The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions

by

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Abstract

Noise-induced hearing loss (NIHL) is becoming increasingly prevalent amongst young adults leading to a whole series of social and public health problems. Genetics and environmental factors frequently influence individual's susceptibility to hearing loss. It is postulated that melanin in the inner ear is related to individual's susceptibility to NIHL. General pigmentation in turn, it is suspected to be related to the amount of pigmentation in the inner ear. The amount of melanin in the inner ear is said to modulate the endocochlear potential and provide an otoprotective effect. The current study aimed to determine the relationship between the contralateral suppression of otoacoustic emissions (CSOAE) in individuals with different hair and eye colour, and temporary emission shift (TES) after short-term noise exposure.

Method: The hearing sensitivity of young adults were determined by using pure tone audiometry followed by CSOAE's and distortion product otoacoustic emissions (DPOAE) before listening to music for one hour individually. Pure tone audiometry and DPOAE's were repeated after short-term music exposure to determine the amount of TES and temporary threshold shift (TTS). Twenty-five normal-hearing adults, ranging from 18 to 28 years (Mean age: 21.64, SD: 1.80) were recruited for the current study. A quasi-experimental repeated within subject's measurement design was used to compare the CSOAE in subjects with different hair and eyes colour with TES after noise exposure for one hour.

Results: No statistically significant difference was measured between the participants with brown eyes and brown hair, and the participants blue eyes and blond hair, efferent suppression as measured by CSOAE's. The blue eyes with blond hair had a temporary threshold shift (TTS) at 4000 Hz as well as a TES in at 2000 Hz after short-term noise exposure.

Conclusion: CSOAE's were therefore unable to predict which group of individuals were more susceptible to NIHL after short-term noise exposure.

Keywords

Contralateral suppressions of transient evoked otoacoustic emissions

Distortion product otoacoustic emissions

Melanin

Noise-induced hearing loss

Music overexposure

Pigmentation

Young adults

Pure tone audiometry

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List of Abbreviations and Acronyms

ANOVA	Analysis of variance
BBN	Broadband noise
CSOAE	Contralateral Suppression of Otoacoustic Emissions
daPa	deka-pascal
dB	Decibel
dB HL	Decibel Hearing Level
dB SL	Decibel Sensation Level
DP	Distortion Level
DPOAE	Distortion Product Otoacoustic Emissions
Hz	Frequency
IHC	Inner hair cells
kHz	Kilohertz
LAeq	Equivalent Continuous A-Weighted Noise Level
M	Mean
ml	Millimetres
MOC	Medial Olivocochlear
mPa	Megapascal
ms	Milliseconds
NF	Noise Floor
NIHL	Noise Induced Hearing Loss
NLo	Number of Sweeps Accepted and Processed
OAE	Otoacoustic Emissions

OHC	Outer hair cells
PTA	Pure Tone Average
PTS	Permanent Threshold Shift
SD	Standard Deviation
SNR	Signal to Noise Ratio
SOC	Superior Olivary Complex
SPL	Sound Pressure Level
SPSS	Statistical Package for the Social Sciences
TEOAE	Transient Evoked Otoacoustic Emissions
TES	Temporary Emission shift
TTS	Temporary Threshold Shift
WHO	World Health Organization
µs	Microseconds

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Chapter 1: Introduction

Hearing loss is one of the most prevalent disabilities occurring in modern society (Mannström, Kirkegaard, & Ulfendahl, 2015). There is an increased awareness in the development of noise-induced hearing loss (NIHL) due to noise exposure during recreational activities. NIHL is known to affect 16% of adults with hearing loss worldwide (Milon et al., 2018). NIHL is an irreversible hearing impairment in the higher frequency region caused by repeated or a single exposure to loud sounds. The impact of hazardous noise exposure is known to cause a temporary hearing deterioration and permanent hearing damage to the sensory cells of the cochlea (Bidelman, Schneider, Heitzmann, & Bhagat, 2016; Daniel, 2007; Keppler et al., 2014; Konings, Laer, & Camp, 2009; Otsuka, Tsuzaki, Sonoda, Tanaka, & Furukawa, 2016; Sliwinska-Kowalska & Davis, 2012). An intensity level of 85 dBA and higher are hazardous and can be potentially harmful when exposed for longer than eight hours a day (Daniel, 2007; Konings et al., 2009; Sliwinska-Kowalska & Davis, 2012). The severity of NIHL increases as duration of exposure and exposure level increases (Degeest, Clays, Corthals, & Keppler, 2017; Hong, Kerr, Poling, & Dhar, 2013). The increased prevalence in NIHL due to occupational and recreational exposure leads to an increase in social and public health complications in the society (Degeest et al., 2017; Hong et al., 2013; Kurabi, Keithley, Housley, Ryan, & Wong, 2017).

NIHL is eventually affecting the progression of age-related hearing loss due to earlier damage to the cochlea. It is estimated by the World Health Organisation (WHO) (2018) that 1.1 billion young adults between the ages of 12 to 35 years are at risk for developing hearing loss due to recreational noise exposure. Recreational activities such as music concerts, nightclubs, music from earphones, gyms and more hobbies may have a potentially damaging effect on our hearing sensitivity (Clark, 1991; Jin, Mannström, Järlebark, & Ulfendahl, 2007). Young adults main source of noise exposure can be linked to discotheque noise (Sliwinska-Kowalska & Davis, 2012). Discotheque intensity levels at music concerts and nightclubs are recorded between 84 to 120 dBA (Beach, Williams, & Gilliver, 2013; Daniel, 2007; Schmuziger,

Fostiropoulos, & Probst, 2006; Whitfield, 1998). Chronic exposure to loud sounds causes the destruction of hair cells in the cochlea which affect the higher frequency region (Beach et al., 2013). NIHL is known to cause non-auditory problems in addition to auditory alterations (Barros, Frota, Atherino, & Osterne, 2007). Auditory and non-auditory problems may affect social and occupational behaviour. Early detection of NIHL is crucial due to being one of the most preventable hearing losses (Keppler et al., 2014). The WHO established guidelines in which noise levels at entertainment events may not exceed 100 dBA if individuals are exposed for a maximum of four hours (Berglund, Lindvall, & Schwela, 1999).

The aforesaid guidelines notwithstanding, an individual's susceptibility to noise has a significant impact on the resulting NIHL. Some individuals have more resilient ears in comparison to other. Therefore, it is important to identify factors influencing an individual's susceptibility to NIHL due to the increasing prevalence of hearing loss amongst young adults. It is uncertain that individuals that are exposed to the same specified level of noise will develop the same degree of hearing loss. Multiple possible factors may influence an individual's susceptibility to NIHL and their recovery following noise exposure. NIHL is a complex disease that is influenced by the interaction of various factors. The possible factors that influence susceptibility to NIHL includes environmental factors or genetics (Bidelman et al., 2016; Daniel, 2007; Jacoszek, Pollak, Płoski, & Ołdak, 2017; Kalatzis & Petit, 1998; Lin et al., 2017; Mannström et al., 2015; Sadhra, Jackson, Ryder, & Brown, 2002). A variety of genetic factors can modulate an individual's susceptibility to noise trauma including their genetic profile, blood pressure, cholesterol level, gender and age (Basinou, Park, Cederroth, & Canlon, 2017; Da Costa, Castro, & Macedo, 2008; Otsuka et al., 2016; Sliwinska-Kowalska & Davis, 2012). Genetic vulnerability and duration of exposure to discotheque noise is a source of concern for a young individual's susceptibility to hearing loss (Sadhra et al., 2002). Inter-individual susceptibility can be attributed to genetic variation even when individuals are exposed to an equal amount of noise (Imam & Hannan, 2017). For example, it has been suggested that white males are more likely to develop hearing loss in comparison to other races and gender (Helzner et al., 2005). Men are also known to be more susceptible to hearing loss in comparison to women (Lauer & Schrode, 2017).

Limited information is available regarding genetic factors influencing NIHL due to inability to collect data from families or twins that are exposed to equal noise conditions (Konings et al., 2009). Bidelman et al. (2016) and Patuzzi and Thompson (1991) suggests that the susceptibility and severity of NIHL may be attributed to inter-individual variability which can be influenced by the tonic activity of the auditory pathways as a result melanin levels in the cochlea. Bartels, Ito, Trune, and Nuttall (2001) reported on work of Bonaccorsi (1965) for the examination of middle-aged individual's temporal bones post-mortem to evaluate the relationship between iris pigmentation and pigmentation in the inner ear. Poorer hearing thresholds have been associated with darker eye colour in adults in comparison with adults with light eye colour in a study done by Hannula, Bloigu, Majamaa, Sorri, and Maki-Torkko (2012). However, Hannula et al. (2012) based their conclusion on the pure-tone results of individuals without noise exposure. Mujica-mota, Schermbrucker, and Daniel (2015) also referred to work from Bonaccorsi (1965) that the iris pigmentation is associated with the otic melanocytes which are the melanocytes that are in the stria vascularis in the inner ear. Thus, it can be postulated that the amount of melanin present in the eyes may be reflective of the melanin present in the cochlea and influencing the tonic activity of the auditory pathway. The conclusion suggested that brown eyed participants had more melanin in their inner ear compared to blue eyed participants, leading to the assumption that brown eyes participants may be less susceptible to NIHL.

Pigmentation abnormalities and hearing loss in humans have been well known to be interconnected (Garber, Turner, Creel, & Witkop, 1982; Mahdi et al., 2012). It is suggested that hearing loss in individuals with auditory-pigmentary syndromes, namely Waardenburg syndrome and Tietz syndrome may be influenced by the lack of melanin that is crucial for endocochlear potential (Jin et al., 2007; Kalatzis & Petit, 1998). Waardenburg syndrome is commonly recognized by pigmentary deficits of the iris and hair colour with the distinct characteristic of a white forelock. Waardenburg syndrome is also predisposed to sensorineural hearing loss (Bartels et al., 2001; Ni et al., 2013; Tomita & Suzuki, 2004). Patients with Waardenburg syndrome present with blue eyes and skin discolouration (Mujica-mota et al., 2015; Read & Newton, 1994). Ni et al. (2013) and Smith, Kelley, Kenyon, and Hoover (2000) described Tietz

syndrome as a congenital auditory-pigmentary disorder which is characterized by severe sensorineural hearing loss and generalized skin hypopigmentation.

The absence in melanocytes is caused by disordered migration of melanoblasts from the neural crest cells which may be observed in hair, skin, and eyes colour (Tomita & Suzuki, 2004). Auditory-pigmentary syndromes are linked to the absence of melanocytes in the inner ear which leads to the deficiency of endocochlear potential and cochleosaccular degeneration (Read & Newton, 1994). The melanin levels in albinism are due to genetic deficits which are caused by the absence of tyrosinase activity preventing melanin production (Gill & Salt, 1997). Driscoll et al. (2009) and Carlin and McCroskey (1980) explained that albinism is associated with abnormal cochlear functioning that can be attributed to exhausted melanin levels. Humans, therefore, demonstrate a positive association between the number of melanocytes in their skin and inner ear (Lin et al., 2017).

Melanin is present in numerous locations in the body such as the inner ear, and including the scala vestibule, modiolus, utricle and saccule (Carlin & McCroskey, 1980; Da Costa et al., 2008; de Jong, Adelman, & Gross, 2017; Driscoll et al., 2009; Gill & Salt, 1997; Lin et al., 2012; Mahdi et al., 2012; Tachibana, 1999; Yamaguchi, Brenner, & Hearing, 2007). Melanin originates from cells, namely melanocytes. Melanocytes develop through precursor cells called melanoblasts that originate in the neural crest cells. Melanoblasts produce to melanocytes, migrate to suitable sites, and separate into melanocytes. Pigmentation of hair and eyes depend solely on the functioning of the melanocytes (Yamaguchi et al., 2007). Strial melanocytes and melanin pigmentation are an important factor for the normal structural development and cochlear functional mechanisms of the stria vascularis (Gill & Salt, 1997; Jin et al., 2007). Steel and Barkway (1989) explained that neural crest cells produce melanocytes and a lack of melanocytes influence the development and production of endocochlear potentials. Melanocytes are critical for the creation of cellular networks between marginal and basal cells. Marginal cells form a continuous layer facing the endolymph in the scala media which is interconnected to the basal cells next to the spiral ligaments and cochlear wall. Melanocytes interact with the epidermis of these

surrounding cells which is essential for the production of melanin (Yamaguchi et al., 2007). Mujica-Mota et al. (2015) explained that there are melanocytes in the eyes, skin, hair, inner ears and meninges of humans and that otic melanocytes are found in the inner ear.

Brown eyes are known to contain more melanin than blue eyes after examination of the iris and tissue of the eyes (Menon et al., 1992). Carlin and McCroskey (1980) study correlated with that of Menon et al. (1992) which explained that eye colour is on a continuum. The continuum ranges from brown eyes at the one extreme end with a larger amount of melanin, in contrast to blue eyes at the other end which lacks melanin. Jin et al. (2007) reported that a deficient or abnormal melanocyte development in marginal or basal cells affects the integrity of the stria vascularis.

Melanin is known to be involved in calcium ion homeostasis in the cochlea (Bush & Simon, 2007; Driscoll et al., 2009; Ohlemiller, Lett, & Gagnon, 2006). Calcium and protein bindings are responsible for calcium homeostasis, adhesion to cells, migration, and activation of signaling pathways. These binding proteins are found in the inner ear and also in the retina (Jacoszek et al., 2017). The endocochlear electrical potential is dependent upon high calcium concentrations for the transduction process of sound by the hair cells in the inner ear (Driscoll et al., 2009). Calcium is required for the compound action potential which is the synchronous discharge between the hair cells and nerve fibres which lead to excitatory postsynaptic potentials in the auditory nerve. Different levels of melanin content in the inner ear may thus modulate the auditory-neural transduction process due to the effect of calcium on the cochlear hair cell functioning (Gill & Salt, 1997; Jin et al., 2007; Mujica-mota et al., 2015). Driscoll et al. (2009) in addition to Mahdi et al. (2012) reported that melanin influences the ion gradient between the endolymph and perilymph. The absence of melanin in the inner ear may be associated with reduced endocochlear potential (Lin et al., 2017). It is suggested that calcium is released upon acoustic stimulation triggering melanin to regulate the cochlear calcium homeostasis. Melanin provides the inner ear in the course with protection against an influx of calcium. The absence of melanin may lead to higher levels of calcium due to the lack of modulation of calcium, which causes a

continuous noise exposure to the hair cells (Mujica-mota et al., 2015). Therefore, in individuals with less melanin, would have less modulation of calcium and in effect have prolonged stimulation when sound is transmitted to the hairs cells. Keefe, Ellison, Fitzpatrick, and Gorga (2008) supported the conclusion drawn by Lin et al. (2017) that the absence of melanin may result in a reduction in outer hair cells (OHC) motility. Therefore, a decrease in endocochlear potential occurs, causing a reduction in cochlear amplification. A reduction in cochlear amplification decreases the efficient transduction of sound to the inner hair cells (IHC) and auditory nerve, ultimately to the auditory cortex. Melanin is consequently postulated to play a protective role in the auditory system, against ototoxicity and damage due to noise trauma (Ardq, Aktan, Kara, & Sanli, 1998; Bartels et al., 2001; Carlin & McCroskey, 1980; Driscoll et al., 2009; Garber et al., 1982; Hannula et al., 2012; Lin et al., 2012; Mujica-mota et al., 2015; Murillo-Cuesta et al., 2010; Ohlemiller et al., 2006; Xiong, He, Lai, & Wang, 2011).

Hood, Poole, and Freedman (1976) found a correlation between the amount of melanin in the iris and susceptibility to the noise-induced temporary threshold shift. Increased auditory thresholds after noise exposure that recovers gradually are known as a temporary threshold shift (TTS) (Balatsourasa et al., 2005; Barros et al., 2007). It is suggested that metabolic overstimulation after noise may be the cause of TTS (Kurabi et al., 2017). Hood et al. (1976) measured TTS of participants after continuous noise exposure for three minutes. The study used randomly selected hospital staff and categorized participants according to their eye colour. The results indicated that lighter eyed participants had a larger TTS when stimulated at an higher intensities of 120 dB HL in comparison to darker eyed participants when stimulated at 80 dB HL. Carlin and McCroskey (1980) focused on a possible link between the effect of noise exposure and melanin in the inner ear after examination of 100 industrial workers' hearing sensitivity. In agreement with Hood et al. (1976), Carlin and McCroskey (1980) reported a correlation between individuals eye colour and hearing thresholds after working in a noisy industrial setting for 17.6 to 22.1 years.

The amount of TTS is typically attributed to individual susceptibility, duration of noise exposure as well as the intensity that the individual is exposed to (Barros et al., 2007). Xiong et al. (2011) and Barrenas (1997) investigated the relationship between NIHL and the suggested protective effect of melanin in pigmented and albino guinea pigs by exposing the animals to impulse noise. The two studies had differing results after exposing the animals to impulse noise for 72 hours. Xiong et al. (2011) utilized auditory brainstem response to measure TTS and observed fewer TTS in the pigmented guinea pigs in comparison to the albino guinea pigs. Therefore, suggesting that the pigmentated animals were more protected against the noise exposure than albino animals. Barrenas (1997) investigated differences between red, black and albino guinea pigs and found that the red guinea pigs had more OHC damage in comparison to albino and black guinea pigs. Barrenas (1997) suggested there is a more complex interaction between pigmentation and noise susceptibility which may lead to the difference in the results. Tachibana (1999) found that albino guinea pigs had lower cell volume melanocytes, derived from the neural crest cells, in comparison to pigmented guinea pigs. The results suggest that the lack of melanocytes results in less protection against noise exposure in comparison to more pigmented animals with more melanocytes (Tachibana, 1999; Xiong et al., 2011). Tachibana (1999), Henry and Haythorn (1975), and Tomita and Suzuki (2004), all suggested that the lack of melanocytes in the inner ear can be attributed to the irregular development of melanocytes or migration from the neural crest cells.

Noise exposure is hypothesized to reduce the flexibility of the OHC influencing hearing sensitivity (Vinck, Van Cauwenberge, Leroy, & Corthals, 1999). A direct measure of the cochlear OHC can be provided by otoacoustic emissions (OAE). Damage to OHC due to overexposure to noise leads to a reduction in OAE amplitude (Keppler, Dhooge, Maes, D'Haenens, Philips, et al., 2010). Sensory cells in the inner ear are sensitive to high sound pressure levels (SPL) which affect OHC, followed by the IHC functioning, and transduction to the auditory nerve (Barros et al., 2007). OAE's are produced due to movement of the structures of the OHC that generate soft sounds that are reflected through the middle ear from the cochlea (Kemp, 2002). OAE's arise due to a cochlear mechanism that provides hearing sensitivity and frequency responsiveness, known as the cochlear amplifier which is the OHC. Evoked OAE's are generated upon acoustic

stimulation of a functional cochlea, and emissions from the OHC are reflected back through the ear canal (Giraud, Collet, Chéry-Croze, Magnan, & Chays, 1995; Smith, Ichiba, Velenovsky, & Cone, 2017). OAE's is a reflection of different parts of the basal membrane due to stimulation of OAE's at varying frequencies (Hallenbeck & Dancer, 2003). OAE's provide important frequency specific information concerning the status of the cochlear OHC and functioning (Barros et al., 2007; Kemp, 2002; Seixas et al., 2004). Subtle changes in the OHC induced by noise can be monitored by OAE's (Balatsourasa et al., 2005; Shupak et al., 2007). An objective measure such as OAE's can be used to gain insight into the cochlea's pre-neural and biomechanical features of processing acoustic information (Cacace, McClelland, Weiner, & McFarland, 1996). OAE measurements are the recommended tool for monitoring noise-induced hearing damage due to recreational noise (Adnadjevic, Bockstael, Nadon, Thomas, & Botteldooren, 2016).

Numerous studies stated that an earlier indication of cochlear damage is provided by OAE's in comparison to behavioural pure tone threshold audiometry (Balatsourasa et al., 2005; Seixas et al., 2004; Skarżyński, 2014; Vinck et al., 1999). OAE's is an appropriate diagnostic tool to determine cochlear changes from noise exposure that is not yet evident in pure tone audiometry (Moepeng, Soer, & Vinck, 2017). Pure tone audiometry is unable to selectively evaluate changes in the OHC as OAE's are able to (Müller & Janssen, 2008). Contralateral suppression of OAE's (CSOAE) occur once stimulation of one ear causes afferent stimulation of the response in the contralateral ear (Chang, Song, Kim, & Koo, 2013; De Ceulaer, Yperman, Daemers, Van Driessche, Somers, Offeciers, & Govaerts, 2001; van Zyl, Swanepoel, & Hall, 2009). CSOAE's is the consequences of a response mechanism of the auditory system (Sun, 2008). Afferent input received from the superior olivary complex (SOC) is projected through the medial part of the SOC to the medial olivocochlear (MOC) bundle. Afferent input is further projected to the contralateral ear through the nerve fibres (Bidelman et al., 2016; de Boer & Thornton, 2007; Wagner et al., 2005). The MOC bundle is known as the efferent branch of the auditory feedback system that originates in the brainstem and terminates predominantly in the OHC of the opposite cochlea (Brownell, 1990; Jacobson, Kim, Romney, Zhu, & Frisina, 2003; Killan, Brooke, Farrell, & Merrett, 2017; Maison, Micheyl, & Collet, 1998; Mertes & Leek, 2016; Smith et al., 2017; Wagner &

Heyd, 2011). The MOC bundle is responsible for the motility of the OHC which influences the motion of the basilar membrane and IHC, subsequently, the cochlear micromechanics, also known as die MOC reflex (Otsuka et al., 2016; Smith et al., 2017; van Zyl et al., 2009). The MOC reflex inhibits the responses from the cochlear nerve due to the OHC receiving innervations from the medial part of the SOC through MOC complex bundles (Otsuka et al., 2016). OAE's are sensitive to changes induced by the MOC reflex in the OHC (Smith et al., 2017). The MOC reflex of the contralateral ear is mediated by the uncrossed efferent fibres of the MOC bundle. The MOC bundle controls the cochlear amplification and reduces cochlear amplifier gain through its synapse with OHC, resulting in a reduction in the mechano-electrical transduction of noise and causing inhibition of auditory nerve responses (Lichtenhan, Wilson, Hancock, & Guinan, 2016; Smith et al., 2017). MOC reflex can be measured indirectly during acoustic stimulation by measuring the amplitudes of OAE's in the contralateral ear (Smith et al., 2017; Wagner et al., 2005). The MOC functions to reduces the sensitivity of the cochlea to the auditory stimuli provided contralaterally (Wolter, Harrison, & James, 2010). The reduction in amplitude of one to four dB SPL is expected (Robinette & Glattke, 2007).

The strength of the MOC reflex is considered a predictor of the TTS (Otsuka et al., 2016; Zheng, Henderson, McFadden, & Hu, 1997). The MOC reflex is activated by acoustic stimulation and induces an inhibitory effect on OHC motility (Jacobson et al., 2003; Otsuka et al., 2016). The MOC reflex is the suppression effect of response acoustic stimulation and serves as a cochlear defensive mechanism from acoustic damage (Otsuka et al., 2016). It is also suspected that the role of the MOC efferent system is inhibition of the cochlear amplifier gain to aid listening in noise, slowing age-related hearing loss and protection against acoustic overexposure (Bidelman et al., 2016; Canale et al., 2014; Fuente, 2015; Jacobson et al., 2003; Kaf & Danesh, 2013; Killan et al., 2017; Lichtenhan et al., 2016; Maison et al., 1998; Mertes & Leek, 2016; Otsuka et al., 2016; Patuzzi & Thompson, 1991; Smith et al., 2017; Stuart & Cobb, 2015; Wagner et al., 2005; Wagner & Heyd, 2011). The protection provided by the MOC reflex can be attributed to the reduction in the interruptions of electromechanical transduction of the OHC (Patuzzi & Thompson, 1991). The MOC reflex measurements are postulated to be valuable for screening an individual's susceptibility to acoustic

trauma. A weakened MOC reflex is said to suggest susceptibility to NIHL (Mishra & Lutman, 2013; Wagner et al., 2005).

OAE's have been utilized previously to investigate the relationship between differing levels of pigmentation and OAE's. To examine the relationship between of different levels of melanin and OAE's, Driscoll et al. (2009) measured more spontaneous OAE's in African American individuals in comparison to Asian and Caucasian individuals. It was further concluded that African American individuals' OAE amplitudes were larger in comparison to OAE's of Caucasian and albino Caucasian individuals. These findings suggest that higher levels of melanin (as estimated by skin colour) in a participant resulted in greater OAE amplitudes. OHC is especially susceptible to acoustic overstimulation (Barros et al., 2007; Keppler et al., 2014). Thus, suggesting that the functioning of the OHC in the cochlea may be modulated by melanin (Driscoll et al., 2009). A noticeable shift can be measured in OAE's before fluctuations in behavioural pure tone audiometric thresholds may be identified (Seixas et al., 2004). A reduction in OAE response amplitude is known as a temporary emission shift (TES). A TES measurement may provide a better indication of the effect of noise exposure on the cochlea than other audiometric results (Otsuka et al., 2016). An TES after noise exposure can occur at 2000 to 6000 Hz (Barros et al., 2007). Due to the effectiveness, objective, non-invasive manner and reliability of OAE's, it may be the best method of evaluating the correlation between eye and hair colour on cochlear functioning (Balatsourasa et al., 2005; Barros et al., 2007; Doosti, Lotfi, Moosavi, Bakhshi, & Talasaz, 2014; Sun, 2008; Wolter et al., 2010).

A systematic review on the association between eye colour and NIHL concluded that six out of nine studies stated that light-eyed people had a greater permanent hearing loss following noise exposure of five to eight years (Mujica-mota et al., 2015). The remaining three articles stated that the connection was only weakly established, with eye colour only being a modest risk factor. One of the three articles were aimed to develop a noise susceptibility risk profile (Thomas & Williams, 1990). Aviators were divided into a resistant group that was exposed to aircraft noise but did not exhibit a hearing loss, and a group of susceptible individuals who had a hearing loss and who

were exposed to the same aircraft noise. The study reported that eye colour as a weak but persistent association, although they also found higher levels of calcium in the resistant group in comparison to their susceptible group. Da Costa et al. (2008) reported on industrial workers (mean age: 32 years) that were exposed to an excessive noise ranging, from 81 to 96 dBA for a period of two years to 42 years, to determine the association of iris pigmentation and susceptibility to NIHL. The study focused on pure tone thresholds at 3000 to 6000 Hz due to the susceptibility of noise-induced hair cell damage at these frequencies. The dark-eyed participants had a better right ear pure tone average (PTA) of 17.2 dB HL compared to the light-eyed group of 25.1 dB HL PTA in the right ear. The study concluded that blue eyed workers with less than ten years of experience had similar PTA averages than workers with brown eyes with more than ten years of experience. Thus, the results of Da Costa et al. (2008) suggests that noise exposure has a more detrimental effect on the hearing sensitivity of individuals with lighter pigmented iris.

It may be hypothesized from the studies reviewed by Mujica-mota et al. (2015) that participants with a combination of brown eyes and dark-hair will be more protected against excessive noise than the participants with a combination of blue eyes and light hair. Nonetheless, a study to determine the risk factors that cause NIHL was carried out by Hannula et al. (2012) which contradict this hypothesis. Hannula et al. (2012) stated that participants with a combination of dark hair and brown eyes were not more protected against NIHL. This conclusion was made after audiological testing was conducted with adults between the ages of 54 to 66 years who had been exposed to occupational or recreational noise. The participants completed a questionnaire on the type and frequency of noise exposure. The study divided 314 individuals without otological risk factors but with a history of noise exposure according to eye colour. The results indicated that more participants had hearing impairments in the dark-eyed group (50%) in comparison to the light-eyed group (30%) in the frequency region of 500 to 4000 Hz. The study did not indicate the duration of noise exposure, intensity level or if all participants were exposed to an equivalent dose of noise. The conclusion of the study was based on a group of randomly selected participants pure tone audiometry results. The study concluded that the combination of dark-eyed and dark-haired participants which presented with more pigmentation had poorer hearing

thresholds. It was consequently hypothesized by Hannula et al. (2012) that participants with light-eyes are more protected in comparison to the dark-eyed participants.

Driscoll et al. (2009) research study, in addition to Mujica-mota et al. (2015) found a correlation between OAE's and different hair and eye colour. Driscoll et al. (2009) suggest that higher levels of melanin, according to racial heritage in a participant, results in greater OAE amplitudes in an African population in comparison to albino Caucasian population. These findings correlated those with Hood et al. (1976) that the amount of pigmentation in the skin and eyes influences TTS. The conclusion by Da Costa et al. (2008) was based on the evaluation of pure tone thresholds of 3000, 4000 and 6000 Hz in contrast to Hannula et al. (2012), who based their conclusion on the thresholds at a lower frequency range of 500 to 4000 Hz. NIHL is recognized by a notch in the higher frequency region at 4 kHz and often affecting 3000 to 6000 Hz range (Imam & Hannan, 2017; Konings et al., 2009). These specific frequencies are of importance when investigating the effect that noise exposure has on hearing sensitivity (Morata, 2007). In addition, the most inter-individual differences can be observed at 4000 to 8000 Hz after noise exposure (Helzner et al., 2005; Moepeng et al., 2017). Therefore, both OAE's and pure tone audiometry may be utilized to investigate the relationship between susceptibility to NIHL and melanin.

Driscoll et al. (2009) explained that cochlear micromechanics, such as regulation of calcium ion in the inner ear, are controlled by the amount of melanin. Melanin in the inner ear is theorized to protect the inner ear from damaging levels of calcium when there is a flood of calcium during acoustic overstimulation (Mujica-mota et al., 2015). OAE measurements are sensitive to the metabolic changes within the OHC after noise exposure. Thus, using an OAE provides an objective measurement to record changes such as the mechanical and anatomical aspect of the OHC during processing acoustic information (Cacace et al., 1996). The variations of the cochlear micromechanics can be observed in the CSOAE measurements (Otsuka et al., 2016). The difference between OAE amplitude with and without contralateral acoustic stimulation provides an objective, non-invasive method to examine MOC reflex activity (Jacobson et al.,

2003; van Zyl, Swanepoel, & Hall, 2009). CSOAE's provides an indication of the functioning of the MOC efferent system and its ability to protect the inner ears against noise damage (Stuart & Cobb, 2015). CSOAE's is therefore likely to be a tool capable of prediction of an individual's susceptibility to NIHL (Stuart & Cobb, 2015). Increased awareness of risk factors such as these may lead to protective measures when subjected to excessive noise.

Pigmentation is influenced by genetics which has been identified as a risk factor that may influence an individual's susceptibility to NIHL. Numerous studies have investigated the correlation between the amount of melanin-based on eye and hair colour, and the effect that it has on OHC (Da Costa et al., 2008; Hannula et al., 2012; Mujica-mota et al., 2015). Currently, there is still limited conclusive evidence on whether melanin has otoprotective abilities (Bartels et al., 2001). To the researcher's knowledge, no studies have investigated the association between eye and hair colour on CSOAE's after noise exposure. CSOAE's may provide information about the micromechanical aspects of the OHC and the inhibitory effect of the MOC reflex after acoustic stimulation. The aim of the current study was to determine the relationship between the CSOAE in individuals with different hair and eye colour, and TES after short-term noise exposure.

Chapter 2: Methodology

2.1. The main aim

The aim of the study was to determine the relationship between the contralateral suppression of otoacoustic emissions (CSOAE) in individuals with different hair and eye colour, and temporary emission shift (TES) after short-term noise exposure.

2.2. Research design

The research was conducted using a quantitative research design with quasi-experimental repeated within the subject design to compare the CSOAE in subjects with different hair and eyes colour with TES after short-term noise exposure. Quantitative research was used to determine the relationship between the measurable variables to predict occurrence (Leedy & Ormrod, 2005). Therefore, the research design allowed the researcher to make a conclusion of a causal relationship, i.e. The relationship between different hair and eye colours' TES after short-term music exposure. The study was quasi-experimental in nature due to single measurement of CSOAE's and repeated measurement of distortion product otoacoustic emissions (DPOAE) after music exposure. Quasi-experimental research is based on a real-world view due to within subject measurements. Therefore, the researcher could not control other confounding variables and alternative explanations that could not be ruled out (Leedy & Ormrod, 2010).

2.3. Ethical considerations

The ethical approval for the research study was firstly obtained from the Research and Ethics Committee at the Department of Speech-Language Pathology and Audiology, University of Pretoria. Once departmental ethical clearance was obtained, ethical approval was sought after and granted from the Research Ethics Committee of the Faculty of Humanities (Appendix A), University of Pretoria. The permission for students of the Department of Speech-Language Pathology and Audiology to participate in the study was obtained from the Director of Student Affairs (Appendix B) and the Head of Department of Speech-Language Pathology and Audiology (Appendix C). Ethical issues in research with human participants can be categorized into groups, namely: protection from harm, voluntary and informed consent, the right to privacy, confidentiality and anonymity, plagiarism, data collection and referrals (Leedy & Ormrod, 2014).

2.3.1. Protection from harm

The researchers attempted not to cause research participants unnecessary psychological or physical harm (Leedy & Ormrod, 2014). There were no risks associated with the participation in the present study that would affect their daily living. Participants were treated in a respectful manner. The participants were informed about the goals of the study and given the opportunity to learn more about the foundation of the study. Before the commencement of the study, the participants were informed that at any point should she/he feel discomfort or physical pain, they have the choice to withdraw from the study immediately. The permissible time weighted average according the equal energy rule is 91 dBA for two hours before permanent noise damage occurs (Helleman & Dreschler, 2015). Therefore, the study used half of the time and a 90-dBA intensity to ensure that there was no risk of permanent hearing damage.

2.3.2. Voluntary and informed participation

The participants were given information regarding the study to make a fully informed decision to take part in the current research study. The participant letter (Appendix D) contains: i) background information regarding the purpose of the study, ii) participant candidacy and selection process, iii) requirements to be a candidate to participate in the study and time frame of data collection session, iv) test venue and v) possible risks and benefits associated with the study. If the participants had any inquiries regarding the research study, they were given the opportunity to ask questions before consenting to and participating in the research study. The participants were required to complete an informed consent form (Appendix E), indicating their participation in the current study was completely voluntary and provide verbal consent. Written and verbal consent was obtained from research participants before the commencement of data collection procedures. The researcher informed each participant that they have the right to withdraw at any point during the study (Leedy & Ormrod, 2014).

2.3.3. Confidentiality and anonymity

The participants were informed that all the information collected by the researcher would be kept confidential. Leedy and Ormrod (2014) stated that the privacy of the participant should be respected. Confidentiality of the participants was achieved by allocating a unique code that substituted the participant name to ensure anonymity. Although the identity of the participants was known to the researcher, their data was collected and stored using an alphanumeric code and the identity of the participants and their results were kept confidential. In this manner, confidentiality was ensured by the researcher and the participants are protected during data analysis. The researcher must be able to guarantee participant anonymity and undertake to keep all information confidential (Leedy & Ormrod, 2014). The participants' identity was known to the researcher, but the research report will protect the participant's identity from being known and their identity was kept confidential.

2.3.4. Plagiarism

The research study, dissertation, and scientific article are the researcher's original work. During the use of secondary information, acknowledgment and referencing were used that the researcher using APA 6th Edition reference guidelines. The plagiarism policy of the University of Pretoria can be viewed in Appendix F.

2.3.5. Data storage

The University of Pretoria policy states that data obtained from the research project must be securely stored for a minimum of 15 years (Appendix G). Data collected during the research study was stored electronically on a CD and hard copy at the Department of Speech-Language Pathology and Audiology, University of Pretoria. Data files does not include identifying information of participants.

2.3.6. Referrals

If the potential participant was identified with a hearing loss or condition necessitating otologic management (e.g. otitis media), participants were given a referral letter (Appendix H). Participants were also provided with the contact information of their local audiologist or Ear-, Nose- and Throat Specialist for management of the condition.

2.4. Study participants and selection criteria

Twenty-five normal-hearing adults, between the ages of 18 and 28 years (Mean age:21.64 years, SD:1.80) were recruited for the current study. The participants were selected based on a non-probability purposive sampling method. Purposive sampling was used when subjects were chosen for a specific purpose as they have specific features (Leedy & Ormrod, 2005). The participants were comprised of acquaintances, friends, family in the proximity of the University of Pretoria and students of the Department of Speech-Language Pathology and Audiology, University of Pretoria. The participants consisted of twenty females and five males.

The participants were divided into two groups, namely group A were 12 participants with brown eyes and brown hair colour (Mean age: 21.42 years, SD: 1.79), and group B were 13 participants with blue eyes and blond hair colour (Mean age: 21.85 years, SD: 1.86). The participant selection procedure was used to determine candidacy to participate in the research study.

The participant selection procedure included the participants being provided with an information letter before the commencement of the study (Appendix D). After the information letter was provided and volunteers chose to participate in the study, the volunteers had to complete an informed consent letter (Appendix E) to participate in the present study. Individuals with brown eyes and hair, and individuals with blue eyes and blond hair had to adhere to the selection criteria that can be seen in Table 1 to be included in the study. A brief case history (Appendix I) and audiological assessment were conducted to determine if the participants had normal hearing sensitivity, non-smokers and have not been exposed to noise 24 hours prior to participating in the study. The results required for each audiological test as well as the equipment used is described in Table 1 and Table 2, respectively. Upon completion of the data collection, a pass letter was provided to each participant to confirm the tests conducted and the results obtained (Appendix J).

In summary, the participants of group A must have presented with the following features; a young adult between the ages of 18 and 30 with brown eyes and hair, non-smokers, no excessive cerumen, Jerger Type A tympanograms, and normal hearing sensitivity. Group B also had to be young adults between the ages of 18 and 30 years of age with blond hair and blue eyes, non-smokers, no excessive cerumen, Jerger Type A tympanograms, and normal hearing sensitivity.

2.5. Participation inclusion criteria

Table 1 explains the inclusion criteria used during selection of participants, the apparatus used during participant selection in addition to the rationale for the inclusion criteria.

Table 1: Participation selection material and apparatus

Inclusion criteria	Rationale	Equipment
The participants had to have either brown hair and brown eyes, or blond hair and blue eyes.	The participants were divided into group A or group B according to eye and hair colour. Colour is differentiated on a continuum ranging from dark to light colours. Blue eyes are at the one end of the continuum which is lacking melanin whereas brown eyes are at the other extreme end of the continuum with more melanin (Carlin & McCroskey, 1980). Thus, participants were divided into different groups according to perceived amount of melanin in hair and eyes.	Subjective judgement was used, and potential participants were asked to indicate what their original hair colour was.
The participants had to be between the ages of 18 to 30 years.	The specific age range was selected as decline in CSOAE's is reported with increased age (Jacobson et al., 2003; Robinette & Glattke, 2007). The function of the medial olivocochlear (MOC) efferent system, even with normal audiometric results, deteriorates with age (Kim, Frisina, & Florida, 2002).	Not applicable.
No excessive cerumen or abnormalities detected in the ear canal and tympanic membrane.	Otoacoustic emissions (OAE) testing can only be measured assuming normal outer as well as middle ear structures and functioning (Kemp, 2002; Stach, 2010). Cerumen could have caused an obstruction in the ear canal leading to a false positive result during OAE measurements. Thus, no abnormalities in the ear canal or middle ear was ensured.	Heine Mini 3000 otoscope with reusable specula.
Jerger Type A tympanogram.	The middle ear pressure must have been between +100 daPa and -100 daPa and middle ear compliance between 0.3 ml to 1.75 ml (Jerger, 1970). OAE measurements are dependent on normal middle ear functioning for the stimulus to be transmitted to the cochlea (Kemp, 2002). Jerger Type A tympanogram with present acoustic reflexes was typically indicative of normal middle ear functioning (Katz, Chasin, English, Hood, & Tillery, 2015).	Y-226 Hz probe tone GSI Tympstar Middle Ear Analyzer: Comprehensive middle ear measurements.
Acoustic ipsilateral and contralateral reflexes had to be present.	Acoustic reflexes were present when recorded between 70 to 90 dB HL at 500 to 4000 Hz (Stach, 2010). CSOAE's relies on the normal functioning of the acoustic reflexes due to making use of the ipsilateral and contralateral pathways of the acoustic reflexes (Guinan, 2006).	226 Hz probe tone GSI Tympstar Middle Ear Analyzer: Comprehensive middle ear measurements.

Bilateral normal hearing sensitivity was determined by behavioural pure tone thresholds.	The normal hearing sensitivity of ≤15 dB HL was determined by air- and bone conduction using behavioral pure tone audiometry at octave intervals of 250 to 8000 Hz using the modified Hughson-Westlake method of threshold measurement (Stach, 2010). Normal hearing sensitivity was required for participants to participate in the study as a hearing loss ≥30 dB HL influences the amplitude and presence of OAE's (Kemp, 2002).	Audiometer: GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota).
The participant may not have a history of smoking.	Smoking reduces blood supply to inner ear causing damage to these structures (Helzner et al., 2005). Previous studies have found an association between cigarette smoking and hearing loss in young adults (Negley, Katbamna, Crumpton, & Lawson, 2007). Outer hair cells (OHC) may be damaged without resulting in damage to behavioural hearing sensitivity (Daniel, 2007; Vinay, 2010).	Not applicable.

(CSOAE: contralateral suppression of otoacoustic emissions; daPa: deka pascal; dB HL: decibel hearing level; Hz: hertz; ml: millimeters; MOC: medial olivocochlear; OAE: otoacoustic emissions; OHC: outer hair cells).

2.6. Equipment for participant selection

Table 2 displays a summary of the equipment used during the participant selection protocol according to the sequence of the tests conducted.

Table 2: Summary of equipment used for participant selection

Equipment	Description
Heine Mini 3000 otoscope with reusable specula.	Heine Mini 3000 otoscope was used to visually inspect the external ear canal and tympanic membrane.
226 Hz probe tone GSI Tympstar Middle Ear Analyzer: Comprehensive middle ear measurements. *(GSI Tympstar was calibrated annually, immediately prior to data collection* (*South African National Standards [SABS] 0154-1/2, 2018)*	Acoustic immittance measurements were used to examine middle ear function. Acoustic immittance measured the middle ear pressure, compliance and ear canal volume when a probe was placed in the ear canal (Stach, 2010). Acoustic reflexes were measured after the probe placement in the ear canal. Acoustic reflexes were measured ipsilaterally and contralaterally at 500 to 4000 Hz.
Audiometer: GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota) *(GSI 61 Clinical Audiometer was calibrated annually* (South African National Standards [SANS] 10154-1:2012, 2018; South African National Standards [SANS] 10154-2:2012, 2018)*).*	<u>Behavioral pure tone audiometry:</u> Air- and bone conduction audiometry were used to determine the hearing threshold using the Hughson-Westlake method (Stach, 2010). The thresholds were determined by presenting various intensities at octave intervals and including half-octaves of 3000 and 6000 Hz. The thresholds were defined as the lowest intensity the participant responded to 50% of the time (Stach, 2010). <u>Speech detection:</u> Speech detection was used to determine the lowest level at which a participant can perceive a speech signal (Stach, 2010). During the test, the participant was required to respond to spondaic words using monitored pre-recorded speech presented. Testing started at 40 dB HL when the hearing was normal and speech detection threshold should be at approximately the same threshold at the pure tone threshold (Stach, 2010).

(dB HL: decibel hearing level; Hz: hertz)

2.7. Procedure for participant selection

The selection procedure was conducted by a certified audiologist at the Department of Speech-Language Pathology and Audiology, University of Pretoria. The diagnostic test battery was used to determine hearing sensitivity in participants to ensure their candidacy for participation in the current study. The researcher is a Health Professions Council of South Africa certified student audiologist (Registration number: AU S 0006475) that conducted the diagnostic assessment.

2.7.1. Informed consent

The nature of the study was first explained to each potential participant and an information letter (Appendix D) and a consent letter (Appendix E) was provided. The information letter explained the rationale for the research and the procedures that were going to be performed. The participants had the opportunity to ask questions before consenting to the research study. The participants were given an informed consent form (Appendix E) to sign and had to provide verbal consent to participate in the study before the commencement of the selection criteria protocol.

2.7.2. Case history

A brief case history was completed by the participants before commencement of audiometric tests (Appendix I). The case history included the participants age, otological or family history of hearing loss and their subjective judgment of their hair colour. Participants were also asked if they have been exposed to loud noise in the past 24 hours before participation. If participants were exposed in 24 hours prior to data collection, they were excluded from the study to ensure the possibility of cumulative noise exposure.

2.7.3. Otoscopy

A visual examination of the external ear canal with an otoscope was conducted to inspect the external auditory meatus and tympanic membrane (Stach, 2010). During the otoscopic examination, the audiologist can identify possible abnormalities, for example, inflammation, perforations, growths and foreign objects in the ear canal and tympanic membrane. If normal structures of the tympanic membrane were identified participants continued to the diagnostic assessment. The participants who presented with a conductive component were excluded from the study as OAE's are sensitive to middle ear pathologies (Kemp, 2002; Stach, 2010). Otoscopic results were recorded on the data collection sheet (Appendix K). The participants that therefore presented with atypical otoscopic results were referred to necessary medical professional (Appendix H).

2.7.4. Immittance measurements

Immittance measurements allowed the audiologist to assess the mechanical properties of the auditory system of the outer and middle ears (Katz et al., 2015; Stach, 2010). The stapedial muscle was assessed during the test to determine the response that relies on the auditory nerve and middle ear (Sun, 2008). Immittance measurements were performed to exclude the possibility of middle ear pathology and to ensure the integrity of the acoustic reflex arc (Katz et al., 2015).

Tympanometry was conducted using a Y-226 Hz probe tone to assess the middle ear functioning with regards to the compliance, ear canal volume and middle ear pressure (Stach, 2010). Tympanometry results were recorded on the data collection sheet (Appendix K). Participants were required to present with a Jerger Type A tympanogram. The participants that did not present with a Jerger Type A tympanogram were excluded from participation in the study and were referred to the Ear-, Nose – and Throat Specialist (Appendix H). Table 3 displays the normative range of a Jerger Type A tympanogram.

Table 3: Normative range for Jerger Type A tympanogram (Jerger, 1970)

Variables	Measurements
Middle ear pressure	+100 daPa and -100 daPa
Ear canal volume	0.8 ml to 2.0 ml
Middle ear compliance	0.3 ml to 1.75 ml

(daPa: Deka pascal; ml: millimeters)

The acoustic reflex of the stapedius muscle is a response upon stimulation with a loud sound due to reflexive contraction of the stapedial muscle (Stach, 2010). The acoustic reflex threshold was considered present and within normal limits between 70 to 100 dB sensation level (SL) in normal hearing participants (Jerger, 1970). The acoustic reflex threshold was defined as the lowest level the middle ear could identify changes in response to sound (Stach, 2010). The acoustic reflexes offer information regarding hearing sensitivity as well as an assessment of differential auditory pathologies (Sun, 2008). The results of the acoustic reflex were recorded on the data collection sheet (Appendix K). The participants that presented with elevated acoustic reflexes, when reflex were elicited at ≥ 100 dB HL were referred to an audiologist for further assessment (Appendix H) (Jerger, 1970; Stephenson & Laurent, 2012).

2.7.5. Pure tone audiometry

Pure tone audiometry, a behavioural hearing test was conducted to ensure normal hearing sensitivity. The assessment was conducted using an Audiometer: GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota) in a double walled soundproof booth. A pure tone signal was presented through supra-aural TDH-39 headphones in a descending manner according to the Hughson-Westlake method at frequencies 125 to 8000 Hz, including half octave frequencies 3000 and 6000 Hz (Jerger, 1970). The testing started at 30 dB HL and was decreased in 10 dB HL and with a 5 dB HL increase in steps upon the response of the participant until their threshold was reached. Pure tone audiometry was conducted to determine frequency-specific thresholds of audibility. The threshold was defined as the point which the participant could detect the presence of the pure tone signal 50% of the time (Stach, 2010). The pure tone average (PTA) was calculated by determining the mean thresholds of 500, 1000 and 2000 Hz and dividing the mean by three (Stach, 2010).

Bone conduction audiometry was tested at frequencies of 250 to 4000 Hz according to the Hughson-Westlake method with pure tones presented through B-71 bone conductor. Bone conduction thresholds was conducted to cross-check results obtained during immittance measurement (Stach, 2010). Normal hearing sensitivity was accepted as ≤ 15 dB HL at all the frequencies from 125 to 8000 Hz (Moepeng et al., 2017). The thresholds were captured on an audiogram on the data collection sheet (Appendix K). The participants who presented with a hearing loss or an air-bone gap of ≥ 10 dB HL, he/she were referred to an audiologist for further assessment and intervention (Appendix H).

2.7.6. Speech detection

Speech detection was conducted using the GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota). The speech detection threshold was defined as the lowest level at which the participant was able to detect 50% of pre-recorded spondaic words presented to them (Katz et al., 2015). The speech detection procedure was conducted according to Stach (2010) using, pre-recorded spondee words presented through supra-aural TDH-39 headphones at 40 dB HL. When a participant presented with normal hearing sensitivity, the intensity was decreased in 10 dB HL increments with each response until the threshold was obtained. Speech detection thresholds were accepted as normal when correlated ± 7 dB of the PTA (Hall & Mueller, 1997). Speech detection thresholds were captured on the data collection sheet (Appendix K). If participants' speech detection thresholds were not in correlation with their PTA, they were referred to an audiologist for additional assessment (Appendix H).

2.8. Equipment for data collection

Table 4 displays a summary of the equipment used during data collection in the sequence that the tests were performed.

Table 4: Summary of equipment utilized for data collection

Equipment	Description
Sound level meter (RION NA-42). (*Sound level meter was calibrated annually, immediately prior to data collection according to the SANS 61672-1 protocol* (South African National Standards [SANS] 61672-1:2003, 2018)	The sound level meter allowed the recording of the acoustic signal in a room. The sound level meter was used to determine the accumulated sound levels in the double-walled soundproof booth during the duration of the music sample exposure period. The sound levels were measured in equivalent Continuous A-weighted noise level (LAeq). LAeq measurements were used to obtain a measurement of noise over time that is applied when the noise level varies over time (Fahy & Walker, 1998).
DP Echoport ILO V6 (Otodynamics Ltd., England) (DP *Echoport ILO V6 was calibrated annually, immediately prior to data collection according to the SANS 60942 protocol* (South African National Standards [SANS] - 60942, 2018).*)*	<u>CSOAE:</u> CSOAE's were used to determine the amount of efferent suppression in each participant by measuring transient evoked otoacoustic emissions (TEOAE) with and without a contralateral masker. CSOAE were measured when a probe was inserted into the test ear and a second probe inserted into the contralateral ear. TEOAE's were recorded from 1 to 4 kHz when a stimulus tone was presented at 80 dB sound pressure level (SPL) and the masker at 65 dB SPL. TEOAE's were elicited using the recording parameters are defined in Table 5. <u>DPOAE:</u> DPOAE's were used to determine the functioning and integrity of the OHC in the cochlea (Stach, 2010). DPOAE's were elicited by the simultaneous presentation of two primary frequency tones through a probe inserted into the ear canal. DPOAE's were measured at the F2 frequencies (F1/F2 ratio: 1.22): 0.842 to 8 kHz. The two intensities at which tones were presented was set to 65 dB SPL (L1) and 55 dB SPL (L2). Recording parameters are defined in Table 6.

(CSOAE: contralateral suppression of transient evoked otoacoustic emissions; DPOAE: distortion product otoacoustic emissions; kHz: kilohertz; LAeq: equivalent continuous A-weighted noise level; OHC: outer hair cells; SPL: sound pressure level; TEOAE: transient evoked otoacoustic emissions).

2.8.1. Measurement of contralateral suppression of transient evoked otoacoustic emissions (CSOAE)

CSOAE were recorded in responses to a very sudden click stimulus (Hall, 2000). Bilateral measurements of CSOAE were measured in each of the participants before short-term music exposure. TEOAE are frequency specific measures of the cochlea functioning as the signals are elicited by a click stimulus which is divided into frequency bands (Kemp, 2002). A click stimulus consists of a broad frequency range which stimulates a large portion of the cochlea simultaneously.

CSOAE's were recorded using the DP Echoport ILO V6 (Otodynamics Ltd., England). A more pronounced inhibition can be seen in the amplitude and phase of TEOAE's in comparison to DPOAE's (Mishra & Lutman, 2013). Contralateral stimulation causes a reduction in the TEOAE amplitudes (Hall, 2000). Stimulus level of 65 dBA is used for effective activation of the MOC bundle (Stuart & Cobb, 2015). A linear mode was used that causes all four clicks in the sequence to have the same polarity and amplitude (Robinette & Glattke, 2007). The masker consisted of a broadband noise suppressor presented at 60 dB SPL. The click stimuli at a rate of 50 clicks/second were presented by 80 microseconds (μs) rectangular electrical pulse (Mishra & Lutman, 2013). Two hundred and sixty sweeps were accepted before termination of the test. The noise rejection level was 49.5 dB SPL (Stuart & Cobb, 2015).

The software calculated the noise and emission response amplitudes in the frequency bands centered on 1.4, 2, 2.8 and 4 kHz. Noise detection was calculated by determining the root mean square of the non-stimulus intervals, while artifact rejection was based on weighted averaging. To determine the presence or absence of TEOAE response the following factors relating to the recording parameters should be taken into consideration. TEOAE response measurements include the overall correlation of the two waveforms obtained from time averaging, known as the reproducibility, and the response in relation to noise, known as the signal to noise ratio (SNR). Results were filtered and analyzed into half-octave bands centered at 1, 1.4, 2, 2.8 and 4 kHz. This region is known to be a sensitive region of the cochlea (Kemp, 2002).

TEOAE may be considered present when the reproducibility is ≥ 70% or greater (Hood, Berlin, Hurley, Cecola, & Bell, 1996; Stuart & Cobb, 2015). SNR must have been ≥ 6 dB SPL to guarantee independent measurement was higher than the noise to ensure that appropriate noise immunity is achieved (Mishra & Lutman, 2013; Stuart & Cobb, 2015). Thus, the responses with an SNR of ≥ 6 dB SPL and reproducibility of ≥ 70% in 1 to 4 kHz frequency band was accepted as present. The recording parameters for CSOAE's can be seen in Table 5.

Table 5: Contralateral suppression of Transient-evoked Otoacoustic Emissions (CSOAE) setup on DP Echoport ILO V6 (Otodynamics Ltd., England)

Masker level	60 dB SPL
Masker type	Narrowband noise
Masker On/Off NLo count	60
Stimulus level	65 dB SPL
Stimulus type	Linear
Sweeps	260
Frequency band	1.4, 2, 2.8 and 4 kHz

(dB: decibel; dB SPL: decibel sound pressure level; kHz: kilohertz; NLo: number of sweeps accepted and processed)

2.8.2. Measurement of Distortion Product Otoacoustic Emissions (DPOAE)

DPOAE were measured using the DP Echoport ILO V6 (Otodynamics Ltd., England). DPOAE occurs when the lower frequency tone is known as f_1 joining with the higher frequency tone, f_2 causing a distortion of the nonlinear interaction between the two primary tones (Dreisbach & Siegel, 2001). Two mechanisms from which OAE's arise are known as the linear reflection and nonlinear distortion of the cochlea. Bilateral DPOAE were measured before and after short-term music exposure to determine the response of the cochlea as well as the TES.

The $2f_1$-f_2 DPOAE's were elicited by stimulation of two primary tones (f1 and f2) with a f_2/f_1 frequency ratio of 1.22 and f2 ranging from 1001 to 7996 Hz. (Lopez-Gonzalez, Guerrero, Rojas, Osuna, & Delgado, 1999; Smith et al., 2017). The stimulus level at which tones were presented was set to 65 dB SPL (L_1) and 55 dB SPL (L_2) for f_1 and f_2 separately (Dreisbach & Siegel, 2001). The stimulus level was selected as higher level stimuli may cause a false high DPOAE response (Adnadjevic et al., 2016).

The 65/55 dB SPL level is known to lead to the optimal DPOAE response. The noise rejection level was preset at 6 megapascal (mPa) (49.5 dB SPL) by the ILO V6 software. Each frequency was recorded amplitude at the $2f_1$-f_2 frequency which is the response of the cochlea at an f_2 frequency (Stach, 2010). The recorded emissions and noise amplitudes were converted to pressure level and divided into frequency bands of half octaves with the center frequencies at 1, 2, 3, 4, 6 and 7.96 kHz.

DPOAE testing was conducted by sealing off the participant's ear canal with the appropriate probe tip. Probe placement was verified by the automatic protocol of the DP Echoport ILO V6. The same probe was used for the pre-exposure measurement and post-exposure repeated measurements. A probe was placed in the participants' ipsilateral ear which consists of two microphones. The sound source and sensitive microphone were used to record the response of the OAE. The distortion source is generated by intermodulation distortion of overlapping f_1 and f_2 traveling waves. The reflecting source is the product of the distortion source after displacing on the center frequency region on the basilar membrane and is reflected (Smith et al., 2017). The point at which the OAE is generated on the basilar membrane is located between f_1 and f_2, closer to f_2 (Lopez-Gonzalez et al., 1999). The difference between the emissions level (DP) and the noise floor (NF) is known as the DP – NF, otherwise known as the SNR. To be clinically acceptable, the measurement needs to be secured by the result of minimum interference from ambient noise in the test environment. DP amplitude must exceed the noise floor by 6 dB SPL (Negley et al., 2007). The research study used the SNR ≥ 6 dB SPL to interpret DPOAE's after the baseline measurement as well as after the short-term music exposure (Wagner, Heppelmann, Vonthein, & Zenner, 2008). Recording parameters are defined in Table 6.

Table 6: Distortion Product Otoacoustic Emissions (DPOAE) Setup on DP Echoport ILO V6 (Otodynamics Ltd., England)

Frequency ratio	f2/f1: 1.22
Stimulus intensity ratio	65/55 dB SPL
Noise floor	≤ 0 dB SPL
Noise artifact rejection	6 mPa
Frequency bands	1, 1.5, 2, 3, 4, 6 and 8 kHz.

(dB SPL: decibel sound pressure level; kHz: kilohertz; mPa: megapascal).

2.9. Procedure for participant data collection

The participants that adhered to the selection criteria received the same administered tests. The data collection session consisted of a baseline measurement of the following: CSOAE's, which were TEOAE's with and without contralateral noise, and DPOAE. Hereafter, participants were exposed to music followed by repeat DPOAE measurement and pure tone audiometry. An overview of the participant selection and data collection session can be seen in Table 7. Pure tone determination was used for participant selection and for baseline measurement before and repeated after short-term music exposure.

Table 7: Structure of the data collection session

Participant selection procedure	Data collection procedure	Session task	Estimated Time (min)
X		**Otoscopy and immittance measures**	10
X	X	**Pure tone threshold measurement (125 - 8 kHz)**	15
X		**Speech detection**	10
	X	**CSOAE's and DPOAE's**	5
	X	**Music exposure**	60
	X	**DPOAE's**	5
	X	**Pure tone threshold measurement (125 - 8 kHz)**	15
		Total	**120**

(CSOAE: contralateral suppression of otoacoustic emissions; DPOAE: distortion product otoacoustic emissions; kHz: kilohertz; min: minutes).

2.9.1. Baseline measurement

Before the music exposure, pure tone audiometry, CSOAE's and DPOAE's measurements were taken to determine the integrity of their cochlear OHC. CSOAE's and DPOAE's were measured using an DP Echoport ILO V6 (Otodynamics Ltd., England). The probe was calibrated before each session using the 1 cm^3 calibration cavity provided by Otodynamics. The measurements were conducted by sealing a probe tip in the participant's ear canals while the participant sat calmly. Probe placement was verified using the system's automatic probe seal protocol for both measurements. The same probe was used during the repeated measurements after the short-term music exposure for each participant. CSOAE's were recorded bilaterally

according to the parameters in Table 5 followed by ipsilateral DPOAE's according to the setup in Table 6. TEOAE testing was conducted by sealing off the participant's ear canal with the appropriate size probe tip. In the test ear, a TEOAE without noise was measured. Hereafter, a second probe was placed in the contralateral ear and the narrowband noise was presented continuously in the contralateral ear while TEOAE measurements were repeated without removing the probe from the test ear. The absolute TEOAE amplitudes with and without noise were subtracted from each other in order to determine the amount of efferent suppression (Keppler, Van Reybrouck, & Dhooge, 2015) The latency of the TEOAE once the suppressor signal is presented in the contralateral ear ranges from 8 to 15 milliseconds (ms) (Hall, 2000). DPOAE measurements were conducted in the participants' ipsilateral ear according to the parameters in Table 6. DPOAE and pure tone audiometry measurements conducted at the beginning of the session served as the baseline measurements.

2.9.2. Music exposure

The music sample was used to simulate real-world noise exposure for the participants. The music sample provided the participants with a familiar stimulus which is more comfortable rather than white noise or broadband noise (BBN). The mechanical changes of the Organ of Corti can occur when stimulated at extreme intensities over a short period of time. For temporary changes to occur the noise intensity should be above 130 dB SPL (Konings et al., 2009). Thus, the participants were exposed to continuous discotheque music at 90 dBA for one hour in a double-walled soundproof booth. A significant reduction in OAE's amplitudes was measured during a study using 90 dB SLP BBN during noise exposure (Vinck et al., 1999). A study conducted by Keppler et al. (2010) used one hour of music exposure using MP3 players during which the exposure intensity varied from 71 dBA SPL to 102 dBA SPL. Therefore, the music exposure intensity level correlated with previous studies. All the participants were exposed to one hour of the same music sample in a double-walled soundproof booth.

Music sample used for noise exposure

The music sample was compiled from local radio hits that were previously used during a study done by Van Niekerk (2016). Van Niekerk (2016) presented the music sample at an intensity of 99.6 dBA SPL for one hour. The music sample consisted of 16 pop songs compiled from a list of local radio hits of Jacaranda (94.2FM) and Five FM. The song list can be seen in Appendix L. The compilation lasted 61 minutes and 48 seconds which was reported at one hour similar to the method used in previous studies of Keppler et al. (2010). The music sample was chosen due to the probability that the participants would enjoy the type of music due to the familiarity with the music. The music was presented by using an ASUS K541U laptop with a 2RCA audio cable that was connected to the GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota). The music was transduced through free-field via the audiometer with two ear level speakers with the participants seated one meter from the speakers mounted in the two front corners of the booth at a 45 degrees azimuth.

A sound level meter (RION NA-42) was placed at ear level of the participants to monitor music stimuli levels in the room. The dial reading on the audiometer was set so that the noise exposure LAeq dBA level over the complete duration of the music sample was 90 dBA. LAeq is the equivalent of continuous sound with contains the same amount of energy as the sound varies over time (Fahy & Walker, 1998). The measurement using a sound level meter (Model RION NA-42) was completed before the commencement of data collection to ensure that the music sample was kept constantly at 90 dBA on each test day. Each participant was instructed to indicate if the music intensity exceeded their comfortable loudness level. None of the participant's complained of loudness discomfort during the music exposure. Figure 1 displays the set-up in the double-walled soundproof booth during the music exposure. The set-up was the same for all participants during the music exposure.

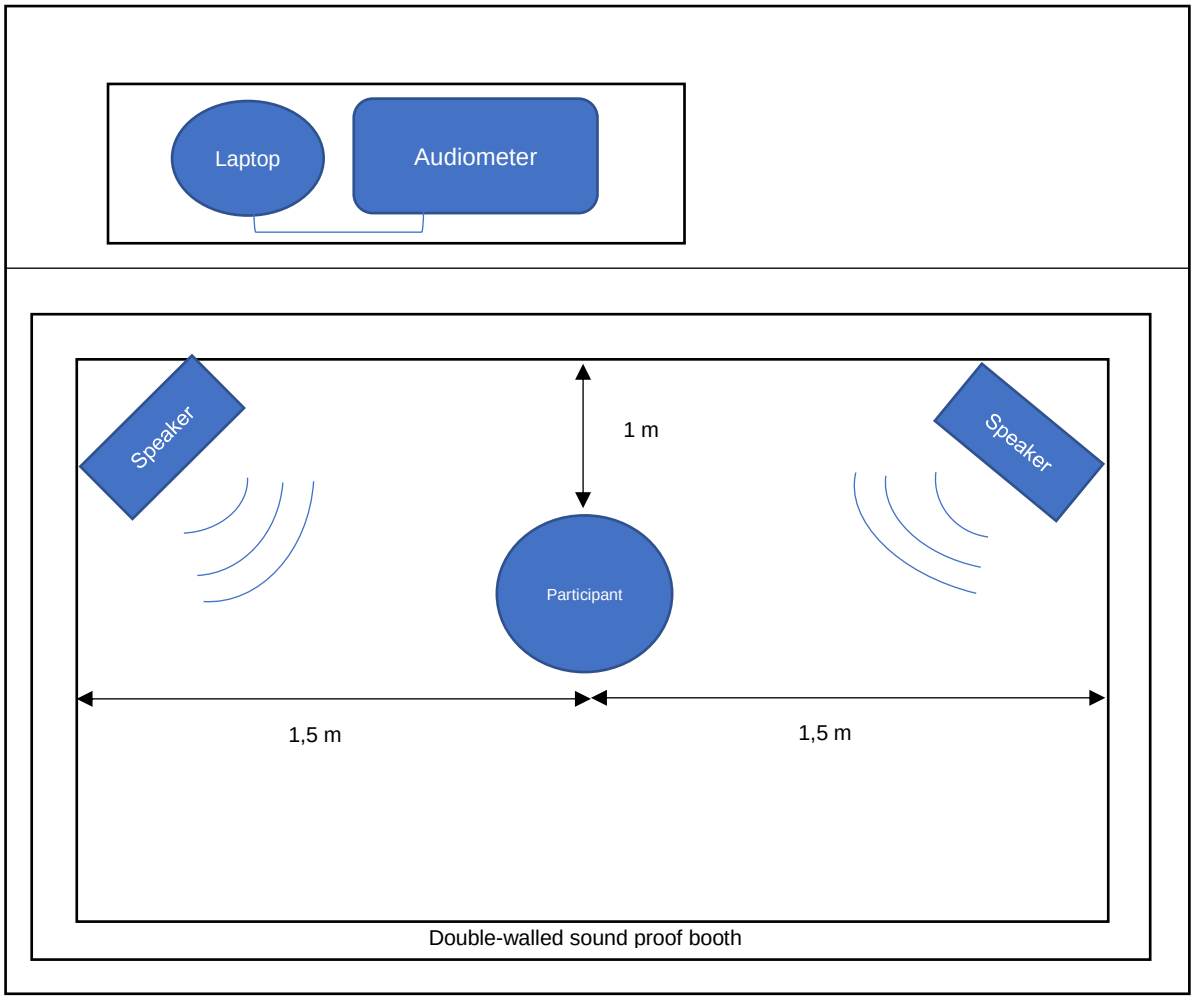

Figure 1: Experimental set-up in the double-walled soundproof booth for music exposure

2.9.3. Post-exposure measurements

The post-exposure measurements commenced within five minutes after the termination of music exposure. Ipsilateral DPOAE measurements and pure tone audiometry were repeated after the music exposure, which served as the post-exposure measurements. DPOAE recording parameters can be seen in Table 6. Post-exposure DPOAE responses and pure tone thresholds were subtracted from the baseline data to calculate the difference between measurements. The difference between the pre- and post of DPOAE response amplitudes constitutes the TES and for pre- and post-pure tone thresholds, the TTS (Balatsourasa et al., 2005; Barros et al., 2007).

2.10. Data analysis

The Statistical Package for the Social Sciences (SPSS) version 25 was used for the statistical testing of all data (Armonk, New York). Amplitudes, SNR and audiometric results were described using descriptive statistics to determine the mean values and standard deviations. The Shapiro Wilk test was used to evaluate normality of distribution. If the results were normally distributed a paired Sample t-test was used compares differences in the means between DPOAE and CSOAE response level and SNR, as well as TTS after music exposure of the two groups. One-way analysis of variance (ANOVA) was used to analyze the influence of two categorical independent variables on one dependent variable, namely the DPOAE response level between the two groups. If the results were not normally distributed, the data were analyzed using the two-tailed Wilcoxon Sign Rank test or a Friedman's test of analysis of variance (ANOVA). Two-tailed Wilcoxon Sign Rank test was used to compare repeated measurements in a single sample, for example, DPOAE or pure tone results before and after music exposure (Williams, Sweeney, & Anderson, 2006). Friedman's ANOVA was used to determine if there was a statistically significant difference between the distribution of three or more related groups, namely differences between CSOAE test frequencies. All results were categorized according to individual related factors, namely as gender (females vs males), ear (left vs right), and brown eyes and hair vs blue eyes and blond hair. In all analyses, a 95% significance level ($p<0.05$) was selected.

2.11. Reliability and validity

Kirk and Muller (1986) explained that reliability is concerned with the repeatability and consistency of the findings in a study. Reliability can be explained as the degree to which other researchers will find the same results after conducting the same test and analysis according to the same method. Validity refers to the extent to which the test measures truly reflect the concept it is proposed to measure (Kirk & Muller, 1986). Reliability and validity were ensured for the current study by the following features:

- An independently experienced audiologist interpreted OAE results.
- The use of objective testing procedures.
- The use of the same testing equipment for all participants.
- The use of the same test environment and double walled soundproof booth to ensure that outside noise does not influence the results at the University of Pretoria.
- The use of controlled exposure to noise to ensure that noise was kept of safe exposure level according to the equal energy rule.
- The equipment used during the present study have been calibration according to the appropriate SANS protocol annually (South African National Standards [SANS] - 60942, 2018; South African National Standards [SANS] 0154-1, 2018; South African National Standards [SANS] 10154-1:2012, 2018; South African National Standards [SANS] 10154-2:2012, 2018; South African National Standards [SANS] 61672-1:2003, 2018).
- The same normative data was used for within participant comparison.
- The use of the same size probe tip for DPOAE measurements for baseline and post-exposure measurement.

Chapter 3: Research article

The article was submitted to the journal of European Archives of Oto-Rhino-Laryngology on the 25th of October 2018. The submission letter from the European Archives of Oto-Rhino-Laryngology can be seen in Appendix M.

The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions

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Compliance with ethical standards

Conflict of interest

All authors declare that there are no conflicts of interest

Ethical approval

All procedures performed in the present study were in accordance with the World Medical Association Declaration of Helsinki 1964. Ethical approval was obtained from the Faculty of Humanities, University of Pretoria.

Informed consent

Written and verbal consent was obtained from each participant before commencement of the current study.

Abstract:

Genetics and environmental factors frequently influence individual's susceptibility to hearing loss. It is postulated that melanin in the inner ear is related to individual's susceptibility to NIHL. General pigmentation in turn, suspected to be related to the amount of pigmentation in the inner ear. The amount of melanin in the inner ear is said to modulate the endocochlear potential and provide an otoprotective effect. **Aim:** The study aimed to determine the relationship between the contralateral suppression of otoacoustic emissions (CSOAE) in individuals with brown eyes and hair, and blue eyes and blond hair, and temporary emission shift (TES) after short-term noise exposure.

Method and materials: The hearing sensitivity of young adults were determined by using pure tone audiometry followed by CSOAE's and distortion product otoacoustic emissions (DPOAE) before listening to music for one hour individually. Pure tone audiometry and DPOAE's were repeated after music exposure to determine the amount of TES and temporary threshold shift (TTS). **Results:** No statistically significant difference between efferent suppression was measured by CSOAE's between the participant groups. A larger TTS at 4000 Hz and TES at 2000 Hz was evident in the blue eyes and blond hair group after short-term music exposure. **Conclusion**: CSOAE's were therefore unable to predict which group of individuals were more susceptible to NIHL after short-term noise exposure.

Keywords: Contralateral suppressions, noise-induced hearing loss, melanin, music exposure, otoacoustic emissions.

Introduction

Noise induced hearing loss (NIHL) is an irreversible hearing impairment due to a single or repeated exposure to loud sounds in the higher frequency region from 2 kHz to 6 kHz. NIHL is becoming more prevalent in the modern society and is said to affect 16% of adults worldwide (Mannström et al., 2015). The impact of hazardous noise exposure is known to cause a temporary hearing deterioration or permanent hearing damage to the sensory cells of the cochlea (Bidelman et al., 2016; Keppler et al., 2014; Otsuka et al., 2016; Sliwinska-Kowalska & Davis, 2012). The severity of NIHL increases as the duration and intensity level increases (Degeest et al., 2017; Sliwinska-Kowalska & Davis, 2012). The increased occurrence of NIHL due to occupational and recreational exposure led to more social an public health complications in the society (Degeest et al., 2017). Therefore, NIHL is eventually affecting the progression of age-related hearing loss due to the early damage to the cochlea. It is estimated by the World Health Organisation (WHO) (2018) that 1.1 billion young adults between the ages of 12 to 35 years are at risk for developing hearing loss due to recreational noise exposure. Young adults' main source of noise exposure can be linked to discotheque noise (Sliwinska-Kowalska & Davis, 2012). Recreational activities such as music concerts, nightclubs, MP3 players, gyms, shooting, and more hobbies may have a potentially damaging effect on our hearing sensitivity (Mannström et al., 2015). Discotheque intensity levels at concerts and nightclubs have been recorded between 84 to 120 dBA (Daniel, 2007; Vinck et al., 1999). The WHO established guidelines in which noise levels at entertainment events may not exceed 100 dBA if individuals are exposed for a maximum of four hours (Berglund et al., 1999). It is important to identify factors influencing an individual's susceptibility with the increase in prevalence of NIHL. There are multiple possible factors that may influence an individual's susceptibility to NIHL and their recovery following noise exposure. These possible factors include environmental factors or genetics (Bidelman et al., 2016; Daniel, 2007; Mannström et al., 2015). Melanin is primarily influenced by genetics that has been recognized to be present in the inner ear. The amount of pigmentation in the inner ear has been related

to general pigmentation and eye colour (Garber et al., 1982). The stria vascularis contains melanin producing cells that play a crucial role in the production of endocochlear potentials and provides an otoprotective effect. It is suggested that hearing loss in individuals with auditory-pigmentary syndromes, namely Waardenburg syndrome and Tietz syndrome may be influenced by the lack of melanin (Jin et al., 2007). Waardenburg syndrome and Tietz syndrome is characterized by hypopigmentation and hearing loss. The susceptibility to NIHL can be attributed to inter-individual variability which can influence the tonic activity of the auditory pathways due to melanin levels (Bidelman et al., 2016; Patuzzi & Thompson, 1991). The underlying mechanism of pigmentation influencing the auditory system is still unclear and cannot fully understood. Numerous studies have referred to work of Bonaccorsi (1965) which suggest that brown-eyed individuals had more melanin in their temporal bones in comparison to blue-eyed individuals (Da Costa et al., 2008; Hood et al., 1976; Mujica-mota et al., 2015). Thus, studies were postulated that the brown-eyed population were less susceptible to NIHL.

Melanin is postulated to play a protective role in the auditory system and against noise trauma (Driscoll et al., 2009; Garber et al., 1982; Hannula et al., 2012; Mujica-mota et al., 2015). Melanin is known to be involved with the calcium ion homeostasis in the cochlea (Driscoll et al., 2009). Calcium homeostasis is crucial for the transduction of sound. Endocochlear electrical potential is dependent upon high calcium concentrations which are required for compound action potential leading to excitatory postsynaptic potential in the auditory nerve (Driscoll et al., 2009). The amount of endocochlear melanin is said to regulate the release of cochlear calcium upon noise exposure to protect the auditory system (Mujica-mota et al., 2015). It is postulated that melanin levels in the inner ear may modulate the auditory-neural transduction process due to the role of calcium on the cochlear hair cell functioning (Jin et al., 2007; Mujica-mota et al., 2015). Damage to the hair cells after prolonged noise exposure can occur in the absence of melanin due to higher levels of calcium in the inner ear without modulation (Mujica-mota et al., 2015). Lower levels of melanin can influence the efficient transduction to inner hair cells and auditory nerve. Individuals with darker skin possess higher melanin levels in the cochlea compared to individuals with lighter skin, which in turn influences hearing sensitivity (Driscoll et al., 2009).

Numerous research (Da Costa et al., 2008; Hood et al., 1976; Mujica-mota et al., 2015) have focused on the association between eye colour and susceptibility to NIHL. Da Costa et al. (2008) and Mujica-mota et al. (2015) reported that light-eyed people had a greater permanent hearing loss following noise exposure in comparison to brown-eyed individuals who exhibited with better hearing thresholds after prolonged noise exposure. Thus, noise exposure has a more forceful effect on individuals with lighter pigmented iris colour. Hannula et al. (2012) findings contradicted the previous studies with results indicating that participants with a combination of dark hair and eyes were not more protected against NIHL. Their conclusion stated that individuals with brown eyes were more susceptible to hearing loss. Driscoll et al. (2009) study found a correlation between otoacoustic emissions (OAE) and different skin pigmentation. Hood et al. (1976) reported a relationship between the amount of melanin and susceptibility to the temporary threshold shifts. Increased auditory thresholds after noise exposure that recover gradually are known as a temporary threshold shift (TTS) (Barros et al., 2007). It is suggested that metabolic overstimulation after noise exposure may be the cause of TTS. A difference between individuals in TTS can be attributed to individual susceptibility, the duration of noise exposure as well as the intensity that the individual is exposed to (Barros et al., 2007). Noise exposure reduces the motility of the outer hair cells (OHC) influencing hearing sensitivity (Vinck et al., 1999). A direct measure of the cochlear OHC can be provided by OAE's. OAE's is an objective measurement to record changes in amplitudes, provide insight of the cochlea's pre-neural and biomechanical aspects of acoustic information which can be influenced by melanin (Cacace et al., 1996). Damage to OHC due to overexposure to noise may lead to a reduction in OAE amplitudes (Keppler, Dhooge, Maes, D'Haenens, Bockstael, et al., 2010). A response level shift after noise exposure can be observed by utilizing OAE's. To examine the relationship between different levels of melanin and OAE's, Driscoll et al. (2009) measured more spontaneous OAE's in darker pigmentated individuals, for example, African Americans in comparison to Caucasians and Asians. Their results supported the hypothesis that the functioning of the OHC may be modulated by melanin as estimated by skin colour (Driscoll et al., 2009). Due to the effectiveness, objective and non-invasive manner of OAE's, it may be the best method of evaluating the correlation between eye and hair colour and cochlear functioning (Barros et al., 2007; Blioskas, Tsalighopoulos, Psillas, & Markou, 2018; Sun, 2008).

The response mechanism of the auditory system can be measured with contralateral suppression of OAE (CSOAE) (Sun, 2008). The medial olivocochlear (MOC) bundle is known as the efferent branch of the auditory feedback system which originates in the brainstem and terminates predominantly in the OHC of the opposite cochlea (Jacobson et al., 2003; Maison et al., 1998). The MOC reflex is the suppression effect in response to acoustic stimulation and serves as a cochlear defensive mechanism from acoustic damage (Otsuka et al., 2016). The MOC reflex measurement is postulated to be valuable for screening an individual's susceptibility to acoustic trauma due to weakened MOC effect for those who have a preferential susceptibility to NIHL (Mishra & Lutman, 2013; Wagner et al., 2005). The MOC efferent system has assumed roles by inhibition of cochlear amplifier gain such as aiding listening in noise, slowing age-related hearing loss and protection against acoustic overexposure (Bidelman et al., 2016; Jacobson et al., 2003; Otsuka et al., 2016; Patuzzi & Thompson, 1991). The strength of the MOC reflex is considered a be able to predict threshold shifts (Otsuka et al., 2016). The MOC reflex is activated by acoustic stimulation and induces an inhibitory effect on OHC motility (Jacobson et al., 2003; Otsuka et al., 2016). The protection provided by the MOC reflex can be attributed to the reduction in the interruptions of electro-mechanical transduction of the OHC (Patuzzi & Thompson, 1991). The reduction in amplitude with a contralateral masker was expected as small as 1 to 4 dB SPL reduction in amplitude (Wagner et al., 2005). A noticeable shift can be measured in the OAE's before fluctuations in behavioural pure tone thresholds may be identified (Seixas et al., 2004). A temporary emissions shift (TES) may provide a better indication of the effect of noise exposure on the cochlea than other audiometric results (Otsuka et al., 2016). An emission shift after noise exposure is expected to occur at 2000 to 6000 Hz (Barros et al., 2007).

To the researcher's knowledge, no studies have investigated the relationship between eye and hair colour and CSOAE's after short-term noise exposure. Previous studies have investigated the correlation between the amount of melanin based on eye and hair colour and the effect that it has on OHC or amount of melanin in the temporal bones (Cunningham & Norris, 1982; Hood et al., 1976; Mujica-mota et al., 2015). CSOAE's can provide information about the protective function of the efferent system

while OAE's provides information on whether the protective reflex maintains over time with acoustic stimulation. Numerous studies stated that an earlier indication of cochlear damage is provided by OAE's in comparison to behavioural pure tone threshold audiometry (Seixas et al., 2004; Vinck et al., 1999). The study aimed to determine the relationship between the CSOAE's in individuals with different hair and eye colour, and TES after short-term noise exposure.

Subjects and method

Participants

Twenty-five normal hearing adults, between the ages of 18 and 28 years were selected for the current study. All participants volunteered to participate in the study, which was approved by an institutional review board. The study consisted of twenty females and five males (Mean age: 21.64 years; SD 1.80). Participants were non-smokers, should have no history of ear diseases and in generally good health. Additional inclusion criteria were defined. A normal bilateral otoscopic examination, Jerger type A tympanograms measured with a Y-226 Hz probe tone utilising a GSI Tympstar Middle Ear Analyzer (Grason-Stadler, Eden Prairie, Minnesota). Jerger Type A tympanograms were defined as a static compliance of 0.3 to 1.75 mmho and a peak pressure of +100 to -100 daPa (Jerger, 1970). Normal ipsilateral and contralateral acoustic reflexes elicited at 85 to 100 dB SPL at frequencies 0.5, 1, 2 and 4 kHz. Furthermore, pure tone thresholds were obtained using the modified Hughson-Westlake procedure (Carhart & Jerger, 1959) on a GSI 61 clinical audiometer with supra-aural TDH-39 headphones (Grason-Stadler, Eden Prairie, Minnesota). Participants were selected based on normal behavioural pure tone air conduction thresholds ≤15 dB HL at octave intervals from 125 to 8000 Hz and including half octave frequencies of 3000 and 6000 Hz were included. The speech detection threshold of the participants had to be within close proximity of ± 7 dB of the pure tone averages (PTA) of the participants at 0.25 to 2 kHz (Hall & Mueller, 1997). All testing was conducted in a double-walled soundproof booth. Participants were grouped in two categories: group A consisted of individuals with brown eyes and hair, and group B was individuals with blue eyes and blond hair. In the study, 47 % of the participants had brown eyes and brown hair and 53 % of the subjects had blue eyes and blond hair.

Baseline testing

Baseline testing of pure tone audiometry, CSOAE and distortion product otoacoustic emissions (DPOAE) precede a one hour by music exposure session at 90 dBA. After the exposure, DPOAE measurements and pure tone audiometry were repeated. All testing was conducted in a double-walled soundproof booth.

Otoacoustic emissions

An Otodynamics DP Echoport ILO V6 was used for both CSOAE and DPOAE measurements. Probe calibration was performed at the beginning of each session using a 1 cm^3 calibration cavity. For CSOAE measurements, a linear mode of stimulation was used with a rate of 50 clicks/sec using an 80μs rectangular electrical pulse. Clicks were evoked with an intensity of 65 dB SPL with and without masker in the contralateral ear. The contralateral stimulus consisted of a continuous broadband noise presented at a stimulus level of 60 dB SPL to elicit efferent activity (Backus, Guinan Jr, Backus, Lilaonitkul, & Aharonson, 2004). The noise rejection level was set at 49.5 dB SPL (Stuart & Cobb, 2015). To evaluate the level of suppression, transient evoked OAE (TEOAE) responses without a broadband noise were measured followed by a measurement with contralateral broadband noise stimulation. The absolute TEOAE suppression was determined by subtracting the amplitude with contralateral stimulation from the amplitude with contralateral stimulation in each ear specifically (Keppler et al., 2015). The obtained TEOAE response levels and noise amplitudes were analysed in half-octave frequency bands centred at 1, 1.4, 2, 2.8 and 4 kHz. To determine the presence or absence of TEOAE responses, the following factors relating to the recording parameters were taken into consideration (Mishra & Lutman, 2013; Stuart & Cobb, 2015). Measurements were present when a stimulus stability of 90% and a reproducibility of 75% were present. Signal to noise ratio (SNR) must have been ≥ 6 dB (Mishra & Lutman, 2013). Following CSOAE measurements, ipsilateral 2f1-f2 DPOAE's were evoked by stimulation generation of two primary tones (f1 and f2) with an f2/f1 frequency ratio of 1.22 and f2 ranging from 1001 to 7996 Hz. The stimulus tone level combination L1/L2 was set to 65/55 dB SPL, to ensure that the optimal SNR was achieved (Dreisbach & Siegel, 2001). The noise rejection level was set at 49.5 dB SPL (Keppler, Dhooge, Maes, D'Haenens, Bockstael, et al., 2010). The obtained DPOAE responses were converted and reported into half-octave frequency with the center

frequencies at 1, 2, 3, 4, 6, 7.96 kHz. DPOAE results were deemed present if four or more frequencies had a DP amplitude exceeded the noise floor by 6 dB, therefore a SNR of ≥ 6 dB SPL (Wagner et al., 2008).

Music exposure

Following the baseline measurements, each participant was exposed to continuous discotheque music set at an intensity of 90 dBA for one hour in a double-walled soundproof booth. A sound level meter (RION NA-42) was placed at ear level of the participants to monitor noise in the room. The noise exposure LAeq dBA level over the complete duration of the music sample was measured at 90 dBA. The music was presented by using an ASUS K541U laptop that was connected to the GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota) to ensure the intensity was kept consistently at 90 dBA. The music was transduced through free-field via the audiometer's two GSI speakers with the participants seated one meter from the two speakers mounted in the front corners of the booth.

Post-exposure measurements

Post-exposure measurements commenced within five minutes after the end of music exposure. Ipsilateral DPOAE measurements and pure tone audiometry were repeated after the music exposure, which served as the post-exposure measurements. Post-exposure DPOAE responses and pure tone thresholds were subtracted from the baseline data to calculate the difference between measurements. The difference between the pre- and post of DPOAE amplitudes constitutes the TES and for pre- and post-pure tone thresholds, the TTS.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 25 was used for the statistical analysis of all data. Amplitudes, SNR and audiometric results were described using descriptive statistics to determine the mean values and standard deviations.

The Shapiro Wilk test was used to evaluate normality of distribution (Williams et al., 2006). If the results were normally distributed, a paired sample t-test was used to compare differences in the mean values between DPOAE and CSOAE response level, and SNR as well as audiometric shift after music exposure of the two groups. One-way ANOVA was used to analyse the influence of two categorical independent variables on one dependent variable, namely the DPOAE response level between the two groups (Williams et al., 2006). If the results were not normally distributed, the data was analysed using the two-tailed Wilcoxon Sign Rank test and Friedman's test of analysis of variance. Wilcoxon Sign Rank test was used to compare repeated measurement in a single sample, DPOAE and pure tone results before and after music exposure. Friedman's test of analysis of variance was used to determine if there was a statistically significant difference between the distribution of three or more related groups of CSOAE frequencies. All results were categorized according to individual related factors namely gender (females vs males), ear (left vs right) and hair and eye colour (brown eyes and hair vs blue eyes and blond hair). In all analyses, a 95% level of significance ($p<0.05$) was used.

Results

Impact of hair and eye colour on the contralateral suppression of transient evoked otoacoustic emissions

Table 8 displays the mean absolute TEOAE amplitude with noise and amount of suppression per test frequency for participants with brown eyes and hair, and for participants with blue eyes and blond hair.

Table 8: The mean efferent suppression (dB SPL) per frequency for group with brown eyes and hair, and group with blue eyes and blond hair

Frequency (Hz)	1001	1414	2000	2828	4000
Brown eyes and brown hair (n=24 ears)	8.34 ±4.04	9.67 ±3.30	6.84 ±3.13	4.26 ±4.63	0.94 ±4.84
Absolute TEOAE amplitude with noise (dB SPL ± SD)	0.46	0.53	0.54	0.57	0.38
Amount of suppression (dB)					
Blue eyes and blond hair (n=26 ears)	7.50 ±5.84	7.52 ±4.35	7.57 ±3.61	4.74 ±3.64	1.23 ±5.98
Absolute TEOAE amplitude with noise (dB SPL ± SD)	0.48	0.54	0.38	0.44	0.29
Amount of suppression (dB)					

(dB: decibel; dB SPL: decibel sensation level; Hz: frequency; SD: standard deviation; %: percentage)

The mean absolute efferent suppression decreased from the mid frequencies towards the higher frequency region in all participants. The two groups exhibited the same degree of efferent suppression with similar mean suppression at each frequency. The mean efferent suppression decreased in the higher frequencies from 2.8 kHz in comparison to the lower frequency region. No statistically significant difference in suppression could be measured between the two groups ($z=-0.30$-1.60; $p>0.05$). However, a significant difference was observed between the degree of suppression between the individual test frequencies.

A Friedman's analysis of variance (ANOVA) with Bonferonni correction of the difference suppression values at different test frequencies in the total participants group indicated a statistically less suppression for 4000 Hz compared to 2828 Hz ($p<0.03$) and highly significant difference between the following frequencies: 4000 and 2000 Hz ($p<0.001$); 4000 and 1001 Hz ($p<0.001$); 4000 and 1414 Hz ($p<0.001$); 2828 and 2000 Hz ($p<0.001$); 2828 and 1001 Hz ($p<0.001$) and 2000 and 1001 Hz ($p<0.001$). The other frequencies namely 2000 and 1001 Hz, 2000 and 1414 Hz and 1001 and 1414 Hz did not indicate a statistical significance ($p>0.05$).

The mean efferent suppression was symmetrical between the left and right ears did not indicate a statistical significance (z=-0.22-0.66; p>0.05).

Differential impact of short-term music exposure n pure tone audiometric thresholds

Table 9 provides the baseline measurements in pure tone thresholds, post-exposure pure tone thresholds, and the TTS in the brown eyes and brown hair participants, and blue eyes and blond hair participants.

Table 9: Mean pure tone thresholds in participants with brown eyes and hair and participants with blue eyes and blond hair after music exposure at each frequency (n=50 ears)

Frequency (Hz)	**125**	**250**	**500**	**1000**	**2000**	**3000**	**4000**	**6000**	**8000**
Brown eyes and hair (n=24 ears)									
Baseline pure tone threshold (dB HL ± SD)	1.25 ±2.66	1.87 ±3.55	1.04 ±2.54	0.42 ±1.41	0.83 ±2.40	0.42 ±1.41	0.42 ± 1.41	1.46 ± 3.12	0.83 ±2.82
Pure tone threshold after music exposure (dB HL ± SD)	1.87 ±3.55	2.5 ±3.10	1.46 ±2.75	0.63 ±1.69	0.63 ±1.69	0.42 ±1.41	1.25 ±2.66	1.86 ±2.88	1.25 ±3.69
Threshold shift (dB)	**0.63**	**0.63**	**0.42**	**0.21**	**0.21**	**0**	**0.83**	**0.42**	**0.42**
Blue eyes and blond hair (n=26 ears)									
Baseline pure tone threshold (dB HL ± SD)	1.92 ±3.49	0.77 ± 1.84	0.96 ± 2.83	0.58 ± 1.63	0.38 ±1.36	0.38 ±1.36	0.96 ±2.83	3.27 ±3.99	2,12 ±3.21
Pure tone threshold after music exposure (dB HL ± SD)	2.31 ±4.30	1.54 ±2.75	1.35 ±4.14	0.58 ±2.16	1.54 ±3.68	1.35 ±2.68	4.04 ±4.69	5.00 ±5.48	3.65 ±5.20
Threshold shift (dB)	**0.38**	**0.77**	**0.38**	**0**	**1.15**	**0.96**	**3.08 ***	**1.73**	**1.54**

*(dB HL: decibel hearing level; Hz: frequency; SD: standard deviation; * indicative of significance)*

The mean PTA before and after noise exposure were the same in participants with brown eyes and hair, and participants with blue eyes and blond hair, namely 1.00 dB HL. The participants with blue eyes and blond hair had a larger mean pure tone threshold in the higher frequencies from 2000 to 8000 Hz after the music exposure in comparison to participants with brown eyes and hair. The blue eyes with blond hair group displayed a significant larger TTS from baseline measurement to post-exposure

at 4 kHz (z=-2.17; p<0.05). The brown eyes with brown hair group indicated a mean TTS of 0.83 dB HL which is smaller than the participants with blue eyes with blond hair group with a mean TTS of 3.00 dB HL. The mean TTS at all other frequencies between the two groups exhibited no statistically significant differences (z=-0.07-1.87; p>0.05).

The mean TTS at 4 kHz was much larger in the left ear than in the right ear but was not statistically significant (z=-1.46; p>0.05). The mean TTS did not indicate a significant difference between the left and right ear at frequencies 125 to 8000 Hz (z=-0.20-1.75; >0.05).

Differential impact of short-term noise exposure on distortion product otoacoustic emissions

Table 10 displays the mean baseline SNR measurement, post-exposure SNR measurement and reduction in SNR at each frequency in all the participants.

Table 10: Mean baseline and post-exposure distortion product otoacoustic emissions (DPOAE) signal to noise ratio (SNR) in all participants (n=50 ears)

Frequency (Hz)	**1001**	**1501**	**2002**	**3003**	**4004**	**6006**	**7996**
Mean baseline SNR (dB SPL ± SD)	16.52 ±5.67	20.9 ±5.23	20.9 ±4.72	18.05 ±4.55	21.36 ±4.32	21.70 ±6.07	11.06 ±10.8
Mean post-exposure SNR (dB SPL ± SD)	12.43 ±6.78	18.35 ±6.26	18.65 ±6.18	15.61 ±5.12	18.38 ±4.52	19.12 ±7.41	5.67 ±14.32
Mean difference between baseline and post-exposure SNR (dB SPL ± SD)	4.09 ±2.89 **	2.55 ±1.80 **	2.25 ±1.59 **	2.44 ±1.73 **	2.98 ±2,10 **	2.58 ±1.82 **	5.81 ±3,11 **

*(dB SPL: decibel sensation level; DPOAE; distortion product otoacoustic emissions; Hz: frequency; SD: standard deviation; SNR: signal to noise ratio; * indicative of significance)*

The mean DPOAE SNR reduction indicated a highly significant difference (t=3.73-6.93; p<0.001) at each frequency. The mean TES also indicated a highly significant difference at each frequency in all participants (t=3.46-7.08; p<0.001). The difference in the mean TES is shown in Figure 2 between participants with brown eyes and hair, and participants with blue eyes and blond hair from 1001 to 7996 Hz (* indicative of significance).

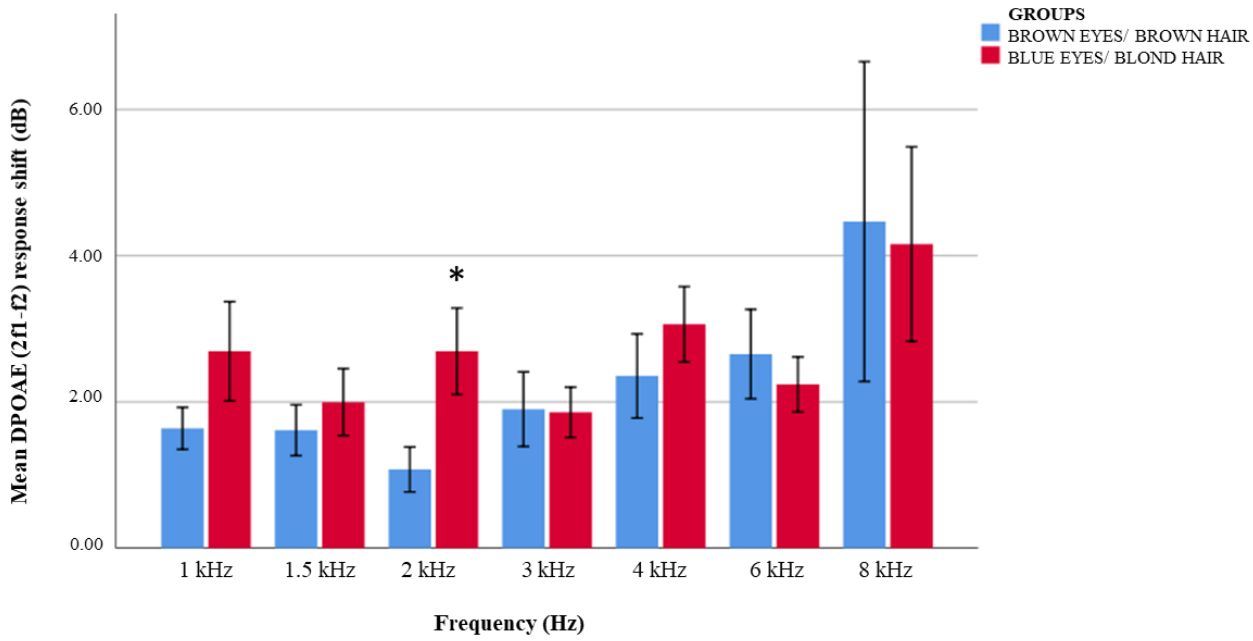

Figure 2: The mean distortion product otoacoustic emissions (DPOAE) half octave response level shift after music exposure in participants with brown eyes and hair and participants with blue eyes and blond hair (n=50 ears)

The Wilcoxon signed rank test indicated a statistically significant difference in TES at 2 kHz between the two groups (z=-1.05; p<0.05) with a mean shift of 2.69 dB SPL. At the other frequencies tested the TES from baseline to post-exposure did not indicate statistically significant difference (z=-0.30-2.41; p>0.05) between the participants with brown eyes and hair, and participants with blue eyes and blond hair. A significant difference between groups was also displayed for the SNR of the DPOAE's at 2 kHz (F=4.77; p<0.05).

The baseline measurement and post-exposure measurement of TES indicated symmetrical shifts between the left and right ear. For each of the frequencies, there was no statistically significant difference between the left and right ears ($z=-0.14$-1.55; $p>0.05$).

Discussion

The current study explored the relationship between CSOAE's of individuals with blue eyes and blond hair, and brown eyes and hair, and their TES after one hour of music exposure at 90 dBA. Statistically significant TTS and TES were recorded after music exposure in individuals with blue eyes and blond hair, in comparison to individuals with brown eyes and hair. No significant difference in efferent suppression was observed between participants with brown eyes and hair, and participants with blue eyes and blond hair.

Differential impact of short-term music exposure on pure tone audiometric thresholds

The present study found a mean TTS ranging from 0 to 0.83 dB HL in the brown eyes and hair, and 0 to 3.08 dB HL in the blue eyes and blond hair. Pure tone audiometry has been widely used to evaluate the correlation between iris pigmentation and NIHL in previous studies (Da Costa et al., 2008; Hannula et al., 2012). The current study found that participants with blue eyes and blond hair showed a statistically larger TTS, by 2.25 dB HL, at 4 kHz after music exposure than the participants with brown eyes and hair. The reduction in pure tone thresholds at 4 kHz is typical of NIHL and was evident in the blue eyes and blond hair group after music exposure. The current study did not find significant differences between groups at other test frequencies other than 4000 Hz. Previous studies found significant TTS at 2000 to 6000 Hz in comparison to the current study, which only recorded a significant TTS at 4000 Hz. The results of the current study did not suggest that music exposure of one hour at 90 dBA causes a significant TTS at each frequency within the noise sensitive region of 2000 to 6000 Hz. Bhagat and Davis (2008) found audiometric results were not indicative of noise trauma after 30 minutes of noise exposure and were rather revealed in OAE results

that in noise exposed individuals. Significantly better PTA's have been recorded in dark-eyed individuals in comparison to light eyed individuals after noise exposure (Bhagat & Davis, 2008; Da Costa et al., 2008; Mujica-mota et al., 2015). Dark eyed individuals have been reported to have from 1 to 5 dB HL better hearing thresholds in the noise susceptible frequency region (Da Costa et al., 2008). Previous studies based their conclusion on individuals with long-term noise exposure, ranging from two to 42 years (Da Costa et al., 2008; Mujica-mota et al., 2015). Although the current study used short-term music exposure with participants with normal hearing, the same trends were observed which the individuals with dark eyes and hair which had better hearing thresholds after short-term music exposure. Therefore, it is suggested that the blue eyes and blond hair may be more susceptible to acoustic overstimulation even after only a short-term noise exposure.

Differential impact of short-term noise exposure on distortion product otoacoustic emissions

Due to the effectiveness of OAE's and the objectivity it provides, most studies including the present study used OAE's in addition to pure tone audiometry. Studies utilized DPOAE to measure changes in amplitudes after noise exposure (Hood et al., 1996; Keppler, Dhooge, Maes, D'Haenens, Philips, et al., 2010; Vinck et al., 1999). The current study measured significant differences in all participants' TES and DPOAE SNR at each frequency. A reduction of 2.25 to 5.81 dB SPL was measured in the DPOAE SNR in all participant in the current study with the largest shifts at 4004 to 7996 Hz. Although pure tone audiometry did not display the same results after one-hour noise exposure at each frequency, DPOAE's were able to monitor slight changes in the amplitudes of emissions after noise exposure. The present study found a significantly larger mean TES of 2.69 dB SPL at 2000 Hz in participants with blue eyes and blond hair than in participants with brown eyes and hair. A study reporting on DPOAE's after exposure to impulse noise, reported a significant shift at 4004 to 7996 kHz while the lower frequencies remained stable (Veuillet et al., 2001). Studies have described a trend of TES at 3049 to 7996 Hz after acoustical overstimulation (Keppler et al., 2014; Vinck et al., 1999). Research investigating noise exposure and TES varied from an exposure intensity of 85 to 103 dB SPL for 30 minutes to five hours (Bhagat &

Davis, 2008; Keppler, Dhooge, Maes, D'Haenens, Philips, et al., 2010; Vinck et al., 1999). The degree of shift is highly dependent on the intensity and duration of exposure. Individuals have shown a mean TES of 7 to 10 dB SPL after exposure to broadband noise, music in nightclubs and MP3 players (Hood et al., 1976; Keppler, Dhooge, Maes, D'Haenens, Bockstael, et al., 2010; Vinck et al., 1999). The current study observed a smaller TES after music exposure in comparison to previous research although the affected frequency region correlated (Keppler, Dhooge, Maes, D'Haenens, Bockstael, et al., 2010; Vinck et al., 1999). The larger TTS and TES in the blue eyes and blond hair group were only measured at one test frequency per test, namely a TTS at 4kHz and a TES at 2 kHz.

Mujica-mota et al. (2015) reviewed numerous studies and concluded that although differences between lighter and darker pigmented individuals were noted, it is considered only a modest risk factor. Studies have suggested that individuals with lighter pigmentation were only at risk when exposed to high noise levels over a prolonged period of time (Da Costa et al., 2008; Hannula et al., 2012; Hood et al., 1976). The differences in results and degree of shifts between the blue eyes and blond hair, and the brown eyes and hair's TTS and TES can be attributed to the following three reasons. Firstly, the intensity of the noise exposure in previous studies was greater in comparison to the present study using a 90-dBA exposure level. A louder exposure level may cause larger shifts in the same duration of exposure. Secondly, the duration of noise exposure of previous studies was five to six hours or even years in comparison to the single hour in the present study. According to the equal energy rule, when the intensity increases by three dB it leads to the doubling in sound energy and the reducing duration in half which is acceptable to be exposed to (Jiang, Zhao, Guderley, & Manchaiah, 2016). As soon as the intensity becomes too loud or duration longer, it may cause larger shifts and more permanent damage. Due to the reduced duration, despite similar exposure levels, although shifts were recorded, it was much smaller in comparison to previous studies. Lastly, the TES and TTS can be attributed to inter-individual variability.

There is an agreement between studies that intrinsic and extrinsic factors influence individuals susceptibility and auditory health (Bhagat & Davis, 2008; Bidelman et al., 2016; Da Costa et al., 2008; Hannula et al., 2012; Keppler et al., 2014; Patuzzi & Thompson, 1991). The differences in TTS and TES between individuals with blue eyes and blond hair, and brown eyes and hair may be attributed to an intrinsic factor such as pigmentation that is influenced by genetics. The present study supports the hypothesis of Bonaccorsi (1965) that there may be a correlation between the amount of pigmentation in the iris and temporal bone due to the visible difference between the blue eyes and blond hair and brown eyes and hair in the current study. Although differences between the blue eyes and blond hair, and the brown eyes and hair groups are seen, the results are unlikely to be attributed to pigmentation alone. Factors such as other genetic factors and environmental factors should not be excluded when examining individuals' susceptibility to NIHL.

Impact of hair and eye colour on the contralateral suppression of transient evoked otoacoustic emissions

The current study aimed to determine whether a correlation existed between CSOAE's and TES and TTS after music exposure at 90 dBA in participants with more pigmentation in comparison to participants with less pigmentation. The current study could not identify a difference in efferent suppression as measured by CSOAE's in participants with brown eyes and hair, and participants with blue eyes and blond hair. The present study, however, did observe stronger efferent suppression from 1 to 2 kHz in all participants. This finding correlated with that of Otsuka et al. (2016) who recorded stronger efferent suppression from 1 to 3 kHz in comparison to the higher frequency region in young adults with normal hearing sensitivity. Therefore, the results suggest that MOC efferent system may be stronger at 1 to 2 kHz and weaken in the NIHL sensitive area (Bidelman et al., 2016). The current study observed the same trend of decrease in CSOAE amplitude at ≥ 2.8 kHz with a larger TES measured at 2000 Hz in individuals with less pigmentation compared to those with more pigmentation. The TES was only observed in the group with blue eyes and blond hair although their amount of efferent suppression during CSOAE's was equivalent between the two groups. A previous study used CSOAE's to measure the MOC reflex to assess the risk of hearing

loss among orchestra musicians which indicated significant TTS at 4000 Hz as well as a decrease in efferent suppression at 4000 Hz (Otsuka et al., 2016). Numerous studies have concluded the inability of the CSOAE to reliably predict TTS or TES (Keppler et al., 2014; Otsuka et al., 2016). It is postulated that MOC reflex may provide a protective factor in non-traumatic sound and that the role of the MOC reflex is still vague (Fuente, 2015). The current study is unable to identify a relationship between the strength of efferent suppression, TTS and TES after music exposure in individuals with blue eyes and blond hair and individuals with brown eyes and hair. However, the present study effectively utilized OAE's and pure tone audiometry to measure differences between blue eyes and blond hair and brown eyes and hair after acoustic overstimulation.

A limitation of the current study was that pigmentation was quantified based on hair and eye colour determine the susceptibility to NIHL. Thus, limiting the ability to determine the effect of ethnicity/race on susceptibility to NIHL. The current study aimed to determine the correlation between CSOAE's in different hair and eye colours, and their TES after music exposure, therefore, purposefully excluding race/ethnicity to effectively control variables. Previous studies have investigated the effect of ethnicity/race on hearing loss by using OAE's and pure tone audiometry (Boothalingam et al., 2018; Helzner et al., 2005). The differences between ethnicities were seen in the noise susceptible region of 4000 to 8000 Hz, regardless of gender (Helzner et al., 2005). It is acknowledged that individuals from different races and ethnicities have a differing predisposition for NIHL. Pigmentation distribution to skin, hair, and eyes are influenced by genetics and a lack of pigmentation have been observed in genetic disorders (Costin & Hearing, 2007). The current study excluded race/ethnicity that is also influenced by genetics to have less variables influencing current study' outcome. Pigmentation syndromes have indicated that there is a correlation between skin colour and sensorineural hearing loss (Read & Newton, 1994). Therefore, race/ethnicity should not be excluded when investigating the effect of pigmentation on hearing sensitivity after noise exposure due to its possible influence on individuals' susceptibility to NIHL.

Research regarding genetic factors influencing individual's susceptibility to NIHL has been limited due to the inability to determine cumulative recreational noise exposure across a lifetime. NIHL is becoming more prevalent in the modern society and is preventable. Previous studies mainly focused on acoustic overstimulation in a laboratory or occupational setting rather than a recreational setting (Patuzzi & Thompson, 1991). Future research should focus on monitor the effect of recreational noise exposure on individuals hearing sensitivity. Individuals are differently susceptible to NIHL; therefore, some individuals may have more resilient ears in comparison to others against noise exposure. The underlying mechanism of this phenomenon is not yet understood The use of OAE's allows the objective and non-invasive monitoring of changes in OHC when measured directly after exposure to identify individuals that are more susceptible to NIHL (Veuillet et al., 2001). Inter-individual susceptibility should not be underestimated, and awareness should be created to identify possible risk factors to NIHL. The current study population included normal hearing young adults with less cumulative exposure to occupational and recreational noise. Identifying the influencing factors of inter-individual susceptibility will aid audiologists to better prevent and treat individuals susceptible to NIHL. Efforts should be made to better understand the underlying mechanisms influencing NIHL.

Conclusion

No clear relationship was determined between CSOAE's of individuals with blue eyes and blond hair, and brown eyes and hair, and their TES after one hour of music exposure. There was a significant TES at 2000 Hz in the individuals with blue eyes and blond hair after noise exposure in comparison to individuals with brown eyes and hair. In addition, a larger TTS was measured at 4000 Hz in the individuals with blue eyes and blond hair in comparison to the individuals with brown eyes and hair. No statistically significant difference between the two participants groups could be measured with CSOAE's. Therefore, CSOAE's were unable to predict which group of individuals were more susceptible to NIHL after music exposure. To effectively identify factors that influence individual susceptibility to NIHL, more variables must be identified and monitored over a longer exposure duration.

Chapter 4: Discussion, clinical implication and conclusion

Noise induced hearing loss (NIHL) is becoming an epidemic of the modern age due to young adults exposing themselves to louder recreational and/or occupational noise regularly. Numerous attempts have been made to identify factors which influences an individual's susceptibility to NIHL. Genetics has been identified to influence an individual's resilience to NIHL. Pigmentation which is influenced by genetics and has been postulated to be influencing susceptibility to NIHL. The influence of pigmentation on susceptibility to NIHL has been widely examined by past research by monitoring individuals otoacoustic emissions (OAE) and pure tone thresholds after noise exposure (Driscoll et al., 2009; Hood et al., 1976; Mujica-mota et al., 2015). Previous research concluded that pigmentation may be a possible risk factor for an individual's susceptibility to NIHL (Mujica-mota et al., 2015; Thomas & Williams, 1990). Numerous studies have investigated the correlation between the amount of melanin-based on eye and hair colour, and the effect that it has on outer hair cells (OHC) (Da Costa et al., 2008; Hannula et al., 2012; Mujica-mota et al., 2015). Currently, limited research is available that the researcher is aware of regarding the use of contralateral suppression of otoacoustic emissions (CSOAE) to examine susceptibility to NIHL in differently pigmentated individuals. CSOAE has been known to reflect the functioning of the efferent pathway and provide protection against acoustic stimulation (Jacobson et al., 2003).

The aim of the study was to determine the relationship between the CSOAE's in individuals with blue eyes and blond hair, and brown eyes and brown hair, and their temporary emission shift (TES) after short-term noise exposure. The short-term music exposure consisted of each participant listening to a music sample for one hour at 90 dBA in a double walled soundproof booth. The results of the current study found no evidence that CSOAE's are able to predict the TES in distortion product otoacoustic emissions (DPOAE) after short-term music exposure. However, individuals with blue eyes and blond hair did indicate larger TES and temporary thresholds shifts (TTS) after acoustical overstimulation in comparison to the brown eyes and hair. Therefore, less pigmented individuals presented to be more susceptible to NIHL than more pigmented individuals.

4.1. Summary of results

Differential impact of short-term noise exposure on pure tone audiometric thresholds and distortion product otoacoustic emissions

Pure tone audiometry is currently the gold standard in the evaluation of hearing sensitivity (Torre & Grace, 2014). Previous research has used pure tone audiometry to examine the otoprotective effect of melanin in addition to monitoring changes in hearing sensitivity after noise exposure (Da Costa et al., 2008; Hood et al., 1976; Mujica-mota et al., 2015; Thomas & Williams, 1990). The current study also used pure tone audiometry and recorded a TTS ranging from 0 to 3 dB HL after music exposure in all participants. A significant shift at 4000 Hz was observed with a larger TTS of 3 dB HL in the blue eyes and blond hair group, in comparison to the brown eyes and hair in the present study. Previous studies reported on individuals that had years of occupational noise exposure rather than limited cumulative noise exposure, or a one hour of music over-exposure at 90 dBA as was the case in the present study. Most studies report a deterioration of 5 to 20 dB HL at the noise sensitive frequencies of 3000 to 8000 Hz, with the largest shift between 3000 to 4000 Hz (Barros et al., 2007; Jin, Nam, & Sung, 2005; Keppler et al., 2014; Müller, Dietrich, & Janssen, 2010; Sadhra et al., 2002; Santos et al., 2007; Vinck et al., 1999). The smaller TTS measured at each frequency, with a statistically significant shift at a single frequency in the present study is in contrast to previous research that reported larger TTS at all frequencies. The differences in TTS between studies can be attributed to the duration and type of exposure. The present study used an intensity of 90 dBA which caused a significant TTS in the noise susceptible frequency region. Therefore, the intensity level of the music exposure in the present study was sufficient, and the differences in the amount of TTS may be attributed to the duration and type of noise.

The present study provides evidence of the possible contribution of pigmentation on susceptibility to NIHL, albeit limited to one frequency, as the significant TTS was only measured in only blue eyes and blond hair individuals. A difference in behavioural pure tone averages (PTA) between individuals with less pigmentation in comparison with individuals with more pigmentation has been reported in previous research as well as

in the present study (Da Costa et al., 2008; Garber et al., 1982; Hood et al., 1976; Thomas & Williams, 1990). Individuals with less pigmentation have been known to have poorer pure tone thresholds and the current study observed the same trend. The larger TTS in individuals with blue eyes and blond hair suggests that the less pigmented individuals are more susceptible to acoustic overexposure in comparison to individuals with more pigmentation such as individuals with brown eyes and hair. The current research study acknowledged that there are noticeable differences between groups pure tone audiometry after noise exposure, however, the confounding genetic and environmental factors influencing susceptibility to NIHL should not be excluded.

In addition to TTS, a decrease in OAE's has been observed in studies after acoustical stimulation at a high intensity (Bhagat & Davis, 2008; Keppler et al., 2010; Vinck et al., 1999). The current study observed a significant TES, as well as signal to noise ratio (SNR) reduction, in all participants after music overexposure. The TES in SNR ranged from 2.25 to 5.81 dB sound pressure level (SPL) in all participants. Most studies, including the present study, report a significant TES from 2002 to 7996 Hz (Keppler, Dhooge, Maes, D'Haenens, et al., 2010; Vinck et al., 1999). Previous studies reported a significant decrease of 2.1 to 10 dB SPL was seen in DPOAE amplitudes, after exposure to an average of 90 to 109 dBA for three to 12 hours (Jin et al., 2005; Müller et al., 2010; Santos et al., 2007; Vinck et al., 1999). The individuals with blue eyes and blond hair showed a significantly larger TES at 2002 Hz compared to those with brown eyes and brown hair. NIHL is reportedly been more evident in individuals with less pigmentation (Henderson, Subramaniam, & Boettcher, 1993). The current study observed the same trend in the individuals with less pigmentation that showed a larger TES than individuals with more pigmentation in their eyes and hair. It is postulated that DPOAE measures may be more sensitive to inter-individual variability to noise overexposure, and small changes induced by noise than pure tone audiometry (Seixas et al., 2004).

Impact of hair and eye colour on the contralateral suppression of transient evoked otoacoustic emissions

It is suspected that CSOAE provides protection against acoustic stimulation (Bidelman et al., 2016; Otsuka et al., 2016; Patuzzi & Thompson, 1991; Wagner et al., 2005). The present study recorded a mean efferent suppression measured with CSOAE's ranging from 0.34 to 0.46 dB SPL in all participants. The most efferent suppression was observed in the lower frequency from 1001 to 2000 Hz rather than in the higher frequency region of 2828 to 4000 Hz. Numerous studies, including the present study, recorded the least amount of efferent suppression measured with CSOAE's at 4000 Hz and stronger efferent suppression in the mid frequency range (Bidelman et al., 2016; Otsuka et al., 2016; Wagner et al., 2005; Wagner & Heyd, 2011). In normal hearing individuals, the average amount of efferent suppression measured with CSOAE's reported ranges from 0.93 to 4 dB SPL (Giraud et al., 1995; Müller et al., 2010; Wagner et al., 2005). Individual variability is evident in the amount of enhancement and suppression of CSOAE's which can occur at any frequency (Giraud et al., 1995; Müller et al., 2010). The current study could not identify pigmentation of eye and hair as a factor which influences efferent suppression as measured by CSOAE's.

The relationship between contralateral suppression of transient evoked otoacoustic emissions, distortion product otoacoustic emissions, and pure tone audiometric thresholds after short-term noise exposure

No relationship could be determined between CSOAE's, TES, and TTS after short-term music exposure. Therefore, the efferent strength of the medial olivocochlear (MOC) as measured by CSOAE's was not able to predict susceptibility to NIHL in the current study. Previous studies investigated the relationship between the protective effect of the efferent system and TTS and TES after noise exposure (Keppler et al., 2014; Müller et al., 2010; Otsuka et al., 2016; Snihur & Hampson, 2011). The TES, however, is known to predict TTS and ultimately predicting susceptibility to NIHL. Both, DPOAE's and pure tone thresholds, indicated a shift after noise exposure in the noise susceptible frequency region of 2000 to 6000 Hz in the total participant sample.

DPOAE deemed more sensitive as a significant TES was measured at each frequency after noise exposure but no significant TTS was measured after noise exposure for the total participant sample. When stimulating the cochlea at low intensities the OHC provides feedback by its contraction and amplification of the vibratory traveling wave (Brownell, 1990). The TES at each frequency suggests that eliciting a response even at a low-level primary tone can detect OHC impairment. The differences in the measurements can be attributed to the inability of behavioural audiometry to identify early signs of a music-induced shift in audiometric results. The current study used CSOAE's in addition to DPOAE's and pure tone audiometry to predict susceptibility to NIHL. However, based on the results, it is recommended that DPOAE's be used to monitor the impact of music exposure.

The influence of gender and ear symmetry on contralateral suppression of transient evoked otoacoustic emissions, distortion product otoacoustic emissions, and pure tone audiometric thresholds

Gender and ear differences have been identified in numerous audiological measures such as pure tone audiometry, spontaneous OAE's and evoked OAE's (Bhagat & Davis, 2008; Boothalingam et al., 2018; Snihur & Hampson, 2011). Studies have shown highly significant differences in males' click-evoked OAE's amplitudes, especially in DPOAE amplitudes (Snihur & Hampson, 2011). Investigation regarding gender differences after noise exposure, have frequently reported better pure tone thresholds of 3 to 5 dB HL in females in the noise susceptible region (Boothalingam et al., 2018; Le Prell, Spankovich, Lobariñas, & Griffiths, 2013; Snihur & Hampson, 2011). The current study did not aim to investigate gender differences after noise exposure, although differences in efferent suppressions measured with CSOAE's were evident. No gender differences were recorded in DPOAE's and pure tone audiometry in the current study. There was however a difference between genders, female participants had statistically stronger efferent suppression in comparison to the male participants. However, it must be noted that the current participant sample did not comprise of an equal number of males and females (80% female and 20% males). The study sample may have influenced the conclusion regarding gender in the assessment of gender influence on the assessment battery.

The left ear has been reported to be more vulnerable to TTS and permanent hearing damage in comparison to right ear (Boothalingam & Lineton, 2012; Helleman & Dreschler, 2015; Sliwinska-Kowalska & Davis, 2012). Right ear advantage has been proposed to influence hearing sensitivity and auditory processing (Driscoll, Kei, & McPherson, 2002). Some researchers reported stronger efferent suppressions in the right ear due to less inhibition from the MOC system while other studies found no asymmetry in efferent suppressions (Otsuka et al., 2016; Snihur & Hampson, 2011). DPOAE's and efferent suppression as measured with CSOAE's were symmetrical, despite the larger TTS at 4000 Hz in the left ear after noise exposure.

4.2. Clinical implication

NIHL is becoming more prevalent in the modern society and is fully preventable hearing loss. Inter-induvial variability has been identified as a factor that influences susceptibility to NIHL. Every effort should be made to determine precise genetic factors influencing susceptibility to NIHL. The identification of factors influencing NIHL assists an audiologist to better prevent and treat the individuals susceptible to NIHL. Due to inconclusive results of previous research, the present study attempted to find a correlation between CSOAE's, TTS and TES in individuals with different eyes and hair colours. The present study implies that individuals with pigmentation may be more susceptible to NIHL after noise overexposure.

The current study utilized DPOAE's to objectively monitor differences between individuals with different hair and eye colours after short-term music exposure. DPOAE have frequently been used to monitor changes in cochlear functioning, especially after noise exposure (Cacace et al., 1996; Jin et al., 2005). DPOAE's was effectively used in the present study to identify a greater TES, therefore greater susceptibility to acoustical overstimulation, in blue eyes and blond hair. The current study provided supportive evidence that DPOAE's can by utilized to monitor subtle change of the OHC. Therefore, DPOAE's should be regarded as the monitoring tool of choice for noise exposure.

The clinical purpose of the study was to develop a predictive model which can be used to identify individuals more susceptible to NIHL, based on their hair and eye colour. The present study found statistical differences in TES and TTS between blue eyes and blond hair, and brown eyes and hair after short-term noise exposure. However, no significant differences in efferent suppression as measured by CSOAE's were recorded between blue eyes and blond hair, and brown eyes and hair. Therefore, CSOAE's were unable to predict which individuals were more susceptible to NIHL after short-term noise exposure.

The present study reported on numerous studies that recorded noise levels in clubs, shooting ranges and music concerts. Discotheque intensity levels at music concerts and nightclubs are recorded between 84 to 120 dBA (Beach, Williams, & Gilliver, 2013; Daniel, 2007; Schmuziger, Fostiropoulos, & Probst, 2006; Whitfield, 1998). These levels may have damaging effects on an individual's hearing. The clinical purpose of the study was to develop a predictive model which can be used to identify individuals that are more susceptible to NIHL. For example, results indicated that individuals with blue eyes and blond hair had a larger TES and TTS after one hour of music exposure at 90 dBA. Therefore, individuals with blue eyes and blond hair are more at risk to NIHL and should be made aware of the possible risk. To prevent permanent damage, individuals that more susceptible should be made aware and an individualized hearing conservation programme can be implemented. Recreational activities should implement hearing conservation programmes and promote hearing protecting.

4.3. Critical evaluation: Strengths and limitations of the current study

4.3.1. Strengths of the current study

- The present study investigated the correlation between hair and eye colour and CSOAE's, and DPOAE shifts after music exposure. There is limited research available regarding the utilization of CSOAE to predict susceptibility among different pigmentated individuals. Previous research that was conducted on the operative effect of pigmentation on NIHL was performed by using predominately pure tone audiometry.

The current study aimed to utilize an audiological tool that is less frequently used, namely CSOAE's.

- The use of music rather than broadband noise (BBN) with the modest intensity level of 90 dBA lends strength to the study by its ethical means of inducing a TTS that has the ability to recover rapidly. Music is more familiar and pleasurable in comparison to BBN that has been used in previous studies (Vinck et al., 1999). Music exposure provides the same effect on the OHC in comparison to the BBN exposure. Although, BBN is known to cause a greater TTS and TES, and therefore put individuals at greater risk for permanent damage. Music provides a more real-world context and safer mode of inducing a TTS and TES in young adults. The intensity and spectral variation of the music results in a less consistent stimulation of the OHC and consequently less impact on the OAE results (Vinck et al., 1999).
- The experimental protocol utilized pure tone audiometry which is the gold standard, OAE 's a less frequently used test such as CSOAE. The comprehensiveness of the protocol lends strength to the reliability to the experimental design of the current study.
- The use of OAE's after music exposure was beneficial due to the objectivity and the sensitivity it provides to monitor subtle changes in cochlear functioning. A strict OAE inclusion criteria with regards to presence of OAE's was used, with regards to amplitude, noise floor and SNR. The use of strict selection criteria lends strength to the internal validity of the current study which centred on efferent suppression as measured by CSOAE's.
- The strict selection criteria lend strength to the current study due to individuals limited cumulative noise exposure and no otologic risk factors. Normal hearing participants with no history of family hearing loss or otological diseases limited the possibility of confounding factors influencing TTS.
- The study controlled for extraneous variables by using a dose-controlled music exposure within a double walled soundproof booth on normal hearing young adults. The quasi-experimental nature of the study was selected in attempt to limit extraneous variables that may have otherwise influenced TTS and TES.

4.3.2. Limitations of the current study

- A possible limitation of the current study was the small sample size of ear (n=50 ears). The small sample size limits the generalization to the larger population. Future studies should aim to compile a larger study population.
- The groups were not matched according to age or gender. The study was compiled with an uneven number of females and males. A trend was observed where males presented with poorer efferent suppressions as measured by CSOAE's. A conclusion could not be drawn due to the uneven number of females and males in the present study. Individuals should be matched according to age and gender to compare effectively.
- The present study depends on subjective perception of daily noise exposure. Future studies should include a comprehensive inventory of noise exposure to determine individual daily noise dose. Although by doing so more confounding factors may be added, a more realistic real-world view of an individual's noise exposure may be obtained. Also, variables that influence inter-individual susceptibility may be identified in doing so.
- Larger TTS and TES have been recorded in studies with increased intensities, different types of noise and duration of exposure, namely Jin, Nam, and Sung (2005), Keppler et al. (2010), and Vinck et al. (1999). According to the equal energy rule, and for ethical reasons, increasing intensity, different types of noise and duration may have led to larger TTS, TES and efferent suppression as measured by CSOAE's but may have caused permanent hearing damage (Helleman & Dreschler, 2015). If participants were exposed to music for a longer duration a more forceful effect of noise at more frequencies would be visible, and a larger TTS.
- The current study quantified pigmentation based on hair and eye colour without consideration of the effect of ethnicity/race as well. Previous studies have investigated the effect of race/ethnicity on hearing loss by using OAE's and pure tone audiometry (Boothalingam et al., 2018; Helzner et al., 2005; Lin et al., 2012). The differences between ethnicities have been observed in human and animal studies, especially in the noise susceptible region of 4000 to 8000 Hz, regardless of gender (Barrenas, 1997; Helzner et al., 2005; Mujica-mota et al., 2015). Skin pigmentation should be included in future investigation of the effect of pigmentation on susceptibility to NIHL.

- The current study used hair and eye colour to infer levels of melanin in the inner ear and recognize that it is not a direct reflection of the amount of melanin in the inner ear. Although, previous studies based their conclusion of the correlation between pigmentation and NIHL on hair and eye colour and postulated that hair and eye colour to be an indirect measure of melanin in the inner ear (Mujica-mota et al., 2015).

4.4. Future studies

A large-scale longitudinal study is needed to determine if melanin influences susceptibility to NIHL. Individuals should be categorized into groups according to eye-, skin- and hair colour. To effectively identify factors that influence individual susceptibility to NIHL more variables must be identified and monitored over years of recreational and/or occupational noise exposure. Iris pigmentation has been mostly used to examine the effect of melanin in the inner ear and there have been findings that individuals with darker skin colour display better hearing thresholds (Boothalingam et al., 2018; Driscoll et al., 2009; Helzner et al., 2005; Lin et al., 2012). Future studies should investigate the link between race/ethnicity, hair and eye colour and its effect on the auditory system.

There is currently still no clear relationship between the protective effect of the MOC and TTS or permanent threshold shift (PTS). Numerous studies have postulated about the role of the MOC efferent system in slowing age-related hearing loss, inhibition of cochlear amplifier gain to aid listening in noise, and protection against acoustic overexposure (Bidelman et al., 2016; Canale et al., 2014; Fuente, 2015; Jacobson et al., 2003; Kaf & Danesh, 2013; Killan et al., 2017; Lichtenhan et al., 2016; Maison et al., 1998; McFadden, Henselma, & Zheng, 1999; Mertes & Leek, 2016; Otsuka et al., 2016; Patuzzi & Thompson, 1991; Smith et al., 2017; Stuart & Cobb, 2015; Wagner et al., 2005; Wagner & Heyd, 2011). Future research could aim to determine the relationship between the MOC and the suspected protective effect it provides.

The younger population is frequently exposed to high intensity of noise or music for a prolonged duration that may cause NIHL at a young age. Some individuals may have more resilient ears in comparison to others. The present study aimed to identify young adults that may be susceptible to hearing loss based on iris and hair pigmentation. Due to inter-individual differences, each young adult may be differently affected by noise exposure. Future research could aim to identify additional variables that may influence susceptibility to NIHL, especially in the younger population.

Jin et al. (2005) identified electrocochleography of being capable of providing more specific information and sensitivity in comparison to DPOAE for detecting noise-induced shifts. It is suggested that electrocochleography may also show alterations in cochlear functioning. These alternations will not only be limited to the OHC but give an indication of the auditory pathway's response to noise exposure. Electrocochleography has therefore, been utilized to monitor subtle changes in cochlear functioning after noise exposure. Future studies should investigate the effectiveness of utilizing electrocochleography to monitor changes in hearing sensitivity in occupational and recreational settings.

Gender differences in the auditory system has been acknowledged with the conclusion that females present with better hearing sensitivity (Boothalingam et al., 2018; Snihur & Hampson, 2011). The current study found significantly stronger efferent suppressions as measured by CSOAE's in females in comparison to males. It is suspected that the MOC systems plays a crucial role in providing protection against acoustic overstimulation, inhibition of the cochlear amplifier gain to aid listening in noise and slowing age-related hearing loss (Bidelman et al., 2016; Canale et al., 2014; Fuente, 2015; Jacobson et al., 2003; Kaf & Danesh, 2013; Killan et al., 2017; Stuart & Cobb, 2015). Therefore, future research should aim to investigate whether males present as an at-risk population due to their biological predisposition to NIHL.

The present study found that the left ear showed a larger TTS in comparison to the right ear. Previous research (Boothalingam et al., 2018; Driscoll et al., 2002; Snihur & Hampson, 2011) reported better audiometric results and OAE results in the right ear rather than the left. The underlying phenomenon influencing the difference in ear symmetry is not fully understood. Studies have suggested it can be attributed to handedness and the stronger contralateral pathways in the MOC pathways (Garber et al., 1982; Guinan, 2006; Hannula et al., 2012; Wagner & Heyd, 2011). The present study found similar results and suggests future research should investigate the underlying mechanism influencing the differences in ear symmetry.

4.5. Conclusion

No clear relationship was determined between CSOAE's of individuals with blue eyes and blond hair, and brown eyes and hair, and their TES after one hour of music exposure. There was a significant TES at 2000 Hz, and a significant TTS at 4000 Hz in the individuals with blue eyes and blond hair after short-term music exposure. CSOAE's was unable to predict the individuals that were more susceptible to NIHL after short-term music exposure. To effectively identify factors that influence individual susceptibility to NIHL more variables must be identified and monitored over a longer exposure duration.

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Appendices

Appendix A: Letter of ethical approval of the Faculty of Humanities

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Research Ethics Committee

8 March 2018

Dear M Klopper

Project:	**The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions**
Researcher:	**M Klopper**
Supervisor:	**Dr L Biagio de Jager and Prof BHME Vinck**
Department:	**Speech-Language Pathology and Audiology**
Reference Number:	**14006601 (GW20180114HS)**

Thank you for your response to the Committee's letter of 20 February 2018..

I am pleased to inform you that the above application was **approved** by the **Research Ethics Committee** on 7 March 2018. Data collection may therefore commence.

Please note that this approval is based on the assumption that the research will be carried out along the lines laid out in the proposal. Should the actual research depart significantly from the proposed research, it will be necessary to apply for a new research approval and ethical clearance.

We wish you success with the project.

Sincerely

Prof Maxi Schoeman
Deputy Dean: Postgraduate Studies and Ethics
Faculty of Humanities
UNIVERSITY OF PRETORIA
e-mail: tracey.andrew@up.ac.za

cc: Dr L Biagio de Jager and Prof BHME Vinck (Supervisors)
Dr J van der Linder (HoD)

Appendix B: Letter of approval of the Director of Student Affairs

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Department of Speech-Language Pathology and Audiology

December 2017

Attention: Director of Student Affairs

RE: PERMISSION TO CONDUCT A RESEARCH STUDY WITH STUDENT PARTICIPANTS FROM THE UNIVERSITY OF PRETORIA

I, Marike Klopper, am a final year audiology student at the Department of Speech-Language Pathology and Audiology at the University of Pretoria. I would like to request your permission to invite participants from the university student body to participate in a research project that I am undertaking as a requirement for my undergraduate degree.

I am conducting a research project entitled: **The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions.** This research project will be conducted with the main objective to determine the relationship between contralateral suppression of otoacoustic emissions in individuals with different hair and eye colour, and temporary emission shift after noise exposure. It is suspected that different hair and eye colours have an impact on our ears and the ears ability to protect itself against noise. There is a lack of research in this area of Audiology thus I wish to investigate correlation between hair and eye colour and the susceptibility to noise induced hearing loss.

Participant candidacy: Normal hearing individuals above the age of 18 to 30 (male and female) with good general health.

Design and procedure: Testing will take place at the Department of Speech-Language Pathology and Audiology, University of Pretoria. Candidacy will be determined first using diagnostic audiology to confirm normal hearing, followed by noise exposure for one hour long and afterwards a measurement of contralateral suppression will be measured. A hearing screening after the session is concluded will be conducted to ensure no harm was

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Fakulteit Geesteswetenskappe
Departement Spraakpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Thuthuduo ya Polelo-Molemi le Go Kwa

done.

Session task	Estimated Time (min)
Otoscopy and immittance measures	10
Pure tone threshold estimation (125- 8 kHz)	15
Speech detection	10
Contralateral suppression of transient evoked otoacoustic emissions and distortion product otoacoustic emissions	5
Noise exposure	60
Distortion product otoacoustic emissions	5
HearScreen™	5
Total	**110**

Ethical Considerations: The participant will only participate after they have given the consent. All information about the participant and information collected during this research will be kept confidential and only the researchers will have access to such information. Participants will be given a numeric code to ensure anonymity. This code will be used during data analysis and the code will only be known to the researcher and the supervisors. Confidentiality will be guaranteed. The data collected will be stored for 15 years for research purposes.

Risks and benefits: There are no risks of participating as the participant will not be harmed in anyway. There will be no direct benefits to the participants.

Should you have any queries, concerns or wish to obtain additional information regarding any aspect of this study, feel free to contact me at any point.

Thank you in advance for your time and cooperation.

Yours sincerely,

Faculty of Humanities
Fakulteit Geesteswetenskappe
Lefapha la Bomatho

Klopper

Researcher

Ms. Marike Klopper

Tel: 081 363 2585

Email: marike95@gmail.com

Supervisor

Dr. Leigh Biagio de Jager

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PERMISSION FOR THE USE OF INFORMATION OF STUDENTS FROM THE UNIVERSITY OF PRETORIA AT DEPARTMENT OF SPEECH-LANGUAGE PATHOLOGY AND AUDIOLOGY

I, NRM MADIBA give permission that the participants from the University of Pretoria may be contacted and used as participants for the research project titled: **The**

correlation between hair and eye colour on contralateral suppression of otoacoustic emissions - Pending ethical approval from the faculty.

Signature

13/12/2017

Date

Faculty of Humanities
Department of Speech-Language Pathology and Audiology
Fakulteit Geesteswetenskappe
Departement Spraak-Taalpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Puolokolotši ya Polelo-Maleme le Go Kwa

Appendix C: Letter of approval of the Head of Department of Speech-Language Pathology

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Department of Speech-Language Pathology and Audiology

March 2018

Attention: Head of Department of Speech-Language Pathology and Audiology

RE: PERMISSION TO CONDUCT A RESEARCH STUDY WITH STUDENT PARTICIPANTS FROM THE UNIVERSITY OF PRETORIA

I, Marike Klopper, am a final year audiology student at the Department of Speech-Language Pathology and Audiology at the University of Pretoria. I would like to request your permission to invite participants from the Department of Speech-Language Pathology and Audiology student body to participate in a research project that I am undertaking as a requirement for my undergraduate degree.

I am conducting a research project entitled: **The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions.** This research project will be conducted with the main objective to determine the relationship between contralateral suppression of otoacoustic emissions in individuals with different hair and eye colour, and temporary emission shift after noise exposure. It is suspected that different hair and eye colours have an impact on our ears and the ears ability to protect itself against noise. There is a lack of research in this area of Audiology thus I wish to investigate correlation between hair and eye colour and the susceptibility to noise induced hearing loss.

Participant candidacy: Normal hearing individuals above the age of 18 to 30 (male and female) with good general health.

Fakulteit Geesteswetenskappe
Departement Spraak-Taalpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Phatholotši ya Polelo-Maleme le Go kwa

Design and procedure: Testing will take place at the Department of Speech-Language Pathology and Audiology, University of Pretoria. Candidacy will be determined first using diagnostic audiology to confirm normal hearing, followed by noise exposure for one hour long and afterwards a measurement of contralateral suppression will be measured. A hearing screening after the session is concluded will be conducted to ensure no harm was done.

Session task	**Estimated Time (min)**
Otoscopy and immittance measures	10
Pure tone threshold estimation (125- 8 kHz)	15
Speech detection	10
Contralateral suppression of transient evoked otoacoustic emissions and distortion product otoacoustic emissions	5
Noise exposure	60
Distortion product otoacoustic emissions	5
HearScreen™	5
Total	**110**

Ethical Considerations: The participant will only participate after they have given the consent. All audiological information collected during this research will be kept confidential and only the researchers will have access to such information. Participants will only provide their age and indicate with yes or no if they have a family history of hearing loss and if they are smokers, no other personal information will be required from students. Students must be between the ages of 18-30 to participate in the study. They should not have a history of hearing loss in their family due to normal hearing sensitivity is required for the study. If a history of hearing loss in the family is present, they may present with a hearing loss as well and effect the results of the study. Non-smokers are selected due to smoking may cause damage to hearing as well. Participants will be given a numeric code to ensure anonymity. This code will

Design and procedure: Testing will take place at the Department of Speech-Language Pathology and Audiology, University of Pretoria. Candidacy will be determined first using diagnostic audiology to confirm normal hearing, followed by noise exposure for one hour long and afterwards a measurement of contralateral suppression will be measured. A hearing screening after the session is concluded will be conducted to ensure no harm was done.

Session task	**Estimated Time (min)**
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Noise exposure	60
Distortion product otoacoustic emissions	5
HearScreen™	5
Total	**110**

Ethical Considerations: The participant will only participate after they have given the consent. All audiological information collected during this research will be kept confidential and only the researchers will have access to such information. Participants will only provide their age and indicate with yes or no if they have a family history of hearing loss and if they are smokers, no other personal information will be required from students. Students must be between the ages of 18-30 to participate in the study. They should not have a history of hearing loss in their family due to normal hearing sensitivity is required for the study. If a history of hearing loss in the family is present, they may present with a hearing loss as well and effect the results of the study. Non-smokers are selected due to smoking may cause damage to hearing as well. Participants will be given a numeric code to ensure anonymity. This code will

PERMISSION TO USE STUDENTS FROM THE DEPARTMENT OF SPEECH-LANGUAGE PATHOLOGY AND AUDIOLOGY.

I, Dr. J. van der Linde give permission that the participants of the Department of Speech-Language Pathology and Audiology may be contacted and used as participants for the research project titled: The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions.

Signature

07 - 03 - 2018

Date

Faculty of Humanities
Department of Speech-Language Pathology and Audiology
Fakulteit Geesteswetenskappe
Departement Spraak-Taalpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Phatholotši ya Polelo-Maleme le Go kwa

Appendix D: Participant information letter

Dear Participant,

PARTICIPATION IN RESEARCH STUDY

Thank you for considering becoming a participant in this research study. Information regarding the study and the procedure during the study will follow in this detailed letter. Please read all the information carefully and complete the consent form that follows, if you choose to participate in the research.

Information regarding the research study:

The study aims to determine the relationship between the contralateral suppression of otoacoustic emissions in individuals with different hair and eye colour, after noise exposure. It is suspected that different hair and eye colours have an impact on our ears and the ears ability to protect itself against noise. There is a specific reflex in our brain is known for the ability to protect our ears against noise damage as well as slowing age related hearing loss. The study will use a test called otoacoustic emissions which provides information of our hair cells in our inner ears. The test is sensitive in measuring the functioning of the outer hair cells in our inner ear before any other test can determine a change in hearing. Our otoacoustic emissions reflects a part of our organ of hearing. There have been interesting findings in people that differ in eye and hair colour will exhibit differences in their responses from their inner ears. This study aims to determine which people will be more susceptible to noise induced hearing loss compared to others due to the colour of their hair and eyes using their otoacoustic emissions.

Participant candidacy and selection process:

For a participant to be eligible to participate in this research study the following is required: normal hearing sensitivity in adults that are between the ages of 18 and 30 years. Participants with no history of middle ear infection, family hearing loss, good general health or history of smoking. Participants must have the combination of blue eyes with blond hair, or brown eyes with brown hair to be suitable for participation. To determine if the participant is a candidate for participation, a brief interview followed

by an audiological assessment will be done. The interview will consist of questions about case history and activities that may influence the results of the study. Audiological test results must be normal for participants in this study.

The audiological assessment during the selection process will contain the following procedures:

- Examination of the ear canal and the eardrum.
- Pure tone audiometry: to determine hearing thresholds and that they are in normal range. This requires the participant to respond when a tone is presented to them in a sound proof booth
- Transient Evoked Otoacoustic Emissions (TEOAE) as well as Distortion Product Otoacoustic Emissions (DPOAE) will be conducted to measure the integrity of the outer hair cells. During OAE measurements a sound will be presented into the ear canal. OAE's is when a sound is presented into the ear canal and reflected is the response of the outer hair cells in the inner ear. Both tests are commonly used due to its ability to measure the functioning of the outer hair cells. The participant will not be required to do anything during this test.

Requirements from the participant:

- If you choose to participate in this research and selected based on the criteria, you will be required to take part in one session of audiological testing.

Session task	**Estimated Time (min)**
Otoscopy and immittance measures	10
Pure tone threshold estimation (125- 8 kHz)	15
Speech detection	10
Contralateral suppression of transient evoked otoacoustic emissions and distortion product otoacoustic emissions	5
Noise exposure	60
Distortion product otoacoustic emissions	5
Pure tone threshold estimation (125 – 8 kHz)	5
Total	**120**

Test venue:

The interview, audiological assessment and testing will take place at the Department of Speech-Language Pathology and Audiology at the University of Pretoria.

Possible risks and benefits associated with this study:

This study will not but harmful in any way and there are no risk factors that participants will be exposed to. Participants will not receive direct benefit or receive financial reinforcement for participating in the study but may find the results of the study insightful to their personal knowledge.

The information obtained from this study could help to identify risk factors in the future for noise susceptibility and improve hearing conservation programs.

Confidentiality and anonymity:

Confidentiality of participants personal and audiological results will be ensured. The researcher and research supervisors will be the only persons that can access this data. Participants will be given a code number to ensure anonymity. Information will be kept confidential. The researchers only will know your code number. The results obtained will be stored at the Department of Speech-Language Pathology and Audiology for a minimum period of 15 years as a policy at the University of Pretoria.

Sharing of results:

The knowledge obtained from this research will be reported in the form of a scientific article. This dissertation will be available to professionals in the field of Audiology. If you would like a summary of the findings can be sent to you when the research has been completed.

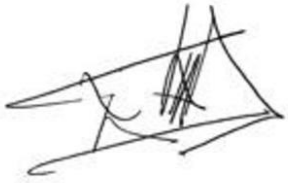
Refusal or withdrawal from the research:

A participant has the right to refuse or withdraw their participation to this study at any time. Your participation is completely voluntary.

Contact details:

If you have any concerns regarding aspects of the research study, please contact us.

Researcher
Ms. Marike Klopper
Tel: 081 363 2585
Email: marike95@gmail.com



Supervisor
Prof Bart Vinck
Tel: +27 12 420 2355
Email: bart.vinck@up.ac.za

Supervisor
Dr. Leigh Biagio de Jager
Tel: +27 12 420 6774
Email: leigh.biagio@up.ac.za

Appendix E: Informed consent form

INFORMED CONSENT TO THE RESEARCH STUDY: The correlation of hair and eye colour on contralateral suppression of otoacoustic emissions.

Please complete the following:

I, _____, hereby confirm that I have read and understood the above stated information on this research study. I have also had the opportunity to ask any questions I had about the study.

I hereby consent to participate in this study. I understand that I do so voluntarily and that I may withdraw from this study at any time. I also understand that the data will be used for research purposes in accordance with the information provided in the information letter.

_____

Signature of participant

_____

Date

_____

Contact number(s)

Appendix F: Plagiarism declaration

DECLARATION OF ORIGINALITY UNIVERSITY OF PRETORIA

The Department of Speech-Language Pathology and Audiology places great emphasis upon integrity and ethical conduct in the preparation of all written work submitted for academic evaluation.

While academic staff teach you about referencing techniques and how to avoid plagiarism, you too have a responsibility in this regard. If you are at any stage uncertain as to what is required, you should speak to your lecturer before any written work is submitted.

You are guilty of plagiarism if you copy something from another author's work (e.g. a book, an article or a website) without acknowledging the source and pass it off as your own. In effect, you are stealing something that belongs to someone else. This is not only the case when you copy work word-for-word (verbatim), but also when you submit someone else's work in a slightly altered form (paraphrase) or use a line of argument without acknowledging it. You are not allowed to use work previously produced by another student. You are also not allowed to let anybody copy your work with the intention of passing if off as his/her work.

Students who commit plagiarism will not be given any credit for plagiarised work. The matter may also be referred to the Disciplinary Committee (Students) for a ruling. Plagiarism is regarded as a serious contravention of the University's rules and can lead to expulsion from the University.

The declaration which follows must accompany all written work submitted while you are a student of the Department of Speech-Language Pathology and Audiology. No written work will be accepted unless the declaration has been completed and attached.

Full names of student: Marike Klopper

Student number: 14006601

Topic of work: The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions.

Declaration

1. I understand what plagiarism is and am aware of the University's policy in this regard. 2. I declare that this thesis (e.g. essay, report, project, assignment, dissertation, thesis, etc) is my own original work. Where other people's work has been used (either from a printed source, Internet or any other source), this has been properly acknowledged and referenced in accordance with departmental requirements.

3. I have not used work previously produced by another student or any other person to hand in as my own.

4. I have not allowed, and will not allow, anyone, to copy my work with the intention of passing it off as his or her own work.

SIGNATURE

Date: 15/01/2018

Appendix G: Declaration of data storage

Principal Investigator's Declaration for the storage of research data and/or documents

I, the Principal Investigator(s), Marike Klopper of the following trial/study titled the correlation between hair and eye colour on contralateral suppression of otoacoustic emissions will be storing all the research data and/or documents referring to the above-mentioned trial/study at the following non-residential address:

Department of Speech-Language Pathology and Audiology,

University of Pretoria

Pretoria

South Africa

I understand that the storage for the abovementioned data and/or documents must be maintained for a minimum of 15 years from the end of this trial/study.

START DATE OF TRIAL/STUDY: 01/01/2018

END DATE OF TRIAL/STUDY: 01/01/ 2019

SPECIFIC PERIOD OF DATA STORAGE AMOUNTING TO NO LESS THAN 15 YEARS:

02/01/2019 until 02/01/2034

Name: Marike Klopper (14006601)

Signature  **Date 15/02/2018**

Appendix H: Participant referral letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Department of Speech-Language Pathology and Audiology

Research Participant Referral Letter

To: _____

Date: dd / mm / yyyy

DOB: dd / mm / yyyy

_____ participated in a research study called: **The correlation of hair and eye colour on contralateral suppression of otoacoustic emissions,** on the _____ at the Department of Speech-Language Pathology and Audiology at the University of Pretoria.

The test battery included:

- Otoscopy
- Immittance measurements
- Diagnostic test battery
- Otoacoustic emissions

Reason for referral:

_____

_____

_____

_____

Fakulteit Geesteswetenskappe
Departement Spraak-Taalpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Phatholotši ya Polelo-Maleme le Go kwa

Thank you for your participation in the research study. Should you require further information kindly contact the researcher, Marike Klopper at 081 363 2585.

Kind regards,

Researcher
Ms. Marike Klopper
Tel: 081 363 2585
Email: marike95@gmail.com

Appendix I: Case history form

Case history form

Please provide your age and indicate the following by marking the questions below with an 'X':

Age: _____

Any history of middle ear infections, surgery to the ears or family history of hearing loss:

Yes	No

Hair colour and eye colour:

Brown hair and brown eyes	Blond hair and blue eyes

If you have smoked for longer than 5 years:

Yes	No

Appendix J: Participant pass letter

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Faculty of Humanities
Department of Speech-Language Pathology and Audiology

Research Participant Pass Letter

To: _____

Date: dd / mm / yyyy

DOB: dd / mm / yyyy

_____ participated in a research study called: **The correlation of hair and eye colour on contralateral suppression of otoacoustic emissions,** on the dd / mm / yyyy at the Department of Speech-Language Pathology and Audiology at the University of Pretoria.

The test battery included:

- Otoscopy
- Immittance measurements
- Diagnostic test battery
- Otoacoustic emissions

According to the hearing test results, you present with normal hearing sensitivity. It is recommended that you have your hearing evaluated annually. Thank you for your participation in the research study. Should you require further information kindly contact the researcher, Marike Klopper at 081 363 2585.

Fakulteit Geesteswetenskappe
Departement Spraak-Taalpatologie en Oudiologie
Lefapha la Bomotho
Kgoro ya Phatholotši ya Polelo-Maleme le Go kwa

Kind regards,

Researcher

Ms. Marike Klopper

Tel: 081 363 2585

Email: marike95@gmail.com

Appendix K: Data collection sheet

THE CORRELATION BETWEEN HAIR AND EYE COLOUR ON CONTRALATERAL SUPPRESSION OF OTOACOUSTIC EMISSIONS

Date of visit:
Randomized participant number:
Age:
Gender
Male ☐ **Female** ☐

RESULTS

1. Otoscopy

Left ear: _____
Right ear: _____

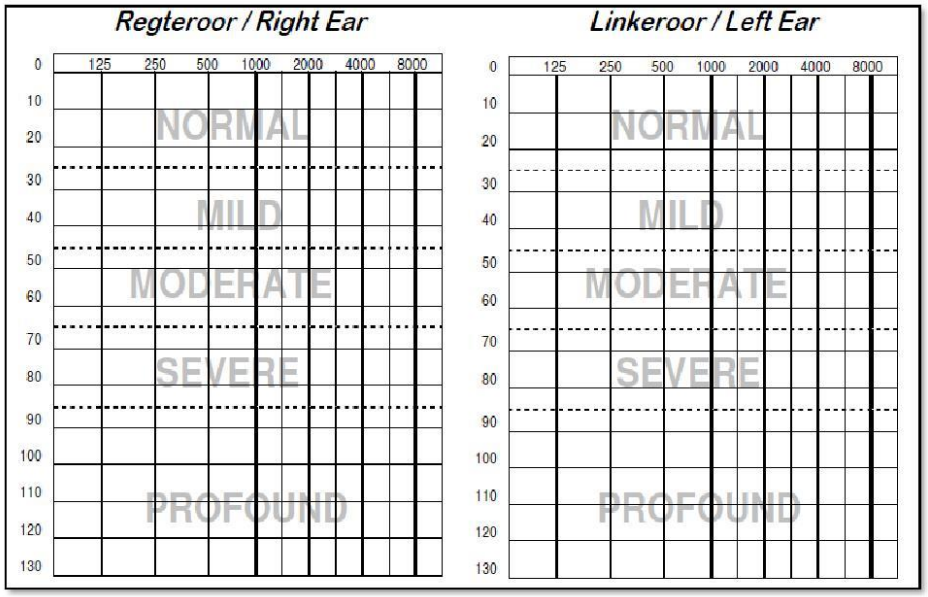
2. Acoustic Immittance Measurements

	Left ear	Right ear
Tympanogram type		
Ear canal volume		
Static compliance		
Ear canal pressure		

Acoustic Reflex Measurements:

	Left ear		Right ear	
	Ipsilateral	Contralateral	Ipsilateral	Contralateral
500Hz				
1000Hz				
2000Hz				
4000Hz				

3. Pure Tone Audiometry



4. Speech Detection

	Left ear	Right ear
Speech detection		

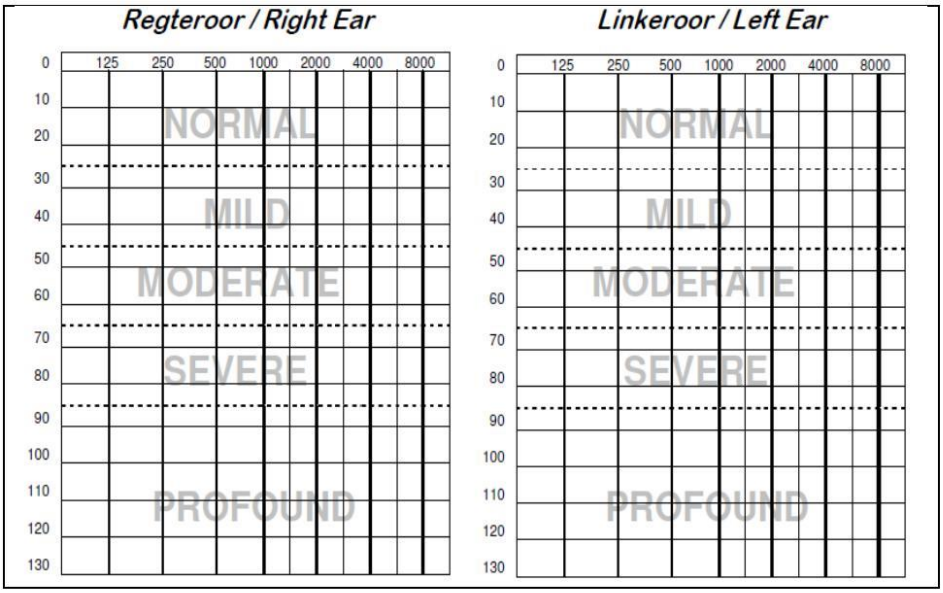
5. Contralateral Suppressions of Transient Evoked Otoacoustic Emissions and Distortion Product Otoacoustic Emissions.

Results will be exported from equipment database management to a computer and printed.

6. Distortion Product Otoacoustic Emissions

Results will be printed from computer with software to perform test.

7. Pure tone audiometry



Appendix L: Table of songs in music sample

Table 11: Songs in the music sample (Van Niekerk, 2016)

Track number	Artist	Song title	Track duration (min)
1	American Authors	Best day of my life	03:18
2	Armin van Buuren feat. Mr. Probz	Another you	03:13
3	George Ezra	Budapest	03:26
4	Lorde	Team	03:20
5	OMI Felix Jaehn Remix radio edit	Cheerleader	03:04
6	Years & Years	Shine	04:13
7	Pharrell Williams	Happy	03:56
8	Bastille	Pompeii	03:32
9	One Republic	Counting Stars	04:43
10	Aloe Blacc	The Man	04:17
11	Ellie Goulding	Lights	03:48
12	Jason Mraz	I'm yours	04:23
13	Coldplay	A sky full of stars	04:30
14	Matthew Mole	Take yours I'll take mine	03:42
15	Nico & Vinc	Am I wrong	04:09
16	Passenger	Let her go	04:14
Total			**61:48**

(min: minutes)

Appendix M: The submission letter from the journal of European Archives of Oto-Rhino-Laryngology

European Archives of Oto-Rhino-Laryngology

The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions

--Manuscript Draft--

Manuscript Number:	
Full Title:	The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions
Article Type:	Miscellaneous (Original Article)
Keywords:	Contralateral suppressions; noise-induced hearing loss; melanin; music exposure; otoacoustic emissions.
Corresponding Author:	Marike Klopper, B. Communication Pathology University of Pretoria Pretoria, Gauteng SOUTH AFRICA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	University of Pretoria
Corresponding Author's Secondary Institution:	
First Author:	Marike Klopper, B. Communication Pathology
First Author Secondary Information:	
Order of Authors:	Marike Klopper, B. Communication Pathology
	Leigh Biagio de Jager
	Bart HME Vinck
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Purpose: The study aimed to determine the relationship between the contralateral suppression of otoacoustic emissions (CSOAE) in individuals with brown eyes and hair, and blue eyes and blond hair, and temporary emission shift (TES) after short-term noise exposure. Method: The hearing sensitivity of young adults were determined by using pure tone audiometry followed by CSOAE's and distortion product otoacoustic emissions (DPOAE) before listening to music for one hour individually. Pure tone audiometry and DPOAE's were repeated after music exposure to determine the amount of TES and temporary threshold shift (TTS). Results: No statistically significant difference between efferent suppression was measured by CSOAE's between the participant groups. A larger TTS at 4000 Hz and TES at 2000 Hz was evident in the blue eyes and blond hair group after short-term music exposure. Conclusion: CSOAE's were therefore unable to predict which group of individuals were more susceptible to NIHL after short-term noise exposure.

The correlation between hair and eye colour on contralateral suppression of otoacoustic emissions

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Funding

None

Compliance with ethical standards

Conflict of interest

All authors declare that there are no conflicts of interest

Ethical approval

All procedures performed in the present study were in accordance with the World Medical Association Declaration of Helsinki 1964. Ethical approval was obtained from the Faculty of Humanities, University of Pretoria.

Informed consent

Written and verbal consent was obtained from participant before commencement of the current study.

Introduction

Noise induced hearing loss (NIHL) is an irreversible hearing impairment due to a single or repeated exposure to loud sounds in the higher frequency region from 2 kHz to 6 kHz. NIHL is becoming more prevalent in the modern society and is said to affect 16% of adults worldwide [1]. The impact of hazardous noise exposure is known to cause a temporary hearing deterioration or permanent hearing damage to the sensory cells of the cochlea [2]–[5]. The severity of NIHL increases as the duration and intensity level increases [5], [6]. The increased occurrence of NIHL due to occupational and recreational exposure led to more social an public health complications in the society [6]. Therefore, NIHL is eventually affecting the progression of age-related hearing loss due to the early damage to the cochlea. It is estimated by the World Health Organisation (WHO) (2018) that 1.1 billion young adults between the ages of 12 to 35 years are at risk for developing hearing loss due to recreational noise exposure. Young adults' main source of noise exposure can be linked to discotheque noise [5]. Recreational activities such as music concerts, nightclubs, MP3 players, gyms, shooting, and more hobbies may have a potentially damaging effect on our hearing sensitivity [1]. Discotheque intensity levels at concerts and nightclubs have been recorded between 84 to 120 dBA [8], [9]. The WHO established guidelines in which noise levels at entertainment events may not exceed 100 dBA if individuals are exposed for a maximum of four hours [10]. It is important to identify factors influencing an individual's susceptibility with the increase in prevalence of NIHL. There are multiple possible factors that may influence an individual's susceptibility to NIHL and their recovery following noise exposure. These possible factors include environmental factors or genetics [1], [2], [8]. Melanin is primarily influenced by genetics that has been recognized to be present in the inner ear. The amount of pigmentation in the inner ear has been related to general pigmentation and eye colour [11]. The stria vascularis contains melanin producing cells that play a crucial role in the production of endocochlear potentials and provides an otoprotective effect. It is suggested that hearing loss in individuals with auditory-pigmentary syndromes, namely Waardenburg syndrome and Tietz syndrome may be influenced by the lack of melanin [12]. Waardenburg syndrome and Tietz syndrome is characterized by hypopigmentation and hearing loss. The susceptibility to NIHL can be attributed to inter-individual variability which can influence the tonic activity of the auditory pathways due to melanin levels [2], [13]. The underlying mechanism of pigmentation influencing the auditory system is still unclear and cannot fully understood. Numerous studies have referred to work of Bonaccorsi (1965) which suggest that brown-eyed individuals had more melanin in their temporal bones in comparison to blue-eyed individuals [14]–[16]. Thus, studies were postulated that the brown-eyed population were less susceptible to NIHL.

Melanin is postulated to play a protective role in the auditory system and against noise trauma [11], [16]–[18]. Melanin is known to be involved with the calcium ion homeostasis in the cochlea [18]. Calcium homeostasis is crucial for the transduction of sound. Endocochlear electrical potential is dependent upon high calcium concentrations which are required for compound action potential leading to excitatory postsynaptic potential in the auditory nerve [18]. The amount of endocochlear melanin is said to regulate the release of cochlear calcium upon noise exposure to protect the auditory system [16]. It is postulated that melanin levels in the inner ear may modulate the auditory-neural transduction process due to the role of calcium on the cochlear hair cell functioning [12], [16]. Damage to the hair cells after prolonged noise exposure can occur in the absence of melanin due to higher levels of calcium in the inner ear without modulation [16]. Lower levels of melanin can influence the efficient transduction to inner hair cells and auditory nerve. Individuals with darker skin possess higher melanin levels in the cochlea compared to individuals with lighter skin, which in turn influences hearing sensitivity [18].

Numerous research [14]–[16] have focused on the association between eye colour and susceptibility to NIHL. Da Costa et al. (2008) and Mujica-mota et al. (2015) reported that light-eyed people had a greater permanent hearing loss following noise exposure in comparison to brown-eyed individuals who exhibited with better hearing thresholds after prolonged noise exposure. Thus, noise exposure has a more forceful effect on individuals with lighter pigmented iris colour. Hannula et al. (2012) findings contradicted the previous studies with results indicating that participants with a combination of dark hair and eyes were not more protected against NIHL. Their conclusion stated that individuals with brown eyes were more susceptible to hearing loss. Driscoll et al. (2009) study found a correlation between otoacoustic emissions (OAE) and different skin pigmentation. Hood et al. (1976) reported a relationship between the amount of melanin and susceptibility to the temporary threshold shifts. Increased auditory thresholds after noise exposure that recover gradually are known as a temporary threshold shift (TTS) [19]. It is suggested that metabolic overstimulation after noise exposure may be the cause of TTS. A difference between individuals in TTS can be attributed to individual susceptibility, the duration of noise exposure as well as the intensity that the individual is exposed to [19]. Noise exposure reduces the motility of the outer hair cells (OHC) influencing hearing sensitivity [9]. A direct measure of the cochlear OHC can be provided by OAE's. OAE's is an objective measurement to record changes in amplitudes, provide insight of the cochlea's pre-neural and biomechanical aspects of acoustic information which can be influenced by melanin [20]. Damage to OHC due to overexposure to noise may lead to a reduction in OAE amplitudes [21]. A response

level shift after noise exposure can be observed by utilizing OAE's. To examine the relationship between different levels of melanin and OAE's, Driscoll et al. (2009) measured more spontaneous OAE's in darker pigmentated individuals, for example, African Americans in comparison to Caucasians and Asians. Their results supported the hypothesis that the functioning of the OHC may be modulated by melanin as estimated by skin colour [18]. Due to the effectiveness, objective and non-invasive manner of OAE's, it may be the best method of evaluating the correlation between eye and hair colour and cochlear functioning [19], [22].

The response mechanism of the auditory system can be measured with contralateral suppression of OAE (CSOAE) [22]. The medial olivocochlear (MOC) bundle is known as the efferent branch of the auditory feedback system which originates in the brainstem and terminates predominantly in the OHC's of the opposite cochlea [23], [24]. The MOC reflex is the suppression effect in response to acoustic stimulation and serves as a cochlear defensive mechanism from acoustic damage [4]. The MOC reflex measurement is postulated to be valuable for screening an individual's susceptibility to acoustic trauma due to weakened MOC effect for those who have a preferential susceptibility to NIHL [25], [26]. The MOC efferent system has assumed roles by inhibition of cochlear amplifier gain such as aiding listening in noise, slowing age-related hearing loss and protection against acoustic overexposure [2], [4], [13], [23]. The strength of the MOC reflex is considered a be able to predict threshold shifts [4]. The MOC reflex is activated by acoustic stimulation and induces an inhibitory effect on OHC motility [4], [23]. The protection provided by the MOC reflex can be attributed to the reduction in the interruptions of electro-mechanical transduction of the OHC [13]. The reduction in amplitude with a contralateral masker was expected as small as 1 to 4 dB SPL reduction in amplitude [26]. A noticeable shift can be measured in the OAE's before fluctuations in behavioural pure tone thresholds may be identified [27]. A temporary emissions shift (TES) may provide a better indication of the effect of noise exposure on the cochlea than other audiometric results [4]. An emission shift after noise exposure is expected to occur at 2000 to 6000 Hz [19].

Previous studies have investigated the correlation between the amount of melanin based on eye and hair colour and the effect that it has on OHC's or amount of melanin in the temporal bones. No studies have investigated the relationship between eye and hair colour and CSOAE's after short-term noise exposure. CSOAE's can provide information about the protective function of the efferent system while OAE's provides information on whether the protective reflex maintains over time with acoustic stimulation. Numerous studies stated that an earlier indication of cochlear damage is provided by OAE's in comparison to behavioural pure tone threshold audiometry [9], [27]. The study aimed to determine the relationship between the CSOAE's in individuals with different hair and eye colour, and TES after short-term noise exposure.

Method

Participants

Twenty-five normal hearing adults, between the ages of 18 and 28 years were selected for the current study. All participants volunteered to participate in the study, which was approved by an institutional review board. The study consisted of twenty females and five males (Mean age: 21.64 years; SD 1.80). Participants were non-smokers, should have no history of ear diseases and in generally good health. Additional inclusion criteria were defined. A normal bilateral otoscopic examination, Jerger type A tympanograms measured with a Y-226 Hz probe tone utilising a GSI Tympstar Middle Ear Analyzer (Grason-Stadler, Eden Prairie, Minnesota). Jerger Type A tympanograms were defined as a static compliance of 0.3 to 1.75 mmho and a peak pressure of +100 to -100 daPa [28]. Normal ipsilateral and contralateral acoustic reflexes elicited at 85 to 100 dB SPL at frequencies 0.5, 1, 2 and 4 kHz. Furthermore, pure tone thresholds were obtained using the modified Hughson-Westlake procedure [29] on a GSI 61 clinical audiometer with supra-aural TDH-39 headphones (Grason-Stadler, Eden Prairie, Minnesota). Participants were selected based on normal behavioural pure tone air conduction thresholds ≤15 dB HL at octave intervals from 125 to 8000 Hz and including half octave frequencies of 3000 and 6000 Hz were included. Speech detection threshold must be within normal limits of the pure tone averages (PTA) of 0.25 to 2 kHz. All testing was conducted in a double-walled soundproof booth. Participants were grouped in two categories: group A consisted of individuals with brown eyes and hair, and group B was individuals with blue eyes and blond hair. In the study, 47 % of the participants had brown eyes and brown hair and 53 % of the subjects had blue eyes and blond hair.

Baseline testing

Baseline testing of pure tone audiometry, CSOAE and distortion product otoacoustic emissions (DPOAE) precede a one hour by music exposure session at 90 dBA. After the exposure, DPOAE measurements and pure tone audiometry were repeated. All testing was conducted in a double-walled soundproof booth.

Otoacoustic emissions

An Otodynamics DP Echoport ILO V6 was used for both CSOAE and DPOAE measurements. Probe calibration was performed at the beginning of each session using a 1 cm^3 calibration cavity. For CSOAE measurements, a linear mode of stimulation was used with a rate of 50 clicks/sec using an 80μs rectangular electrical pulse. Clicks were evoked with an intensity of 65 dB SPL with and without masker in the contralateral ear. The contralateral stimulus consisted of a continuous broadband noise presented at a stimulus level of 60 dB SPL to elicit efferent activity [30]. The noise rejection level was set at 49.5 dB SPL [31]. To evaluate the level of suppression, transient evoked OAE (TEOAE) responses without a broadband noise were measured followed by a measurement with contralateral broadband noise stimulation. The absolute TEOAE suppression was determined by subtracting the amplitude with contralateral stimulation from the amplitude with contralateral stimulation in each ear specifically [32]. The obtained TEOAE response levels and noise amplitudes were analysed in half-octave frequency bands centred at 1, 1.4, 2, 2.8 and 4 kHz. To determine if whether a TEOAE response was present, the following factors relating to the recording parameters were taken into consideration [25], [31]. Measurements were present when a stimulus stability of 90% and a reproducibility of 75% were present. Signal to noise ratio (SNR) must have been ≥ 6 dB [25]. Following CSOAE measurements, ipsilateral $2f_1$-f_2 DPOAE's were evoked by stimulation generation of two primary tones (f1 and f2) with an f2/f1 frequency ratio of 1.22 and f2 ranging from 1001 to 7996 Hz. The stimulus tone level combination L1/L2 was set to 65/55 dB SPL, to ensure that the optimal SNR was achieved [33]. The noise rejection level was set at 49.5 dB SPL [21]. The obtained DPOAE responses were converted and reported into half-octave frequency with the center frequencies at 1, 2, 3, 4, 6, 7.96 kHz. DPOAE results were deemed present if four or more frequencies had a DP amplitude exceeded the noise floor by 6 dB, therefore a SNR of ≥ 6 dB SPL [34].

Music exposure

Following the baseline measurements, each participant was exposed to continuous discotheque music set at an intensity of 90 dBA for one hour in a double-walled soundproof booth. A sound level meter (RION NA-42) was placed at ear level of the participants to monitor noise in the room. The noise exposure LAeq dBA level over the complete duration of the music sample was measured at 90 dBA. The music was presented by using an ASUS K541U laptop that was connected to the GSI 61 Clinical Audiometer (Grason-Stadler, Eden Prairie, Minnesota) to ensure the intensity was kept consistently at 90 dBA. The music was transduced through free-field via the audiometer's two GSI speakers with the participants seated one meter from the two speakers mounted in the front corners of the booth.

Post-exposure measurements

Post-exposure measurements commenced within five minutes after the end of music exposure. Ipsilateral DPOAE measurements and pure tone audiometry were repeated after the music exposure, which served as the post-exposure measurements. Post-exposure DPOAE responses and pure tone thresholds were subtracted from the baseline data to calculate the difference between measurements. The difference between the pre- and post of DPOAE amplitudes constitutes the TES and for pre- and post-pure tone thresholds, the TTS.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) version 25 was used for the statistical analysis of all data. Amplitudes, SNR and audiometric results were described using descriptive statistics to determine the mean values and standard deviations. The Shapiro Wilk test was used to evaluate normality of distribution. If the results were normally distributed, a paired sample t-test was used to compare differences in the mean values between DPOAE and CSOAE response level, and SNR as well as audiometric shift after music exposure of the two groups. One-way ANOVA was used to analyse the influence of two categorical independent variables on one dependent variable, namely the DPOAE response level between the two groups [35]. If the results were not normally distributed, the data was analysed using the two-tailed Wilcoxon Sign Rank test and Friedman's test of analysis of variance. Wilcoxon Sign Rank test was used to compare repeated measurement in a single sample,

DPOAE and pure tone results before and after music exposure. Friedman's test of analysis of variance was used to determine if there was a statistically significant difference between the distribution of three or more related groups of CSOAE frequencies. All results were categorized according to individual related factors namely gender (females vs males), ear (left vs right) and hair and eye colour (brown eyes and hair vs blue eyes and blond hair). In all analyses, a 95% level of significance ($p<0.05$) was used.

Results

Impact of hair and eye colour on the contralateral suppression of transient evoked otoacoustic emissions

Table 1 displays the mean absolute TEOAE amplitude with noise and amount of suppression per test frequency for participants with brown eyes and hair, and for participants with blue eyes and blond hair.

Insert Table 1

The mean absolute efferent suppression decreases from the mid frequencies towards the higher frequency region in all participants. The two groups exhibited the same degree of efferent suppression with similar mean suppression at each frequency. The mean efferent suppression decreased in the higher frequencies from 2.8 kHz in comparison to the lower frequency region. No statistically significant difference in suppression could be measured between the two groups ($z=-0.30$-1.60; $p>0.05$). However, a significant difference was observed between the degree of suppression between the individual test frequencies.

A Friedman's analysis of variance (ANOVA) with Bonferonni correction of the difference suppression values at different test frequencies in the total participants group indicated a statistically less suppression for 4000 Hz compared to 2828 Hz ($p<0.03$) and highly significant difference between the following frequencies: 4000 and 2000 Hz ($p<0.001$); 4000 and 1001 Hz ($p<0.001$); 4000 and 1414 Hz ($p<0.001$); 2828 and 2000 Hz ($p<0.001$); 2828 and 1001 Hz ($p<0.001$) and 2000 and 1001 Hz ($p<0.001$). The other frequencies did not exhibit a statistical significance ($p>0.05$) at 2000 and 1001 Hz, 2000 and 1414 Hz and 1001 and 1414 Hz.

The mean efferent suppression was symmetrical between the left and right ears did not indicate a statistical significance ($z=-0.22$-0.66; $p>0.05$).

Differential impact of short-term music exposure n pure tone audiometric thresholds

Table 2 provides the baseline measurements in pure tone thresholds, post-exposure pure tone thresholds, and the TTS in the brown eyes and brown hair participants, and blue eyes and blond hair participants.

Insert Table 2

The mean PTA before and after noise exposure were the same in participants with brown eyes and hair, and participants with blue eyes and blond hair, namely 1.00 dB HL. The participants with blue eyes and blond hair had a larger mean pure tone threshold in the higher frequencies from 2000 to 8000 Hz after the music exposure in comparison to participants with brown eyes and hair. The blue eyes with blond hair group displayed a significant larger TTS from baseline measurement to post-exposure at 4 kHz ($z=-2.17$; $p<0.05$). The brown eyes with brown hair group indicated a mean TTS of 0.83 dB HL which is smaller than the participants with blue eyes with blond hair group with a mean TTS of 3.00 dB HL. The mean TTS at all other frequencies between the two groups exhibited no statistically significant differences ($z=-0.07$-1.87; $p>0.05$).

The mean TTS at 4 kHz was much larger in the left ear than in the right ear but was not statistically significant ($z=-1.46$; $p>0.05$). The mean TTS did not indicate a significant difference between the left and right ear at frequencies 125 to 8000 Hz ($z=-0.20$-1.75; >0.05).

Differential impact of short-term noise exposure on distortion product otoacoustic emissions

Table 3 displays the mean baseline SNR measurement, post-exposure SNR measurement and reduction in SNR at each frequency in all the participants.

Insert Table 2

The mean DPOAE SNR reduction indicated a highly significant difference (t=3.73-6.93; p<0.001) at each frequency. The mean TES also indicated a highly significant difference at each frequency in all participants (t=3.46-7.08; p<0.001). The difference in the mean TES is shown in Figure 1 between participants with brown eyes and hair, and participants with blue eyes and blond hair from 1001 to 7996 Hz *(* indicative of significance)*.

Insert Figure 1

The Wilcoxon signed rank test indicated a statistically significant difference in TES at 2 kHz between the two groups (z=-1.05; p<0.05) with a mean shift of 2.69 dB SPL. At the other frequencies tested the TES from baseline to post-exposure did not indicate statistically significant difference (z=-0.30-2.41; p>0.05) between the participants with brown eyes and hair, and participants with blue eyes and blond hair. A significant difference between groups was also displayed for the SNR of the DPOAE's at 2 kHz (F=4.77; p<0.05).

The baseline measurement and post-exposure measurement of TES indicated symmetrical shifts between the left and right ear. For each of the frequencies, there was no statistically significant difference between the left and right ears (z=-0.14-1.55; p>0.05).

Discussion

The current study explored the relationship between CSOAE's of individuals with blue eyes and blond hair, and brown eyes and hair, and their TES after one hour of music exposure at 90 dBA. Statistically significant TTS and TES were recorded after music exposure in individuals with blue eyes and blond hair, in comparison to individuals with brown eyes and hair. No significant difference in efferent suppression was observed between participants with brown eyes and hair, and participants with blue eyes and blond hair.

Differential impact of short-term music exposure on pure tone audiometric thresholds

The present study found a mean TTS ranging from 0 to 0.83 dB HL in the brown eyes and hair, and 0 to 3.08 dB HL in the blue eyes and blond hair. Pure tone audiometry has been widely used to evaluate the correlation between iris pigmentation and NIHL in previous studies [14], [17]. The current study found that participants with blue eyes and blond hair showed a statistically larger TTS, by 2.25 dB HL, at 4 kHz after music exposure than the participants with brown eyes and hair. The reduction in pure tone threshold at 4 kHz is typical of NIHL and was evident in the blue eyes and blond hair group after music exposure. The current study did not find significant differences between groups at other test frequencies other than 4000 Hz. Previous studies found significant TTS at 2000 to 6000 Hz in comparison to the current study, which only recorded a significant TTS at 4000 Hz. The results of the current study did not suggest that music exposure of one hour at 90 dBA causes a significant TTS at each frequency within the noise sensitive region of 2000 to 6000 Hz. Bhagat and Davis (2008) found that in noise exposed individuals that the audiometric results were not indicative of noise trauma after 30 minutes of noise exposure and were rather revealed in OAE results. Significantly better PTA have been recorded in dark-eyed individuals in comparison to light eyed individuals after noise exposure [14], [16], [36]. Dark eyed individuals have been reported to have from 1 to 5 dB HL better hearing thresholds in the noise susceptible frequency region [14]. Previous studies based their conclusion on individuals with long-term noise exposure, ranging from two to 42 years [14], [16]. Although the current study used short-term music exposure with participants with normal hearing, the same trend was observed which the individuals with dark eyes and hair had better hearing thresholds after short-term music exposure. Therefore, it is suggested that the blue eyes and blond hair may be more susceptible to acoustic overstimulation even after only a short-term noise exposure.

Differential impact of short-term noise exposure on distortion product otoacoustic emissions

Due to the effectiveness of OAE's and the objectivity it provides, most studies including the present study used OAE's in addition to pure tone audiometry. Studies utilized DPOAE to measure changes in amplitudes after noise exposure [9], [37], [38]. The current study measured significant differences in all participants' TES and DPOAE SNR at each frequency. A reduction of 2.25 to 5.81 dB SPL was measured in the DPOAE SNR in all participant in the current study with the largest shifts at 4004 to 7996 Hz. Although pure tone audiometry did not display the same results after one-hour noise exposure at each frequency, DPOAE's were able to monitor slight changes in the amplitudes of emissions after noise exposure. The present study found a significantly larger mean TES of 2.69 dB SPL at 2000 Hz in participants with blue eyes and blond hair than in participants with brown

eyes and hair. A study reporting on DPOAE's after exposure to impulse noise, reported a significant shift at 4004 to 7996 kHz while the lower frequencies remained stable [39]. Studies have described a trend of TES at 3049 to 7996 Hz after acoustical overstimulation [3], [9]. Research investigating noise exposure and TES varied from an exposure intensity of 85 to 103 dB SPL for 30 minutes to five hours [9], [36], [38]. The degree of shift is highly dependent on the intensity and duration of exposure. Individuals have shown mean TES of 7 to 10 dB SPL after exposure to broadband noise, music in nightclubs and MP3 players [9], [15], [21]. The current study observed a smaller TES after music exposure in comparison to previous research although the affected frequency region correlated [9], [21]. The larger TTS and TES in the blue eyes and blond hair group were only measured at one test frequency per test, namely a TTS at 4kHz and a TES at 2 kHz.

Mujica-mota et al. (2015) reviewed numerous studies and concluded that although differences between lighter and darker pigmented individuals were noted, it is considered only a modest risk factor. Studies have suggested that individuals with lighter pigmentation were only at risk when exposed to high noise levels over a prolonged period of time [14], [15], [17]. The differences in results and degree of shifts between the blue eyes and blond hair, and the brown eyes and hair's TTS and TES can be attributed to the following three reasons. Firstly, the intensity of the noise exposure in previous studies was greater in comparison to the present study using a 90 dBA exposure level. A louder exposure level may cause larger shifts in the same duration of exposure. Secondly, the duration of noise exposure of previous studies was five to six hours or even years in comparison to the single hour in the present study. According to the equal energy rule, when the intensity increases by three dB it leads to the doubling in sound energy and the reducing duration in half which is acceptable to be exposed to [40]. As soon as the intensity becomes too loud or duration longer, it may cause larger shifts and more permanent damage. Due to the reduced duration, despite similar exposure levels, although shifts were recorded, it was much smaller in comparison to previous studies. Lastly, the TES and TTS can be attributed to inter-individual variability.

There is an agreement between studies that intrinsic and extrinsic factors influence individuals susceptibility and auditory health [2], [3], [13], [14], [17], [36]. The differences in TTS and TES between individuals with blue eyes and blond hair, and brown eyes and hair may be attributed to an intrinsic factor such as pigmentation that is influenced by genetics. The present study supports the hypothesis of Bonaccorsi (1965) that there may be a correlation between the amount of pigmentation in the iris and temporal bone due to the visible difference between the blue eyes and blond hair and brown eyes and hair in the current study. Although differences between the blue eyes and blond hair, and the brown eyes and hair groups are seen, the results are unlikely to be attributed to pigmentation alone. Factors such as other genetic factors and environmental factors should not be excluded when examining individuals' susceptibility to NIHL.

Impact of hair and eye colour on the contralateral suppression of transient evoked otoacoustic emissions

The current study aimed to determine whether a correlation existed between CSOAE's and TES and TTS after music exposure at 90 dBA in participants with more pigmentation in comparison to participants with less pigmentation. The current study could not identify a difference in efferent suppression as measured by CSOAE's in participants with brown eyes and hair, and participants with blue eyes and blond hair. The present study, however did observe stronger efferent suppression from 1 to 2 kHz in all participants. This finding correlated with that of Otsuka et al. (2016) who recorded stronger efferent suppression from 1 to 3 kHz in comparison to the higher frequency region in young adults with normal hearing sensitivity. Therefore, the results suggest that MOC efferent system may be stronger at 1 to 2 kHz and weaken in the NIHL sensitive area [2]. The current study observed the same trend of decrease in CSOAE amplitude at ≥ 2.8 kHz with a larger TES measured at 2000 Hz in individuals with less pigmentation compared to those with more pigmentation. The TES was only observed in the group with blue eyes and blond hair although their amount of efferent suppression during CSOAE's was equivalent between the two groups. A previous study used CSOAE's to measure the MOC reflex to assess the risk of hearing loss among orchestra musicians which indicated significant TTS at 4000 Hz as well as a decrease in efferent suppression at 4000 Hz [4]. Numerous studies have concluded the inability of the CSOAE to reliably predict TTS or TES [3], [4]. It is postulated that MOC reflex may provide a protective factor in non-traumatic sound and that the role of the MOC reflex is still vague [41]. The current study is unable to identify a relationship between the strength of efferent suppression, TTS and TES after music exposure in individuals with blue eyes and blond hair and individuals with brown eyes and hair. However, the present study effectively utilized OAE's and pure tone audiometry to measure differences between blue eyes and blond hair and brown eyes and hair after acoustic overstimulation.

A limitation of the current study was that pigmentation was quantified based on hair and eye colour determine the susceptibility to NIHL. Thus, limiting the ability to determine the effect of ethnicity/race on susceptibility to

NIHL. The current study aimed to determine the correlation between CSOAE's in different hair and eye colour, and their TES after music exposure, therefore, purposefully excluding race/ethnicity to effectively control variables. Previous studies have investigated the effect of ethnicity/race on hearing loss by using OAE's and pure tone audiometry [42], [43]. The differences between ethnicities were seen in the noise susceptible region of 4000 to 8000 Hz, regardless of gender [43]. It is acknowledged that individuals from different races and ethnicities have a differing predisposition for NIHL. Pigmentation distribution to skin, hair, and eyes are influenced by genetics and a lack of pigmentation have been observed in genetic disorders [44]. The current study excluded race/ethnicity that is also influenced by genetics to have less variables influencing current study' outcome. Pigmentation syndromes have indicated that there is a correlation between skin colour and sensorineural hearing loss [45]. Therefore, race/ethnicity should not be excluded when investigating the effect of pigmentation on hearing sensitivity after noise exposure due to its possible influence on individuals' susceptibility to NIHL.

Research regarding genetic factors influencing individual's susceptibility to NIHL has been limited due to the inability to determine cumulative recreational noise exposure across a lifetime. NIHL is becoming more prevalent in the modern society and is preventable. Previous studies mainly focused on acoustic overstimulation in a laboratory or occupational setting rather than a recreational setting [13]. Future research should focus on monitor the effect of recreational noise exposure on individuals hearing sensitivity. Individuals are differently susceptible to NIHL, therefore, some individuals may have more resilient ears in comparison to others against noise exposure. The underlying mechanism of this phenomenon is not yet understood The use of OAE's allows the objective and non-invasive monitoring of changes in OHC's when measured directly after exposure to identify individuals that are more susceptible to NIHL [39]. Inter-individual susceptibility should not be underestimated, and awareness should be created to identify possible risk factors to NIHL. The current study population included normal hearing young adults with less cumulative exposure to occupational and recreational noise. Identifying the influencing factors of inter-individual susceptibility will aid audiologists to better prevent and treat individuals susceptible to NIHL. Efforts should be made to better understand the underlying mechanisms influencing NIHL.

Conclusion

No clear relationship was determined between CSOAE's of individuals with blue eyes and blond hair, and brown eyes and hair, and their TES after one hour of music exposure. There was a significant TES at 2000 Hz in the individuals with blue eyes and blond hair after noise exposure in comparison to individuals with brown eyes and hair. In addition, a larger TTS was measured at 4000 Hz in the individuals with blue eyes and blond hair in comparison to the individuals with brown eyes and hair. No statistically significant difference between the two participants groups could be measured with CSOAE's. Therefore, CSOAE's were unable to predict which group of individuals were more susceptible to NIHL after music exposure. To effectively identify factors that influence individual susceptibility to NIHL, more variables must be identified and monitored over a longer exposure duration.

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Figure 1

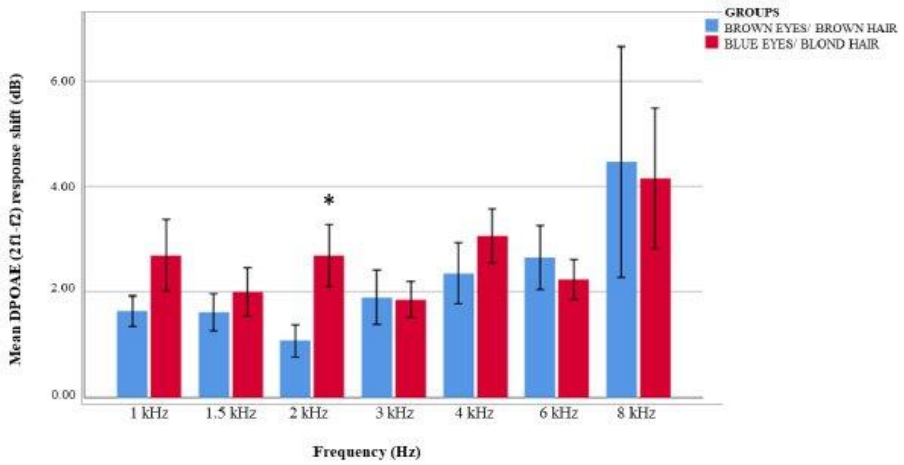

Figure 1: The mean distortion product otoacoustic emissions (DPOAE) half octave response level shift after music exposure in participants with brown eyes and hair and participants with blue eyes and blond hair (n=50 ears)

Table 1: The mean efferent suppression (dB SPL) per frequency for group with brown eyes and hair and group with blue eyes and blond hair

Frequency (Hz)	1001	1414	2000	2828	4000
Brown eyes and brown hair (n=24 ears)					
Absolute TEOAE amplitude with noise (dB SPL ± SD)	8.34 ±4.04	9.67 ±3.30	6.84 ±3.13	4.26 ±4.63	0.94 ±4.84
Amount of suppression (dB)	0.46	0.53	0.54	0.57	0.38
Blue eyes and blond hair (n=26 ears)					
Absolute TEOAE amplitude with noise (dB SPL ± SD)	7.50 ±5.84	7.52 ±4.35	7.57 ±3.61	4.74 ±3.64	1.23 ±5.98
Amount of suppression (dB)	0.48	0.54	0.38	0.44	0.29

(dB: decibel; dB SPL: decibel sensation level; Hz: frequency; SD: standard deviation; %: percentage)

Table 2: Mean pure tone thresholds in participants with brown eyes and hair and participants with blue eyes and blond hair after music exposure at each frequency (n=50 ears)

Frequency (Hz)	125	250	500	1000	2000	3000	4000	6000	8000
Brown eyes and hair (n=24 ears)									
Baseline pure tone threshold (dB HL ±SD)	1.25 ±2.66	1.87 ±3.55	1.04 ±2.54	0.42 ±1.41	0.83 ±2.40	0.42 ±1.41	0.42 ± 1.41	1.46 ± 3.12	0.83 ±2.82
Pure tone threshold after music exposure (dB HL ± SD)	1.87 ±3.55	2.5 ±3.10	1.46 ±2.75	0.63 ±1.69	0.63 ±1.69	0.42 ±1.41	1.25 ±2.66	1.86 ±2.88	1.25 ±3.69
Threshold shift (dB)	**0.63**	**0.63**	**0.42**	**0.21**	**0.21**	**0**	**0.83**	**0.42**	**0.42**
Blue eyes and blond hair (n=26 ears)									
Baseline pure tone threshold (dB HL ±SD)	1.92 ±3.49	0.77 ± 1.84	0.96 ± 2.83	0.58 ± 1.63	0.38 ±1.36	0.38 ±1.36	0.96 ±2.83	3.27 ±3.99	2,12 ±3.21
Pure tone threshold after music exposure (dB HL ± SD)	2.31 ±4.30	1.54 ±2.75	1.35 ±4.14	0.58 ±2.16	1.54 ±3.68	1.35 ±2.68	4.04 ±4.69	5.00 ±5.48	3.65 ±5.20
Threshold shift (dB)	**0.38**	**0.77**	**0.38**	**0**	**1.15**	**0.96**	**3.08 ***	**1.73**	**1.54**

*(dB HL: decibel hearing level; Hz: frequency; SD: standard deviation; * indicative of significance)*

Table 3: Mean baseline and post-exposure distortion product otoacoustic emissions (DPOAE) signal to noise ratio (SNR) in all participants (n=50 ears)

Frequency (Hz)	1001	1501	2002	3003	4004	6006	7996
Mean baseline SNR (dB SPL ±SD)	16.52 ±5.67	20.9 ±5.23	20.9 ±4.72	18.05 ±4.55	21.36 ±4.32	21.70 ±6.07	11.06 ±10.8
Mean post-exposure SNR (dB SPL ±SD)	12.43 ±6.78	18.35 ±6.26	18.65 ±6.18	15.61 ±5.12	18.38 ±4.52	19.12 ±7.41	5.67 ±14.32
Mean difference between baseline and post-exposure SNR (dB SPL ±SD)	4.09 ±2.89 **	2.55 ±1.80 **	2.25 ±1.59 **	2.44 ±1.73 **	2.98 ±2,10 **	2.58 ±1.82 **	5.81 ±3,11 **

*(dB SPL: decibel sensation level; DPOAE; distortion product otoacoustic emissions; Hz: frequency; SD: standard deviation; SNR: signal to noise ratio; * indicative of significance)*