, provided they are previously classified.

CONSENT

All authors declare that written informed consent was obtained from the parents of the patient for publication of this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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