Effects of choir singing experience on neural encoding of sounds in the ageing brain

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Abstract

Several studies have shown that cognitive impairment related to ageing is reflected in changes in auditory event-related potential (ERP) responses (P1, N1) and mismatch negativity (MMN). As well, musical activity, especially a professional one, has been observed to have an effect, and it has been hypothesized that musical engagement may protect the brain from the effects of ageing. The aim of this cross-sectional study was to examine whether a long-term choir singing pursuit could be reflected on the processing of general (P1, N1) or change-related auditory encoding (MMN) in seniors over 60 years of age (N = 54). As a stimuli sinusoidal sounds were used. Deviant stimuli differed from the standard either in their frequency (pitch) or spatial location. Based on previous literature it was hypothesized that the effect of choir singing experience against possible cognitive decline would be seen as decreased P1 and increased N1 amplitudes and, strengthened MMN responses relative to controls. ANOVA analysis revealed a statistically significant interaction between the group and the ERP response for spatial deviants deviating 36° or 72° degrees from the standard tone source on P1 component of the ERP signal. Against the hypothesis, P1 response of choir singers was found to be higher compared to controls with medium effect sizes in all the studied regions of interest (frontal, central or parietal). However, otherwise the groups did not differ significantly from each other. As a conclusion, with the current study design, it was not possible to demonstrate an effect of musical pursuit in the group of choir singers compared to controls in line with the hypothesis. This could possibly be due to the fact that choir singers of the study were a rather heterogeneous group in their musical skills and instead of a profession, music was a hobby for the subjects weakening the possible effect in the group. No effects were observed when groups' ERP responses were examined in relation to age. In a *post hoc* regression analysis it was found that in the group of choir singers longer choir singing experience (in years) was related to better pitch discrimination, i.e., higher MMN response for pitch deviants differing one or two tempered whole tones from the standard tone. The fit of the model was best in central ROI (region of interest). Such an association was not found with easier pitch deviants or with spatial deviants. This suggests that long-term choir singing possible has an impact on auditory pitch change-related information.

Keywords: choir singing, senior adult, ERP, P1, N1, MMN, pitch, location

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Tiivistelmä

Monissa tutkimuksissa on osoitettu, että ikääntymiseen liittyvä kognitiivinen heikkeneminen heijastuu auditoristen tapahtumasidonnaisten herätepotentiaalien (ERP) P1- ja N1-vasteiden sekä poikkeavuusnegatiivisuuden (MMN) muutoksiin. Niin ikään musiikillisen aktiivisuuden, erityisesti ammattimaisen muusikin harjoittamisen, on osoitettu heijastuvan näihin vasteisiin. On esitetty hypoteeseja, että musiikilliseen toimintaan osallistuminen (esim. soittaminen, laulaminen) suojaisi aivoja ikääntymisen vaikutuksilta. Tämän poikkileikkaustutkimuksen tarkoituksena oli tutkia, voisiko pitkäaikainen kuorolauluharrastus heijastua yleisiin (P1, N1) tai muutokseen liittyviin (MMN) auditorisiin herätevasteisiin yli 60-vuotiailla henkilöillä (N = 54). Tutkimuksessa käytetyt ärsykkeet olivat sinimuotoisia ääniä. Poikkeavat ärsykkeet erosivat standardiäänistä taajuudellaan tai sijainniltaan. Perustuen aikaisempiin tutkimuksiin oletettiin, että kuorolauluharrastus suojaa henkilöitä ikääntymiseen liittyvältä kognitiiviselta heikkenemiseltä heijastuen kuoroharrastajien ryhmässä matalampina P1- ja suurempina N1-vasteina, sekä vahvistuneina MMN-vasteina kontrolliryhmään verrattuna. Tilastollisesti merkitsevä vuorovaikutus ryhmän ja ERP-vasteen välillä voitiin ANOVA-analyysissä havaita P1-vasteessa sijainniltaan 36° ja 72° standardista poikkeavien äänten kohdalla. Tutkimushypoteesin vastaisesti kuorolaulajien ryhmässä P1-vaste oli suurempi kuin kontrolliryhmässä; efektikoko oli keskikokoinen kaikilla kolmella tarkastellulla aivoalueella (frontaali-, keski- tai parietaalialue). Muuten ryhmät eivät eronneet toisistaan merkittävästi. Tutkimuksen avulla ei pystytty osoittamaan oletushypoteesin mukaista vaikutusta. Tämä voi selittyä sillä, että tutkimukseen osallistuneet kuorolaulajat olivat taidoiltaan heterogeeninen, ammattilaisten sijasta harrastajista koostuva ryhmä, mikä heikentää ryhmän mahdollista vaikutusta ammattilaisista koostuvaan ryhmään verrattuna. Mitään vaikutusta ei havaittu, kun ryhmien ERP-vasteita tutkittiin ikäryhmittäin (nuoremmat/vanhemman seniorit). Post hoc -regressio-analyysissä havaittiin, että mitä pitkäkestoisempi kuoroharrastus koehenkilöllä oli, sitä parempi oli erotuskyky standardiäänestä yhden tai kahden kokosävelaskeleen poikkeamille (suurempi MMN-vaste). Malli toimi parhaiten keskimmäisessä tutkituista aivoalueista (ROI C). Vastaavaa ei havaittu helpompien, taajuudeltaan tai sijainniltaan standardista poikkeavien äänten kohdalla. Tämä antaa viitteitä siitä, että pitkäaikainen kuorolauluharrastus voi mahdollisesti vaikuttaa auditoriseen sävelkorkeuden muutokseen liittyvän informaation prosessointiin.

Avainsanat: kuorolaulu, seniori, ikääntyminen, ERP, P1, N1, MMN,

sävelkorkeus, äänen sijainti

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1. Introduction

1.1 General introduction

The brain is able to adapt throughout life to the stimuli it receives. Studies have shown that music practice affects the macroscopic structure of the brain (Pantev et al., 2001). Changes can be observed in the cortices that process auditory, tactile, and motor information. For example, changes caused by musical experience can be observed at many stages of the auditory pathway from the brainstem to the primary and surrounding auditory cortices as well to areas involved in higher-level auditory cognition (Herholz & Zatorre, 2012). A classic example relates to structural changes in the motor cortex: neurons that have lost their regular input (e.g., because of an injury to a finger used to play an instrument) become recruited by neighboring brain areas (processing information from the other fingers) (Pantev, 2001). Even a short-term musical practice changes the processing of auditory, tactile, and motor information (Herholz & Zatorre, 2012; Seppänen et al., 2012). Moreover, it has been found that such musical practice is reflected in enhancement of other cognitive processes of the brain, e.g., processing speech in noise (SIN), auditory working memory and auditory attention (Parbery-Clark et al., 2011). The research question in this study is constructed based on the above: because instrumental or vocal musical professions have been found to improve cognitive performance in the ageing brain (O'Brien et al., 2015), could a longtime persisting musical pursuit in senior citizens, such as choir singing, as hobby, also be reflected in the processing of auditory information and the automatic ability to learn and discriminate music-related information. Because cognitive function of the brain is not immune to the degenerative effects brought on by age (e.g., Hedden & Gabrieli, 2004), the potential effects of the musical pursuit in seniors should be viewed against possible age-induced cognitive changes. To investigate the matter, senior choir singers with substantial long choir singing background were compared to a corresponding group of non-musically active control seniors for their cognitive information processing. Methodologically, the focus was set on the possible groupwise differences in subjects' general auditory information processing and automatic ability to learn and discriminate music-related information studied as event-related potentials (ERPs) analyzed from electroencephalography (EEG) data.

1.2 General effects of ageing on cognitive and neural processes

Ageing affects the structure and function of different parts of the brain in different ways. Both cross-sectional and longitudinal studies have shown that many cognitive abilities decline with normal ageing. In longitudinal studies of healthy subjects, only little reduction in cognition (behavior) has been observed before the age of 60 years (Hedden & Gabrieli, 2004). On the other hand, in cross-sectional studies, a linear decrease has been observed throughout lifespan. Decrease has been observed, for example, in the coding of new episodic memories, in executive functions such as working memory, in processing speed, and in sensory processing. In turn, short-term memory, autobiographical memory, and emotional processing have been found to remain relatively stable, or in case of semantic memory, even increased. (Hedden & Gabrieli, 2004; Reuter-Lorenz & Park, 2010).

The change in cognitive performance in normal ageing has been suggested to be due to atrophy of brain tissue and a change in neural signal transmission, leading to reorganization of neural connections and compensatory activity in the brain (Reuter-Lorenz & Park, 2010). However, it is challenging for researchers to really make correct interpretations on age-related cognitive changes. In the first place, one has to face how it is possible to distinguish processes of normal ageing from pathological processes. Next, because age cannot be experimentally manipulated, conclusions regarding the effects on ageing are by default correlational. In addition, studies are often cross-sectional comparisons between groups rather than longitudinal studies, so the interpretations are indirect, although trend in this respect is changing. And last, changes in the brain and mental abilities are age-parallel, making it difficult to associate certain brain changes with certain mental changes (Hedden & Gabrieli, 2004).

Many theories based on neural functions have been proposed for cognitive ageing. One of the most integrative theories is the Scaffolding Theory of Ageing and Cognition (STAC; Park & Reuter-Lorenz, 2009; Reuter-Lorenz & Park, 2010). The aim of the STAC model is to combine the key findings of cognitive neuroscience of ageing and to address both neurocognitive impairment and neuroplasticity in the ageing brain. Figure 1 shows the conceptual model of the STAC, as presented originally by Park & Reuter-Lorenz (2009).

According to STAC, the brain has to adapt to a variety of neural challenges posed by ageing, including amyloid deposition, atrophy, and depletion of white matter and dopamine receptors. In connection with these changes, functional changes also occur in the brain, e.g., dedifferentiation, in which functional specificity of brain areas is decreased, dysregulation of the default network, and decreased activation of medial temporal lobe (MTL) structures. Due to these changes, the brain begins to utilize alternative neural circuits (scaffolds), which may not be as effective as the neural networks in young adulthood, but which, however, allow individuals to maintain higher levels of cognitive function in older age. Such changes include overactivation patterns, mainly in the prefrontal cortex, but also in parietal, mediotemporal, and occipital areas. Although the adaptive neuroplasticity afforded by neurogenesis, synaptogenesis, and angiogenesis decreases with ageing, the model suggests that all mechanisms remain functional and provide credible means to construct alternative neural circuits. Scaffolding mechanism protects cognitive function in the ageing brain, and there is evidence that cognitive engagement and training strengthen the ability to use the mechanism (Reuter-Lorenz & Park, 2010).

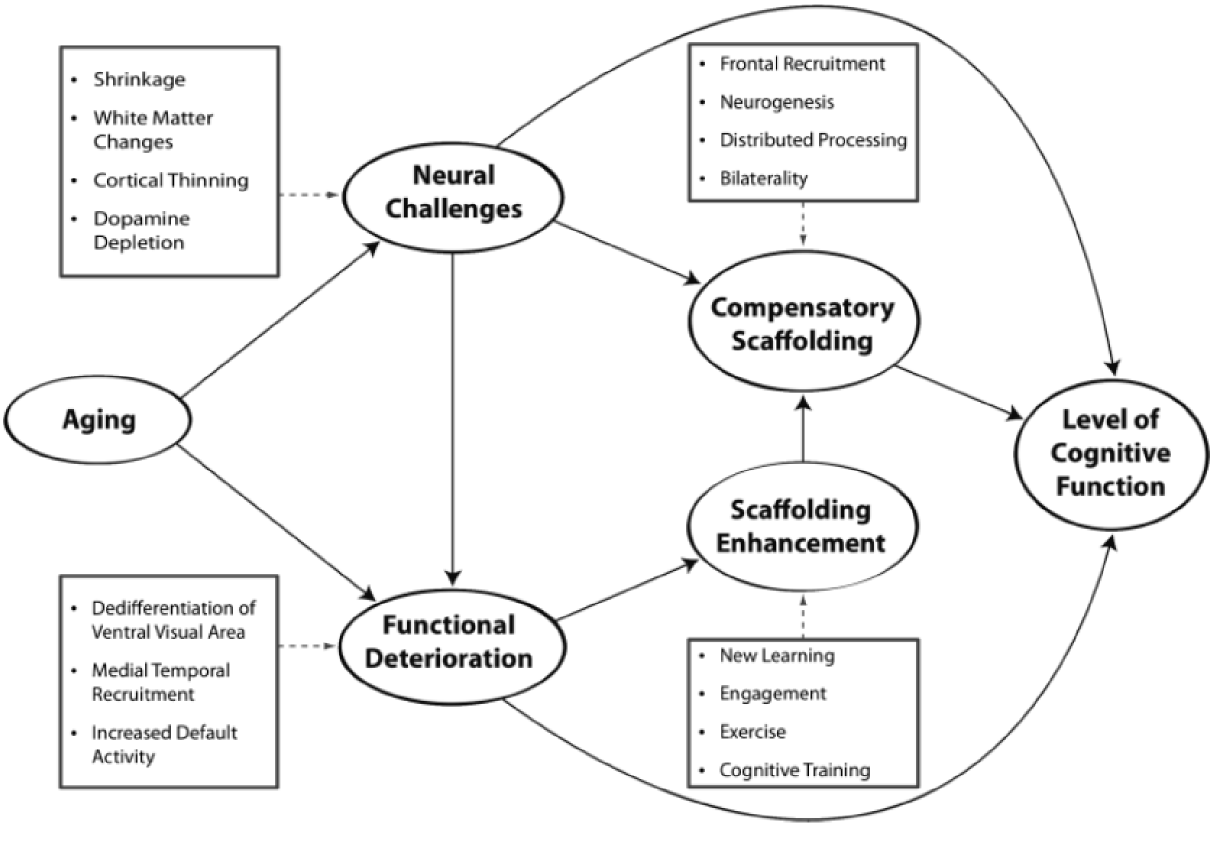

Figure 1. A conceptual model of the scaffolding theory of Ageing and cognition (STAC). Reprint of the original (Park and Reuter-Lorenz, 2009).

Also other challenges exist in studying of the effects of ageing on neural changes. In healthy people, the longitudinal changes in brain volume caused by ageing are not similar across different brain regions. The greatest neural shrinkage is observed in the caudate, cerebellum, hippocampus, and tertiary association cortices. Especially in hippocampus and prefrontal white matter the shrinkage increases with age (Raz et al., 2005). Causes for age-related changes are many. Just to mention few possible, these are molecular biological considerations, vascular health (including hypertension), genomic functioning (e.g., apolipoprotein E 4/4 genotype), neural causes e.g., smaller neuronal populations, less synchronous firing of neurons or decreased neurotransmitter binding leading to decreased activation, and failures of inhibitory connections leading to increased activation (Hedden & Gabrieli, 2004, Raz et al., 2005). Different changes in cognitive processes (e.g., processing speed, episodic memory, working memory, inhibition, and attention) have been affiliated with volume changes in different part of the brain and as a conclusion, there seems not to exist one unitary domain explaining cognitive changes with age (Reuter-Lorenz & Park, 2010).

1.3 Specific effects of ageing on sensory perception and neural processing of auditory information

An essential facet of human life, from its very beginning until the final outbreath, is the continuous flow of developmental changes – abilities, such as auditory information processing, rise and fall. For example, the perception of the acoustic space develops from an initially imprecise representation in infants and young children to a concise representation of spatial positions in young adults. Performance again declines in older adults (Freigang et al., 2015). There are considerably deficiencies in (auditory) cognitive processing related to ageing beginning already from very early sensory–perceptual, encoding phase, and extending to higher-order executive operations (Roth, 2015).

The sensory-perceptual phase includes neural activity in the cochlea and brainstem, executive functions occurring in the level of cortex (PFC, MTL). When considering the effects of age-specific changes on auditory sensory processing at a behavioral level, presbycusis is a term used to refer to an age-related hearing loss (ARHL). Under the term is included all factors and structural changes which are leading to hearing loss in older people. There is no one, generally accepted etiologic factor behind ARHL. The peripheral cochlear part with decreasing hearing threshold has a major role, but as well the central nervous function with all its neurophysiologic signal transduction is affecting it. In general, ageing also affects functioning of central nervous system by slowing down processing speed and decreasing effectivity of the task-related inhibition. These declining functional changes are related, e.g., to an individual's genetics background and the cumulative damage of noise (Roth, 2015). Zendel (2011) suggests that age-related decline in auditory processing associates with central but not peripheral auditory processing. On the other hand, with regard to the therapeutic treatment of age-related auditory processing disorders, central nervous function disorders are addressed easier through interventions than peripheral ones.

Particularly regarding this, in studying the effects of ageing on central auditory processing, EEG recording of ERPs induced by repetitive sound stimuli has long been a research method of choice. ERPs are signals in the EEG- or (MEG; magnetoencephalogram) data manifested after a sensory stimulus. ERPs represents only a tiny fraction of brain processes and EEG-data. Scalp ERPs are thought to arise mainly from postsynaptic potentials of thousands to millions of spatially and temporally aligned cortical neurons called pyramidal cells. The parallel orientation of the pyramidal cells causes a summation of their potentials. This gains the signal, makes it readable and finally enables recording potentials, for example at the scalp (Luck, 2014).

Due to the cortical source of the potentials, ERPs can be categorically also included in the so-called cortical auditory evoked potentials (CAEPs). CAEPs are again divided based on the latency time in short mid-latency (10–50 ms) and long-latency CAEPs (50–500 ms) and in this way separated from brainstem auditory evoked potentials (BAEPs) with very short (1–10 ms) latencies (Supp et al., 2018). A typical CAEP graph has first a positive deflection (P) followed by a negative deflection (N) which are followed again by another deflections (Figure 2). However, visually discernible negative or positive components in the graph are not intrinsic by themselves but instead are formed by summation of many independent underlying cortical processes (Helfrich & Knight, 2019). These processes are affiliated in different psychophysiological functions and age-related changes is observed in these (Näätänen & Picton, 1987). Due to that brain is a conductive medium where electrical potentials of neurons spread and high electric resistance of skull, the scalp distribution of an ERP component is usually very broad and blurred. (Luck, 2014).

A commonly studied deflection pattern is for example P1-N1-P2 complex. Other common ERPs are MMN and P300 (consisting of P3a and P3b). In these examples ERPs basically belong to the long-latency CAEPs. According to Stothart & Kazanina (2016), the P1-N1-P2 complex reflects the information flow from primary auditory to association cortical processing, and the transition from tonotopic auditory processing to more complex spectral processing, and greater sensitivity to top-down regulation.

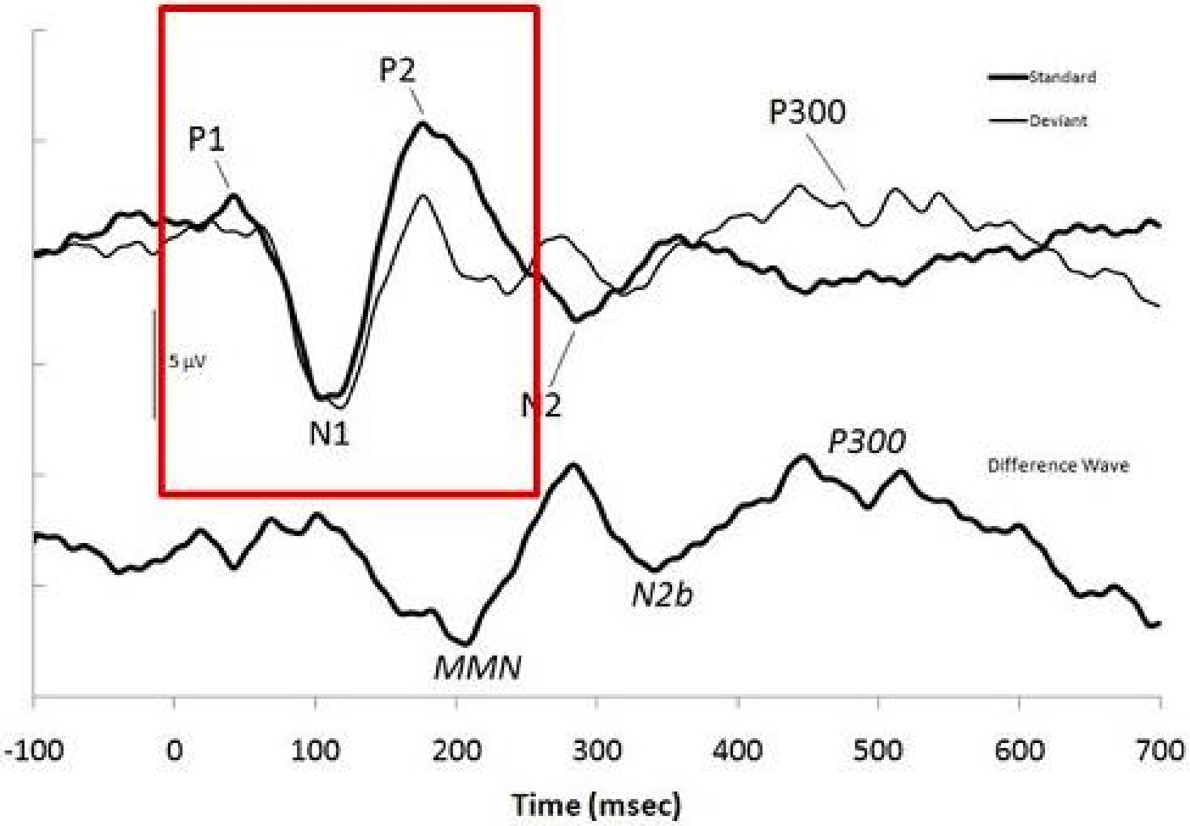

Figure 2. Characteristic illustration of a CAEP and the associated markers, which are introduced more detailed in the body text. Image adopted from Atcherson & Stoody (2012).

Because P1, N1 and MMN are CAEP components central to the current study, these are briefly introduced and considered how a putative stimulus in the acoustic environment or deviations from this stimulus is reflected in these. P1 aka P50 in humans peaks about at 50 ms after the stimulus and is recorded approximately 30–40 to 70–80 ms after the onset of an auditory stimulus (Bertoli et al., 2005; Liegeois-Chauvel et al., 1994). P1 is considered the earliest component (Figure 2) of the long-latency CAEPs (Buján et al., 2019). Findings suggest that the main generator of the P1 component locates in the temporal lobe bilaterally (Korzyukov et al. 2007). P1 is generated in the Heschl’s gyri or transverse temporal gyri which is a part of the primary auditory cortex (Liegeois-Chauvel et al., 1994), and reflects the regulation of sensory information from the peripheral nervous system to the cortex (Stothart & Kazanina, 2016). Luck *et al.* (1994) proposed in the studies based on visual ERPs that P1 would reflect "cost of attention": whenever a subject is paying attention to a target (visual) stimulus instead of paying attention to the, instructed object other than target, a suppression in the amplitude of P1 can been seen. Getzmann et al. (2015) suggested that the phenomenon is not unique to the processing of visual information but is seen in processing of auditory stimuli when a corresponding study design is used.

The second visible component of long-latency CAEPs is N1 which is thought to represent synchronous neural firing in the thalamic-cortical segment of the central auditory system in response to the onset of stimulus (Lister et al., 2016). N1 reaches its maximum amplitude typically between 90 and 130 ms. Similar to the P1 component, research has revealed that N1 has its neural source bilaterally over fronto-central topographical regions of cortex. N1 is generated in the primary auditory cortices (core area) with its generators in the Heschl's gyri as well as in association auditory cortex (Stothart & Kazanina, 2016) which is also named as the tertiary or the parabelt area of the auditory cortex. The N1 component has been found to be moderated by attention. There is an increase in the N1 amplitude when there is a congruence between the object of attention and its location, i.e., a participant is attending to a certain area in visual field and the stimulus is shown in that area (Luck et al., 1994). Luck names this as the "benefit of attention". Similar increase in the N1 amplitude is supposed to occur also in processing of auditory information in an analogous study design (Getzmann et al., 2015).

Mismatch negativity (MMN) is a component that can be seen in the CAEPs after a stimulus differing from the expected one. MMN is an electrophysiological change-detection response of the brain (Näätänen et al., 1978) which is usually studied with oddball paradigm. MMN is elicited when the current auditory input is "found" to differ or "mismatch" from the representation of the preceding, in some way regular, repetitive acoustic events related to, for example pitch, harmony, duration, intensity or position of signal (Näätänen et al., 2012). MMN appears as a negative potential enhancement over the frontocentral scalp areas relative to the standard-stimulus ERP. Functionally, the MMN is remarkably context dependent (Fishman, 2014; Bartha-Doering et al., 2015). Pre-attentive processing of auditory stimuli can extend to very complex relationships between the stimulus features (Paavilainen et al., 2001). It has been suggested that MMN response is elicited at least to a large degree independent of attention although attention or behavioral task can modulate it (Näätänen et al., 1978; Näätänen, 1993; Paavilainen et al., 2001); in other words, the MMN is thought to reflect bottom-up memory-driven processing rather than top-down attentional processing (e.g., Berti et al. 2004) and to provide a measure of auditory neural processing before focused attention (Näätänen & Alho, 1995). In general, the larger the deviation between the stimuli, for example the larger the pitch change, the larger the MMN amplitudes (Tervaniemi et al., 2005). The MMN appears in the ERP scalp distribution (using a nose reference) as a fronto-central negativity with a latency of 100–250 ms (Fishman, 2014; Näätänen et al., 2012). The auditory MMN response is mainly generated by processes which occur bilaterally in auditory and frontal cortices. According to review by Javitt et al. (2018), the initial sources of MMN are localized mainly to primary and secondary auditory regions.

1.3.1 Effects of ageing on general auditory ERPs (P1, N1)

Generally, research findings support an age-related increase in P1 amplitude (e.g., Zendel & Alain, 2014; Alain et al., 2012; Gaeta et al., 1998) and as well in N1 amplitude (Zendel & Alain, 2014; Amenedo, 1998a; Strömmer et al., 2017). For the P1 component, researchers have paid significantly less attention to it than the N1 and MMN components. However, many studies have reported age-related increases in P1 amplitudes (e.g., Harris et al., 2008, Amenedo & Días, 1998b; Golob et al., 2007). In general, the study of the P1 component has been associated with a P1-N1-P2 response, in which ageing-related effects have been observed in the amplitudes of the complex, please see Harris et al. (2008) for the list of studies. However, it is good to notice that whether the amplitude of the P1 response is higher or lower may be related to the experimental set-up of the study. If the task is, for example, to react to stimuli (or changes in stimuli) behaviorally by touching a button, then the higher amplitude of the response is usually related to attention.

Examining the ability to discriminate small differences in pitch of pure sinusoidal tones, Bertoli et al. (2005) could find both in attended and unattended conditions age-related changes in P1 amplitude: young normally hearing subjects had significantly smaller P1 amplitude than older normally hearing subjects both in the presence and absence of contralateral cafeteria noise. More (auditory) processing resources are required with increased age, so in line with STAC model a higher ERP response can be sign of a compensation processes for decreased ability for resolution and/or perception. Another way to interpret the enhanced amplitudes has been to attribute it to age-related deficits in central inhibition (e.g., Tremblay & Ross, 2007) or to a deficit in stimulus adaptation (Zendel & Alain, 2014).

N1 is proposed to reflect auditory detection and discrimination (Tomé et al., 2015). In a systematic review by Tomé et al. (2015), it was interpreted that N1 amplitude at EEG reference point Cz in central midline sagittal plane reaches a stabile level in 30 to 40 years at which it remains for the rest of the life. However, regarding the N1 amplitude at Fz in frontal midline sagittal plane, it was interpreted that there is long-time decrease which finishes just in the beginning of the senior years at 60 years of age and begin an increase thereafter. However, given the number of studies on seniors used in the review of Tomé et al. (2015), the veracity of this conclusion should be treated with caution and confirmed by new studies on seniors. The larger N1 amplitude may be due to reduced neural synchrony caused by decreased inhibitory control of the central auditory nervous system and may not simply a result of age-related delays in neural conduction times (Harris et al., 2008).

1.3.2 Effects of ageing on change-related auditory ERPs (MMN)

Like with the P1 and N1 component, the investigation of the MMN-component has revealed age-related changes in amplitude. In older adults changes in the frequency and duration of the auditory signal are reflected as attenuation of MMN amplitude compared to young adults (Cheng et al., 2013; Strömmer et al., 2017). This attenuation is regarded as a prominent signature in decline of cognitive neural processes in normal ageing as well as in different neuropsychiatric, neurological, and neurodevelopmental disorders (Näätänen et al., 2012; Strömmer et al., 2017), and has been proposed to associate with impaired cognitive performance, especially in verbal memory and executive functions (Strömmer et al., 2017). Gaeta et al. (1998) reported that at different levels of deviance, MMN amplitudes were always smaller in older relative to younger adults. Young, but not older adults showed robust MMNs at the smallest level of deviance. Based on this, Gaeta et al. (1998) suggested that older adults demonstrate less sensitivity to stimulus deviance, so that only highly deviant stimuli are likely to capture their involuntary attention. The lack of age differences in MMN amplitudes was reported by Sur & Golob (2020). However, this referred to an experimental setup in which the sound or tone stimuli were delivered i) rapidly and ii) with obvious pitch or location differences between the standards and the deviants (Sur & Golob, 2020).

A common hypothesis is that declined ability to process frequency changes in auditory information in aged adults reflects disturbances in central auditory processing (Cheng et al., 2013). It is suggested that one of the major mechanisms behind this is faster decay of sensory memory traces or then diminished MMN indicates an impairment in the encoding of sensory information (Freigang et al., 2015). On the other hand, in accordance with an inhibitory deficit hypothesis, it has been proposed that processing of a task-irrelevant stimulation (i.e., impaired inhibition) is sensitive to ageing (Alain & Woods, 1999), resulting in discrimination deficiency with aged people.

1.4 General and specific effects of musical pursuit on cognitive and neural processing of (auditory) information

Neuroimaging studies have revealed that musical stimuli or musical training are linked to wide bilateral network in the frontal, parietal, cerebellar, and limbic/paralimbic areas of the brain during simultaneous cognitive activity (Figure 3). This activity is associated with processing of complex acoustic features such as melody (Alluri et al., 2012), processing of syntactic and semantic information (Koelsch & Siebel, 2005), attention and working memory (Janata et al., 2002; Särkämö et al., 2014), episodic and semantic memory (Janata, 2009; Särkämö et al., 2014), motor and rhythmic processing (Zatorre et al., 2007), executive function and general cognition (Särkämö et al., 2014), experiencing emotion and reward (Koelsch, 2010), and creativity (Benz et al., 2016). Moreover, music can also have a significant impact psychologically (also related to ageing), for example on emotional self-regulation (Juslin & Sloboda, 2010) and on emotional well-being, for example by alleviating agitation, anxiety, depression (Guétin et al., 2009; Raglio et al., 2008), and quality of life (QOL), (Särkämö et al., 2014). Combined EEG (MEG) studies with anatomic studies have revealed neuroplasticity in musicians' brain as enlargement of cortical auditory areas essential for music perception as well superior pre-attentive auditory sensory memory representations (O'Brien et al., 2015).

The lifelong ability to adapt to changing environmental needs is based on the plasticity of the central nervous system. Investigations with structural MRI studies have revealed that musicians on average have 2 to 4 % greater cerebellar volume compared to nonmusicians (Schlaug, 2001), significantly larger anterior corpus callosum (Schlaug, 2001), and increased

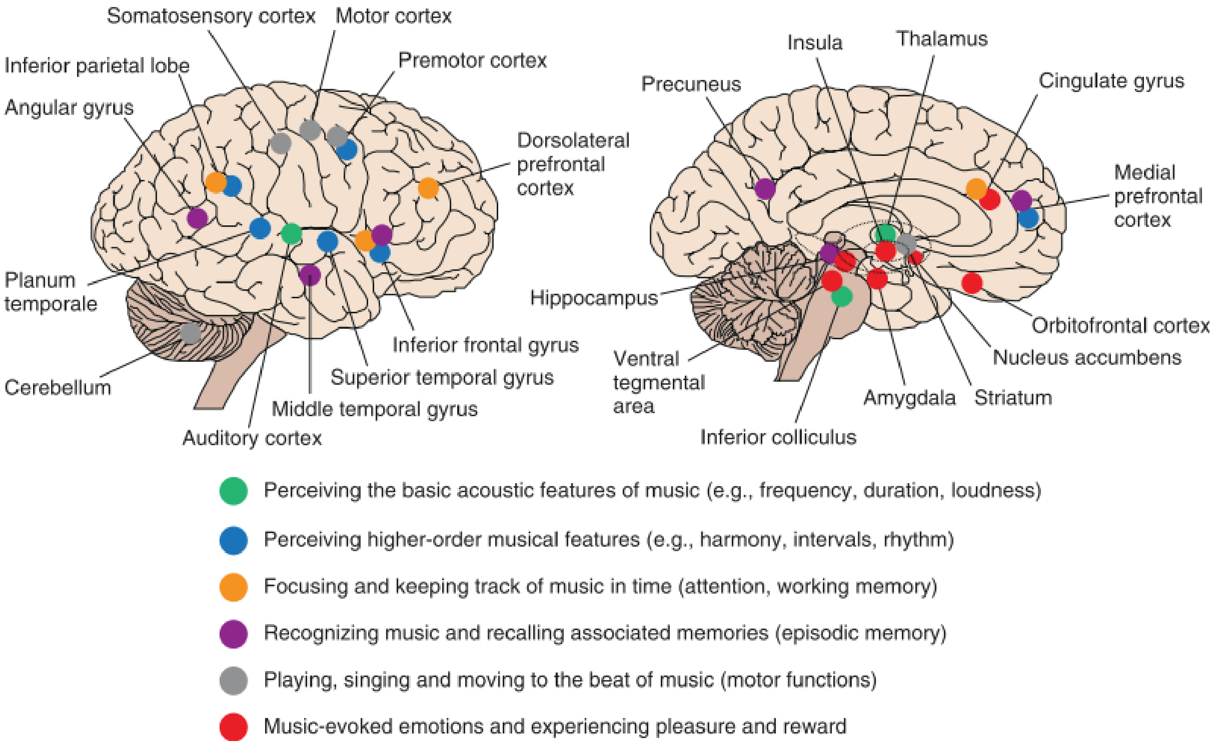

Figure 3. Schematic illustration of key areas associated with music processing based on neuroimaging studies of healthy subjects. Adopted from Särkämö et al. (2013).

gray matter volume in the left Heschl's gyrus (e.g., Schneider et al., 2002) and left inferior frontal gyrus (e.g., Abdul-Kareem et al., 2010). With neurophysiological experiments it has been demonstrated that the musical capacity of musicians achieved by prolonged practice of musical pursuit is associated with increased functional organization in cortical areas of the somatosensory as well of the auditory domains specifically for musical tones, but not for pure sinusoidal tones. Also, it is found that in the somatosensory cortex, plastic changes are specific only for those fingers which are frequently used and stimulated in playing a certain instrument (Pantev et al., 2001). Based on neural evidence it is suggested that musicians employ different brain structures for auditory memory tasks, which improve the memory in musicians (Parbery-Clark et al., 2011).

Regarding the effects of musical pursuit on cognitive performance, musical pursuit requires constant use of pitch information which requires both high cognitive demands and auditory acuity. Schön et al. (2004) and Wong et al. (2007) have in their experiments shown that usage of musical pitch information (in musical training or in musicianship) is facilitating language processing related to frequency-processing (pitch). This may further imply that long-term musical activity may shape basic sensory circuitry consisting of feedback from the cortex to the inferior colliculus such, that accurate pitch information is relayed from subcortical structures (brainstem) to the neocortex to facilitate successful performance of cognitively demanding nonmusical tasks (Wong et al., 2007). Regarding ageing, lifelong musical training of musicians has been found to reduce age-related decline in hearing abilities or auditory processing related for example to speech in noise (SIN) (Parbery-Clark et al., 2011; Zendel, 2011). The perceptual advantage of musicians over controls for understanding SIN is suggested to base on enhancements for auditory-specific cognitive abilities, e.g., auditory working memory and auditory attention, and heightened auditory abilities (Parbery-Clark et al., 2011).

The former may be related to (motor) skills required to learn to play an instrument or other musical activity, where integration of both auditory perceptual and cognitive skills is quintessential. Musicians may exhibit also some unique abilities related to musicianship: an ability to memorize long, and complex bimanual finger sequences (reflected as activity in different motor, sensory and cerebellar cortices) or an ability to induce motor sequences during sight-reading of musical notation, and ability to identify tones with absolute pitch without a reference (Schlaug, 2001).

1.4.1 Effects of musical pursuit on general auditory ERPs (P1, N1)

With CAEP studies is has been shown that musicians with extensive formal and informal auditory training throughout their lifespan have in a many ways enhanced processing and discrimination of musical (auditory) information compared to nonmusicians (O'Brien et al., 2015). For example, musicians are able to discriminate auditory differences at a pre-attentive processing that are undetectable of nonmusicians. Musicians compared to nonmusicians have larger amplitude responses in changes to musical information e.g., melodic contour and interval, rhythmic deviation pitch structure and timbre of the instrument regarding the profession of the musician. As discussed above, long-term musical action affects brain function on many levels through neural plasticity. Examining plasticity with CAEPs, musicians have shown enhanced sensitivity to short-term musical training (Seppänen et al., 2012; O'Brien et al., 2015).

Regarding on P1, the evidence indicates that the effect of musical pursuit on this early component depends on the stimulus type as well as on whether the study condition is passive or active. The effect of musical pursuit on the efficiency of auditory information processing of musical stimuli or harmonic pure tone patterns in passive listening is reflected in a decrease in the amplitude of P1 compared to non-musicians controls (Nikjeh et al., 2009; Zendel et al., 2014). However during active listening, the P1 amplitude has reported to be smaller in musicians, but without a significant difference (Zendel et al., 2014). As well, using mere pure tone or speech (syllable) as stimuli it has not been possible to differentiate musicians and controls (Nikjeh et al., 2009). The decrease in P1 has likewise been shown by magnetoencephalography (Kuriki et al., 2006). The interpretations for the smaller P1 amplitude observed in musicians related to auditory processing is considered more extensively in the discussion.

N1 amplitude has been found to be enhanced in musicians, which has been suggested to be specific to musical stimuli used in experiments or then stimuli specific to the instrument on which the musician has trained (Baumann et al., 2008; Pantev et al., 1998 and 2001). When pure tone (sinusoidal) harmonies are used as stimuli increase related to musicianship in N1 is not detected (Zendel & Alain, 2014).

In summary, differences related in musical pursuit are observed in the exogenous, stimulus input-driven ERP components (Baumann et al., 2008; Nikjeh et al., 2009; Pantev et al., 1998 and 2001; Zendel & Alain, 2014), and the differences between musicians and non-musicians are reported to be similar across the lifespan, suggesting that exogenous auditory ERPs are enhanced in musicians but decline with age at the same rate (Zendel & Alain, 2014). Additionally, it has been found that attention-related, endogenous activity, in the right auditory cortex, was selectively enhanced in older musicians with lifelong musical background in response to a complex sound (Zendel & Alain, 2014).

1.4.2 Effects of musical pursuit on change-related auditory ERPs (MMN)

MMN component of CAEPs have been used more than P1 and N1 to compare musicians and nonmusicians in examining the influence of music training on auditory processing. Findings indicate that musicians relative to nonmusicians have superior pre-attentive auditory sensory-memory traces (MMN) of acoustic features over a range of auditory stimuli such as pure tones, harmonic tones, and speech, enhancing musicians' automatic discrimination of acoustic features (Nikjeh et al., 2009).

Koelsch et al. (1999) used the MMN beside behavioral investigations to determine the pre-attentive pitch discrimination accuracy of violin players during passive and active listening of the stimuli. In this study, the sinusoidal standard stimulus consisted of major chords consisting of three tones including a major third and perfect fifth. The deviant stimulus differed from the standard in the marginally mistuned (<1 %) middle tone. During passive condition, the deviants elicited the MMN only in musicians. In the active behavioral task, where subjects were informed to discriminate deviant chords, violin players detected 80 % of these chords with significant MMN, while nonmusicians detected only 10 % accompanied with lower amplitude in MMN. The study could demonstrate that long-term musical experience modifies automatic, pre-attentative mechanisms of sensory memory.

Tervaniemi et al. (2005) presented evidence that musicians are faster than nonmusicians to behaviorally discriminate the pitch changes in pitch shifts of different sizes. Musicians outperformed nonmusicians in accuracy in the small (0.8 %) and medium (2 %) shifts. However, in a passive condition this was not reflected as a difference in MMN response between musicians and nonmusicians.

In the study of Nikjeh et al. (2009), the latencies of MMN in the group of musicians were shorter for all deviances (harmonic tones, pure tones, and speech) compared to controls. However, no significant differences between groups in MMN amplitudes were observed, although the effect of speech syllable on MMN amplitude was almost significant. As a conclusion, musicians were interpreted to have enhanced sensitivity for acoustic, spectrally rich, i.e., harmonic tones and speech stimuli, changes. In line with study of Nikjeh et al. (2009) Brattico et al. (2001) reported also shorter latencies for pitch deviants in musicians compared to controls without significant differences in amplitudes. In light of these previous studies of the passive listening condition, it appears that the enhanced discrimination ability of a deviant stimulus (pitch change) in musicians is primarily reflected in the shorter latency of the MMN. However, Fujioka et al. (2004) reported also enhanced MMNm amplitudes for musicians: significantly larger MMNm was present in musicians compared to non-musicians, whereas MMNm in the control condition was similar for both groups. The authors suggested that this is an indication of musical training enhancement in the ability to automatically register abstract pitch changes in melodies.

Considering studies already conducted to investigate P1, N1, and MMN responses to different auditory stimuli, the use of spatially anomalous sound sources as stimuli has shown little interest among researchers compared to other stimuli. However, Tervaniemi et al. (2006) studied MMN responses in amateur rock musicians and nonmusicians with spatially differing stimuli in passive listening condition and found that MMN to deviants in location was significant in both groups, but in musicians the response (amplitude) was significantly larger. MMN was maximal in frontal regions and lateralized on the right hemisphere. Also, Vuust et al. (2012) reported significantly enhanced MMN responses of jazz and rock musicians in passive listening condition to stimuli that differed spatially and in pitch. The enhancement of MMN responses especially in jazz musicians was seen to be related to the communication between musicians using auditory cues, which is an essential part of this genre. The authors concluded that in musicians the neural processing of auditory information is plastically shaped based on the environment in which the musical activity exposes.

In addition, although the MMN is automatically elicited, experiments have shown that the MMN parameters closely correlate with subjects' behavioral performance. In this way it has been demonstrated that musical training boosts music related skills. For instance, in behavioral tests musicians have faster reaction times and more accurate hit rates in discriminating changes in pitch or duration (Amenedo & Escera, 2000; Jaramillo et al., 2000; Tiitinen et al., 1994). These enhanced abilities are reflected as well as on enhanced, i.e., increased MMN amplitude (and/or shorter MMN peak latency) (Brattico et al., 2001).

1.5 Effects of choir singing on cognition and neural processing in adults and aged population

There is increasing number of findings supporting that lifestyle related choices can have significant impact on successful ageing, for example older people who engage in activities which stimulate their cognition e.g., reading, learning or game playing or being socially active, show lower rate of cognitive decline or dementia (Zendel, 2011; Middleton & Yaffe, 2009). Continued engagement in stimulating activities can buffer against age-related cognitive decline by maintaining or enhancing a 'cognitive reserve' one has created for example through early educational or occupational achievements (Zendel, 2011). Evidence suggests that musicians experience not as much age-related decline in auditory processing abilities than nonmusicians do (Zendel & Alain, 2014).

It requires singers to perform many simultaneous cognitive tasks for a polyphonic choir to be of good quality, i.e., a choir to sound good. During choir singing activity, a singer must adjust volume, pitch, and timbre of their voices relative to other singers, in a temporally and locally restricted frame. From the brains of the singers, this requires complex, but flexible collaboration of cognitions related to execution of vocal-motor, auditory, visual, spatial, linguistic, and emotional processing in a goal-directed context. Beside this, the performance at the same time is linked to social interplay with other choir singers, during which a choir singer is learning to sing and perform own stems in songs varying in their polyphonic arrangements (Pentikäinen et al., 2021). Grounded on this, it is relevant to hypothesize that choir singing develops or maintains cognitive ability, which could serve as a protection against cognitive decay in old age.

Based on neuroimaging it has been suggested that two different cortical systems, i.e., the parietal-frontal (dorsal) sound production pathway and the temporal-frontal (ventral) auditory perceptual pathway, are required for singing. These systems interact with each other as feedback loops. Also, other neural processes in the prefrontal brain region, limbic system, and cerebellum related to attention, working memory, rhythm, and emotion are involved (Zarate, 2013; Whitehead & Armony, 2018; Pentikäinen et al., 2021).

Previously, two different studies have been published focusing on cognitive benefits of choir singing in older adults (Pentikäinen et al., 2021; Fu et al., 2018). In the study of Pentikäinen et al. (2021), it was showed that compared to the control group choir singers had better verbal flexibility, which probably reflect the verbal-cognitive demands of choir singing. Also, choir singers experienced better social integration and health compared to controls. In line with study of Pentikäinen et al. (2021), Fu et al., 2018 in their pilot study on older adults reported improved performances in phonemic fluency and memory tests after a 12-weeks group singing program. An improved phonemic fluency has been reported also in studies focusing on possible effects of musical pursuit – other than singing – on cognition (Mansens et al., 2018; Hanna-Pladdy & Gajewski, 2012). These concordant findings suggest that cognitive flexibility is an essential function affected positively by different musical pursuit during ageing (Pentikäinen et al., 2021).

The neurocognitive effects of instrumental musical pursuit in relation to ageing have been extensively studied – however, the opposite is true when singing or choir singing research is considered. There is a lack of knowledge of the potential effects of singing and choir singing on brain neuroplasticity and cognitive function in connection with ageing.

1.6 Aim of the study

The purpose of this Master's thesis study is to investigate the possible impact of previous choir singing and other musical engagement on neurocognitive processes in older people. The core aim is to find out whether the groups of subjects (choir singers versus controls) differentiate from each other in the neural processing of auditory information. In practice, this topic is assessed with data obtained by the EEG. From the data ERPs related to i) general (P1 and N1) and ii) to change detection (MMN) of an exogenous auditory stimulus are extracted and studied taking into account background variables.

The research is part of a larger longitudinal research entity and serves as a foundation or baseline (timepoint 0) for a three-year longitudinal choir intervention study. From a broader perspective, the current research is small part of a field of research exploring potential impacts of social activity such as choir singing and evaluating the usability of this kind of activity in society as a cost-effective intervention against the cognitive decline related to ageing and especially among senior citizens.

1.7 Study hypothesis

Many of the senior choir members have practiced choir singing or other musical pursuit for much of their lives, which may be reflected in the processing of auditory information in the brain. When singing in a choir, a choir member has to continuously compare the pitch of own voice with the pitches of other singers, which moreover, are spatially located in several different places. For this reason, the study focuses on these musical factors, i.e., pitch and location of a tone. The first hypothesis for the current study is that choir singers' cognitive processing measured as change-detection at tone pitch and tone location, is more responsive compared to control seniors, and reflected as greater amplitude of MMN in difference wave.

Next, choir singers' longtime engagement in choir singing and other musical pursuit is assumed to affect as well general neural processes of exogenous, obligatory auditory information. Musicianship has been found to have different effects on these early ERP components. There is a lot of reports of N1 enlargement in musicians. However, P1 of musicians has been reported to be reduced in passive listening condition with no interaction with age (Zendel & Alain, 2014), which is explained as enhance automatic inhibitory function or then as stronger adaptation to the stimulus. This gives ground for the following hypothesis: earlier components of brains' event-related potentials, namely P1 and N1, in choir singers' group reflect cognitive resource or capability against the decline brought by ageing compared to the control group. In practice, this is hypothesized to be reflected as decreased amplitude for P1 and increased amplitude N1 components in a group of choir singers compared to the control group.

Additionally, all studied ERPs are compared between two age groups. The age-group of 70-year-old or younger subjects is hypothesized to have better discrimination for deviating tones than the group of over 70-year-old subjects, which is assumed to be seen as higher MMN amplitudes (in difference wave) in the group of younger senior subjects of the study (Cheng et al., 2013; Strömmer et al., 2017). On the other hand, ageing has found to enlarge P1 and N1 components of auditory processing (Alain & Woods, 1999; Amenedo & Diaz, 1998a; Harris et al., 2008; Tomé et al., 2015; Zendel & Alain, 2014). So, in the older age group, P1 and N1 components are assumed to be higher than in the group of younger senior subjects.

The deviant stimuli consisted of tones with varying difference from the standard stimuli. The differences between i) choir singers and controls or ii) younger seniors and older seniors are thought to be more prominent in cases of hard deviants relative to easy deviants. Also, for comparison all the deviant stimuli are clustered together and analyzed separately.

In addition, all of the above responses (P1, N1, and MMN) are examined within the group of choir singers in relation to the choir singing years reported by the choir singers. The hypothesis is that within a group of choir singers, especially the amplitude of the MMN subtraction curve reflects the number of choir singing years. For P1 and N1 responses, the hypothesis is that these responses decrease relative to choir singing years.

2. Material and methods

2.1 Participants

The participants for the study, 29 senior choir singers (age 61 to 83 years; average age 70.9 years; 19 females), were gathered mainly from three different choirs led by trained choir conductors and assembling in adult education centers in Helsinki (HAEC), Espoo (OMNIA) and Vantaa (VAEC), (Table 1). Occasional participants were affiliated to the study cohort from other local choirs. Participation in the choirs of adult education is easy, with no auditions and low tuitions making the participating threshold lower. For these reasons the choirs enable participation regardless of previous musical training or socio-economic status. As controls 25 demographically matched seniors were included (age 60 to 88 years; average age 69.8 years; 19 females), (Table 1). These were recruited from HAEC, VAEC, and advertising in public places and local newspapers. Also, spouses of the senior choir singers were recruited. Importantly, qualification in the control group requires the subject not to engage in choir singing or other musical hobbies but to participate in other activities to a different degree. All the participants were right-handed, Finnish speaking individuals, without any neurological or severe psychiatric illnesses, hearing disabilities or history of drug abuse. All the participants signed an informed consent before the study.

2.2 Recording of brain electrical activity

2.2.1 Procedure and stimuli

The experimental part of the study has been carried out in University of Helsinki at a laboratory of Cognitive Brain Research Unit (CBRU). For recording electrical activity of the brain, the subjects were presented repetitive auditory stimuli, i.e., a passive oddball task measuring auditory sensory memory (mismatch negativity, MMN) while watching a silent movie. The varying conditions were based on a pitch oddball paradigm and a location oddball paradigm. In practice, during the measurement the participants were seated on a comfortable armchair in an electrically and acoustically shielded, anechoic room, enabling the presentation of acoustic stimuli in a three-dimensional space under controlled semi-natural conditions. The participants focused on watching a silent movie shown from a computer screen in front of them (The Circus by Charlie Chaplin, 1928) while hearing a sequence of tones varying in pitch and location played from loudspeakers. The subjects were asked to concentrate on a movie without paying attention to any sounds from the speakers.

The speakers were arranged in a half circle (180°) 63 cm apart (36°) from each other. Each speaker was in front of the test subject at a distance of 1 m so that three of the speakers were on left hand side and three were on right hand side of the test subject (Figure 4). In the experiments six different sinusoidal tone stimulus varying in pitch were used. The pitches were 740 Hz, $F\#_5$; 831 Hz, $G\#_5$; 932 Hz, $A\#_5$; 1047Hz, C_6; 1175 Hz, D_6 and 1319 Hz, E_6. Each stimulus had a duration of 100 ms with an intensity from 70 to 80 dB depending on the subjects hearing threshold. The stimuli were separated by a silent gap of 400 ms.

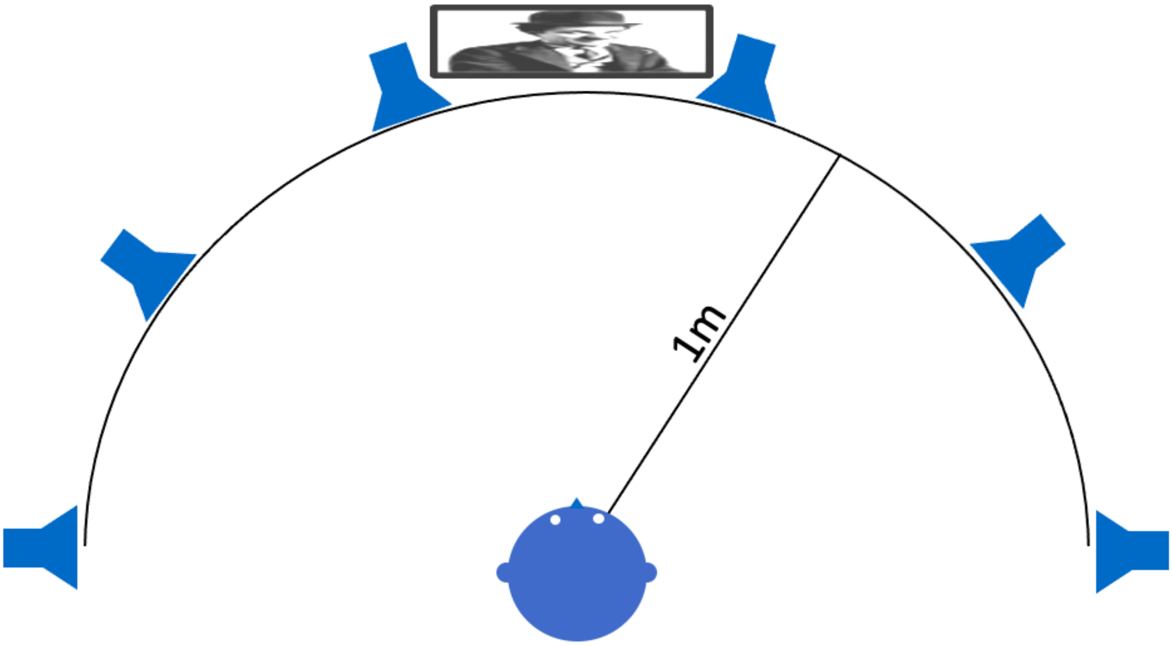

Figure 4. Arrangement of the test setup. Stimuli were played from six loudspeakers located evenly in a semicircle one meter from the subject. In front of the subject was a computer screen from which a silent film was played.

In *pitch oddball* condition, as a standard stimulus only one frequency, either the lowest (740 Hz) or the highest (1319 Hz) of the set, was played continuously from all of the six speakers in a random order. The standard tone setting was balanced across the test subjects. As a deviant stimulus, one of the remaining five tones was occasionally played from a random speaker. The deviant stimuli were further classified as ‘easy’ and ‘hard’ depending on their frequency distance from the standard, easy being the two furthest from the standard and hard being the two closest to the standard. With this setup, the location of the sound was controlled, and the deviants focused on the frequency i.e. the pitch of the tone. The total number of the stimuli was 720, of which one sixth were deviants (120 in total). Each deviant tone was presented equal amount (n=24) of times.

In *location oddball* condition, all of the six tones were played either from the far-right speaker or from the far-left speaker. The standard speaker setting was balanced across the subjects. As deviant stimulus, one of the six sounds occasionally was played from one other randomly selected speaker. The deviant stimuli were classified as 'easy' or 'hard' depending on their distance from the standard speaker, easy being the two furthest from the standard speaker and hard being the two closest to the standard speaker. With this setup, the frequency of the sound was controlled, and the deviants focused on the location of the sound. As in the pitch oddball condition, the total number of the stimuli in location oddball condition was 720, of which one sixth were deviants (120 in total). Each deviant speaker location was presented equal amount (n=24) of times.

2.2.2 EEG recording

The EEG recording was performed with a Biosemi ActiveTwo EEG measurement system (BioSemi, The Netherlands; www.biosemi.com). Each test subject was measured two times for both tasks differing in test conditions (2 x PITCH and 2 x LOCATION). The presentation order of the tasks was balanced across the test subjects. Altogether, the duration of the experiment (2 x 2 x 6min) with preparations was about 45 minutes.

EEG was recorded with a 64-channel cap according to the extended 10–20 electrode system (Jasper, 1958). For referencing, adhesive electrode rings were used to attach the electrodes to the mastoids behind the auricles and near to the lower eyelids in order to monitor eye-blinks and horizontal eye-movements (electro-oculography, EOG). The sampling frequency in EEG recording was 512 Hz.

During the measurements, the behavior of the participants was monitored through video camera from the laboratory control room for ensuring the quality of the measurements. Between each block/individual measurement, a few questions for the participants were presented about the silent movie in order to monitor the activity level. When the situation so required, a short breaks were taken.

2.3 Data preprocessing

The offline data analysis for all the EEG data was performed in MATLAB toolbox EEGlab (ver. 2019.0) by Delorme and Makeig (2004). Preprocessing of the raw EEG data and data analysis was conducted with CBRUplugin ver 2.1b in EEGlab. First, the data was band-pass filtered between 0.5 and 40Hz and subsequently epoched from -100 to 500 ms, time-locked to the onset of each stimulus. Extreme activity (artefacts) was automatically rejected for epochs containing ±500 µV peak to peak activity. Independent component analysis (ICA) was run to correct for artefacts related to eye-blinks and horizontal eye movements. Data from channels with poor signal quality were detected by visual inspection. The participants with more than 9 bad channels were excluded from the study (11/61 subjects in PITCH condition; 12/65 in LOCATION condition). The bad channels of the participants with good enough quality data were individually removed and substituted with data reconstructed by interpolating the data from neighbor channels comprising 7.63 % of the PITCH condition data and 4.42 % of the LOCATION condition data. Next, 9 participants of the PITCH condition and 2 participants of the LOCATION condition were removed from the study, due to poor EEG data quality (more than 50 % of the trials rejected). Finally, epochs were merged, additional low-pass filtering of the epoched data at 30 Hz was run and the baseline was removed between -100 and 0 ms.

After the preprocessing described above, the data comprised 22 senior choir singers in PITCH condition, which were again balanced for HIGH standard tone (n=13) and for LOW standard tone (n=9), and their 19 matched senior controls balanced for HIGH standard tone (n=9) and for LOW standard tone (n=10). In the LOCATION condition the data comprised 28 senior choir singers balanced for RIGHT standard location (n=10) and for LEFT standard location (n=18), and respectively their 23 matched senior controls balanced for RIGHT standard location (n=11) and for LEFT standard location (n=12). Altogether 54 participants (29 choir singers and 25 controls) were included in the study.

2.4 Statistical analysis of data

Statistical analysis of ERP data was applied for three clusters of electrodes, i.e., regions of interest (ROIs): for frontal electrodes (ROI F; F3, Fz, F4), for central electrodes (ROI C; C3, Cz, C4) and parietal electrodes (ROI P; P3, Pz, P4). The time windows for searching the highest peak amplitude for each ERPs were: P1 (25–75 ms, peak direction positive), N1 (75–125 ms, peak direction negative) and MMN (100–250 ms, peak direction positive). For P1 and N1, the mean peak amplitude was determined by calculating the mean of the amplitudes in time window ±15 ms around the peak maximum value. Respectively for the MMN, the value for the subtraction curve (standard minus deviant) was determined by calculating the mean of the amplitudes in time window ±25 ms around the MMN peak maximum value.

For the statistical analysis, deviant trials of each ERP (P1, N1 and subtraction curve of MMN) were further clustered in EASY and HARD deviant classes (see above 2.2.1 Stimuli), and also in a cluster including all the deviant trials (ALL). Deviant and standard ERPs were averaged separately for each participant, condition, and stimuli. Grand-average waveforms were computed for each stimulus, condition, and group.

As the ANOVA assumptions were met, a repeated measures analysis of variance was applied for the variance analysis of the different combinations of data, separately for PITCH and LOCATION conditions, and separately for stimulus types, standard (STANDARD) for early CAEPs P1 and N1, and deviant (EASY, HARD, ALL) for all CAEPs.

The effects of group (choir singers versus controls) and age group (from 60 to 70 years vs. over 70 years) on P1 and N1 amplitudes, and on MMN subtraction curve in each ROI (F, C or P) was examined using ERP and ROI as within-subject factors, and group and age group as between-subject factors for the analysis. With significant ($p < .05$) or almost significant ($p < .10$) results obtained in the repeated ANOVA (meaning significant or almost significant main effect of ROI or ROI x group/age group interaction), subsequent multivariate ANOVA was applied separately on each ROI. Analysis for group x age group were also implemented and results are presented in the tables, but because the sizes of the subgroups became so small, these results have not been interpreted or discussed.

Prior to ANOVA analysis, differences between group of choir singers and controls in some variables relevant to the study were assessed with independent sample t-tests. Tested variables were i) MoCa score, ii) sex, iii) age, iv) lifetime education, as well different activities after 60 years of age estimated as scores in v) non-specific musical activity, vi) non-specific cognitive activity with and vii) without musical activity, viii) and non-specific physical activity.

Within the group of choir singers regression models were generated from the data, in which the dependent variable was the CAEP amplitude (P1, N1 or MMN) for each standard and deviant stimuli (EASY/HARD) in one of the ROIs (F/C/P) and in either condition (PITCH/LOCATION). The independent variable was the number of choir singing years.

All the forementioned statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

In addition, effect sizes for the group differences were calculated by means of Cohen's *d*, taking into account the group sizes, and interpreting the effect as small when $d = .2$, medium when $d = .5$, and large when $d = .8$, (https://www.psychometrica.de/effect_size.html).

As additional remarks for the analysis i) N1 and MMN values were converted to their complements (a larger positive means a larger response), ii) the PITCH and LOCATION conditions had to be analyzed separately, because of limiting sample size and iii) the repeated ANOVA analyses were limited to a maximum of three parallel interactions between group, age group, and response/ROI.

3. Results

When assessing the possible differences between choir singers and controls prior to ANOVA analysis only statistically significant difference between the groups was observed in averagely different engaging in non-specific musical activity after 60 years of age. Choir singers engagement was higher than controls: $t(5) = 7.16$, $p < .001$ (Table 1). The descriptive statistic for the analysis is presented in the Tables 2A and 2B. In the tables the results for group x age group are also presented although it is obvious that the sizes of the subgroups (N varies between 9 and 16) and thus the statistical power are weak to exploit these results. However, the results are tabulated to allow visual examination of subgroup-level phenomena.

Table 1. Summary of independent sample t-test results for background variables between choir singers and controls. Significant differences bolded. An asterisk (*) refers to non-specific activity. *p*-value is 2-tailed.

VARIABLE	GROUP	N	M	STD	t	df	p
MoCa	S	29	25.59	3.145	0.285	51	.777
	C	24	25.33	3.306			
Sex	S	29	1.34	0.484	0.831	52	.41
	C	25	1.24	0.436			
Age	S	29	70.86	4.612	0.676	52	.502
	C	25	69.8	6.856			
Lifetime education	S	26	3.65	1.623	-1.466	40	.15
	C	24	4.5	2.359			
Musical activity *	S	28	8.96	3.383	**7.157**	**50**	**.000**
	C	24	2.42	3.175			
Cognitive activity with musical activity *	S	29	12.48	2.459	1.836	47	.073
	C	25	11.44	4.063			
Cognitive activity without musical activity *	S	27	13.67	3.013	1.119	38	.27
	C	22	11.82	4.031			
Physical activity *	S	25	9.44	2.468	-0.039	34	.969
	C	21	9.48	3.614			

Table 2A. Descriptive statistics including amplitude means and standard STDs (μV) regarding the ERP responses (P1, N1 and MMN) at different ROIs for different stimuli (standard, deviant (ALL, EASY & HARD) in PITCH condition.

PITCH CONDITION				P1								N1								MMN (STD-DEV)					
				Standard		Dev (ALL)		Dev (EASY)		Dev (HARD)		Standard		Dev (ALL)		Dev (EASY)		Dev (HARD)		Dev (ALL)		Dev (EASY)		Dev (HARD)	
ROI	Group	N	Age-class	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD
ROI F	Choir singers	10	60-70 years	2.22	1.48	2.61	1.83	2.42	1.56	2.58	2.29	1.27	0.81	3.60	1.13	5.13	1.57	2.22	1.59	2.27	0.66	3.72	1.04	1.27	0.96
		12	> 70 years	1.72	1.05	2.14	1.31	2.42	2.10	2.08	1.23	1.58	2.07	4.29	2.81	5.24	2.82	3.25	2.55	2.70	1.28	3.74	1.75	2.07	1.64
		22	Total	1.95	1.26	2.35	1.55	2.42	1.83	2.31	1.76	1.44	1.59	3.98	2.19	5.19	2.29	2.78	2.18	2.51	1.05	3.73	1.44	1.71	1.41
	Controls	9	60-70 years	1.60	0.95	1.90	1.30	1.70	1.69	2.23	0.77	1.85	1.72	4.26	1.44	5.73	1.53	3.01	1.77	2.10	1.22	3.41	1.72	1.53	1.33
		10	> 70 years	2.53	1.45	2.49	1.43	2.53	1.50	2.46	1.60	2.03	1.32	3.99	1.83	4.86	1.96	3.29	1.97	2.46	1.11	3.21	1.81	1.64	1.45
		19	Total	2.09	1.30	2.21	1.37	2.14	1.60	2.35	1.25	1.95	1.48	4.12	1.62	5.27	1.78	3.16	1.83	2.29	1.14	3.31	1.72	1.59	1.35
	Total	19	60-70 years	1.93	1.26	2.27	1.60	2.08	1.62	2.41	1.71	1.55	1.32	3.92	1.30	5.41	1.54	2.60	1.68	2.19	0.94	3.57	1.37	1.39	1.12
		22	> 70 years	2.09	1.28	2.30	1.34	2.47	1.81	2.25	1.39	1.79	1.74	4.16	2.37	5.06	2.42	3.27	2.25	2.59	1.18	3.50	1.76	1.87	1.54
		41	Total	2.01	1.26	2.29	1.45	2.29	1.72	2.33	1.52	1.67	1.55	4.05	1.93	5.23	2.04	2.96	2.01	2.41	1.08	3.53	1.57	1.65	1.37
ROI C	Choir singers	10	60-70 years	2.15	1.35	2.50	1.95	2.27	1.89	2.79	2.12	1.26	0.77	3.34	0.93	4.79	1.42	1.76	1.42	1.98	0.49	3.15	1.01	1.06	1.04
		12	> 70 years	1.81	0.91	2.35	1.22	2.70	1.60	2.04	1.15	1.28	1.89	3.80	2.40	4.75	2.46	2.99	2.23	2.44	1.06	3.49	1.38	1.94	1.38
		22	Total	1.97	1.12	2.42	1.55	2.50	1.71	2.38	1.66	1.27	1.46	3.59	1.86	4.77	2.01	2.43	1.97	2.23	0.86	3.33	1.21	1.54	1.29
	Controls	9	60-70 years	1.67	1.04	2.03	1.62	1.85	2.14	2.29	1.06	1.65	1.52	3.97	1.22	5.59	1.46	2.73	1.52	2.12	0.88	3.24	1.44	1.31	0.87
		10	> 70 years	2.53	1.41	2.60	1.51	2.18	1.39	2.58	1.54	1.98	1.10	3.68	1.48	5.02	1.66	3.11	1.62	2.19	1.14	3.21	1.81	1.32	1.17
		19	Total	2.12	1.30	2.33	1.55	2.02	1.74	2.44	1.30	1.83	1.29	3.82	1.33	5.29	1.56	2.93	1.54	2.16	0.99	3.22	1.60	1.31	1.01
	Total	19	60-70 years	1.92	1.21	2.28	1.77	2.07	1.97	2.55	1.68	1.45	1.17	3.64	1.10	5.16	1.46	2.22	1.51	2.05	0.68	3.19	1.19	1.18	0.95
		22	> 70 years	2.14	1.19	2.46	1.33	2.46	1.49	2.29	1.33	1.60	1.59	3.75	1.99	4.87	2.09	3.04	1.93	2.32	1.08	3.36	1.56	1.66	1.30
		41	Total	2.04	1.19	2.38	1.53	2.28	1.72	2.41	1.49	1.53	1.39	3.70	1.62	5.01	1.81	2.66	1.78	2.20	0.91	3.28	1.39	1.43	1.16
ROI P	Choir singers	10	60-70 years	1.28	1.09	1.53	1.78	1.57	1.63	1.68	1.99	0.80	0.51	2.11	0.75	3.04	1.28	1.24	1.22	1.47	0.46	2.82	1.31	0.90	0.93
		12	> 70 years	1.12	0.69	1.42	0.95	1.72	1.26	1.19	0.96	0.69	1.19	2.41	1.71	3.05	1.86	2.00	1.26	1.74	0.91	3.30	2.04	1.73	1.18
		22	Total	1.19	0.88	1.47	1.35	1.65	1.41	1.41	1.50	0.74	0.93	2.27	1.34	3.04	1.58	1.65	1.27	1.62	0.74	3.08	1.73	1.35	1.13
	Controls	9	60-70 years	0.88	0.64	1.28	1.16	1.04	1.50	1.66	1.08	1.01	0.93	2.47	0.96	3.58	1.25	1.80	1.19	1.41	0.60	3.19	1.29	0.61	0.87
		10	> 70 years	1.11	0.61	1.36	0.85	1.18	1.08	1.55	1.10	1.24	0.72	2.11	1.39	3.03	1.54	1.82	1.54	1.34	1.11	2.28	1.68	0.81	1.11
		19	Total	1.00	0.62	1.32	0.98	1.12	1.26	1.60	1.06	1.13	0.81	2.28	1.19	3.29	1.40	1.81	1.35	1.37	0.88	2.71	1.54	0.72	0.98
	Total	19	60-70 years	1.09	0.90	1.41	1.48	1.32	1.55	1.67	1.58	0.90	0.73	2.28	0.85	3.29	1.26	1.51	1.21	1.44	0.52	3.00	1.28	0.76	0.89
		22	> 70 years	1.11	0.64	1.39	0.88	1.48	1.18	1.35	1.02	0.94	1.02	2.27	1.55	3.04	1.68	1.92	1.36	1.56	1.00	2.83	1.92	1.31	1.22
		41	Total	1.10	0.76	1.40	1.18	1.41	1.35	1.50	1.30	0.92	0.89	2.28	1.26	3.16	1.49	1.73	1.29	1.50	0.81	2.91	1.63	1.06	1.10

Table 2B. Descriptive statistics including amplitude means and standard STDs (μV) regarding the ERP responses (P1, N1 and MMN) at different ROIs for different stimuli (standard, deviant (ALL, EASY & HARD) in LOCATION condition.

LOCATION CONDITION				P1								N1								MMN (STD-DEV)					
				Standard		Dev (ALL)		Dev (EASY)		Dev (HARD)		Standard		Dev (ALL)		Dev (EASY)		Dev (HARD)		Dev (ALL)		Dev (EASY)		Dev (HARD)	
ROI	Group	N	Age-class	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD	M	STD
ROI F	Choir singers	12	60-70 years	2.81	1.43	3.08	1.49	2.75	1.78	3.44	1.65	2.58	2.58	3.63	2.89	4.12	2.87	3.09	3.04	1.94	1.30	2.36	1.93	1.77	1.56
		16	> 70 years	2.53	1.38	2.67	1.77	2.79	2.12	2.86	1.38	1.62	1.71	2.48	2.13	2.49	2.45	2.47	2.04	1.10	0.86	1.33	1.16	1.45	0.88
		28	Total	2.65	1.38	2.85	1.64	2.77	1.95	3.11	1.50	2.03	2.14	2.98	2.50	3.19	2.72	2.73	2.48	1.46	1.13	1.77	1.59	1.59	1.20
	Controls	14	60-70 years	1.86	0.91	2.24	0.86	2.37	1.09	1.91	1.24	2.06	1.51	3.09	1.37	3.18	2.09	3.41	1.46	1.29	0.82	1.34	1.30	1.46	0.70
		9	> 70 years	1.92	0.88	2.26	1.56	2.55	1.57	2.13	2.00	2.38	1.33	3.42	1.41	3.32	1.89	3.44	1.93	1.08	1.02	1.01	1.16	1.38	1.28
		23	Total	1.89	0.88	2.25	1.15	2.44	1.27	1.99	1.54	2.19	1.42	3.22	1.36	3.23	1.97	3.42	1.62	1.21	0.88	1.21	1.23	1.43	0.94
	Total	26	60-70 years	2.30	1.25	2.63	1.24	2.54	1.43	2.61	1.61	2.30	2.05	3.34	2.17	3.61	2.48	3.26	2.28	1.59	1.09	1.81	1.67	1.60	1.16
		25	> 70 years	2.31	1.24	2.53	1.68	2.70	1.91	2.60	1.63	1.89	1.60	2.82	1.92	2.79	2.26	2.82	2.02	1.09	0.90	1.21	1.15	1.43	1.02
		51	Total	2.31	1.23	2.58	1.46	2.62	1.67	2.61	1.60	2.10	1.84	3.09	2.05	3.21	2.39	3.05	2.15	1.34	1.02	1.52	1.45	1.52	1.09
ROI C	Choir singers	12	60-70 years	2.59	1.29	2.87	1.26	2.66	1.36	3.20	1.60	2.43	2.36	3.39	2.66	3.71	2.67	3.01	2.83	1.71	1.05	1.96	1.65	1.73	1.11
		16	> 70 years	2.58	1.71	2.71	1.95	2.64	2.22	2.84	2.06	1.38	1.64	2.26	1.86	2.41	2.31	2.40	1.87	1.16	0.66	1.36	1.10	1.52	0.95
		28	Total	2.59	1.52	2.78	1.66	2.64	1.87	3.00	1.86	1.83	2.01	2.74	2.27	2.97	2.51	2.66	2.30	1.40	0.88	1.61	1.37	1.61	1.01
	Controls	14	60-70 years	1.89	1.00	2.14	1.07	2.29	1.37	2.06	0.92	1.94	1.31	2.90	1.46	3.07	1.72	2.97	1.64	1.30	0.80	1.55	1.02	1.25	0.81
		9	> 70 years	1.73	0.86	2.15	1.18	2.25	1.48	1.92	1.60	2.26	1.28	3.22	1.43	3.24	1.66	3.27	1.74	1.07	0.97	0.91	1.28	1.33	1.34
		23	Total	1.83	0.93	2.15	1.09	2.27	1.38	2.01	1.20	2.07	1.28	3.02	1.43	3.14	1.66	3.09	1.65	1.21	0.85	1.30	1.14	1.28	1.02
	Total	26	60-70 years	2.21	1.17	2.48	1.20	2.46	1.35	2.59	1.38	2.16	1.84	3.12	2.07	3.37	2.19	2.99	2.22	1.49	0.93	1.74	1.33	1.47	0.97
		25	> 70 years	2.28	1.50	2.51	1.71	2.49	1.96	2.51	1.93	1.70	1.55	2.61	1.75	2.71	2.10	2.71	1.84	1.13	0.77	1.20	1.16	1.45	1.08
		51	Total	2.24	1.33	2.50	1.45	2.48	1.66	2.55	1.65	1.94	1.71	2.87	1.92	3.04	2.15	2.85	2.03	1.31	0.86	1.47	1.27	1.46	1.02
ROI P	Choir singers	12	60-70 years	1.41	1.01	1.50	0.97	1.51	1.15	1.72	1.20	1.62	1.63	2.25	1.76	2.48	1.77	1.90	1.79	1.27	0.73	1.51	1.35	1.32	0.85
		16	> 70 years	1.41	1.06	1.46	1.20	1.60	1.42	1.63	1.31	0.78	1.07	1.38	1.21	1.52	1.56	1.62	1.33	0.92	0.57	1.12	0.90	1.24	1.14
		28	Total	1.41	1.02	1.48	1.09	1.56	1.29	1.67	1.24	1.14	1.38	1.76	1.50	1.93	1.69	1.74	1.52	1.07	0.66	1.29	1.11	1.27	1.01
	Controls	14	60-70 years	1.04	0.64	1.14	0.92	1.36	1.17	1.21	0.78	1.19	0.78	1.77	1.06	1.97	1.25	1.70	1.26	1.07	0.90	1.22	1.28	0.85	1.07
		9	> 70 years	0.75	0.59	1.02	0.78	1.16	0.86	0.78	0.91	1.37	0.95	2.08	0.86	2.20	1.02	2.34	1.08	0.70	0.60	0.63	0.93	0.89	0.85
		23	Total	0.93	0.63	1.09	0.85	1.28	1.05	1.04	0.84	1.26	0.83	1.89	0.98	2.06	1.15	1.95	1.21	0.93	0.80	0.99	1.17	0.87	0.97
	Total	26	60-70 years	1.21	0.84	1.31	0.94	1.43	1.14	1.44	1.01	1.39	1.23	1.99	1.41	2.20	1.50	1.79	1.50	1.16	0.81	1.35	1.30	1.07	0.99
		25	> 70 years	1.17	0.96	1.30	1.07	1.44	1.25	1.33	1.23	0.99	1.05	1.64	1.13	1.76	1.41	1.88	1.27	0.84	0.58	0.94	0.92	1.11	1.04
		51	Total	1.19	0.89	1.30	1.00	1.44	1.18	1.39	1.12	1.20	1.15	1.82	1.28	1.99	1.46	1.84	1.38	1.01	0.72	1.15	1.14	1.09	1.00

Amplitudes of deviant and standard ERPs (µV) were averaged separately for each participant, condition (PITCH/LOCATION), and stimuli (standard and deviant: EASY/HARD/ALL). Grand-average waveforms were computed for standard, easy and hard deviant stimuli in both condition and plotted in Figure 5 separately for groups (choir singers/controls).

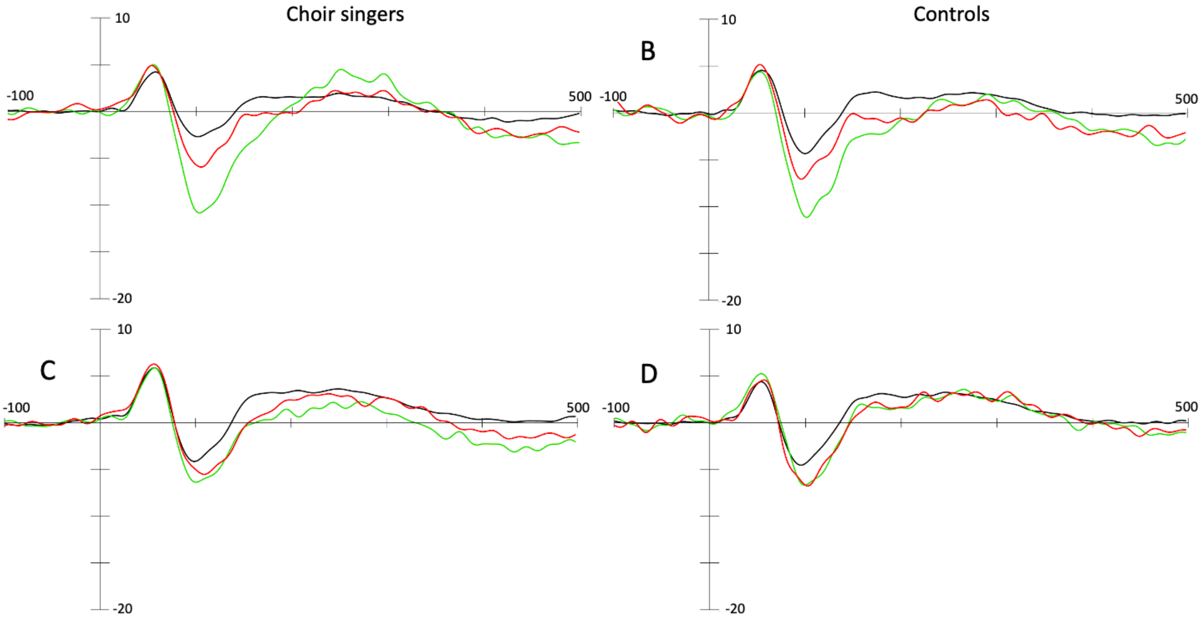

Figure 5. The grand-average topography of ERP responses in PITCH (A and B) and LOCATION (C and D) conditions in frontal region of interest (ROI F). A) senior choir singers (n=22), B) controls (n=19), C) senior choir singers (n=28) and D) controls (n=23). Scales on X-axis: milliseconds, Y-axis: µV. Black color denotes response to standard stimuli, in which in PITCH condition the pitch of the standard tone is locked in the highest or the lowest tone (balanced between subjects), and respectively in LOCATION condition the location of the tones is locked in the far-right or the far-left speaker position (balanced between subjects). Green color denotes response to easy deviant and red color in hard deviant stimuli.

For exploring the variance between the groups, repeated ANOVA analysis was applied. Following the path of significant (or near-significant, $p < .10$) interactions hierarchically downward, the result is that in PITCH condition there is no significant differences between choir singers and controls regarding any effects on inspected stimulus (i.e., standard or deviant: EASY/HARD/ALL) on any of the ERP responses (P1, N1 or MMN), (Table 3).

Table 3. Summary of repeated ANOVA analyses on PITCH condition for standard stimulus and deviants grouped in EASY, HARD and ALL. G = group (choir singers vs. controls), A-G = Age-group (60-70 years vs. over 70 years). No statistically significant effects.

STIMULUS	Between-Subject Factor(s)	Within-subject Factor(s)	F	p	df	Error	Correction for lack of sphericity
STANDARD PITCH	Group	-	1.12	.297	1	37	
		Group x ERP	0.70	.407	1.00	37.0	Greenhouse-Geisser
		Group x ROI	1.91	.173	1.21	44.9	Greenhouse-Geisser
		Group x ERP x ROI	0.20	.715	1.26	46.6	Greenhouse-Geisser
	Age-group	-	0.52	.474	1	37	
		Age-group x ERP	0.00	.985	1.00	37.0	Greenhouse-Geisser
		Age-group x ROI	1.14	.303	1.21	44.9	Greenhouse-Geisser
		Age-group x ERP x ROI	0.11	.795	1.26	46.6	Greenhouse-Geisser
	Group x age-group	-	1.68	.203	1	37	
		G x A-G x ERP	0.53	.470	1.00	37	Greenhouse-Geisser
		G x A-G x ROI	1.10	.313	1.21	44.9	Greenhouse-Geisser
		G x A-G x ERP x ROI	3.55	.056	1.26	46.6	Greenhouse-Geisser
DEVIANTS (ALL)	Group	-	0.04	.843	1	37	
		Group x ERP	0.22	.727	1.42	52.6	Greenhouse-Geisser
		Group x ROI	0.39	.577	1.23	45.5	Greenhouse-Geisser
		Group x ERP x ROI	0.10	.898	1.91	70.7	Greenhouse-Geisser
	Age-group	-	0.30	.589	1	37	
		Age-group x ERP	0.06	.885	1.42	52.6	Greenhouse-Geisser
		Age-group x ROI	0.78	.407	1.23	45.5	Greenhouse-Geisser
		Age-group x ERP x ROI	0.20	.807	1.91	70.7	Greenhouse-Geisser
	Group x age-group	-	0.07	.794	1	37	
		G x A-G x ERP	0.91	.380	1.42	52.6	Greenhouse-Geisser
		G x A-G x ROI	0.32	.620	1.23	45.5	Greenhouse-Geisser
		G x A-G x ERP x ROI	0.74	.474	1.91	70.7	Greenhouse-Geisser
DEVIANTS (EASY)	Group	-	0.18	.676	1	37	
		Group x ERP	0.73	.455	1.57	58.2	Greenhouse-Geisser
		Group x ROI	0.60	.501	1.46	54.2	Greenhouse-Geisser
		Group x ERP x ROI	0.49	.649	2.43	90.0	Greenhouse-Geisser
	Age-group	-	0.00	.958	1	37	
		Age-group x ERP	0.49	.573	1.57	58.2	Greenhouse-Geisser
		Age-group x ROI	0.45	.582	1.46	54.2	Greenhouse-Geisser
		Age-group x ERP x ROI	0.32	.771	2.43	90.0	Greenhouse-Geisser
	Group x age-group	-	0.33	.571	1	37	
		G x A-G x ERP	0.34	.661	1.57	58.2	Greenhouse-Geisser
		G x A-G x ROI	0.86	.398	1.46	54.2	Greenhouse-Geisser
		G x A-G x ERP x ROI	1.21	.309	2.43	90.0	Greenhouse-Geisser
DEVIANTS (HARD)	Group	-	0.04	.837	1	37	
		Group x ERP	0.59	.519	1.56	57.8	Greenhouse-Geisser
		Group x ROI	1.17	.297	1.19	44.2	Greenhouse-Geisser
		Group x ERP x ROI	1.02	.369	2.11	77.6	Greenhouse-Geisser
	Age-group	-	1.30	.262	1	37	
		Age-group x ERP	1.03	.349	1.56	57.8	Greenhouse-Geisser
		Age-group x ROI	0.50	.516	1.19	44.2	Greenhouse-Geisser
		Age-group x ERP x ROI	0.47	.636	2.11	77.6	Greenhouse-Geisser
	Group x age-group	-	0.28	.599	1	37	
		G x A-G x ERP	0.93	.378	1.56	57.8	Greenhouse-Geisser
		G x A-G x ROI	0.07	.837	1.19	44.2	Greenhouse-Geisser
		G x A-G x ERP x ROI	0,423	0,667	2,107	77,593	Greenhouse-Geisser

When following a similar path of interactions in the LOCATION condition, the result was again that there were no significant differences between choir singers and controls regarding any effects on inspected stimulus. However, when the location of deviant was spatially closer to the standard location, i.e., hard deviant, almost significant (*p*=.076) effect was found on interaction between group and response (ERP), see Table 4. Age did not have an effect.

Table 4. Summary of repeated ANOVA analyses on LOCATION condition for standard stimulus and deviants grouped in EASY, HARD and ALL. G = group (choir singers vs. controls), A-G = Age-group (60-70 years vs. over 70 years). No statistically significant effects.

STIMULUS	Between-Subject Factor(s)	Within-subject Factor(s)	F	p	df	Err	Correction for lack of sphericity
STANDARD LOCATION	Group	-	1.24	.272	1	47	
		Group x ERP	2.00	.164	1.00	47.0	Greenhouse-Geisser
		Group x ROI	0.37	.600	1.27	59.8	Greenhouse-Geisser
		Group x ERP x ROI	1.01	.343	1.35	63.5	Greenhouse-Geisser
	Age-group	-	0.82	.369	1	47	
		Age-group x ERP	0.15	.697	1.00	47.0	Greenhouse-Geisser
		Age-group x ROI	0.02	.936	1.27	59.8	Greenhouse-Geisser
		Age-group x ERP x ROI	0.05	.888	1.35	63.5	Greenhouse-Geisser
	Group x age-group	-	1.46	.240	1	47	
		G x A-G x ERP	1.17	.285	1.00	47.0	Greenhouse-Geisser
		G x A-G x ROI	1.41	.246	1.27	59.8	Greenhouse-Geisser
		G x A-G x ERP x ROI	0.51	.531	1.35	63.5	Greenhouse-Geisser
DEVIANTS (ALL)	Group	-	0.95	.335	1	47	
		Group x ERP	1.00	.350	1.41	66.4	Greenhouse-Geisser
		Group x ROI	0.23	.686	1.25	58.6	Greenhouse-Geisser
		Group x ERP x ROI	0.48	.647	2.3	107.9	Greenhouse-Geisser
	Age-group	-	1.89	.176	1	47	
		Age-group x ERP	0.19	.748	1.41	66.4	Greenhouse-Geisser
		Age-group x ROI	0.53	.509	1.25	58.6	Greenhouse-Geisser
		Age-group x ERP x ROI	0.08	.946	2.3	107.9	Greenhouse-Geisser
	Group x age-group	-	1.98	.166	1	47	
		G x A-G x ERP	0.77	.425	1.41	66.4	Greenhouse-Geisser
		G x A-G x ROI	1.68	.201	1.25	58.6	Greenhouse-Geisser
		G x A-G x ERP x ROI	0.10	.928	2.3	107.9	Greenhouse-Geisser
DEVIANTS (EASY)	Group	-	0.93	.341	1	47	
		Group x ERP	0.40	.590	1.33	62.7	Greenhouse-Geisser
		Group x ROI	0.50	.538	1.37	64.2	Greenhouse-Geisser
		Group x ERP x ROI	0.19	.830	2.03	95.2	Greenhouse-Geisser
	Age-group	-	1.98	.166	1	47	
		Age-group x ERP	0.60	.486	1.33	62.7	Greenhouse-Geisser
		Age-group x ROI	0.44	.570	1.37	64.2	Greenhouse-Geisser
		Age-group x ERP x ROI	0.49	.618	2.03	95.2	Greenhouse-Geisser
	Group x age-group	-	0.95	.335	1	47	
		G x A-G x ERP	0.91	.371	1.33	62.7	Greenhouse-Geisser
		G x A-G x ROI	2.33	.123	1.37	64.2	Greenhouse-Geisser
		G x A-G x ERP x ROI	0.17	.847	2.03	95.2	Greenhouse-Geisser
DEVIANTS (HARD)	Group	-	1.40	.243	1	47	
		Group x ERP	2.99	.076	1.36	64.1	Greenhouse-Geisser
		Group x ROI	0.18	.716	1.19	56.0	Greenhouse-Geisser
		Group x ERP x ROI	1.26	.292	2.47	116.0	Greenhouse-Geisser
	Age-group	-	0.36	.550	1	47	
		Age-group x ERP	0.04	.911	1.36	64.1	Greenhouse-Geisser
		Age-group x ROI	0.80	.397	1.19	56.0	Greenhouse-Geisser
		Age-group x ERP x ROI	0.51	.639	2.47	116.0	Greenhouse-Geisser
	Group x age-group	-	0.84	.365	1	47	
		G x A-G x ERP	0.19	.739	1.36	64.1	Greenhouse-Geisser
		G x A-G x ROI	0.60	.469	1.19	56.0	Greenhouse-Geisser
		G x A-G x ERP x ROI	0.85	.450	2.47	116.0	Greenhouse-Geisser

A further exploring of this interaction between group and ERP response for hard deviant stimulus revealed a statistically significant main effect (*p*=.015) on P1 component of the ERP signal (Table 5). Additionally, choir singers had higher P1 response to difficult (hard) deviants at all studied ROIs compared to controls (Table 6; Figure 6). Again, age had no significant effect.

Table 5. Summary of repeated ANOVA analyses on LOCATION condition for different ERPs (P1, N1 and MMN) when stimulus is HARD deviant. G = group (choir singers vs. controls), A-G = Age-group (60-70 years vs. over 70 years). Statistically significant effects bolded.

ERP FOR HARD DEVIANTS	Between-Subject Factor(s)	Within-subject Factor(s)	F	p	df	Error	Correction for lack of sphericity
P1	Group	-	**6.34**	**.015**	1	47	
		Group x ROI	1.37	.259	1.82	85.7	Greenhouse-Geisser
	Age-group	-	0.37	.548	1	47	
		Age-group x ROI	0.05	.943	1.82	85.7	Greenhouse-Geisser
	Group x age-group	-	0.87	.769	1	47	
		G x A-G x ROI	2.01	.145	1.82	85.7	Greenhouse-Geisser
N1	Group	-	0.73	.397	1	47	
		Group x ROI	0.74	.432	1.34	63.1	Greenhouse-Geisser
	Age-group	-	0.03	.860	1	47	
		Age-group x ROI	1.11	.315	1.34	63.1	Greenhouse-Geisser
	Group x age-group	-	0.65	.424	1	47	
		G x A-G x ROI	0.11	.812	1.34	63.1	Greenhouse-Geisser
MMN	Group	-	1.25	.270	1	47	
		Group x ROI	0.57	.511	1.42	66.8	Greenhouse-Geisser
	Age-group	-	0.12	.728	1	47	
		Age-group x ROI	0.39	.608	1.42	66.8	Greenhouse-Geisser
	Group x age-group	-	0.15	.698	1	47	
		G x A-G x ROI	0.09	.854	1.42	66.8	Greenhouse-Geisser

Table 6. Result of multivariate ANOVA analysis on LOCATION condition for different ROIs (F, C and P) when considering the P1 ERP-component on hard deviant stimuli. G = group (choir singers vs. controls), A-G = Age-group (60-70 years vs. over 70 years). Statistically significant effects bolded.

P1 FOR HARD DEVIANTS	Between-Subject Factor(s)	ROI	F	p	df	Error
	Group	F	6.66	**.013**	1	47
		C	4.91	**.032**	1	47
		P	4.77	**.034**	1	47
	Age-group	F	0.16	.687	1	47
		C	0.29	.593	1	47
		P	0.67	.416	1	47
	Group x age-group	F	0.83	.366	1	47
		C	0.05	.829	1	47
		P	0.29	.594	1	47

Table 7. Means and standard deviations for P1 amplitudes (μV) in different ROIs (F, C and P) in choir singers and controls on LOCATION condition for the hard deviant stimuli.

	CHOIR SINGERS		CONTROLS		
ROI	M	DEV	M	DEV	p
F	3.11	1.50	1.99	1.54	.012
C	3.00	1.86	2.01	1.20	.032
P	1.67	1.24	1.04	0.84	.043

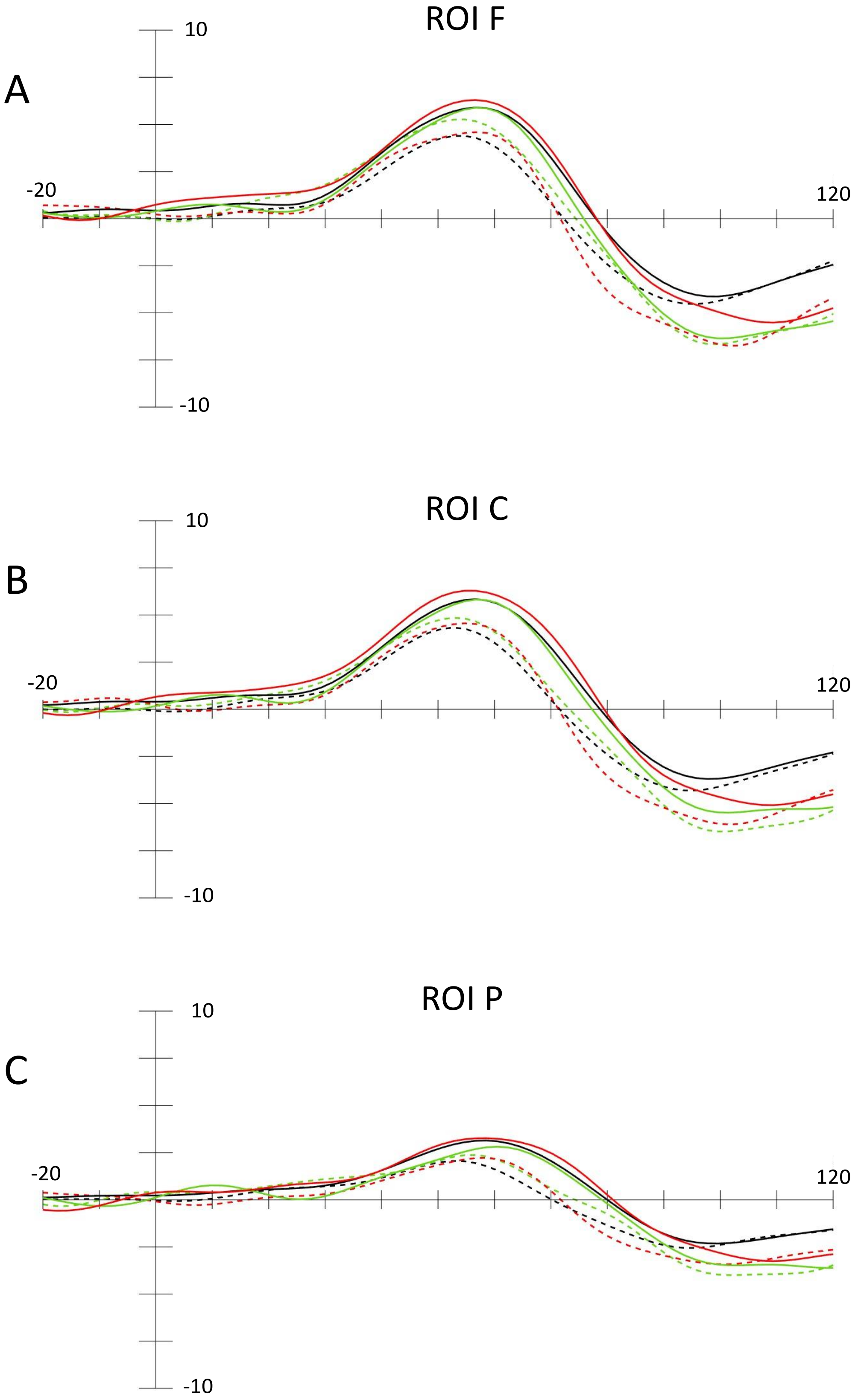

Figure 6. The grand-average topography of P1 response in LOCATION condition in frontal ROI F (A), in central ROI C (B), and in parietal ROI P (C). Responses of senior choir singers (n=28) presented in solid lines and of controls (n=23) presented in dashed lines. Scales on X-axis: milliseconds, Y-axis: μV. Blue color denotes grand-average response to standard stimuli, in which the location of the tones is locked in the far-right or the far-left speaker position (balanced between subjects). Green color denotes grand-average response to easy deviant and red color in hard deviant stimuli.

In the Table 7 the mean amplitudes with standard deviations for both groups are presented. The differences between choir singers and controls corresponds medium effect sizes for each ROI. d_{Cohen} = .74 for frontal electrodes (ROI F), d_{Cohen} = .62 for central electrodes (ROI C), and d_{Cohen} = .59 for parietal electrodes (ROI P).

When responses (P1, N1, and MMN) were examined within a group of choir singers relative to the choir singing years reported by subjects, it was observed that the MMN response to hard deviants in PITCH-condition reflects the number of choir singing years in each ROI statistically very significantly. The results are reported in Table 8. The fit of the model was best in ROI C: F (1,20) = 11.70; p = .003 and R^2 = .369. The higher the number of choir singing years, the stronger the MMN response amplitude, B = 0.046; 95% CI = [0.018; 0.074], β = 0.607; t = 3.42; p < .003 (Figure 7).

The N1 response to easy deviants in the LOCATION condition also reflected the number of choir singing years in ROI F. In this case, the fit of the model was F(1,26) = 5.069; p = .033 and R^2 = .163. The higher the number of choir singing years, the lower the N1 response, B = -0.070; 95% CI = [-0.133; -0.006]; β = -0.404; t = -2.51; p < .033.

Table 8. Summary of parameters of the regression models, in which the dependent variable was the ERP amplitude (P1, N1 or MMN) for standard and deviating stimuli in one of the ROIs (F/C/P) and in either condition (LOCATION/PITCH) and the independent variable was the number of choir singing years. R2 = R square. Beta = standardized beta coefficient. In case of statistically significant regression models the values are bolded.

			LOCATION				PITCH			
ERP	**STIMULUS**	**ERP**	**F(1,26)**	**p**	**R2**	**Beta**	**F(1,20)**	**p**	**R2**	**Beta**
P1	STD	F	0.014	.908	.001	0.023	0.029	.866	.001	−0.038
		C	0.369	.549	.014	0.118	0.086	.773	.004	0.065
		P	1.210	.281	.044	0.211	0.603	.446	.029	0.171
	EASY	F	0.015	.905	.001	0.024	1.128	.301	.053	0.231
		C	0.046	.832	.002	0.042	1.906	.183	.087	0.295
		P	0.000	.990	.000	−0.002	1.048	.318	.050	0.223
	HARD	F	1.939	.176	.069	−0.263	0.129	.723	.006	−0.080
		C	0.259	.615	.010	−0.099	0.088	.769	.004	−0.066
		P	0.232	.634	.009	0.094	0.087	.771	.004	0.066
N1	STD	F	3.399	.077	.116	−0.340	1.253	.276	.059	−0.243
		C	3.296	.081	.113	−0.335	1.492	.236	.069	−0.263
		P	2.732	.110	.095	−0.308	1.751	.201	.080	−0.284
	EASY	**F**	**5.069**	**.033**	**.163**	**−0.404**	1.234	.280	.058	−0.241
		C	4.149	.052	.138	−0.371	0.615	.442	.030	−0.173
		P	3.054	.092	.105	−0.324	0.016	.901	.001	−0.028
	HARD	F	3.771	.063	.127	−0.356	0.101	.754	.005	0.071
		C	2.835	.104	.098	−0.314	0.554	.465	.027	0.164
		P	0.631	.434	.024	−0.154	1.370	.256	.064	0.253
MMN	EASY	F	3.302	.081	.113	−0.336	0.140	.712	.007	0.083
		C	3.249	.083	.111	−0.333	0.845	.369	.041	0.201
		P	2.454	.129	.086	−0.294	0.712	.409	.034	0.185
	HARD	F	0.249	.622	.009	−0.097	**10.145**	**.005**	**.337**	**0.580**
		C	0.001	.977	.000	−0.006	**11.695**	**.003**	**.369**	**0.607**
		P	0.670	.421	.025	0.158	**10.766**	**.004**	**.350**	**0.592**

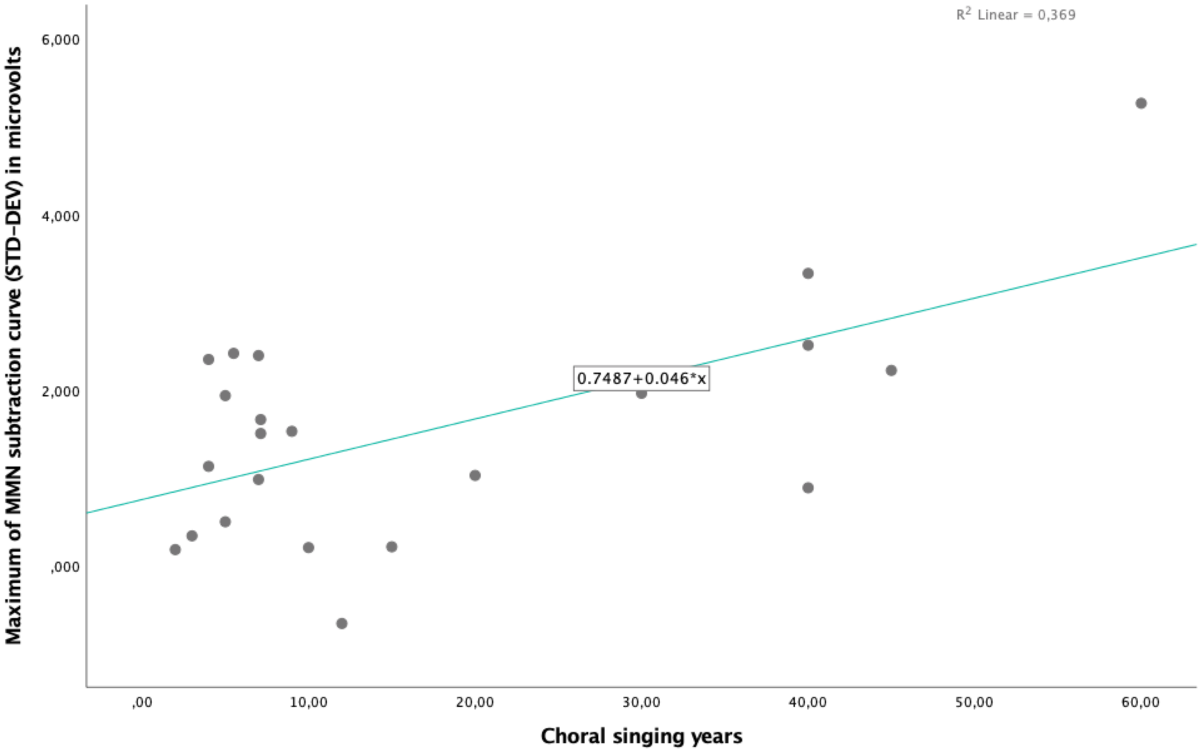

Figure 7. The scatter plot of maximum amplitude of MMN subtraction curve in choir singers (N=22) for hard deviants in PITCH condition in central ROI C against choir singing years with regression line.

4. Discussion

The main purpose of this study was to examine the possible effects of long-term choir singing on i) early general and ii) subsequent change-related auditory encoding by comparing healthy senior choir singers over the age of 60 and their healthy matched control subjects. In addition, the possible effects of age on the above-mentioned auditory encoding were also investigated. The cognitive processing was assessed by using a passive oddball paradigm, with a setup in which subjects were unattentively listening standard tones and their frequential or spatial deviants, which provided means to study age-related changes in auditory processing while minimizing the contribution of attentative factors (Harris et al., 2008).

At the general level, senior choir singers' group and the control group did not show significant differences in their auditory processing of sinusoidal standard tones or deviants with different pitch or tone location. However, P1 response to the spatially deviating tones (hard deviants in LOCATION condition) reached the statistical significance level of .05 in all the ROIs included in the study – P1 amplitude in choir singers was larger than in the control group. So, at least in the current study design it seems that lifetime choir singing has no other major effects on the studied CAEPs (P1/N1/MMN) in auditory signal processing across different brain regions ROI (F/C/P). No significant group-distinguishing results were found when the age groups (60-70 years and over 70 years) or their interaction with subject group was examined.

4.1 The effects of choir singing on early obligatory auditory ERP responses

Regarding the early obligatory ERP components (P1 and N1) in PITCH and LOCATION conditions the study revealed a statistically significant difference between choir singers and controls only in the LOCATION condition, which was used to examine the auditory signal processing differences in detecting sound locations deviating from the standard sound source (meaning speaker on either the far-left or far-right). In this condition it was found that the early long-latency P1 response of choir singers was greater than that of controls in all the studied ROIs (F, C and P). No other differences were found between the groups regarding the P1 and N1. Nor could statistically significant difference in any response be found between two age groups of subjects (from 60 to 70 years in age, and over 70 years of age).

For choir singers, ability to discriminate different choral sounds spatially is key to successful performance. Could the current finding, in relation to the early pre-attentative P1 response, refer to the more sensitive resolution of spatial auditory information that has developed through the practice of music and especially through decades choir singing in the group of choir singers? For this to be the case, a logical assumption follows that this would also be reflected in the MMN response, however, this does not happen. Of course, it should not be ignored that there is a theoretical possibility that the elevated response could describe some innate neural processing pattern in the group of choir singers. Such a possibility could more reasonable explain the observed CAEP pattern in which an elevated P1 response is observed in relation to stimulus discrimination without seeing any reflections in the MMN response.

Logically, it seems relevant that specifically singing in a choir has developed such neural activity related to distinguishing (deviant) sound locations in choir singers relative to controls – in a choir, individuals have to listen to their own voice in relation to other stems that are spatially (and in frequency) different. As well, singers' own voices have to be in line with the singers in the same stem.

Because of the general concordance of CAEP responses, the initial assumption is that effect of musical pursuit and ageing on the processing and CAEP responses of spatial auditory information is similar to the effects of other relevant auditory information. In general, based on previous studies, it can be assumed that on passive condition with sinusoidal stimuli the musical pursuit should decrease the amplitude of P1 relative to controls (Zendel & Alain, 2014; Nikjeh et al., 2009). On the other hand, the effect of ageing on these early components has been found to be the opposite (Zendel & Alain, 2014). Since higher P1 amplitudes were observed in choir singers, this would suggest, contrary to the research hypothesis, that musical pursuit, i.e., choir singing, would be reflected on increase of P1 amplitude for spatial deviants. Even if there seems to be a lack of studies on effects (of ageing) on P1 and N1 responses on spatially deviant auditory stimuli to which the result could be contrasted, the finding would be unique in a kind compared to the putative P1 response. Ageing has usually been found to elevate P1 responses, however, no differences in P1 amplitudes were observed between age groups. How could the observation then be explained?

Would it be possible that the higher P1 amplitude of choir singers would reflect the increased, compensatory neural processing associated with a difficult spatially deviating stimulus? This to be the case, one could assume that the phenomenon would also be reflected on easier deviants – according to earlier reports easier spatial deviants produce a higher response than difficult deviants (e.g., Sonnadara et al., 2006). In fact, for easier deviants the amplitude of P1 seems in visual examination to be lower on average in the LOCATION condition (Table 2). For the N1 response, the amplitudes are in line with previous research results: easier spatial deviants may produce a higher response than difficult deviants, even if statistical difference is not reached between the groups (Table 2). Thus, the observation of a statistically significant difference in the amplitude of P1 amplitudes between groups for hard deviants is difficult to explain as compensatory neural processing. It is also difficult to understand how the observed differences between the groups in P1 response would not be reflected in differences in MMN responses.

An additional challenge to the interpretation is the spatial quality (i.e., the amount of difference in the direction of the tone) of the actual deviant stimulus. When compared to previous study designs (e.g., Colin et al., 2002; Deouell et al., 2006), it is obvious that the difficulty level of the hard spatial deviant actually is rather mild. So, differences between easy and hard spatial deviants can be arbitrary regarding the size of the difference or angle between the standard and deviant tone sources (in minimum 36 degrees), and in the light of previous studies (Deouell et al., 2006) the spatial resolution in healthy adult subjects easily is in the order of 10 degrees. It would be interesting to still conduct an additional analysis where the spatial stimulus classes (easy and hard) would be dismantled, and the analysis would be made with five different deviating classes. However, the number of stimuli per deviant speaker in the experimental setup (in maximum 36 deviant trials) and the quality of the EEG data, as well small groups would weaken the statistical power. However, such an analysis could provide more rationale for the experimental design arrangement in which spatial discrimination capacity should be examined. In this way, the data omitted from the analysis when EASY and HARD deviant classes were constructed, would also be included in the analysis.

Could the phenomenon be explained groupwise difference in attentive behavior? Attentive behavior has been reported to increase N1 but not P1 amplitude. However, in older subjects the attention dependent increase in N1 amplitude has been milder than in younger subjects (Zendel & Alain, 2014). Contrary to the N1, a suppression in the amplitude of P1 is usually seen as “cost of attention” (Luck et al., 1994; Getzmann et al., 2015). So, it can be theorized that if for some reason control subjects would have behaved differently as a group in the experimental situation paying more attention to stimuli during the experiment, this would be seen at the group level as attenuated P1 amplitude increasing the difference between groups. This to be the case, it would be assumed that the N1 amplitude in control group would also increase with attention. In fact, the response of the control group N1 to hard spatial deviants is higher (mostly in ROI F) relative to the group of choir singers (Table 2), but with no significant effect. On the other hand, this phenomenon would also be expected to be seen similarly in the case of easy deviants, but this is not the case.

Is it possible to explain the phenomenon so that the sound sources of easy deviants are on the opposite side of the subject's centerline as the standard and the sound sources of hard deviants on the same side, which would then be reflected as a difference in CAEPs between the groups? This to be the case, the difference in P1 (CAEP) between the groups could have been arisen because of groupwise different behavioral (attentional) or neural response to the deviant tone stimuli located on the same or different side than the standard tone from the centerline of the subject. However, such speculation seems unfounded in the light that no other differences between the groups in ERP responses was revealed.

When exploring the variance between the groups in PITCH condition no significant differences between choir singers and controls (or between age-groups) on early ERP responses (P1, N1) or MMN was found regarding any effects on inspected standard or deviant stimuli. In fact, this result regarding the P1 amplitudes is in line with study results of Nikjeh et al. (2009) using pure tones as stimuli. N1 amplitude has found to be enhanced in musicians more in relation to specific musical components of the stimuli (Baumann et al., 2008; Pantev et al., 1998 and 2001), and the non-musical sinusoidal stimulus type may affect the N1 responses in a way that the group of choir singers does not benefit from their musical experience in relation to the stimuli.

The fact that no differences are observed when comparing age groups is most likely due to the fact that the difference between the age groups is marginal: the subjects in both groups under review are seniors. In addition, due to such grouping, the groups also become more heterogeneous as both age groups include subjects with and without musical experience, possibly increasing the within-group variance of P1 and N1 responses: musical experience should have (at least in theory) the opposite effect on P1 and N1 amplitudes than the age. This makes it difficult to interpret age-related phenomena. In fact, when looking at Table 2, a tendency can be seen that the P1 and N1 amplitudes of the older choir singers group could be lower than the younger ones.

When the group of choir singers was examined closer, it was found that with age group distribution, the experience of singing in the choir is unevenly distributed among the groups of younger, from 60 to 70 years old, and older, over 70 years old. In the group of older choir singers, the experience measured in choir singing years was on average 23.9 years (SD = 18.1 years; LOCATION condition) compared to 7.8 years (SD = 5.2 years) in the group of younger choir singers. Respectively regarding the PITCH condition older singers' choir years on average were 25.4 years (SD = 19.2 years) compared to 8.0 years (SD = 5.5 years) in the group of younger singers. This means that in the group of older choir singers there is over three times more singing experience (measured in years) than in the group of younger singers ($t = 3.82$; $p = .001$). In addition, in the group of younger choir singers, more than 2/3 have started choir singing only in senior age (average 59 years, M = 61 years), while in the group of older choirs less than half had started choir singing so late (average 51 years, M = 61 years), the difference is almost significant ($t = 2.09$; $p = .052$). It might be speculated that the beginning age of choir singing may reflect the degree of dedication or devotion to singing or choir singing. This again, could reflect on the plastic development of neural processes. So, when attempting to investigate the potential effects of choir singing against cognitive decline in old age using such a subject population, it is good to evaluate whether the group of subjects selected in the group of choir singers validly represent the group attributes (choir singing experience) or then is too heterogeneous.

This observation about the unequal distribution of singing experience in the group of choir singers was explored *post hoc* with an additional regression analysis. In this analysis a longer choir singing experience associated with a lower N1 response to easy spatial deviations in ROI F, which may be indicating that experienced choir singers become habituated to this class of stimuli. However, given the exploratory nature of the additional regression analysis and the fact this was the only statistically significant result regarding the spatial deviants, it is not worthwhile to draw major conclusions from this observation. The *p*-value (.033) is relatively low considering that no multiple comparison was performed. However, based on the results of regression analysis a larger data could provide certainty as to whether the amount of choir singing experience is reflected in the MMN and N1 responses of easy deviants.

4.2 The effects of choir singing on MMN responses

Why there was not an analogical response for MMN as we see for P1 in the LOCATION condition? It would be assumed that the effect would be similarly reflected specifically in the MMN response on hard deviants. However, this was not the case (*p* = .270; see Table 5).

The results regarding the MMN responses both in PITCH and LOCATION conditions indicate that the group of choir singers is neither statistically significantly better in distinguishing pitch deviations nor spatial deviations. However, it cannot be ruled out that the lack of statistical group difference in the MMN amplitude results from a relatively small MMN amplitudes combined with a large within-group variation (see Tables 2A and B). However, by visually inspecting (Tables 2A and B) the MMN amplitudes (or difference waves) are bigger in both conditions for choir singers and regarding the quality of the deviant (EASY/HARD), the amplitudes of MMN responses are more prominent for easy deviants and the difference between the groups is smaller in hard deviants, even though the statistical significance is not met. Again, if we assume that the hypotheses for current study based on previous literature are correct, then a question that arises for the curious is whether the experimental design or the groups (taking account groupwise heterogeneity in the auditory cognition) were carefully chosen for producing possible differences between groups of subjects?

When looking at the PITCH condition, a potential reason that no difference in MMN amplitudes was observed between the groups may be that the selected frequency differences are relatively coarse, in other words too simple to produce a groupwise difference between choir singers and controls (Nikjeh et al., 2009; Tervaniemi et al., 2005). At its smallest, the differences between the standard and the deviating tones were 12.3% (740 Hz – 831 Hz) and 10.9% (1319 Hz – 1175 Hz). The naming of the pitch deviants as easy and hard may be misleading, a more sensible designation would probably be easy and moderate instead of easy and hard. For example, Tervaniemi et al. (2005) in their data presented evidence that professional musicians outperformed behaviorally (actively attending) nonmusicians in discriminating the pitch changes more accurately in the small (0.8 %) and medium (2 %) pitch shifts, but not in large (4 %) pitch shift. However, in a passive, non-attending condition this was not reflected as a difference in MMN between the groups. The authors questioned whether the auditory perceptual task was too simplistic to reveal MMN amplitude differences between musicians and nonmusicians in passive condition. Also, the authors suggested that musical expertise may not exert necessarily neural processes on pre-attentive but merely at attentive levels. On the other hand, the studies of Koelsch et al. (1999) focusing on pitch difference detection in musicians and nonmusicians used marginally mistuned (<1 %) middle tone in a major sinusoidal chord in a passive condition. The deviants elicited the MMN only in musicians. In light of above, it seems rather obvious that the frequency differences selected for the experimental setup were not small enough to reveal statistically significant differences between MMN-responses of amateur choir singers and their matched controls. However, an exploratory *post hoc* regression analysis conducted for choir singers brings another perspective to this discussion. The regression study found that the longer a singer had sung in a choir, the better he or she was able to learn to distinct a difficult pitch deviation from the standard tone (higher MMN response). No such a relation was found regarding the choir singing years and MMN response to easy pitch deviants. Even if this result is in line with previous research results (e.g., Koelsch et al., 1999), it can only be considered preliminary and further studies with a larger number of subjects would be needed to confirm the finding. Also, for this reason, no correction (e.g., Bonferroni correction) for multiple comparisons (Table 8) included in the regression analysis was not performed. To sum up the discussion about the percentual size of pitch difference, perhaps a conclusion can be drawn that the problem may not be over 10 % pitch differences between the tones. Rather the challenge with the current study might be that for a large proportion of choir singers, the musical experience is not deep enough to be reflected on CAEPs in passive condition, which could then be seen as a group-level difference when comparing choir singers and controls.

When looking at the LOCATION condition, analogous problems to the PITCH condition in the experimental setup, i.e., too coarse spatial differences between standard and deviating loudspeakers, may be the cause for not to see differences between the groups. Like it was earlier noted, there was possibly a tendency for MMN responses to be larger for choir singers than for controls with both easy and hard spatial deviants, even though the statistical significance was not met. In the previous studies (Colin et al., 2002; Deouell et al., 2006) with rather small amounts of nonmusician subjects (healthy adults; N=8 and 12 respectively) it has been shown that a spatial deviation of 10 or 20 degrees from the standard sound elicits a MMN response in a passive condition. In the study of Deouell et al. (2006) MMN response corresponded linearly to the degree of spatial change with a resolution of at least 10 degrees. Perhaps smaller gradual differences in the spatial position of loudspeakers in the experimental setup could have guaranteed larger and statistically significant group differences, especially when the subject groups in the study did not differ in terms of degree of professionalism.

In the group of choir singers, no statistically significant correlation between the change detection of auditory stimuli deviating spatially from the standard (MMN) and the choir singing years could be found. However, there were indications that easy spatial deviants would produce a greater response than hard deviants, but this was not statistically affirmed. The reason for the lack of differences may be in the experimental setup or too large groupwise heterogeneity in auditory cognition. Also, positioning of the standard loudspeaker – instead of the far-left or far-right position – in front of the subject would eliminate the open question of whether the detection of spatially deviant stimuli is related to the fact that the deviant is located on the same/different side of the subject's centerline.

4.3 Review of results in a broader theoretical framework of STAC model

The STAC model (Reuter-Lorenz & Park, 2010) predicts that the brain will have to build new compensatory neural pathways (scaffolds) mainly in the prefrontal cortex to compensate for the decline in cognitive processing caused by ageing. According to the prediction of the model, these scaffolds may not be as effective as the original neural networks and therefore an increased activity in older adults is seen (larger amplitudes of CAEPs) because of recruitment for regulating incoming auditory stimuli – in other words reduced auditory inhibition, or then deficit in stimulus adaptation (Zendel & Alain, 2014). Another assumption of the model is that cognitive engagement, e.g., musical pursuit, has parallel effects with the former: engagement further strengthens the compensation, i.e., increases the neural activity reflected for example on CAEPs.

So, against the STAC model the larger amplitude of P1 with the hard deviants in the LOCATION condition could reflect more effective (compensatory) processing of spatial auditory sensory information changes on the feature level (Grimm et al., 2012) in the group of choir singers related to their musical engagement. On the other hand, it could reflect more effective neural compensation for the reduced perception brought about by ageing compared to controls. In line with the STAC model, the greatest (over)activation (amplitudes) were found in frontal region of interest (ROI F). But is it justified to interpret with current data that the brains of choir singers have better adapted i.e., changed through plasticity, to spatial auditory information processing? Is the larger P1 amplitude in choir singers reflecting scaffold-related activity, allowing choir singers to maintain higher levels of cognitive function on pre-attentive level compared to controls? This to be the case, we could expect to see an increase also in the N1 component of choir singers, however N1 responses do not differentiate between the groups. Regarding the N1 this is probably due to the fact that sinusoidal tones were used as stimuli in the current study instead of musical stimuli (Zendel & Alain, 2014). Also, P1 has been found in fact to be smaller in musicians (Zendel & Alain, 2014), which is explained as enhance automatic inhibitory function or then as stronger adaptation to the stimulus, which has served as reference for the hypothesis of the current study. In other studies finding the smaller P1 for musicians it has been suggested as a representation of a modification of central auditory processing facilitated by musical pursuit (O'Brien et al., 2015) or reflection of reduced processing demands or then conversely, increased processing efficiency (Nikjeh et al., 2009). In previous research (Bertoli et al., 2005; Nikjeh et al., 2009; Lister et al., 2011; Zendel & Alain, 2014) larger P1 amplitudes has been reported related to the ageing. In the context of the current study, similar interpretation seems however unfair because then we would not expect to see differences between the groups related to ageing. As well, no differences between the groups nor age groups were found for MMN in either condition, which may be due to the simplicity of the used stimuli. In summary, the higher P1 amplitude of choir singers cannot be explained inconsistently with the STAC model or previous research findings.

Zendel & Alain (2014) reported an increase in the N1 and P1 amplitudes associated with ageing. In line with current results no enhance in N1 response was observed in the musicians, which the researchers justified by the use of a sinusoidal stimulus. On the other hand, contrary to the assumption of the STAC model the amplitude of P1 was lower in musicians compared to controls regardless of age. The authors highlight the division of (auditory) neural processing into two components, namely, exogenous, obligatory (bottom-up), and endogenous, attention-related (top-down) components. Authors suggested that increase in early ERP responses could reflect generally that, in older people endogenous activity compensates for decreased exogenous activity. The authors suggest that being a musician affects ageing related changes in auditory processing especially via exogenous processing, which was enhanced in musicians, but decreased at equally rate in musicians and non-musicians, indicating a persistent pattern of differentiation (Zendel & Alain, 2014).

Based on previous it seems more logical to interpret that it is in fact controls of the current study who has a better ability to meet this challenge of ageing. Unfortunately, there is no such more specific information on the cognitive activity of the controls, so the question cannot be answered. The effect of the experimental setup on the problem of *p*-significance should neither be ignored: because the experimental setup was specifically designed to study the MMN response, as additional components P1 and N1 carry a higher risk of finding a random statistically significant result and are not a truly group-differentiated response.

However, among choir singers, it was found that the greater the experience of choir singing, the stronger the MMN response to frequency-deviating stimuli. Although this result can only be considered preliminary, it signals that choir singing may have an effect on enhanced processing of auditory information according to the STAC model, but the study design of the current study was unable to bring this to light. Regression analysis also found that higher number of choir singing years reflected on lower N1 response, which again contradicts the hypothesis. Of course, this single result must be treated with caution, as its statistical certainty ($p = .033$) was not very high given the preliminary nature of the analysis.

Finally, the STAC model should not be interpreted so that cognitive engagement automatically – in the same way than scaffolds against cognitive decline of ageing – increases the number of neural scaffolds (being then reflected on increased ERP amplitudes). Different CAEP responses should be interpreted without losing the big picture. It should also be remembered that the components have been found to be the result of independent neural processes (Näätänen & Picton, 1987), so given the limitations of the study and the complexity of the neural processes, the interpretation of the results in the light of STAC is not simple. So it may be that generally reported decrease in P1 response for deviant stimuli in musicians is not in concordance with STAC model. In fact, it would be useful to study the development of the P1 response (and other responses) in different groups (musicians, choir singers, controls) in a longitudinal setting to better determine baseline abdominal levels - whether or not classical cognitive activity maintains baseline P1 levels or so that it drops the P1 response below the assumed baseline?

4.4 Methodological limitations

When evaluating the results of the study, it is important to pay attention to its methodological limitations as well. The subjects belong to a natural sample of older adults participating in choir singing and no experimental control of the subjects' previous (long-time) choir training, skill level, musical goals and motivation have been implemented. For this reason, these factors can have an impact on results. On the other hand, because of the same reason, it is more realistic that possible findings would also be more generalizable if the aim is to assess the potential positive effects of amateur musical pursuit on cognitive decline caused by ageing. The subjects are a part of the study population of Pentikäinen et al. (2021).

Obviously, the relatively small sample size (N=54) also contributes to the fact that hardly any statistically significant results could be found in the study, thereby undermining the generalizability of results and related discussion. In particular, no statistically valid study could be performed for the age-related subgroups of choir singers and controls. In fact, the study originally included EEG data from a total of 69 subjects, but data from 6 subjects had to be rejected due to poor quality of data in the initial inspection, and additionally data from 9 subject had to be omitted due to too low a proportion of deviant trials (see Materials and Methods). Because of this, data were eventually obtained from a total of 54 subjects. And yet, unfortunately, no good quality data were obtained from all test subjects for both PITCH and LOCATION conditions, which further reduces the availability of data for more comprehensive analysis – for example comparing the two conditions within the same analysis.

The cross-sectional study setup is also limited, which is why the results are only correlative, which further means that no real conclusion about the causality of findings and the long-term effects of choir singing on brains' neural processing can be made (see also Pentikäinen et al., 2021). As it is earlier mentioned, this study serves as a part of a baseline study for three years longitudinal follow-up study. During this period the subjects are annually tested and maybe this project is able to reveal some causalities linked to long-lasting choir singing pursuit. It would be interesting if these studies also could include a group of young choir singers and group of young, non-musically active controls for comparison, in order to make it easier to interpret age-related (albeit cross-sectional) conclusions about changes in studied CAEPs.

One shortcoming of the study is also that responses' latencies were not examined beside the amplitudes, which is often the case in the published studies. On the other hand, this may not be so detrimental when interpreting the current results. For example Bertoli et al., 2005 did not found significant effect of subject group on P1 and N1 latencies, and all the observed changes in amplitude and latency of the P1, N1, as well in the MMN were comparatively small or absent. Neither could Tremblay et al. (2004) in their experiment using a pure-tone stimulus found latency differences for older subjects even though P1 amplitude was found to increase with age. And yet, Lister et a*l.* (2016) in their study studying of older subjects could not find difference between P1 amplitude and latencies in subjects with probable MCI and CNOA when using pure tone or speech as stimuli. Of course, we are not able to fully validate with this that the differences between the groups in latencies would not have been observed in the current study, but probably in light of the previous study, the differences would have been marginal. Finally, as Luck and Gaspelin (2017) pointed out that analyzing latencies beside amplitudes can double the familywise error rate (as will analyzing the data from two components instead of just one), it is also justified to limit the number of ANOVAs performed and focus solely on amplitude studies.

Musical activity is relatively easy to control, but what about hobbies that activate auditory cognition, e.g., computer gaming or ornithology? The potential heterogeneity in the study control group in this respect may manifest as greater variability in neural responses and thus as difficulty producing desired phenomena in the experimental setup. This also highlights that, in terms of reviewing the results, it would be better to have more accurate information about the lifelong amount of cognitively demanding pursuits or other non-musical activities in the control group. For example, the use a questionnaire to assess the above would have allowed a regression study to be performed in the control group as well and provide an additional perspective for evaluating the effect of lifelong auditory cognitive-activating hobbies (other than musical pursuit) on a more general level. Regarding the contribution of musical pursuit to the phenomenon observed in the regression analysis (experience in choir singing correlates with an increased MMN response for pitch deviants), a similar analysis with the control group would yield slightly more control/reference and thus reliability in interpreting the analysis, even if the experimental setup would be cross-sectional. Of course, neither is such a setup without problems: the cognitive activities of the control group are heterogeneous and leisure/work-related cognitive activities overlap partly in both study groups.

In connection with the above, the question arises as to whether a sinusoidal stimulus is best in an effort to assess differences in the potential effects of choir singing on auditory information processing in the brain. The sinusoidal stimulus is more neutral than the stimulus with a musical character and therefore it relates to studying phenomena at a more general level linked to auditory information processing (also relevant to this study). On the other hand, in many studies, a difference between groups has been observed specifically related to the non-sinusoidal stimulus (Baumann et al., 2008; Pantev et al., 1998 and 2001). So, it can be speculated that if, for example, the same sounds used in the current study were human voice samples, the differences between the choir singers and controls might have been more significant and relatively larger effect sizes would be observed. On the other hand, in this regard, the responses of the choir members might appear to be too pronounced. Therefore, perhaps the golden mean for the stimuli could be a similar sounds produced by some "rarer" instrument (e.g., a bassoon) than that used in this study, which would have been more equitable for the groups being compared. The use of sinusoidal sound as a universal stimulus, of course, facilitates comparisons between experiments, but as noted few times earlier, experimental setups vary considerably between studies, which maintains the difficulty of comparing different studies and interpreting results.

As a general note, the research field could benefit from replicating studies in different laboratories. In any case, the choice of a better suitable stimulus for the experimental design would be useful and perhaps for the research field, utilizing such a new universal experimental stimulus (e.g., standardized human voice or instrumental samples) alongside sinusoidal sound could add value in the long run in understanding auditory signal processing phenomena.

Last, no multiple comparison (e.g., Bonferroni correction) was performed on the *t*-test results of the regression analysis, which would result in lower *p*-values observed. This was not done because the nature of the study was more exploration of the data *post hoc* than a main part of the actual study. Therefore, it is wise to consider the results of regression analysis with caution. Although this cannot be ascertained in the current study, it seems plausible that the cognitive qualities or skills developed with choir singing correlate positively with the number of choir singing years. The issue should be examined in an experimental setup in which the temporal duration of the choir singing pursuit (in years), the quality of the practice, and the dedication to the rehearsal would be evaluated in relation to the neural responses.

4.5 Conclusions

The central finding of the current study is that at the general level years of continuous choir singing activity does not have major effects on auditory signal processing (P1/N1/MMN). Senior choir singers and matched controls did not show significant differences on group level in auditory processing of sinusoidal tones differing in pitch or tone location. However, a statistically significant difference between groups was observed in the early P1 response in terms of spatial resolution related to tone cluster deviating 36 or 72 degrees from the standard. The reason for the group difference in the P1 response remained unclear and the finding contradicts previous findings.

In *post hoc* analysis it was found that in the group of choir singers, the number of choir singing years positively correlated with the resolution of tone cluster deviating one or two tempered whole tones from the standard, eliciting higher MMN responses in more experienced choir singers. Preliminarily it seems plausible that lifelong choir singing is reflected in better pitch discrimination ability, but result should be verified with further studies.

It would be interesting to study neural responses in an experimental design that takes into account the intensity and dedication of music practice. Added value to such research would also be provided by a longitudinal experimental set-up in which the possible development of neural responses induced by choir singing could be studied in relation to the ageing and accumulation of musical experience. In general, large-scale studies with randomized controlled trial (RCT) and longitudinal cohort designs would be more than welcomed on the research field. In particular, this wish is addressed in studies aimed at understanding the impact of non-professional musical pursuit on cognitive processing. Also, there is a major lack of research on the auditory processing of spatial stimuli. Location-related ERP research seems to be secondary in the studies focusing primarily on visual or audiovisual investigation.

Last, one topic of interest for research related to the possible effects of choir singing on processing of auditory location and pitch information would be the simultaneous study of these aspects. In fact, such a study is underway with the data (from same subjects) being recorded at same time than the current data. It will be interesting whether in such a combined setup with increased complexity, relatively large spatial differences in tone location or pitch differences of more than 10% between the tones are able to produce change-related responses.

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