Original Research Article

Detecting Visuoperceptive Defects in Adult Disabled Readers with the TETRA Analyzer™. Pathological Sample and Comparison with Normal Readers

ABSTRACT

Aims: To investigate visuoperceptive abnormalities in adult dyslexics with the TETRA Analyzer™, and to compare the results with those obtained with normal adult readers.
Study design: Epidemiological study.
Place and Duration of Study: Service of Neuro-Ophthalmology, University of Turin, Italy, between April 2016 and October 2017.
Methodology: Ocular dominance, spatial relationship perception, and interocular visual input have been tested in 25 adolescent and adult dyslexic readers (age 11-34 years) with the TETRA Analyzer™. The TETRA Analyzer™ is a set of 4 exams devised to evaluate ocular dominance (Domitest M), spatial relationship perception (Eidomorphometry), interocular sensory pattern (Domitest S), and their effect of the reading performance (Reading Performance Test, REPORT). Results have been compared with the normative data of a sample of adult subjects ("mature readers") gathered in a previous investigation.
Results: Compared to mature readers, a higher proportion of adult dyslexics showed dominance instability (20% vs 4%). Average spatial relationship anisotropy was up to threefold higher in adult dyslexics (3.54% vs 1.32% in mature readers), and a greater proportion of patients showed unbalanced binocular sensory interaction (28% vs 12.7%), even if this difference was not significant (p=.07).The distribution of the interocular inhibition was bimodal, resembling that of immature readers (children). The reading rate of non words was strongly affected by the inter-letter spacing (R^2=0.50, P=.01), in support of an involvement of these alterations in affecting the lexical function.
Conclusion: Defective visuoperceptive functions can play a role not only in children but even in adult dyslexic. The resemblance of dominance, spatial relationship perception and especially interocular inhibitory pattern of adult dyslexics and immature readers may be the sign that a stunt or delay of the normal development of these visuoperceptive functions takes place in a subpopulation of dyslexic subjects.

Keywords: Adults, Spatial Relationship Perception; Crowding; Dyslexia; Interocular Inhibition; Ocular Dominance; TETRA Analyzer™

1. INTRODUCTION

Developmental dyslexia is a specific reading disability that affects approximately 4-10% of the scholar population [1,2], It occurs despite adequate instruction and education, normal intellective capacities and socio-cultural situation, and is not caused by reduced visual acuity or psychiatric pathologies [3].

Even if developmental dyslexia is basically a neuropsychiatric condition, there is a wealth of research showing that a proportion of dyslexic children exhibit to a certain extent also defective visual functions (see Aleci, 2013 for a comprehensive review [4]). These alterations involve motion perception (e.g. [5,6]), frequency doubling sensitivity [5], visual persistence time [e.g. [7-9], crowding [10-18], ocular dominance [19-26], and, as we have recently posited, may affect even interocular inhibitory interaction [27,28].

In this scenario unstable ocular dominance, enhanced crowding, and abnormal interocular input interaction deserve particular consideration: indeed, they seem especially effective in accounting for the

lexical difficulties that characterize dyslexic children, i.e. frequent omissions, positional changes of syllables, the confusability of characters, as well as the sensation of jumping and moving letters.

According to a wealth of research, a consistent proportion of dyslexic children suffer from fixation instability due to unstable ocular dominance. In these subjects the visual axes oscillate around the letters and the syllables, hampering their recognition and positional encoding [19,20,22, 24, 25, 29]. Upon this basis, it is reasonable to assume that texts with larger inter-letter distance help prevent unstable dominant readers from positional errors. Proof of this, suspected dyslexics with unstable ocular dominance are found to be more prone than stable dominants to non-words errors with small text print size (therefore when the distance between the characters is made smaller [30]).

In addition, a reinforcement of crowding, that is the inhibitory effect that two flanking stimuli (e.g. two letters) have on a central target (a third character), is found to affect the lexical task in a proportion of patients [6, 10-15, 17, 18]. In a previous paper we posited that increased crowding is caused by abnormal spatial relationship anisotropy (SRA), responsible for a perceptual contraction of the visual space along the horizontal axis: indeed, we found that spatial relationship anisotropy in school-age disabled readers (3rd-5th grade) is almost double compared to normal age-matched children [17]. This effect would make characters perceptually closer, thereby more prone to reciprocal lateral masking. If it were the case, in visual dyslexics the reading rate is expected to improve by making the distance between letters wider, while non-visual dyslexics (as well as normal readers) would be insensitive to this perceptive modification. Evidence to this hypothesis has been recently provided [17,18].

Finally, abnormal inhibitory interaction may contribute to make reading difficult as well. There is evidence that the visual input to one eye tends to suppress the processing of the same input in the contralateral eye [31,32]. Indeed, we have postulated that excessive interocular inhibition increases the probability of "perceptual blinks", that are time intervals when the left-right suppression takes places simultaneously, generating a period of no-perception. This might have a relevance when dealing with sequential scanning of lexical strings, and could account for omissions, errors, and in general reduced reading speed [27]. As a matter of fact, in a recent paper we found that the reading rate of school age disabled readers with strong interocular inhibition was sensitive to inter-letter spacing (even if an explanation for this phenomenon still needs to be provided [33]).

In order to detect and measure unstable ocular dominance, significant SRA, abnormal interocular inhibitory pattern, as well as the effect of these variables on the reading performance of disabled readers, the TETRA Analyzer™ has been devised [4, 27, 28]. Evidently, the TETRA Analyzer™ is not intended for diagnosing developmental dyslexia. Instead, it aims at detecting those visuoperceptual abnormalities we suppose could help explain the reading difficulty in a proportion of patients (that we will therefore call "visual dyslexics").

Despite research and attention on developmental dyslexia is mostly focused on the pediatric population, many dyslexic children reach adolescence and then adulthood without being diagnosed [34]. In spite of this, so far relatively few effort has been made to investigate the traits of dyslexia in adults, and in particular if and (in case) how the visuoperceptive alterations reported in children persist in adult disabled readers.

It is therefore worthwhile to evaluate ocular dominance, spatial relationship anisotropy and the distribution of the interocular inhibitory pattern in this class of patients. As a first step, in our past study we have provided normative data examining a sample of normal adolescent and adult subjects (we will refer to as "mature readers" [28]).

To summarize the results of that paper:

- in mature readers the proportion of right dominants is slightly higher compared to left dominants. The great majority of the sample showed strong, stable dominance, in line with other surveys [e.g. 35, 36].

-SRA is lower in adult normal readers compared to children (we will refer to as “immature readers”). This finding suggests that spatial relationship perception along the x,y coordinates tends to balance out with the development of the visual system.

-In the adult sample binocular sensory input is overall balanced, with almost one third of subjects who did not show binocular input asymmetry. The distribution of the interocular inhibition shows a large positive skew, with the median inhibitory effect consistently lower than in children. In sum, compared to immature readers, in mature readers the binocular sensory processing is more balanced and the reciprocal inter-inhibitory effect is reduced.

-Finally, like in children, also in adults the reading rate is insensitive to changes of inter-letter spacing, confirming that fixation, parafoveal crowding, and interocular inhibition, if normal, do not affect the visual processing of words.

The aim of this paper is to examine the same parameters in a sample of adolescent and adult disabled readers, and to analyze the results in the light of our previous findings in mature [28] and immature readers [17].

2. MATERIAL AND METHODS

The exams making up the TETRA Analyzer™ have been already described [17, 27, 28, 37]. For detailed information the reader can refer to the aforementioned publications. In brief:

The Domitest M is a modified version of the pinhole test [38]. The observer is asked to look binocularly at a target displayed on a background through a hole in a cardboard placed in front of his/her face. The target is flanked at each side by a graduated scale. The degree of dominance lateralization (Value of Dominance) is expressed by the angular value the observers report when their dominant eye is occluded. By repeating the procedure 5 times dominance stability can be graded as stable, partly stable, and unstable.

The Eidomorphometry™ is a psychophysical test developed to measure spatial relationship perception (SRP). We define SRP as the function able to detect the difference in the extent of bidimensional shapes (ellipses) along the x/y cardinal coordinates. The test evaluates the SRP by estimating the discrimination threshold between circles and ellipses, with the eccentricity of the targets expressed as percent interaxis ratio (IR). The amount of the spatial relationship anisotropy (SRA) is computed as the difference between the discrimination threshold of horizontal ellipses (Horizontal Threshold, HT) and vertical ellipses (Vertical Threshold, VT), so that the higher is this difference (HT-VT), the higher the SRA. The effect of the resulting perceptual spatial contraction on the lexical string is illusorily reduced inter-letter spacing, thereby increased crowding between adjoining letters.

In the Domitest-S two streams of stimuli are presented dichoptically: within each sequence the null stimuli are checkerboard-like patterns, whereas the target is a checkerboard pattern whose matrices are arranged so as to form a “X”. The observer is asked time after time to report the target embedded in the left or in the right stream. The Imbalance Value (IBV, ranging from -1 to +1) quantifies the asymmetry between the left/right input based on the proportion of L/R correct responses. In turn, the Inhibitory Interocular Index (III, ranging from 0 to 2) depends on the overall proportion of correct responses, and quantifies the interocular inhibition.

Finally, as quoted in our previous study: “the Reading Performance Test (REPORT™) checks the effect of the three abovementioned variables on reading. Words and non-words samples are randomly presented at different values of inter-letter spacing (from 0.2 50 0.51 deg at a reading distance of 40 cm) and the reading rate as well as the number of errors is computed. The REPORT computes the correlation coefficient r between reading rate and inter-letter spacing” [28].

The visuoperceptive functions considered in this study and supposedly involved in the reading disability of adult dyslexics are summarized in Table 1 of our last paper [28]. For convenience the table is duplicated here:

Table 1. The parameters considered as potential markers of visuoperceptive impairment during reading

Visuoperceptive function	Related test	Marker
Spatial Relationship Perception	Eidomorphometry™	Abnormal Spatial Relationship Anisotropy [SRA]
Ocular Dominance	Domitest-M™	Unstable dominance Abnormal dominance lateralization
Binocular Sensory Input	Domitest-S™	Abnormal Imbalance Value [IBV] Increased Interocular Inhibitory Index [III]
Presumed involvement of the three variables in the lexical task	REPORT™	Positive correlation between reading rate and inter- letter spacing ($p<.05$)

2.1 Participants

Twenty-five young adult disabled readers (14 males, 11 females, 11-34 years, median 16 years) have been recruited from the Department of Ophthalmology, University of Turin. Participants were diagnosed as dyslexics at school age. At that time the diagnosis of dyslexia had been conducted according to its operational definition, 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, normal IQ and visual acuity, and no behavioral problems or auditory impairment [3].

All recruited subjects were not affected by ophthalmological or systemic diseases. In all cases BCVA vas ≥60/60 (Table 2).

Table 2. The recruited samples of adult dyslexic. Demographics

PAT NB	PAT	SEX	AGE	BCVA	REFR. RE	REFR. LE
1	E.F.	M	15	60/60	/	/
2	I.A.	F	15	60/60	/	/
3	A.S.	F	16	60/60	/	/
4	S.R.	F	16	60/60	-2,25	-2,50
5	G.L.	M	19	60/60	-0,75	-0,75
6	S.G.	F	14	60/60	/	/
7	N.E.	M	13	60/60	/	/

8	G.Z.	M	13	60/60	/	/
9	L.R.	F	21	60/60	/	/
10	M.O.	M	11	60/60	/	/
11	L.S.	M	17	60/60	-1	-1
12	I.A.	F	18	60/60	-4	-4
13	S.A.	F	15	60/60	/	/
14	C.A.	F	21	60/60	/	/
15	P.D.	M	19	60/60	/	/
16	B.F.	M	18	60/60	-4.25	-4.25
17	A.L.	F	16	90/60	/	/
18	S.S.	M	15	72/60	/	/
19	A.F.	M	12	60/60	-0,25	-0.75
20	L.G.	F	16	60/60	/	/
21	F.M.	M	11	60/60	/	/
22	F.M.M.	M	21	60/60	-2	-2
23	H.P.	M	34	60/60	-0,50	-1
24	M.M.	M	20	60/60	/	/
25	S.L.	F	14	60/60	-1.25	-1.25

**Refraction is spherical equivalent.*

In order to exclude a potential learning effect, each exam has been repeated after 30 minutes and data collected from the second administration have been considered and analyzed.

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.

3. RESULTS AND DISCUSSION

After data have been collected, Tukey's test [38] has been performed for each variable to detect multiple outliers.

3.1 Ocular dominance

The prevalence of stable dominance was 80%; the proportion of right motor dominants was higher compared to the left, being respectively 64% and 16%. The remaining 20% of subjects did not show any dominance laterality.

In the stable dominant dyslexics the degree of lateralization (median Value of Dominance) computed as an absolute value was 5.0 (IQR=2.0).

The frequency distribution of the value of dominance in the adult dyslexic population was normal (KS=0.16, P=.06: Fig. 1, left panel). The parametric distribution is even more evident as an absolute value

KS: 0.13, P>.10: Fig. 1, right panel). The median absolute value of dominance in the whole sample (non dominant subjects included) was 4.0 (IQR: 4.0).

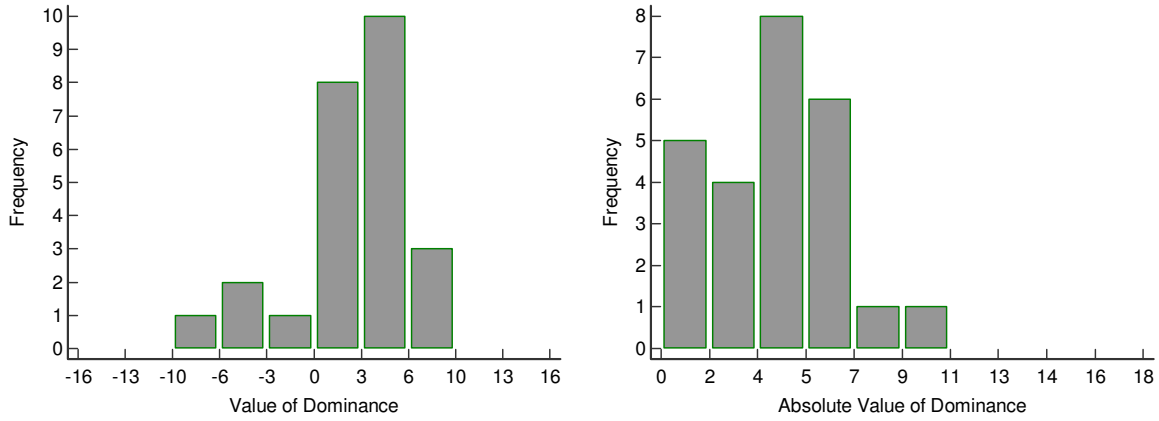


Fig. 1. Frequency distribution of dominance lateralization (value of dominance) in the adult dyslexic population

Left: negative values refer to left dominance, positive values express right dominance

3.2 Spatial relationship perception

Three observations were detected as outliers and removed. No correlation was found between horizontal threshold, vertical threshold or spatial relationship anisotropy and the age of the dyslexic subjects (HT: R^2= 0.03, P= .43; VT: R^2=0.009, P= .86; SRA: R^2=0.009, P= .65). SRP thresholds and anisotropy are reported in table 3 and depicted in Fig. 2.

Table 3. Summary statistics of HT, VT, and SRA in the adult dyslexics

Threshold	Mean	SD	Median	IQR
HT	7.09	±2.34	7	3
VT	3.48	±1.82	3	3
SRA	3.54	±2.77	3	5

From Table 3 it is evident that the average vertical threshold in the adult dyslexic population was lower compared to the horizontal threshold (paired t –test: $P < .001$), generating a small but significant anisotropy.

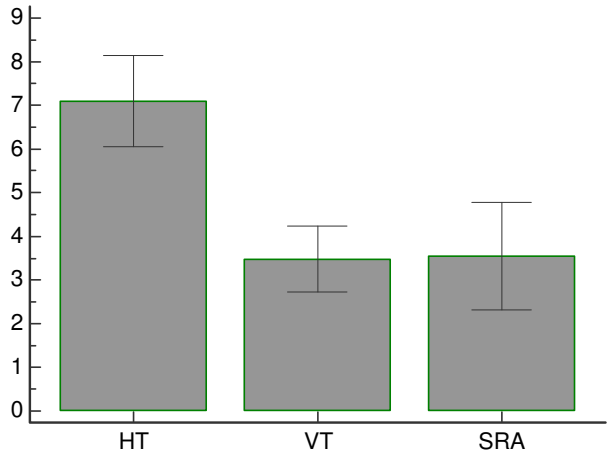


Fig. 2. Frequency Horizontal and vertical threshold, and SRP-related anisotropy in the sample of adult dyslexics. adult dyslexic population. Average values

Vertical axis: interaxis ratio (IR%). The bars refer to the confidence interval (CI 95%)

3.3 Binocular sensory interaction

In the adult dyslexic sample the sensory mismatch is directed more to the right than to the left, mimicking the distribution of the ocular dominance: subjects with higher detection frequency for stimuli presented to the right eye were, in fact, 13 (52%) vs 7 (28%) with better detection rate for stimuli presented to the left eye. The binocular sensory input was found perfectly balanced (IBV=0) in 20% of cases (5 subject out of 25). Twenty-eight per cent of the cases showed IBV equal or higher than 0.2.

The frequency distribution of the IBV (as absolute value) in the adult dyslexic population departed from normality (KS=0.33, P< .001: Fig. 3, left panel). The median absolute BV was 0.10 (IQR: 0.20).

The distribution of the reciprocal interocular inhibition as expressed by the III in the recruited adult dyslexic sample was bimodal, showing two clusters: one on the left, that is localized at a lower level of inhibition, and the other on the right (stronger inter-inhibitory effect: Fig. 3, right panel). Median III of the weak and strong inhibitory cluster was, respectively, 0.3 (IQR: 0.3) and 1.15 (IQR: 0.2).

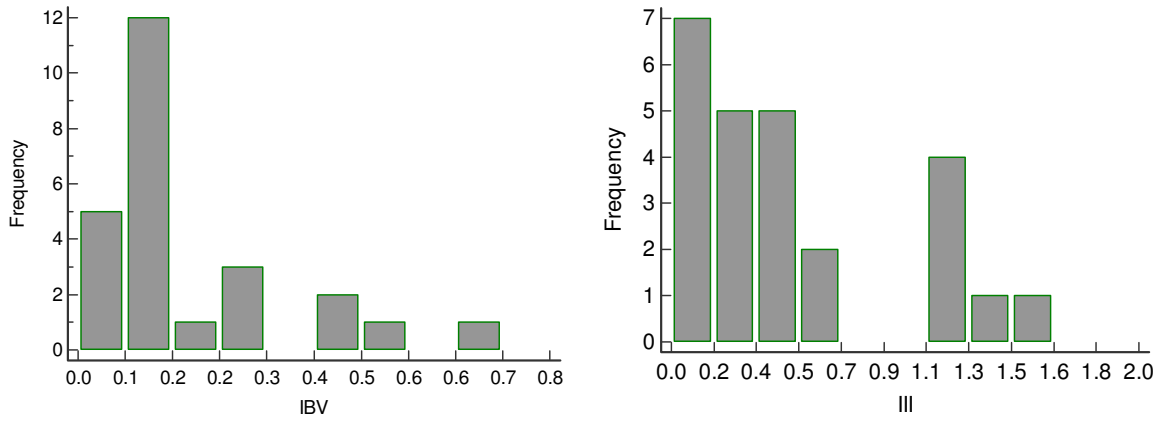


Fig. 3. Left: sensory balance (IBV) in the adult dyslexic population (absolute values). Right: distribution of the interocular inhibitory index

As in mature readers, in the adult dyslexic sample the interocular inhibitory effect does not correlate with age (R2= 0.003, P= .35).

3.4 Reading rate and inter-letter spacing

As expected, average reading rate was higher for words compared to non-words (4.23 syl/sec ±0.94 vs 2.16 syl/sec ±0.59: Fig. 4).

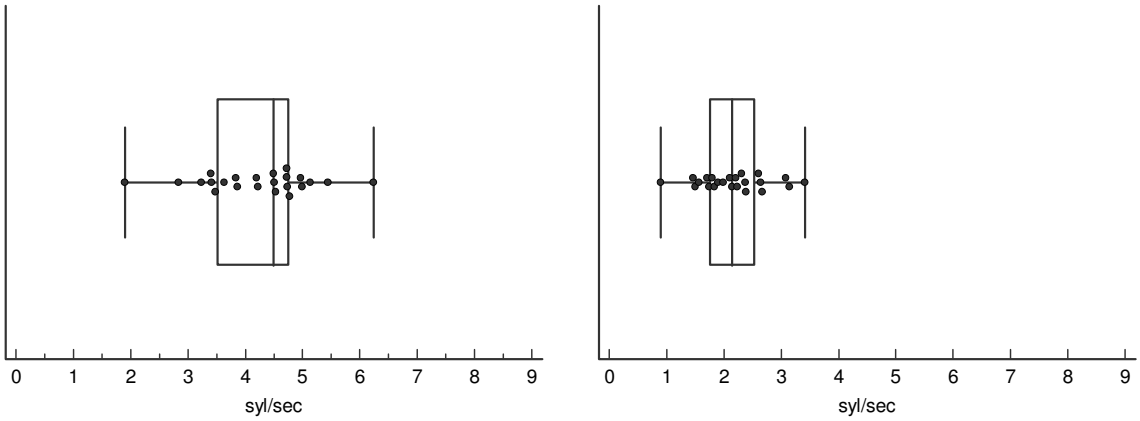


Fig. 4. Reading rate in the recruited sample of adult disabled readers. Box-and-whisker plots *Left: words. Right: non words*

No correlation was found between age and reading rate for words (R2= 0.004, P=.34) and non-words (R2= 0.08, P= .18).

To better understand the effect spacing has on the lexical fluency, the reading rate has been normalized by dividing the value measured at each inter-letter distance by the value measured at the reference spacing (0.4 deg). In the adult dyslexic population the normalized reading rate was insensitive to changes of inter-letter spacing when words were administered (R^2= 0.25, P= .11), whereas with non words the regression model was significant (R^2= 0.50, P= .01: Fig.5).

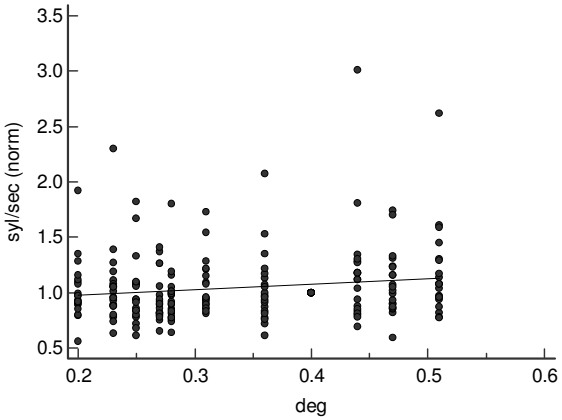


Fig. 5. Reading rate of non words as a function of inter-letter spacing in the adult dyslexic sample

3.5 Comparison with adult normal readers

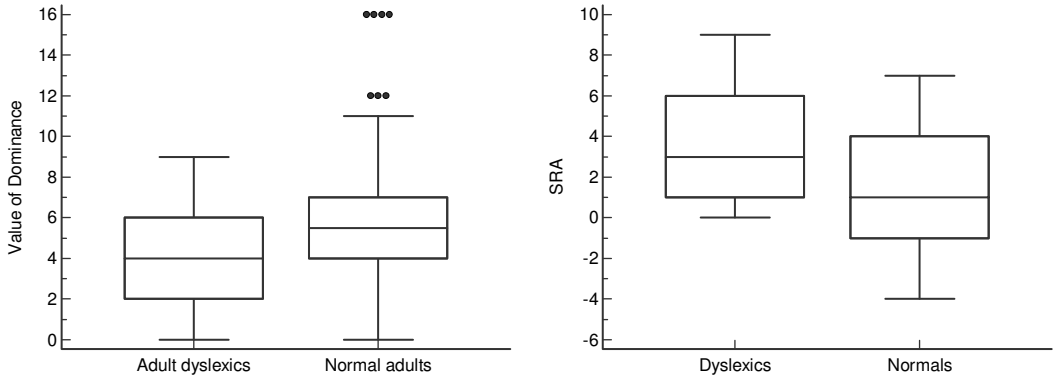
Compared to the adult ("mature") readers of our recent study [28], a higher proportion of unstable ocular dominants characterized the sample of adult dyslexics (20% vs 4%; Fisher's exact test: P< .001). In addition, in the pathological group ocular dominance was less lateralized (average Value of Dominance: 4.0 [IQR: 4.0] in patients vs 5.5 [IQR: 3,0] in controls: Mann-Whitney (U)= 825.00, P= .021: Fig. 6, upper left panel).

On the contrary, even if a greater proportion of dyslexics showed imbalanced binocular input (80% vs 69.2%), this difference was not significant (Fisher's exact test: P= .32).

Average spatial relationship anisotropy is up to threefold higher in patients compared to normal readers (3.54 vs 1.32 in normal adults as we found in our last study [28], or 1.13 as we reported in a previous experiment [37], Fig. 6, upper right panel). One-way ANOVA revealed significant between-group differences related to spatial relationship thresholds (F=33.6, P< .001). In particular HT and SRA was higher in adult dyslexics compared to controls (Tukey-Kramer: q(4.06) = 9.54 (P< .001), and q(4.06) = 5.37 (P< .01), while the sensitivity along the vertical axis did not differ in the two groups (Tukey-Kramer: q[4.06] = 3.97, P> .05).

A greater proportion of patients showed unbalanced binocular sensory interaction (28% vs 12.7%), even if this difference was not significant (Fisher's exact test: p=.07). As a confirmation of the same degree of binocular input asymmetry in the two samples, the median IBV was the identical..

The distribution of the Interocular Inhibition Index in the adult dyslexic population differs significantly from that of the normal readers, as rather than being skewed to the left (i.e. toward low interocular inhibition values) it is bimodal, with a group showing weak interocular suppression (weak III subpopulation), and a class with high Interocular Inhibition Index (high III subpopulation). The median interocular inhibition, indeed, turned out to be even lower in the normal readers than the median III of the weak III dyslexic subpopulation. However, probably due to the small size of the weak III subpopulation this finding lacks to achieve statistical significance Mann-Whitney (U)= 755.50, P= .42: Fig. 6, lower panels).



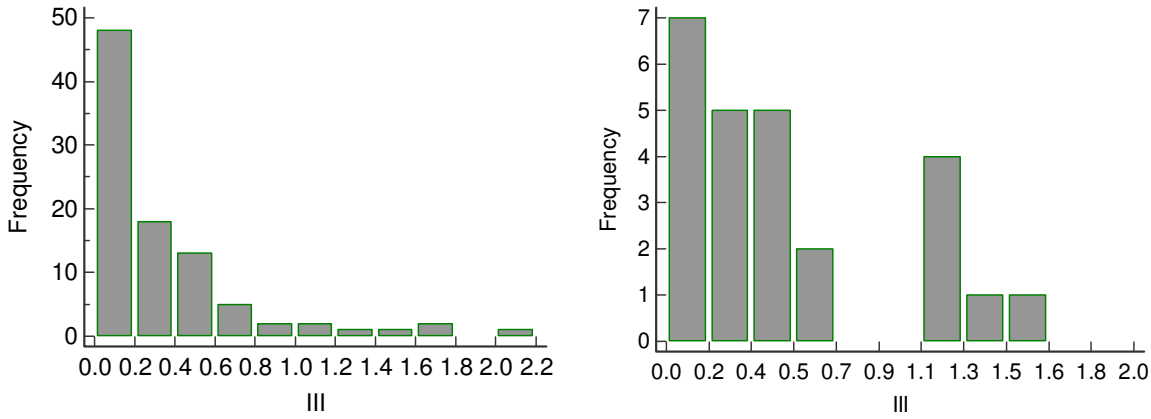


Fig. 6. Ocular dominance, spatial relationship perception, and interocular inhibition. Comparison between mature readers* (left) and adult dyslexics (right)

See text for explanations

**From Aleci et al, 2017*

Finally, mature readers were insensitive to inter-letter spacing from 0.2 to 0.51 deg width, whereas in dyslexic adults the reading rate improved as the distance between characters was made larger. This effect was evident when the reader had to make use of the sub-lexical (i.e. when non words were administered), whereas the trend was not significant when the lexical route could be recruited (i.e. when words were presented). Compensatory strategies involving the lexical route could account for this discrepancy.

3.6 Discussion

The presence (or persistence) of visuoperceptive alterations supposedly involved in reading disability as far as we know has not yet been investigated in adult dyslexics. Shaywitz et al in their Connecticut Longitudinal Study dating back 1999 evaluated not only phonological and academic skills, but also, to a certain extent, the visual spatial performance in a sample of 95 grade 9-12 subjects. In this survey the role of the visuospatial performance (limited to the Visuomotor Integration and Embedded Figure Test) in the reading disability in this age class was judged small [40].

Yet, the phonological factor may play a less important role in transparent languages, like Spanish, Portuguese or Italian), and in turn the visuoperceptive involvement could be more consistent in these cases compared to opaque languages like English. In this study such visuoperceptive involvement has been analyzed for the sensorial parameters that we consider representative causal factors of dyslexia.

According to our results, adult dyslexics do not seem to have overcome the perceptive problems reported to affects them since school age: it follows that the compensation of their disability, documented in many cases [e.g.41, 42] will probably rely on different, putatively non-visual strategies.

As a matter of fact, and in line with the previous literature (e.g. [19, 22, 25, 26]), unstable ocular dominance affects to a higher extent not only dyslexic children but also adult disabled readers: this suggests that abnormal fixation of letters and syllables due to this binocular alteration continues to hamper the lexical task even into adulthood.

In addition, the way adult dyslexics process the spatial relationships is anisotropic, as their sensibility to this function along the horizontal axis is lower in patients than in mature readers; in turn, discrimination threshold along the vertical axis remains roughly the same. In a previous study [17], we found a similar pattern in a pediatric sample (mean age 8.4 years), with HT and SRA higher in dyslexic children compared to age-matched controls (Fig. 7).

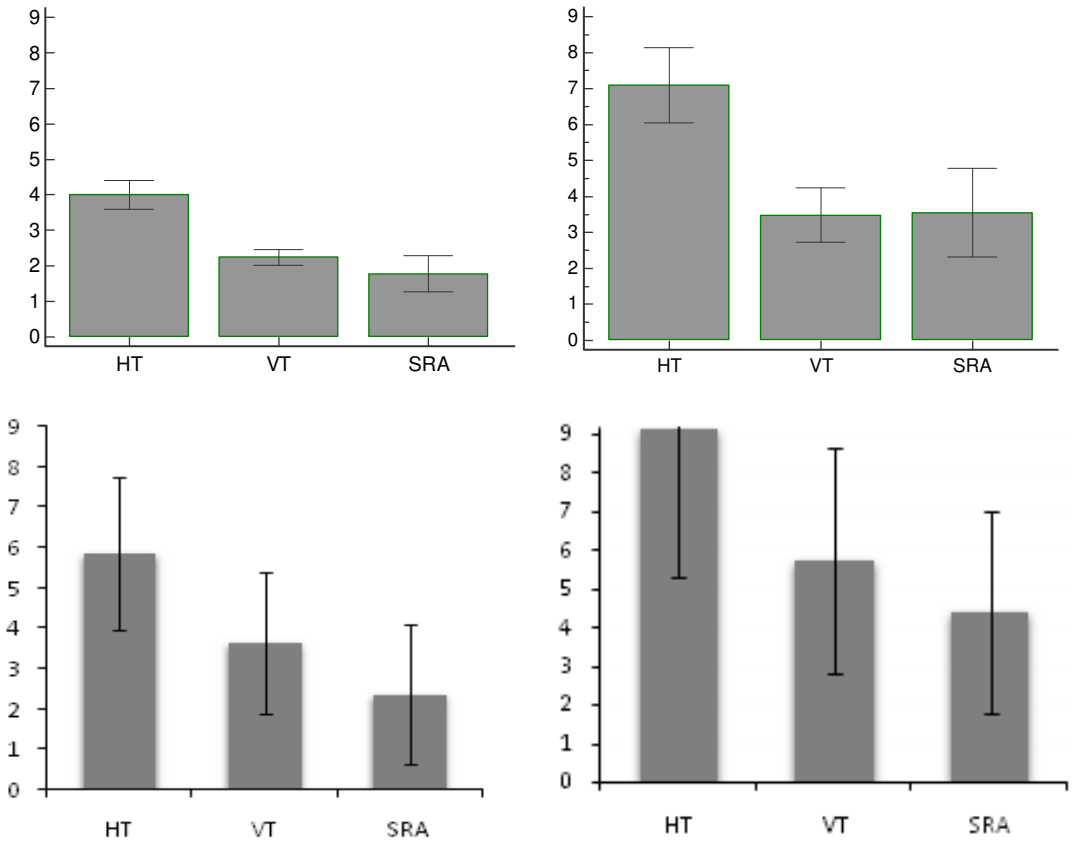


Fig. 7. Spatial relationship perception in mature readers[1] (upper left), adult dyslexics (upper right), normal and dyslexic children[2] (bottom left and right).

[1] *From Aleci et al, 2017 [28]*

[2] *From Aleci et al, 2012 [17]*

Taken together, these data suggest that spatial relationship perception, thereby lateral masking, tends to improve in adult normal subjects. Reduction of crowding in adult age, indeed, has been documented in literature ([42]. Interestingly, the value of anisotropy measured in this study in the sample of adult dyslexics is not statistically different from the same value we had previously estimated in immature readers [17] (Welch test: P= .41). This finding leads us to suppose that a developmental halt of this function takes place in disabled readers.

Finally, the interocular inhibitory pattern of adult dyslexics differs significantly from that of mature readers, as the latter shows a consistent proportion of subjects with interocular inhibition close to zero, whereas in the frequency distribution of the adult disabled readers two different clusters (weak and strong inhibition) are evident. This finding is in line with the results obtained with a coherent motion-based paradigm by Li et al [31]. In this respect, indeed, the frequency pattern of the adult dyslexic population is similar to the pattern of the immature readers. Interestingly, even if both clusters are still present in adult dyslexics, they peak at a lower interocular inhibition index compared to immature readers (0.3 and 1.15 vs 0.73 and 1.33).

Upon this basis we hypothesize that with the normal development of the visual system the interocular inhibition decreases, so that the cluster of strong interocular inhibition in immature readers tends to disappear in mature readers, while the remaining cluster of weak interocular inhibition tends to zero (see Fig. 6). In sum, this aspect, again, suggests incomplete maturation in the binocular interaction in the dyslexic population, with a consistent reciprocal inhibitory effect that persists into adulthood. (Fig 8).

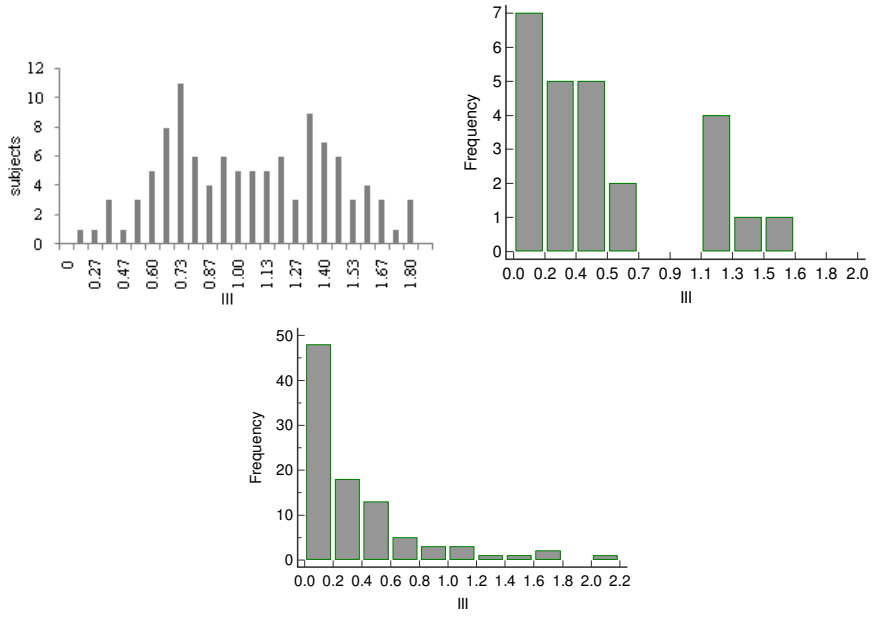


Fig. 8. S Comparison between the distribution of the interocular inhibitory pattern in immature readers[1], adult dyslexic subjects, and mature readers[2]

[1] *From Aleci et al, 2014 [27]*

[2] *From Aleci et al, 2017 [28]*

The effect of unstable ocular dominance, abnormal crowding, and increased interocular inhibitory interference, in isolation or combined to a various extent, would be revealed by the improvement of the reading rate as a function of the distance between characters. This correlation, indeed, was not present in the population of mature and immature readers, in which these three parameters were normal. Contrary to dyslexic children, adult patients were sensitive to changes of the inter-letter spacing only when non words were administered, whereas the effect did not take place with words. To account for this difference we hypothesize that in adults compensatory, phonological-based mechanisms may have occurred.

4. CONCLUSION

According to this study, developmental dyslexia in adulthood retains the defective visuoperceptive traits described in school age patients. The compensation of the reading disability in many adult subjects would therefore rely on different, higher-order mechanisms. Contrary to the phonological deficit, that proved to be resistant to intensive phonological rehabilitation administered during childhood [44], visual rehabilitation seems to provide evident results (e.g.: [21, 45]).

Undoubtedly a better comprehension of the visual dynamics involved in dyslexia and of the way such dynamics persist during the development of the individual will allow researchers to develop novel and effective rehabilitative strategies. The treatment of the visuoperceptive alterations, in turn, could enhance the abovementioned compensatory mechanisms in adolescence, eventually helping adult patients perform better in their academic skills.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

This study was approved by the University of Turin as part of a bachelor's thesis (ref n. 786927, registration November, 4st, 2016).

All authors hereby declare that the experiment has been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

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