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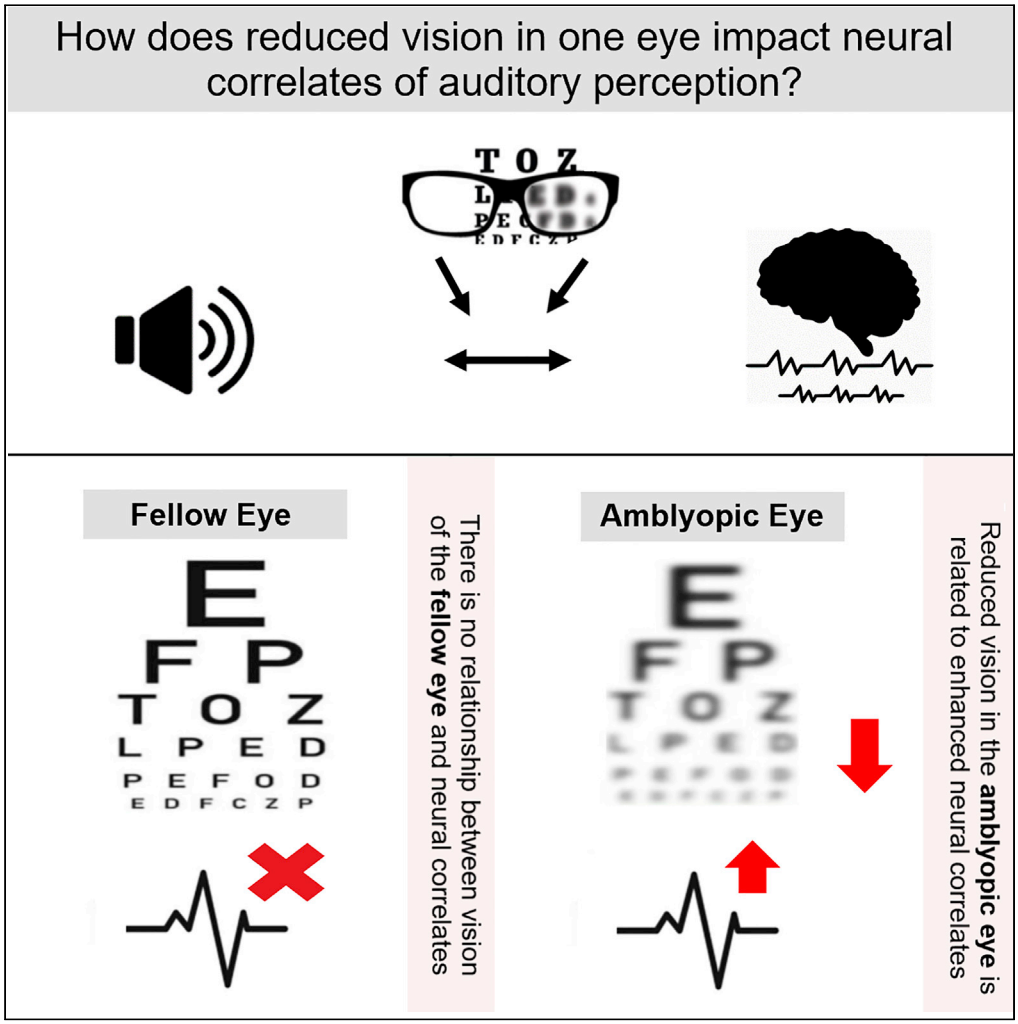

Matin Mortazavi, Kiera Aigner, Jessica E. Antono, Christina Gambacorta, Mor Nahum, Dennis M. Levi, Julia Föcker

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Highlights

Profound vision loss leads to enhanced neural signals in response to auditory stimuli

Individuals with amblyopia show enhanced auditory event-related potential (ERP) responses compared to controls

Enhanced auditory ERP responses correlated with reduced vision in the amblyopic eye

Neural plastic changes occur even after moderate vision loss in humans with amblyopia

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Intramodal cortical plastic changes after moderate visual impairment in human amblyopia

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SUMMARY

Early blindness results in alterations in the neural responses to auditory stimuli. Here we show that even moderately reduced vision in one eye early in life is sufficient to induce neural plastic changes in voice processing. We asked individuals with reduced visual acuity in one eye due to amblyopia to attend to vocal cues during electroencephalogram recording. We found enhanced frontal auditory responses at 125 ms–225 ms, which were correlated with reduced vision in the amblyopic eye, but not the fellow eye. Our results indicate intramodal reorganization, typically observed after congenital profound visual deprivation.

INTRODUCTION

The capacity of the human brain to reorganize has been observed after individuals faced prolonged periods of visual deprivation (see Fine and Park, 2018; Singh et al., 2018; for reviews). Extensive reorganization of the human brain has been documented in early blind and congenitally blind individuals who showed improved auditory localization skills compared to sighted individuals (Lessard et al., 1998; see Hötting and Röder, 2009 for a review), especially when auditory stimuli were presented at peripheral locations (Röder et al., 1999b; Topalidis et al., 2020; Voss et al., 2004). Other studies documented similar auditory localization skills in blind and sighted individuals when participants were asked to point to the auditory sounds with their index finger but worse performance in blind individuals when participants were instructed to verbally report the location of the sound, suggesting that an auditory deficit is present only for external auditory representation, but not for a body-centered representation (Vercillo et al., 2018; but see Amadeo et al., 2019 in auditory bisection tasks).

Studies using event-related potentials (ERPs) to investigate the time course of enhanced auditory processing in blind individuals showed increased and earlier activity in the time range of auditory N1 ERP component (100 ms post-onset of the auditory stimulus), as well as an advanced recovery of the auditory N1 amplitude in congenitally blind, compared to sighted individuals when auditory stimuli were presented successively (Röder et al., 1999a, 1999b; Topalidis et al., 2020). The auditory N1 is a negative potential with neural sources localized in several brain regions comprising the auditory cortex, temporal and parietal association cortices, and motor/premotor areas. Modulations of the auditory N1 have been interpreted as a reorganization of the auditory cortex in blind individuals as well as enhanced excitability of the auditory cortex which might result in their superior auditory skills. Tonotopic areas of the auditory cortex were enlarged and the N1 component had an earlier peak latency in blind individuals compared to sighted individuals (Elbert et al., 2002).

Recent findings provide further evidence for intramodal reorganization by reporting increased power in theta-to-beta frequency bands (4–30Hz) in the right auditory and frontal cortex of blind individuals which has also been related to their fast auditory processing and segmentation skills (Lubinus et al., 2021). To summarize, the studies outlined above document a variety of intramodal neural plastic changes after visual deprivation from birth in response to auditory stimulation, affecting the time course, the amplitude, and the frequency bands of electrophysiological signals (see also for further principles of neural plastic changes after visual sensory deprivation reported in Fine and Park, 2018).

Auditory processing after abnormal visual experience

Recent studies suggest that even a "short" period of visual sensory deprivation starting from birth (e.g., 9–294 days) enhances the processing of auditory information (De Heering et al., 2016). Those findings have

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been interpreted in line with the hypercompensation account, suggesting a superior performance in some cognitive and perceptual tasks in blind individuals compared to sighted controls by "sharpening" the remaining senses (Lewald, 2002). For instance, individuals born with dense cataracts removed within 10 months responded faster to auditory stimuli in a redundant task paradigm and showed shorter dwell times when the modality switched from visual to auditory, compared to switches from auditory to visual stimuli. This was interpreted as a higher attentional salience for simple auditory targets in cataract reversal individuals (De Heering et al., 2016). In another experiment, individuals with different severity of visual impairments were asked to localize their position in a dark room by using auditory cues (Després et al., 2005). Results indicated that individuals with amblyopia as well as late blind individuals performed better than sighted individuals. This could suggest that even those with mild visual impairment weighted the auditory cue information to a higher extent in this self-localization task compared to sighted individuals (Després et al., 2005).

Evidence for compensatory neural plastic mechanisms in individuals with amblyopia have also been documented by investigating resting-state brain activity. Whereas decreased similarity in brain activity (regional homogeneity; ReHo) was observed in subcortical and frontal brain areas, the increased similarity was also found in auditory areas such as the left superior temporal gyrus (Lin et al., 2012), possibly pointing to compensatory plasticity in amblyopia.

These findings suggest that even a short period of visual sensory deprivation can elicit compensatory changes in the auditory modality and that moderate vision loss can change the neural activation patterns of the auditory modality.

On the other hand, it might be argued that some functions depend on visual information, as vision is the leading sense and informs the other modalities about relevant principles, also known as the cross-sensory calibration hypothesis (Gori et al., 2008). According to this hypothesis, reduced vision in one eye might result in an "impaired" calibration process in which vision cannot inform the remaining modalities, and thus, auditory performance might be impaired in individuals with amblyopia. In line with this hypothesis, two recent experiments on auditory localization reported lower performance in individuals diagnosed with amblyopia compared to normally sighted controls (Richards et al., 2019a). Participants were either asked to indicate whether a second click occurred to the left or to the right relative to the first click (Experiment 1) or participants had to indicate the perceived direction of the sound source (Experiment 2). Results showed that the minimum audible difference was greater in individuals with amblyopia compared to neurotypical observers. Individuals with amblyopia had more sound localization errors compared to normally sighted controls. Several factors might account for these findings such as the nature or timing of the reduced vision (e.g. cataract versus strabismus or anisometropia). However, it could be also associated with the "response modality": Specifically, impaired visuomotor skills have been frequently reported in the literature in individuals with amblyopia (Grant et al., 2007; Niechwiej-Szwedo et al., 2011; Suttle et al., 2011). Thus, aligning a visual cursor with the perceived sound location might be a challenging task for individuals with amblyopia. Consequently, baseline differences in the ability to precisely indicate the location of even one specific sound source might be responsible for the observed group differences.

Enhanced attentional control after abnormal visual experience

Other studies have pointed to the fact that enhanced perceptual functions after visual sensory deprivation might be related to enhanced attentional skills in blind individuals (see also Collignon and De Volder, 2009, see Collignon et al., 2006; Kujala et al., 1997; for enhanced divided attention abilities). For instance, enhanced top-down attentional control functions were suggested in blind individuals (see also Collignon and De Volder, 2009, see Collignon et al., 2006; Kujala et al., 1997) referring to the ability to better ignore irrelevant distractions. In line with this assumption, Stevens et al. (2007) reported that the recorded preparatory functional brain activity to an auditory cue extracted from the medial occipital areas in blind individuals predicted their task performance. It might be speculated that early visual deprivation might also elicit the reorganization of brain networks including the fronto-parietal network, the connectivity to sensory brain areas as well as between sensory brain areas, such as auditory and occipital areas (Bavelier and Neville, 2002).

To summarize, whereas some studies have reported enhanced auditory processing abilities in individuals diagnosed with amblyopia, others reported impaired performance. Different factors, such as the nature and time of onset and duration of the visual deprivation, the experimental design, and response modality

likely contribute to those results. Furthermore, these findings suggest that whereas some systems show enhanced performance and thus provide evidence for compensatory changes in the intact modalities, other findings suggest that those systems which depend on visual information might lead to lower performance in individuals with reduced vision (Röder, 2012).

Audio-visual processing after abnormal visual experience

A few studies have investigated multisensory processing after visual sensory deprivation (see also Richards et al., 2019b for a review; Röder and Kekunnaya, 2021 for a review). Animal studies have shown that whereas the number of multisensory neurons in cats that were raised in complete darkness does not differ from sighted control animals, the response properties of those neurons differed between dark-reared and sighted control cats (Wallace et al., 2004). For instance, the principle of multisensory integration such as a higher neural response to multisensory stimuli compared to the sum of unisensory stimuli was not observed in dark-reared cats. Furthermore, the size of the receptive fields in visually deprived cats did not reduce as is usually observed in typical developing cats, but was similar to newborn cats (Wallace and Stein, 1997) which might lead to an immature response pattern of multisensory information. Correspondingly, brain imaging studies in humans have demonstrated a lack of multisensory integration in auditory regions in cataract reversal individuals whose cataract has been removed after 3–24 months of age: the blood-oxygenation-level-dependent (BOLD) response extracted from auditory regions, such as superior temporal areas was similar in response to audio-visual stimuli and auditory stimuli (Guerreiro et al., 2015). In the visual cortex, the BOLD response to visual stimuli was higher compared to audio-visual stimuli. The authors argue that the possible suppression of visual information might affect crossmodal integration in different brain areas. It has been suggested that irrelevant information from the "deprived retina" might be suppressed in order to reduce any "interference during auditory processing" (p. 1503, Guerreiro et al., 2015). These inhibitory interactions might explain the lack of multisensory integration in the auditory cortex or other brain areas. In line with the argument that congenital visual deprivation impairs multisensory interactions, another study reported that individuals whose cataracts were removed after the age of five months were more precise than normally sighted controls in reporting a specific color of a target, even when a distractor sound was presented (Putzar et al., 2007).

Deficits in multisensory integration in individuals with amblyopia have been reported in the context of audio-visual perception, such as the McGurk effect (Narinesingh et al., 2014) and the sound flash illusion (Narinesingh et al., 2017). The sound flash illusion is the misperception of the number of perceived light flashes when presented simultaneously with a number of sounds, e.g., one light flash which is accompanied by two sounds is usually perceived as two light flashes. A broader audio-visual integration window has been observed when testing the sound flash illusion in individuals with amblyopia (Narinesingh et al., 2017). Typically, the longer the time interval (SOA) between the auditory and the visual stimulus, the more reduced the perceived illusion in sighted individuals. However, in individuals with amblyopia, audio-visual integration was stable over time irrespective of the increased SOA duration, especially under binocular viewing conditions.

On the other hand, it has been shown that the *temporal* integration of auditory and visual events might be intact in human amblyopia, which was observed via the temporal ventriloquist effect (see Richards et al., 2018). In this task, participants have to detect the order of onsets of two light flashes. Auditory clicks which were presented 100 ms after the onset of the second light flash improved performance in both individuals with amblyopia and sighted controls as they "pull" the onset of the visual stimulus "forward in time" and thus increase the perceived time interval between the first and second light.

Taken together, it might be suggested that instead of a "primary multisensory deficit" to integrate audio-visual information, the abnormalities of multisensory processing in amblyopia might be related to "reduced temporal resolution in unisensory perception or in the mechanism for cross-modal matching (i.e., nonintegrative comparison of unisensory features)" (p.129, Richards et al., 2018 for further discussion).

Present study

It is yet not clear whether a period of profound sensory deprivation is a requirement for the induction of intramodal plastic changes. In other words, is a moderate reduction of visual acuity in one eye or the impaired binocular interactions between the two eyes during development, as occurs in amblyopia due to strabismus (turned eye) or anisometropia (unequal refractive error), also accompanied by the previously

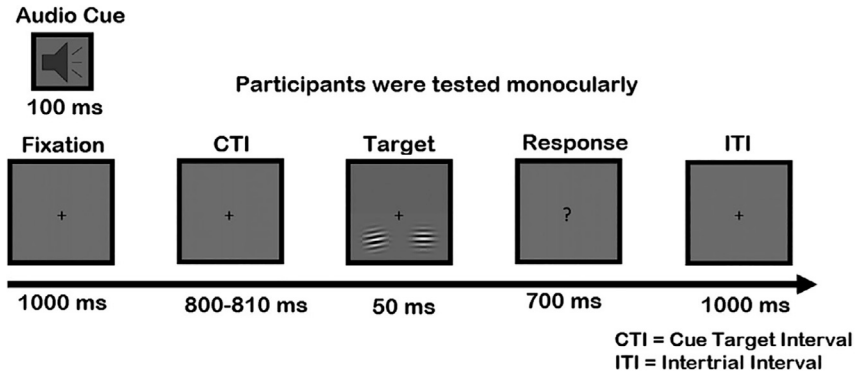

Figure 1. Experimental paradigm (Mortazavi et al., 2021); © 2020 The Authors
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reported cortical reorganizations observed in blind individuals or those with early cataracts? This is important because amblyopia due to strabismus and/or anisometropia is a very common cause of visual loss in infants and young children (Levi, 2020).

In the present study, we addressed this question by using the same dataset as reported in Mortazavi et al. (2021). In this experimental design, an auditory cue (vocal "left" vs "right") indicated that participants should focus their attention on either the right or the left side of the screen (see Figure 1). After an interval of 800–810 ms, two Gabor patches were presented which were oriented horizontally in most of the trials (80%). However, in 20% of the trials, one of the Gabor patches differed in orientation by its rotation of 5° to the right or to the left side. In half of these trials, those rare target Gabor patches occurred at the cued side (validly cued) whereas in the other half they occurred at the uncued side (invalidly cued). Individuals with amblyopia and neurotypical observers were asked to identify and respond to target Gabors at the cued location as fast and as correctly as possible while performing the task monocularly. The contrast of the visual Gabor stimuli was optimized for the amblyopic individuals to ensure a good response rate in this group.

In Mortazavi et al. (2021) we focused on the ERP responses to the visual Gabor patch stimuli. However, in the current study, the ERP responses to auditory cue stimuli were investigated. We compared auditory ERP activities with early and mid/later latencies, pertaining, respectively, to early auditory perception and higher cognitive functions and attentional orientation, between neurotypical observers and individuals with amblyopia. Furthermore, we examined the correlations between early neural responses and the visual acuity of the amblyopic and the fellow eye to understand whether reduced visual acuity is associated with higher neural responses, which could suggest intramodal compensatory neural plastic changes in the brain. The auditory N1 was investigated as an early predominantly sensory ERP component along with later ERP components, namely the P2 and Anterior Directing Negativity (ADAN), which represent post-perceptual processes and cognitive attentional mechanisms. The ADAN is an ERP component appearing 300–500 ms after the onset of the cue over fronto-central sites (Eimer et al., 2002). It typically reflects enhanced negativity to ERPs at electrodes contralateral to the attentional shift by the cue and originates from brain areas that belong to the fronto-parietal brain network, such as the lateral premotor cortex and the frontal eye fields. We used the ADAN component as the neural representation of the capacity of the participants to attend to possible target Gabor patches at the cued location and we also calculated the Attention Modulation index (AMI) as the behavioral measure for this capacity (see also Mishra et al., 2011; Treue and Maunsell, 1996). AMI was calculated as follow: AMI = (responses in validly cued trials - responses in invalidly cued trials)/(responses in validly cued trials + responses in invalidly cued trials). A positive AMI index indicates that participants focus their attention on the cued site while ignoring the Gabor patches at the non-indicated site (see similar calculations in Mishra et al., 2011; Treue and Maunsell, 1996). A larger AMI index also indicates the ability to suppress the irrelevant information at the non-indicated location, and thus indicates greater suppression ability. An AMI index of 1 indicates that participants have successfully ignored Gabor patches at the non-indicated site. Differences in ADAN and AMI between amblyopic individuals and neurotypical observers might reflect differences in attentional modulation in space.

Table 1. Descriptive statistics

Attention Modulation Index (AMI)	Mean	SE
Individuals with amblyopia		
Amblyopic eye	0.92	0.02
Fellow eye	0.95	0.02
Neurotypical observers		
Left eye	0.97	0.01
Right eye	0.98	0.01

Please note that these effects remain similar after excluding the 64 years old individual diagnosed with amblyopia and her 38 years old female neurotypical observer counterpart from the two groups. Main effect of *Eye*: $F(1,10) = 0.55$, $p = 0.472$, $\eta_p^2 = 0.053$; *Eye*Group*: $F(1,10) = .13$, $p = 0.718$, $\eta_p^2 = 0.014$; *Group*: $F(1,10) = 1.84$, $p = 0.204$, $\eta_p^2 = 0.156$.

RESULTS

Comparable attention modulation index in adults with amblyopia and neurotypical observers

The response rate was comparable between individuals with amblyopia and neurotypical observers (response rate: main effect of *Group*: $F(1,12) = 0.33$, $p = 0.573$, $\eta_p^2 = 0.027$; see also Mortazavi et al., 2021). To examine attentional modulation in both groups, AMI was calculated for each participant and each eye (see introduction for a description of AMI). Results indicate a comparable AMI index between the amblyopic observers and neurotypical observers and between both eyes suggesting similar performance in ignoring irrelevant Gabor patches at the non-indicated site (*Eye*: $F(1,12) = 1.18$, $p = 0.29$, $\eta_p^2 = 0.09$; *Eye*Group*: $F(1,12) = 0.52$, $p = 0.484$, $\eta_p^2 = 0.042$; *Group:* $F(1,12) = 2.39$, $p = 0.147$, $\eta_p^2 = 0.167$; see descriptive statistics presented in Table 1).

Enhanced frontal auditory event-related potentials in the time range of 125–225ms (N1) in individuals with amblyopia compared to neurotypical observers

Second, we analyzed whether individuals with amblyopia differed in neural responses to the auditory cue from neurotypical observers as has been previously found in congenitally blind individuals. The following ANOVA compared early auditory neural responses between individuals with amblyopia and neurotypical observers: *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Side* (contralateral activity versus ipsilateral activity), *Cluster* (anterior, central, posterior), and *Group* (amblyopes versus neurotypical observers) on the peak amplitude of the auditory N1 ERP component (defined as the most negative ERP peak in the time-range 125–225ms post-onset of the auditory cue). This analysis investigated whether an enhancement of early auditory neural responses, found previously in congenitally blind individuals, is also observed in individuals with amblyopia who, unlike the blind, experienced only a transient period of visual deprivation. As there is an imbalance of visual acuity between the amblyopic eye and the fellow eye, the factor Eye was also included in the ANOVA. ERP average waveforms had a higher peak amplitude of the auditory N1 in individuals with amblyopia compared to neurotypical observers at the central (Cz, C1, C2 electrodes) and anterior electrode clusters (AFz, AF3, AF4 electrodes), consistent with the typical central-anterior topography of this component in the ERP literature (Figure 2).

A significant *Group* by *Cluster* interaction effect indicated significantly larger peak amplitudes in the amblyopes ($M = -5.37$ μV, $SE = 0.87$) compared to the control sample ($M = -2.5$ μV, $SE = 0.87$) only in the anterior cluster ($F(2,24) = 6.1$, $p = 0.007$, $\eta_p^2 = 0.34$; post-hoc t-test: $t(12) = 2.55$, $p = 0.025$, d = 3.58; see Figure 2 anterior cluster; see Figure 3 central cluster). To investigate if this higher anterior auditory N1 in the amblyopic group is driven by a higher activity level when performing the task using their fellow/unaffected eye rather than the amblyopic eye, the auditory N1 from each eye of the amblyopes was separately contrasted against the N1 amplitudes of the neurotypical observers averaged across both left and right eye, using t-tests. N1 amplitudes were significantly higher in individuals with amblyopia than the control sample regardless of the eye with which they performed the task, indicating a generally higher auditory neural response in the amblyopes (amblyopic eye: $M = -4.28$ μV, $SE = 0.67$, $t(12) = 2.36$, $p = 0.036$; d = 2.29; fellow eye: $M = -4.63$ μV, $SE = 0.69$, $t(12) = 2.7$, $p = 0.02$; d = 2.71). Furthermore, the difference in auditory N1 amplitudes between the fellow eye and amblyopic eye was comparable to the difference between the left and right eyes in the neurotypical observers, as shown by a statistically non-significant *Eye* by *Group*

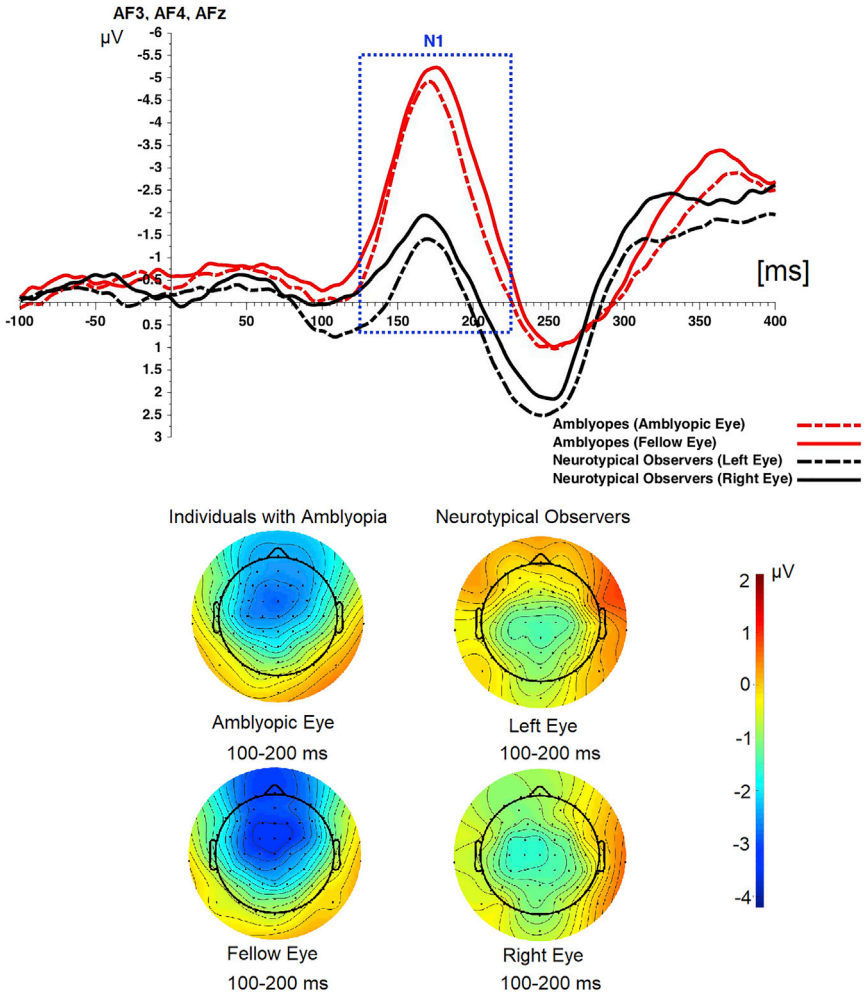

Figure 2. ERP average waveforms were recorded to the auditory cue when individuals with amblyopia were asked to perform the visual target detection task either with their amblyopic eye (red dashed line) or their fellow eye

Neurotypical observers performed the visual target detection either with their right or left eye (black dashed line: left eye, black solid line: right eye). An anterior cluster of electrodes (AF3, AF4, AFz) is shown. Scalp maps in the time range 100 to 200ms post-auditory cue presentation are shown below the waveforms.

interaction ($F(1, 12) = 0.85$, $p = 0.78$; $\eta_p^2 = 0.007$). The auditory N1 was found to be larger when performing the task using the left eye in both experimental groups (main effect of *Eye*: $F(1,12) = 5.85$, $p = 0.032$, $\eta_p^2 = 0.328$).

Our sample of amblyopic individuals included a 64-year-old participant. As aging is known to affect neural signals (Herrmann et al., 2016), we repeated all the statistical analyses on N1's peak amplitude, this time excluding this participant and her match among the neurotypical observers (38-year-old female), to investigate whether our experimental findings were substantially affected by the inclusion of this older participant. All the main experimental findings were replicated after excluding these participants. The same ANOVA was used again on N1 peak amplitudes with the independent factors *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Cluster* (anterior, central, posterior), *Group* (amblyopes versus neurotypical observers), and *Side* (contralateral activity versus ipsilateral activity). Like the ANOVA results from the entire sample reported in the article, a significant *Cluster* by *Group* interaction ($F(2, 20) = 3.99$, $p = 0.043$, $\eta p^2 = 0.6$) was found and the follow-up t-tests indicated an enhanced N1 amplitude for the amblyopes ($M = -5.78$ μV, $SE = 0.99$) compared to neurotypical observers ($M = -2.45$ μV, $SE = 0.89$; $t(10) = -2.5$, $p = 0.032$, d = 3.53).

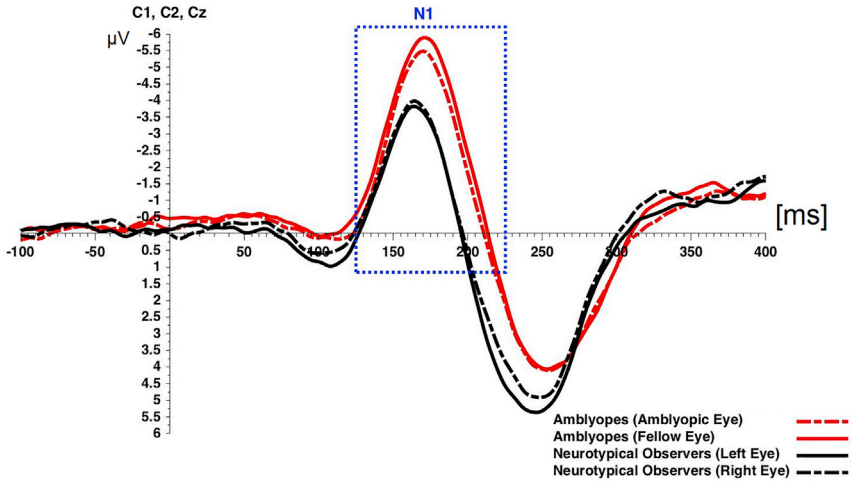

Figure 3. ERPs recorded to the auditory cue when individuals with amblyopia were asked to perform the visual target detection task either with their amblyopic eye (red dashed line) or their fellow eye (red solid line)

Neurotypical observers performed the visual target detection either with their right or left eye (black dashed line: left eye, black solid line: right eye). A central cluster of electrodes (C1, C2, Cz) is shown.

The enhanced auditory N1 is related to reduced vision in the amblyopic eye but not the fellow eye

A link between the enhanced auditory N1 amplitude found in the amblyopes and a poorer visual acuity in the amblyopic eye could serve as a signature of intramodal plastic changes. To investigate this link, a Spearman correlation was run separately for the amblyopic eye and the fellow eye. The results (Figure 4) indicate that increased neural auditory responses are associated with reduced visual acuity in the amblyopic eye ($r = -0.79$, $p = 0.048$, $N = 7$) but not in the fellow eye ($r = 0.2$, $p = 0.67$, $N = 7$). Furthermore, the significant correlation between lower visual acuity and higher N1 peak amplitudes in the amblyopic eye of the patients (r = -0.87, $p = 0.024$, $N = 6$) and the lack of such correlation in the fellow eye of the patients (r = 0.14, $p = 0.78$, $N = 6$) was also replicated in the patients when excluding the 64 years old participant.

Comparable P2 (200–300ms) in individuals with amblyopia and neurotypical observers

The P2 peak amplitude was measured in the time range 200–300ms post-onset of auditory cue using an anterior cluster of electrodes including AF7, AF8, AF3, AF4, F3, F4, F5, and F6 sites. The P2 amplitude was then submitted to an ANOVA with *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Group* (amblyopes versus neurotypical observers), and *Side* (contralateral activity versus ipsilateral activity) as independent variables. The results showed no significant main or interaction effects ($p > 0.1$), revealing a comparable P2 amplitude between the Amblyopes and Neurotypical observers (main effect of *Group*: $F(1, 12) = 0.9$, $p = 0.36$, $\eta_p^2 = 0.07$).

The anterior directing attention negativity is comparable between the individuals diagnosed with amblyopia and neurotypical observers

Beside AMI as a behavioral measure, we also examined attentional modulation in the two groups using auditory neural responses, namely the ADAN ERP component which usually occurs 300–500 ms after the onset of the cue over fronto-central sites (Figure 5). The ADAN is considered to be the voluntary initiation of attentional shifts within an anterior attention system in response to a cue (Nobre et al., 2000; Praamstra and Kourtis, 2010). The amplitude of the ADAN was measured as the mean amplitude of the average ERP waveform in the time range 500–700ms post-onset of the auditory cue. An anterior cluster of electrodes (consisting of F3, F4, F5, F6, AF3, AF4, AF7, AF8 sites) was used to quantify the ADAN, compatible with its typical topography. The ADAN amplitude was submitted to an ANOVA with the following independent variables: *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Group* (Amblyopes versus Neurotypical Observers), and *Side* (Contralateral activity versus Ipsilateral activity). No difference between amblyopes and neurotypical observers was observed (main effect of *Group*: $F(1,12) = 0.02$, $p = 0.886$, $\eta_p^2 = 0.002$; see Figure 5). Also, no group-related interaction terms were found ($p > 0.05$). These findings on

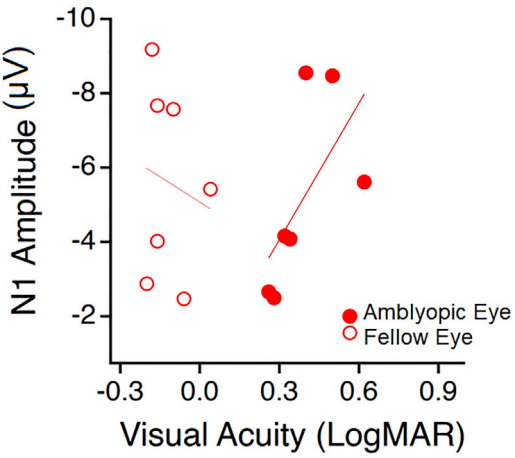

Figure 4. Correlations between peak auditory N1 amplitudes and the visual acuity (LogMAR)

Each point represents one eye from one amblyopic subject. Point and the regression lines are shown separately for the fellow eye and the amblyopic eye.

the neural activity underlying attentional direction mimic the behavioral results from the AMI index, indicating similar attentional modulation outcomes and processes between amblyopes and control subjects.

DISCUSSION

Our results suggest that a period of complete (or profound) visual sensory deprivation is not necessary to elicit intramodal neural plastic changes in the auditory system. We report significantly enhanced neural responses (N1) to auditory stimuli in humans with the common forms of amblyopia due to strabismus and/or anisometropia compared to neurotypical observers. Consistent with our findings, differential and advantageous auditory neural responses have been previously reported for patients diagnosed with dense bilateral cataracts who experienced a limited period of profound visual deprivation, some of whom received surgery only after three months of blindness (Guerreiro et al., 2016). In line with the *neural efficiency hypothesis*, Guerreiro and coauthors (2016) reported more efficient recruitment of auditory areas after the presentation of speech sounds in cataract reversal patients. Correspondingly, enhanced auditory motion processing possibly owing to intramodal plastic changes in the auditory cortex has been reported in individuals who experienced a period of profound visual deprivation from birth and whose cataract has been removed between 2 and 204 months of age (Bottari et al., 2018). Thus, neural changes seem to be maintained in individuals who regained their sight after a limited period of blindness (Guerreiro et al., 2016). This suggests that intramodal changes in the brain might not diminish with sight restoration but might rather be sustained. Here, we show for the first time, that similar intramodal changes are even observed in individuals with amblyopia, who never experienced any period of "blindness" or profound vision loss. Indeed, we showed that the enhanced neural responses to auditory stimuli were associated with reduced visual acuity in the amblyopic eye, but not the fellow eye. At the same time, we observed a comparable attentional modulation on the behavioral level (AMI) in the visual selective attention task between amblyopic individuals and control subjects, which corresponded to comparable levels of auditory neural response (ADAN) to attentional modulation in the two groups.

Cortical intramodal changes in amblyopia such as the ones observed in this study might be related to abnormal binocular interactions between the fellow eye and the amblyopic eye. Levi (2020) postulated that the lack of correlated binocular visual experience early in life may lead to suppression of the amblyopic eye by the fellow eye. Interestingly, studies have shown that suppression in visual area V2 is related to the depth of amblyopia (Bi et al., 2011). Thus, it might be argued that the increased suppression in the visual modality might, eventually, lead to an increased excitatory modulation in the other systems. Hubel and Wiesel (1970) demonstrated weaker and reduced excitatory connections from the amblyopic eye which lead to increased inhibition and changes in ocular dominance. However, it is unclear whether this increased inhibition in one sensory system, such as the visual modality, might lead to stronger excitation in the auditory modality. King et al. (1988) have shown shifts in the auditory maps after inducting strabismus, by deviating one eye. In a study by Rauschecker and Harris (1983), the superior colliculus elicited a more rigorous response in eyelid-sutured than in normal cats. Despite the previous evidence on increased neuronal responses to auditory information after different types of visual sensory deprivation, the causal or

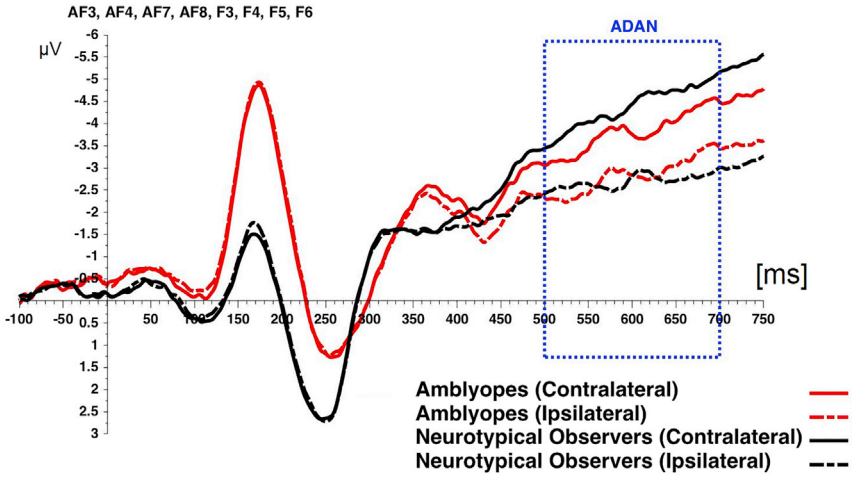

Figure 5. ERPs recorded to the onset of the auditory cue either contralateral or ipsilateral to the cued location separately for the individuals with amblyopia and the neurotypical observers

An anterior cluster of electrodes (AF3, AF4, AF7, AF8, F3, F4, F5, F6) is shown.

correlational role of increased suppression of the amblyopic eye in these neural changes remains to be investigated by future studies.

Recent studies on auditory localization performance in humans diagnosed with amblyopia argue against compensatory performance in the auditory modality (Richards et al., 2018, 2019a). These reported reduced auditory localization skills in individuals with amblyopia compared to sighted controls. The authors suggest that according to the cross-sensory recalibration mechanism, the visual modality is the most informative system which informs the other senses (see also Gori et al., 2008). Thus, vision might be the most informative sense for auditory localization tasks. However, in at least one of the studies participants were asked to use a visual cursor and align it with the perceived direction of the sound, which might be a more difficult task for individuals with amblyopia given their impaired performance in visually guided hand movements, as well as their impaired oculomotor skills (see Levi 2020 for an overview). Our findings on comparable attentional modulation between amblyopes and neurotypical observers, both on behavioral and neural levels, indicate that the enhanced neural signal to the auditory stimulus might not be related to increased attention orientation in individuals with amblyopia. Nevertheless, we cannot completely rule out the possibility that participants with amblyopia might attend to auditory features more than the neurotypical observers. Our findings on neural plastic changes associated with a moderate reduction in visual acuity in human amblyopia encourage further characterization of cortical reorganization following not only profound visual distortions but also moderate visual impairment, using other electrophysiological and imaging methods. How amblyopia impacts fronto-parietal top-down attentional networks in filtering and suppression of irrelevant information during intramodal tasks remains a central question of future research.

Limitation of the study

The statistical results were not corrected for multiple comparisons, due to the moderate size of our sample of amblyopic patients. However, we report effect sizes for all F- and t-tests, where our main finding on the higher N1 amplitudes in amblyopes compared to neurotypical observers show considerable effect sizes. Moreover, the enhanced N1 amplitude in the amblyopes is clearly reflected in the ERP average waveforms. Therefore, we do not think that the main findings are merely a product of type-I error. Further studies with larger sample sizes of amblyopic patients and more elaborate audio-visual attentional paradigms which require participants to also respond to auditory stimuli are needed to examine and build on our findings.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.104871.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.M.L, M.N., C.G., and J.F.; Methodology, D.M.L, M.N., C.G., and J.F; Formal Analysis, D.M.L., M.M., K.M.A., J.E.A., M.N., C.G., and J.F.; Investigation, M.N., and C.G.; Writing – Original Draft, D.M.L., M.M., K.M.A., M.N., C.G., and J.F.; Writing- Review & Editing, D.M.L., M.M., K.M.A., J.E.A., M.N., C.G., and J.F.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Software and algorithms		
EEG-Lab/ERPLab toolbox	Makeig et al., 2004; Lopez-Calderon and Luck, 2014	https://erpinfo.org/erplab
Matlab	Mathworks, Inc., Natick, MA	https://www.mathworks.com/products/matlab.html
R version 4.0.4	R Foundation for Statistical Computing	https://www.R-project.org/
RStudio	RStudio Team, 2020	http://www.rstudio.com/
SPSS version 27	SPSS Statistics for Windows, Version 27.0. Armonk, NY: IBM Corp	https://www.ibm.com/uk-en/analytics/spss-statistics-software

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Julia Föcker (Jfoecker@lincoln.ac.uk).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- This paper does not report original code.
- All data reported in this paper will be shared by the lead contact upon request.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Participants

Thirteen individuals with amblyopia and 10 controls participated in the behavioral experiments. Two subjects (one amblyopic and one neurotypical observer) were excluded due to exceptionally low behavioral performance (less than 30% hit rates). Of the remaining 21 subjects, seven data sets in individuals with amblyopia and seven data sets in neurotypical observers provided sufficient artifact free ERP data for the analyses, therefore we report the data of these 14 participants. The behavioral data in the excluded participants are similar to those of the 7 amblyopes and the 7 controls. The final sample included seven neurotypical observers (age range: 22–38 years, mean 28.7 years, SD: 7.03, one missing age information; one male, six females, 5 indicated a left dominant eye), and seven individuals diagnosed with amblyopia (age range: 27–64 years, mean: 40.8 years, SD: 12.9; one male, six females, mostly right-handed (six right-handed, one unknown). Similar sample sizes have been reported in Bottari et al. (2016) and Röder et al. (2013). Individuals diagnosed with amblyopia were recruited through the clinical coordinator at the School of Optometry Clinic at UC Berkeley, and through the Smith Kettlewell Eye Institute in San Francisco, CA. Neurotypical control subjects were recruited at the University of California, Berkeley.

Before the actual experiment started, all participants took part in an initial screening in the lab that included: LogMAR visual acuity (VA) and isolated VA, near VA, ophthalmoscopy, stereoacuity tests (randot circles and preschool stereotests), fixation- and worth 4-dot-tests. Patients had to manifest the following criteria in order to be included in the study: acuity in amblyopic eye between 20/30 and 20/400, and a minimum of 2-lines interocular difference in acuity with best correction, no ocular pathology or nystagmus, and 20/20 or better in the fixing (non-amblyopic) eye. Patients had to have at least one diopter difference between the two eyes to be categorized as an anisometrope. Patients with an eye-turn were classified as

strabismic, and those with both anisometropia and strabismus were classified as mixed. Most of the patients had anisometropia. Clinical details are listed in Table S1 (Table S2 includes demographics and descriptions in neurotypical observers, supplementary information). Inclusion criteria for neurotypical controls were: normal or corrected-to-normal visual acuity in both eyes (20/20 or better), no ocular pathology, and no previous treatment for amblyopia (see Table S1). Participants had no difficulty perceiving the auditory cue during the training and both groups showed validity effects, thus it was concluded that all participants were able to perceive the auditory cue.

Participants that fulfilled the criteria in assessment 1 came to the lab for a second assessment. In assessment 2 the patients' acuities and suppression were tested with greater precision using computerized algorithms, to measure letter acuity, grating acuity, stereoacuity, and suppression. Contrast sensitivity was measured with a 5 down 1 up staircase in order to determine the threshold for 90% contrast discrimination.

A hole in the card test was applied in order to measure eye dominance in the neurotypical observers.

METHOD DETAILS

General procedure

Auditory stimuli (= cue, female voice, duration 100 ms, mean intensity 86 dB) consisted of the recorded words *Left* or *Right* presented for a 100ms. The audio cues were presented from the computer's central loudspeaker at individually adjusted sound volume (dB was adjusted during the practice block if participants had difficulties hearing the cue properly and remained unchanged after starting the first experimental block). Participants were asked to attend to the auditory cue but not to make any motor response to it. The presentation of the auditory cue was followed by the presentation of two Gabor patches (see Mortazavi et al., 2021), separated by a cue target interval of 800–810 ms.

The temporal jitter of maximal 10ms after CTI was added, as a standard procedure while designing ERP experiments, to reduce overlap between ERP components of the pre- and post-CTI in addition to filtering out low-frequency activity (Luck, 2005). Since the fixation plus CTI period was relatively long in our experiment (1800ms), the probability of a significant overlap between audio ERPs and visual ERPs was lower and thus a shorter temporal jitter (10ms) was deemed sufficient. Moreover, this study focused on the auditory ERPs and these are elicited by the auditory cue at the beginning of the trials, while the CTI or visual ERPs occur at a later stage in a trial. Hence the early audio ERPs are not significantly affected by the length of the jitter at the end of the CTI or the visual ERPs.

Participants were asked to respond to rare visual target Gabor patches as fast and as correct as possible by pressing a specific button with their right index finger (p. 1091, Mortazavi et al., 2021). The target Gabor patch had a slightly different orientation from the more frequently presented non-target Gabor patches (they were tilted 5 degrees to the clockwise and 5 degrees counterclockwise). Target Gabor patches appeared in 20% of the trials. Target Gabor patches at the cued target location were defined as validly cued target trials and Target Gabor patches which are presented at the uncued location were defined as invalidly cued target trials.

The participants, who were instructed to perform as many trials as possible (to maximize the signal/noise ratio for the ERP averaging process), performed on average more than 300 trials in 5 experimental blocks with each eye (M = 316.07, SE = 12.03 trials). Target and non-target (standard) trials were randomized in each block and the right and left auditory cues were randomly interleaved within a block.

QUANTIFICATION AND STATISTICAL ANALYSIS

EEG data acquisition and analysis

Scalp potentials were recorded from 66 electrodes using a BioSemi system (BioSemi, Amsterdam, The Netherlands) and the electrode labels approximated those of the 10–20 system. Scalp and mastoid electrode impedances were maintained below 10Ω. Scalp potentials were referenced to the Cz channel during recording. The recorded scalp activity was amplified with a band pass of 0.1–80 Hz. Signals were digitized at a sampling rate of 200 Hz with a gain of 10,000.

EEG data was analyzed in Matlab (Mathworks, Inc., Natick, MA) using EEG-Lab/ERPLab toolbox (Makeig et al., 2004; Lopez-Calderon and Luck, 2014). The data was first digitally re-referenced to linked mastoids and then bandpass filtered offline with a half amplitude cutoff of 0.1–40Hz (non-causal Butterworth impulse response function, −6 dB/octave).

An epoch consisting of 800ms of activity starting from the onset of the auditory cue was used to study ERP components. The epochs included a 200ms baseline activity and were all baseline corrected using the mean voltage over 200ms pre-event period. Epochs were not used in further analyses if they included a motor response.

The EEG data was screened for noisy channels and were inspected to find artefactual data segments using both visual inspection and automatic artifact detection algorithms. Noisy channels were detected by eye using the channel data scroll and were manually interpolated (replaced by the average of the six nearest spatial neighbor electrodes). Epochs which met any of the following criteria were marked as artefactual: 1) any voltage exceeding $\pm$ 75μv, 2) a difference between two consecutive data points exceeding 50μv, 3) Epochs with ocular artefacts detected by applying the step-like artefact rejection function (window size: 400 ms, step-size: 50 ms, threshold: 20μv) of ERPLab toolbox (Makeig et al., 2004) to the activity of the two fronto-polar channels (FP1 and F1). Follow-up careful visual inspection of epochs after the artifact rejection procedure ensured the absence of artifacts. Participants with many interpolated noisy channels or less than 100 artifact-free trials in each experimental condition were deemed to have low signal to noise ratio and were thus excluded from further ERP and statistical analyses (7 subjects). All the other 14 participants included in this report (7 individuals with amblyopia and 7 neurotypical observers) had enough artifact-free trials (150 on average) in each eye to be able to quantify early and late latency ERP components with high signal-to-noise ratio. The epochs were then averaged for each participant and for each eye. These average activities were used to quantify and study the ERP components of interest.

Visual inspection of a collapsed-localizer as well the grand average (GA) waveforms determined the appropriate time-windows to study the ERP components. A negative ERP component, with main scalp distribution in the anterior and central electrode sites, resembling that of the well-characterized anterior N1 component, was studied using a peak amplitude measure, defined as the most negative ERP peak in the time-range 125–225ms post-onset of the auditory cue. The N1 was quantified for three anterior (AFz, AF3, AF4 electrodes), central (Cz, C1, C2 electrodes), and posterior (Pz, P1, P2 electrodes) clusters, all of which were later incorporated in an ANOVA to better characterize the main scalp distribution of this ERP component in our cohort. The anterior directing attention negativity (ADAN) ERP component was quantified using a mean amplitude measure in the time-window 500–700ms post-cue onset, in an anterior cluster including electrodes sites F3, F4, F5, F6, AF3, AF4, AF7, AF8. The P2 component was studied using a peak-amplitude measure, defined as the most positive peak-amplitude in the time-window 100–200ms post-onset of auditory cue using anterior cluster of electrodes including AF7, AF8, AF3, AF4, F3, F4, F5, and F6 sites.

The peak amplitude of N1 component was submitted to an ANOVA with the following factors: *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Side* (contralateral activity versus ipsilateral activity), *Cluster* (anterior, central, posterior) and *Group* (amblyopes versus neurotypical observers). The cluster factor was incorporated to better capture the topographical dynamics of this early ERP component in our experiment, as early short latency ERP component tend to express variable topography maps depending on the experimental paradigm and the task at hand. The P2 amplitude was submitted to an ANOVA with *Eye* (amblyopic eye versus fellow eye; left eye versus right eye), *Group* (amblyopes versus neurotypical observers), and *Side* (contralateral activity versus ipsilateral activity) as independent variables.

The ADAN amplitude was evaluated using an ANOVA with the factors: *Eye* (amblyopic eye versus fellow eye, left eye versus right eye), *Group* (amblyopes versus neurotypical observers), and *Side* (contralateral activity versus ipsilateral activity).

Behavioural data analysis

The response rate for validly cued trials was calculated as the responses to target Gabor patches at the validly cued location divided by the total number of target Gabor patches presented at the validly cued location (see also Mortazavi et al., 2021). The same procedure was also used for invalidly cued

trials: Responses to the orientation of target Gabor patches presented at the uncued (invalid) location were divided by the total number of targets at the uncued location. An ANOVA was run including the factors *Eye* (left eye in neurotypical observers/amblyopic eye in patients vs. right eye in neurotypical observers/fellow eye in patients), *Cue* (validly cued vs. invalidly Gabor patches) and *Group* (Neurotypical Observers vs. individuals with amblyopia) using response rates as a dependent variable (see also Mortazavi et al., 2021 for further details).

The response rates for validly cued and invalidly cued locations were used to calculate the Attention Modulation Index (AMI) separately for each eye in each group: AMI = (responses in validly cued trials - responses in invalidly cued trials)/(responses in validly cued trials + responses in invalidly cued trials). A positive AMI index indicates that participants correctly filter out information at the uncued location (see similar calculations in Mishra et al., 2011; Treue and Maunsell, 1996).

An ANOVA was run including the factors *Eye* (left eye in neurotypical observers/amblyopic eye in patients vs. right eye in neurotypical observers/fellow eye in patients), and *Group* (neurotypical observers vs. individuals with amblyopia) using the AMI index as a dependent variable.

All statistical analyses in this study were performed using the software packages R (version 4.0.4 in RStudio) and SPSS (version 27).