Spring 2022

Modulation of Electrocochleography Responses by Contralateral Broadband Noise in Young Adults

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MODULATION OF ELECTROCOCHLEOGRAPHY RESPONSES BY CONTRALATERAL BROADBAND NOISE IN YOUNG ADULTS

A Doctoral Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Doctor of Audiology

By

Riley Rickman

May 2022

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Communication Sciences and Disorders

Missouri State University, May 2022

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ABSTRACT

While it is known the auditory efferent system contains two distinct subgroups – the medial olivocochlear nucleus (MOC) and the lateral olivocochlear nucleus (LOC) – not much is known regarding the function of the LOC in humans. This study aims to evaluate the effect of activating the lateral olivocochlear (LOC) neurons via contralateral broad band noise (CBBN) on electrocochleography responses. A ten-minute time-blocked paradigm was utilized to evaluate both the slow and fast effect of the LOC neurons. Recordings were obtained at four points within this ten-minute block both with and without the presence of 50 dB SPL CBBN to observe the difference in action potential (AP) amplitude and latency using three different stimulus presentation rates (11.1, 58.59, and 97.66 clicks/second). Significant enhancement of the AP amplitude was observed at all rates when CBBN was present. This finding supports the theory that the LOC does function to modulate afferent auditory responses in humans.

KEYWORDS: auditory efferent system, electrocochleography, lateral olivocochlear nucleus, enhancement, compound action potential

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INTRODUCTION

The physiology of human audition via the auditory afferent, ascending system is a widely studied and relatively well-understood topic. Conversely, the auditory efferent, descending system and its impact on audition are less understood. The auditory efferent system, specifically the olivocochlear bundle, serves to create feedback loops that modulate the function of the various parts of the inner ear - the outer hair cells (OHCs), inner hair cells (IHCs) - and auditory nerve (AN) (Guinan, 2006; Ciuman, 2010). The two main subparts of the olivocochlear bundle are the medial olivocochlear (MOC) and the lateral olivocochlear (LOC) neurons, both of which originate in the superior olivary complex (SOC) (Guinan, 2006). These neurons have a complex pathway that varies between species, but generally, the MOC neurons more heavily innervate the contralateral ear (White and Warr, 1983), while the LOC neurons more heavily innervate the ipsilateral ear (Raphael and Altschuler, 2003). The MOC neurons are thick, heavily myelinated, and innervate primarily on the base of the OHCs as well as the type II afferent fibers (Liberman and Liberman, 2019). Conversely, the LOC neurons are thin, relatively unmyelinated and synapse on various cochlear structures: the type I afferent fibers that innervate the IHCs, the base of the IHCs, the type II afferent fibers that innervate the OHCs, and, interestingly, the MOC fibers innervating the cochlear structures (Liberman and Liberman, 2019). Furthermore, the MOC neurons release mostly acetylcholine (ACh) (Gisselsson and Orebro, 1960), while the LOC neurons release a number of neurotransmitters, including ACh, gamma-aminobutyric acid (GABA), calcitonin gene-related peptide (CGRP), choline acetyltransferase (ChAT), enkaphalins, and dopaminergic neurotransmitters (Eyebalin, 1993; Maison et al., 2003; Darrow et al., 2006b; Schrott-Fischer et al., 2007).

Between both components of the olivocochlear bundle, there is a much better understanding of the role of the MOC on human audition compared to the LOC. The MOC reflex activation by auditory stimuli has shown to suppress OHC function via both otoacoustic emissions (OAEs) (Sun, 2008; Abdala, Mishra, and Williams, 2009) and the cochlear microphonic (CM) (Jamos et al., 2020). The MOC reflex function has been linked to protection from acoustic trauma, understanding of speech in noise, and assistance in situations requiring selective attention (Guinan, 2018). Again, the function of the LOC reflex is less understood. When LOC neurons were removed in mice, the side with an LOC lesion showed an enhanced AN response, while the side without a lesion showed a suppressed AN response; the researchers theorized the lesioned mice would have a higher degree of difficulty with localization due to a larger discrepancy when attempting to balance interaural intensity differences (Darrow et al., 2006a). Liberman and Gao (1995) ablated the OCB in mice and exposed mice to high degrees of noise. There was a small, but significant, difference in the degree of permanent threshold shift (PTS) between control mice and mice with ablated OCBs (Liberman and Gao, 1995). This finding, along with the fact that frequency of peak OHC loss did not coincide with frequency of peak PTS led researchers to believe that both subsets of the OCB play a role in the protection of the afferent auditory system from acoustic trauma (Liberman and Gao, 1995). Physiologically, the LOC fibers have a slow effect which is most likely due to the thinness and relative lack of myelination (Guinan, 2018). The slow effect is seen when an efferent fiber is continuously stimulated with noise, which increases the spontaneous firing rate of the nerve fiber; afterward, it takes an extended period of time for the nerve fiber to return to its normal spontaneous firing rate and function (Widerhold and Kiang, 1970; Liberman, 1988). In a study conducted by Sridhar et al. (1995), when stimulating a guinea pig OCB with electrical pulses, it could take as long as 40

seconds to reach peak CAP amplitude suppression and anywhere from 90 – 100 seconds for the CAP amplitude to return to its pre-OCB stimulated baseline.

Research has shown modulation of auditory evoked responses when the LOC is stimulated electrically in animals (Groff and Liberman, 2003). These researchers were able to accomplish this by indirectly stimulating the LOC via direct inferior colliculus stimulation; it was attributed to the LOC response due to the known slow effect associated with the LOC and the lack of response from the OHCs (Groff and Liberman, 2003). There is no known research that shows the LOC reflex effect in humans. This study aims to assess the effect of the LOC reflex activation using contralateral broad band noise (CBBN) on the AN response in human subjects to better understand the role of the LOC in human hearing.

LITERATURE REVIEW

The process of human audition is a complex collaboration between multiple physical, mechanical, and neural components. Though all three of these components are dynamic and involved, the focus of this paper will be on the neural aspect of hearing – primarily the auditory efferent system and its modulation of afferent auditory function.

Peripheral Auditory Anatomy and Physiology

The peripheral auditory system consists of three general sections: 1) the external ear, 2) the middle ear, and 3) the inner ear. The pinna of the external ear gathers sound and assists in localization; the external auditory meatus acts as a resonator, essentially "funneling" sound to the tympanic membrane (TM) causing it to vibrate (Musiek and Baran, 2016). The middle ear space is an air-filled cavity bordered laterally by the TM. The TM is connected medially to the ossicular chain, which consists of three small bones: the malleus, incus, and stapes. The stapes inserts into the oval window, which acts as the lateral barrier to the inner ear. The oval window is an opening in the bony labyrinth of the cochlea covered by a thin membrane, and it lies superiorly to the round window which is a second opening of the bony labyrinth. These windows allow for communication between the middle and inner ear. Once sound has been channeled through the components of the external ear, the middle ear acts as a mechanism to overcome the impedance mismatch, transferring energy between the air at the surface of the TM to the fluid of the cochlea. This impedance mismatch is overcome through the difference in area between the TM and stapes footplate within the oval window, the lever action of the ossicles, and the buckling of the TM (Musiek and Baran, 2016).

Following the mechanical action of the middle ear, the stapes will trigger both a mechanical and electrophysiological reaction within the cochlea. The cochlea functions as a frequency analyzer, processing nearly 10 octaves of sound (Dallos, 1992). The cochlea is a snail-shaped bony labyrinth within the petrous portion of the temporal bone. The snail-like shape comes from bone wrapping around a central structure, known as the modiolus, which houses blood vessels and nerve fibers. Located within the bony portion of the cochlea is a membranous labyrinth. This membrane houses three fluid-filled compartments: the scala vestibuli, filled with perilymph; the scala media, filled with endolymph; and the scala tympani, filled with perilymph (Musiek and Baran, 2016). Perilymph is filled with a large amount of sodium ions and intermingles with the cerebrospinal fluid (Raphael and Alexander, 2003). The bony labyrinth has two openings that open to the middle ear space—the oval window opens to the scala vestibuli and the round window opens to the scala tympani. The scala vestibuli is the most superior of the three scalae; it is separated from the scala media, or cochlear duct, by Reissner's membrane (Dallos, 1992). The scala tympani is the most inferior of the three scalae and is separated from the cochlear duct by the basilar membrane (BM) (Musiek and Baran, 2016). The BM is tonotopically organized, with a narrow, stiff base for high-frequency tuning and a wide, flaccid apex for low-frequency tuning (Narayan et al., 1998). The organ of Corti, or organ of hearing, sits along the entire length of the BM. The organ of Corti houses supporting cells, inner hair cells (IHCs), outer hair cells (OHCs), and neural connections to these hair cells (Dallos, 1992). Just above the organ of Corti sits the tectorial membrane - a collagenous, gel-like flap, again spanning the entire length of the cochlear duct.

Hair Cells. In a normal human cochlea, there are 3-5 rows of OHCs, totaling approximately 12,000 cells; additionally, there is a single row of IHCs, totaling approximately

3,500 cells (Liberman and Liberman, 2019). Atop these cells are stereocilia, connected by tip-links and side-links, and basally, there are both afferent and efferent neural connections (Raphael and Altschuler, 2003).

The OHCs are cylindrically shaped sensory cells within the organ of Corti, and their stereocilia are embedded in the underside of the tectorial membrane (Raphael and Altschuler, 2003). The IHCs are goblet-shaped sensory cells within the organ of Corti with free-standing stereocilia (Raphael and Altschuler, 2003). Covering the organ of Corti is the reticular lamina, which acts as a barrier, preventing the +80 mV endolymph of the cochlear duct from interfering with the neutrally charged fluids housed within the organ of Corti (Musiek and Baran, 2016). When a sound triggers the movement of the stapes outward on the oval window, the decrease in pressure within the cochlea causes the cochlear duct to move upward. This deflection of the cochlear duct causes the stereocilia of the OHCs to shear toward the stria vascularis. This shearing causes the +80 mV endolymph of the cochlear duct and the -70 mV OHC to create a voltage gradient, allowing potassium (K^+) to rush into the cell and depolarizing the OHC (Dallos, 1992). The stria vascularis recycles the (K^+) forced out during cellular firing back into the cochlear fluids to allow for re-firing of the OHCs (Brownell, 1990). A similar response and voltage gradient is created with the IHC which has a -40 mV charge (Musiek and Baran, 2016). However, there is a significant difference in the response generated by the depolarization of the two types of hair cells. Depolarization of the OHC results in an electromotile response via the protein prestin, allowing for contraction and expansion of the sensory cell (Dallos et al., 2008). The OHCs are known as the cochlear amplifier, and they are responsible for amplification of low intensity sounds as well as frequency specificity within the cochlea (Dallos et al., 2008). However, the IHCs are considered to have a more passive response (Dallos, 1992). As the

tectorial membrane moves with the traveling wave and OHC contraction, it will shear the stereocilia of the IHCs, depolarizing the cell (Raphael and Altschuler, 2003). Depolarization of the IHCs results in release of the neurotransmitter glutamate, causing firing of the afferent auditory nerve fibers synapsed at the base of the IHC (Kataoka and Ohmori, 1994).

The Auditory Nerve. The vestibulocochlear nerve is the eighth cranial nerve, and it divides into two distinct branches: the vestibular branch and the auditory branch. The auditory nerve (AN) takes sensory information from the cochlea to the cochlear nucleus within the lower brainstem. From the cochlear nucleus, afferent neurons project to the ipsilateral and contralateral superior olivary complex, lateral lemniscus and inferior colliculus. The AN courses from the brainstem to the terminal boutons of the hair cells within the cochlea through a narrow canal called the internal auditory meatus (Musiek and Baran, 2016). There are two distinct types of afferent AN fibers within the cochlea. Type I fibers, or radial fibers, make up 90-95% of all AN fibers; each IHC receives innervation from 10-20 Type I fibers in humans (Musiek, 1992), and 9-30 in cats (Liberman et al., 1990). Type I fibers are large, thick, and densely myelinated.; these fibers are more heavily concentrated on IHCs in the middle region of the cochlea, centered around the 1000 – 2000 Hz range (Kiang, et al. 1982; Musiek and Baran, 2016). Conversely, Type II fibers make up 5-10% of all AN fibers in humans, and each fiber synapses to multiple OHCs; type II fibers are small, thin, and essentially unmyelinated (Kiang et al., 1982). In the basal portion of the cochlea, type II fibers are more densely concentrated and heavily innervate the outer row of OHCs; in the middle and apical regions of the cochlea, type II fibers' innervation focus moves to the middle and inner row of OHCs. The AN is tonotopically organized, with low frequency fibers occupying the center of the nerve and high frequency fibers covering the outside of the nerve (Spoendlin and Schrott, 1989).

The AN is responsible for coding timing, frequency, and intensity information of incoming sounds (Heil and Peterson, 2015). Temporal coding will occur via phase-locking. For low-frequency signals, the AN fibers will fire at the same rate as the frequency that is being coded. However, from about 1000 – 5000 Hz the ability of the AN to phase lock begins deteriorating; at frequencies higher than 5000 Hz, the AN loses the ability to phase lock (Musiek and Baran, 2016). The AN also has intensity coding capabilities through modulation of firing rate, and the main contributor to intensity coding is the spontaneous firing rate of the AN (Heil and Peterson, 2015). Each nerve fiber has one of three spontaneous firing rates: low (0-0.5 spikes/second), medium (0.5-18 spikes/second), or high (>18 spikes/second) (Heil and Peterson, 2015). Essentially, for the neuron to fire in response to a sound (i.e. not spontaneously) the firing rate has to be higher than that of the spontaneous rate. High spontaneous rate nerve fibers respond better to low-intensity sounds and saturate more quickly while low and medium rate nerve fibers respond better to mid-high intensity sounds and take a larger growth in intensity to saturate (Heil and Peterson, 2015). Ultimately, since the high spontaneous rate nerve fibers are already firing rather rapidly on their own, the addition of low-intensity sound will give the nerve enough drive to fire for audition (Heil and Peterson, 2015). The high spontaneous rate nerve fibers are important for hearing sounds near threshold level while the low and medium spontaneous rate nerve fibers respond to moderate to high sounds which makes them vital for speech encoding and understanding (Musiek and Baran, 2016).

Efferent Auditory System

The efferent auditory system is the descending auditory pathway, which modulates afferent neural function and sensory cells through the creation of feedback loops (Guinan, 2006).

The rostral section of the efferent system originates in the auditory cortex and forms a network of connections with the ipsilateral and contralateral inferior colliculus (IC) and medial geniculate body (MGB) (Musiek and Baran, 2016). The anatomy and function of the rostral efferent system is greatly unknown compared to the caudal efferent system. The caudal portion of the system can be generalized to the olivocochlear bundle and its subparts – the medial olivocochlear (MOC) and lateral olivocochlear (LOC) neurons, and both originate from the superior olivary complex (SOC) (Guinan, 2006).

Medial Olivocochlear Neurons. MOC neurons are thick, myelinated neurons that synapse directly to the base of OHCs, as well as to the outer spiral bundle of afferent type II neurons that innervate the OHCs (Liberman and Liberman, 2019). These neurons most heavily innervate the first row of OHCs surrounding the 4 kHz point before slowly falling off more apically and basally on the human cochlea (Liberman and Liberman, 2019). MOC neurons innervate the OHCs of the cochlea on both the ipsilateral and contralateral side of the periolivary region of the medial superior olive (Ciuman, 2010). Studies have demonstrated that approximately 2/3 of MOC fibers innervate the contralateral cochlea while 1/3 innervate the ipsilateral cochlea in rats (White and Warr, 1983) and mice (Maison et al., 2003) However, the exact ratio of crossed versus uncrossed MOC fibers in humans is still unknown. Liberman and Liberman (2019) found the MOC neurons innervating the OHCs is significantly less dense in humans compared to mice, guinea pigs, and rhesus monkeys. This finding suggests the human MOC response may be weaker than that of some animal species (Liberman and Liberman, 2019)

While the innervation of these neurons is rather straightforward, the pathway of excitation is more complex. Eliciting the MOC reflex from the ipsilateral cochlea leads to stimulating afferent auditory fibers that cross the brainstem, stimulating MOC neurons on the

contralateral side. These MOC neurons will again cross the brainstem to the cochlea of origin, creating the ipsilateral, or "double-crossed" pathway. Conversely, if sound is presented to the contralateral cochlea and auditory nerve, interneurons will cross the brainstem and stimulate the MOC fibers on the ipsilateral side. These fibers project to the OHCs of the ipsilateral cochlea, creating the contralateral reflex pathway (Guinan, 2006). Results from animal data suggest that the ipsilateral, crossed MOC reflex is stronger than the contralateral MOC reflex, which coincides to the distribution of crossed versus uncrossed fibers (Liberman, 1988). However, there is no apparent difference in MOC reflex strength comparing ipsilateral versus contralateral stimulation in humans with a broadband stimulus (Lilaonitkul and Guinan, 2009).

When stimulated, the MOC fibers act to modify OHC function. This is mainly seen in the form of suppression, or reduction in gain of the OHC; this suppression is observed through change in OAE or CM response amplitude (Sun, 2008; Jamos et al., 2020). This is accomplished by the neurotransmitter acetylcholine (ACh), which is released by the MOC nerve fiber (Gisselsson and Orebro, 1960). This release triggers two different effects: a fast-effect and a slow-effect (Sridhar et al., 1995). The fast-effect happens within 100 milliseconds and results from the released ACh opening a channel and allowing Ca^{+2} to enter the OHC, activating channels to let K^{+} rush out, putting the OHC in a hyperpolarized state (Guinan, 2018). Conversely, the MOC slow-effect occurs if the MOC neurons are stimulated for an extended period of time (Sridhar et al., 1995). When this happens, there is a physiological change in the protein prestin and the OHC cytoskeleton stiffness, and it can last for several seconds (Dallos et al., 2008). However, this reduction in gain of the cochlear amplifier is mostly seen when the MOC reflex is activated in a quiet environment. Kawase et al. (1993) found that when the MOC reflex is stimulated in a noisy environment, it will result in an enhancement, or increase, in

cochlear amplifier function. There is evidence to support three main functions of the MOC reflex: 1) protection from acoustic trauma, 2) listening in noise, and 3) support in selective attention (Guinan, 2006).

Lateral Olivocochlear Neurons. While the MOC is more extensively studied and understood, the LOC remains somewhat a mystery. Compared to the MOC, the LOC fibers are thinner and relatively unmyelinated (Liberman and Liberman, 2019). These fibers originate from the lateral superior olive (LSO) and group together to travel through the fourth ventricle of the brain (Liberman et al., 1990). These fibers will continue laterally, meeting the vestibular nerve to travel through the IAM before innervating the cochlea alongside the auditory nerve (Raphael and Altschuler, 2003). Once at the cochlear level, these nerve fibers will exit the osseous spiral lamina and "spiral" with MOC nerve fibers beneath the IHCs within the tunnel spiral bundle (TSB) and inner spiral bundle (ISB) (Liberman and Liberman, 2019). Studies reveal two types of LOC nerve fibers – small, thin intrinsic fibers and large, thicker shell fibers (Ciuman, 2010; Warr et al., 1997). The shell neurons split when they enter the organ of Corti, and a single neuron can span 1/5 of the cochlear length, or around 1.4 octaves; the intrinsic neurons do not split upon entering the organ of Corti, and a single neuron covers a relatively small portion of the cochlea – less than 0.6 octaves (Warr et al., 1997). Intrinsic LOC neurons densely innervate approximately 20% of the length of the rat cochlea while shell neurons are scantly dispersed along 80-95% of the length of the rat cochlea, forming "en-passant," or in passing, synaptic terminals along its route with multiple swellings (Warr et al., 1997). In contrast to the finding within the rat cochlea, Brown (1987) found the unidirectional or non-splitting neurons, assumed to be intrinsic, to contain the multiple synaptic terminals or "en-passant" synapses in the ISB and TSB of the guinea pig cochlea. Even though researchers found a difference in dispersion along the cochlear

length, shell neurons only make-up around 15% of all LOC fibers that innervate the rat cochlea, while the other 85% are intrinsic fibers (Warr et al., 1997). The densely packed innervation of intrinsic LOC nerve fibers may be evidence of the intrinsic fibers having more frequency specificity within the cochlea compared to the widely distributed shell neurons (Warr et al., 1997).

Opposite of MOC innervation, the LOC fibers will primarily innervate the ipsilateral cochlea and a smaller portion innervate the contralateral cochlea with some efferent fibers innervating bilaterally (Thompson and Thompson, 1986; Ciuman, 2010). Studies presume that the LOC does respond to sound - given its location within the LSO, but researchers are unsure as to how or if it impacts the afferent auditory system (Guinan, 2018). However, given the distribution of nerve fibers, the ipsilateral LOC reflex is presumably stronger than the contralateral (Guinan, 2018). Researchers found that both types of LOC fibers synapse in the region below the IHC or surrounding border cells, but rarely to the base of the IHC itself in the rat cochlea (Warr et al., 1997). Conversely, Liberman et al. (1990) found evidence of direct contact of the LOC nerve fibers to the IHC cell bodies in the cat cochlea. Furthermore, in the basal portion of the cat cochlea, LOC fibers primarily synapse to the radial fibers, near the modiolus, beneath the IHCs, while apically, there is evidence of efferent synapse on the radial fibers, the IHCs directly, and the OHCs (Liberman et al., 1990). A study by Liberman and Liberman (2019) conducted on human temporal bones found the LOC neurons synapse to a variety of peripheral structures: 1) primarily, the radial type I auditory nerve fibers that innervate the IHCs, 2) the IHCs directly, 3) the type II auditory nerve fibers that innervate the OHCs, and 4) the MOC fibers themselves. These findings are synonymous with the LOC synaptic pattern in the cat cochlea (Liberman et al., 1990). The LOC fibers more densely innervate the apex of the

human cochlea before slowly falling off toward the base (Liberman and Liberman, 2019). Additionally, interspecies comparisons between mice and humans demonstrates that human LOC density may be higher than that of mice given the better development of the human TSB (Liberman and Liberman, 2019). The high threshold, or low spontaneous rate, afferent neurons innervating the modiolar side of IHCs, appeared to have a higher amount of LOC innervation, suggesting a functional component to these LOC neurons (Liberman, 1980; Liberman et al., 1990). Due to the LOC nerve fibers primarily synapsing on the Type I afferent auditory nerve fibers, it has been speculated the function of the LOC nerve fibers is to modulate the firing of the auditory nerve (Maison et al., 2003; Schrott-Fisher et al., 2007).

LOC Neurotransmitters. The human LOC reflex is a complex network of neurotransmitters that is currently not completely understood by researchers. A study conducted on mice found cholinergic, GABAergic and CGRPergic synapses present in the inner spiral bundle (ISB), directly on a relatively small amount of IHCs, and coursing to the OHCs (Maison et al., 2003). Furthermore, Maison et al. (2003) found that terminals presenting with GABA and ACh are colocalized in the ISB of the mouse cochlea, meaning they overlap with one another. Schrott-Fischer et al. (2007) found evidence of choline acetyltransferase (ChAT), GABA, CGRP - a neuropeptide, and enkephalins in the olivocochlear efferents of their human temporal bone study. Researchers concluded some fibers expressing ChAT, which is a cholinergic neurotransmitter, most likely correlated to LOC neurons rather than MOC neurons due to their termination on type I afferent fibers (Eyebalin, 1993; Schrott-Fischer, et al., 2007). This study also found that some fibers expressing enkephalin, CGRP, and GABA activity within the ISB correlated to LOC efferent fibers, and researchers believe the group of neurotransmitters work together to modulate cochlear function (Schrott-Fischer et al., 2007). Additionally, Darrow et al.

(2006b) found evidence of dopaminergic neurotransmitter activity within the LOC of the mouse cochlea. Dopaminergic fibers are only present in the ISB and TSB and innervated the cochlea evenly; researchers believe this even distribution correlates to dopaminergic fibers more strongly influencing high-frequency regions due to the tuning of the mouse cochlea (Darrow et al., 2006b). To further provide evidence for these fibers originating from efferent auditory system, brainstem staining of mice indicated dopaminergic reactivity originating from the LSO (Darrow et al., 2006b). Mulders and Robertson (2004) conducted a similar study on guinea pigs and reported similar findings; dopaminergic LOC fibers originated from the high frequency portion of the LSO and more densely innervated the basal portion of the guinea pig cochlea. Notably, the dopaminergic subgroup only accounted for around 10-25% of the mouse LOC fibers compared to the much more prominent cholinergic fibers, and these fibers were essentially distinct from one another during staining, but some overlap between the two was present (Darrow et al., 2006b). These studies provide evidence for two separate LOC systems based on chemical function – a dopaminergic subgroup and an essentially cholinergic subgroup (Eyebalin, 1993; Maison et al., 2003; Darrow et al., 2006b; Schrott-Fischer et al., 2007). Eyebalin (1993) suggested that the cholinergic LOC neurons are responsible for increasing the firing rate of the afferent neurons these fibers synapse to via the increased release of glutamate. It was also suggested that the release of dopamine within the organ of Corti disrupts communication between the IHCs and the afferent auditory nerve fibers they are attempting to fire, potentially inhibiting AN function (Eyebalin, 1993). Regarding the enkephalin family of neurotransmitters, there is evidence of their involvement with the LOC system of animals in the presence of noise (Eyebalin, 1993).

Fast vs. Slow Effect of the LOC. As previously mentioned, the MOC reflex has both

"fast" and "slow" effects. However, the LOC nerve fibers have an extremely slow effect, which takes a longer amount of time to stimulate the fibers and a longer amount of time for the effect of the fibers to decay (Liberman, 1988). This is more than likely due to the lack of myelination among the nerve fibers (Guinan, 2018). Liberman (1988) found when an efferent fiber with essentially no spontaneous discharge was continuously stimulated with noise for several minutes, the spontaneous firing rate increased and took several minutes post-noise exposure to return to its original inactive state. Additionally, low frequency efferent fibers fired for an extended period following the removal of the acoustic stimulation compared to high frequency efferent fibers (Widerhold and Kiang, 1970; Liberman, 1988). To further investigate this phenomenon, Sridhar et al. (1995) delivered various patterns of electric stimulation to the OCB of a guinea pig to evaluate the fast and slow efferent effect. The slow effect was observed in three different experimental paradigms: 1) when presenting electrical pulses every 1.5 seconds, 2) when presenting electrical pulses intermittently, and 3) when presenting electrical pulses continuously. In each trial, it would take as many as 40 seconds for the CAP amplitude to reach peak suppression and as much as 90 – 100 seconds for the CAP amplitude to return to normal (Sridhar et al., 1995). However, those researchers did not differentiate stimulation of the MOC or LOC, but ACh is a neurotransmitter associated with both systems.

Function of the Lateral Olivocochlear Neurons. While increasing evidence in the literature describing the role of the MOC in the auditory system function, the LOC remains less understood. However, studies have shown promising results for the LOC contributing to the localization of sound and protection from acoustic trauma.

LOC and Localization. One study provided evidence of the LOCs role in localization through ablation of the LSO in mice (Darrow et al., 2006a). Ablation was proven through loss of

ACh receptors in the IHCs, but not the OHCs, and a change in CAP response without a change in distortion product otoacoustic emission (DPOAE) response. Interestingly, when the LSO was destroyed unilaterally, there was a bilateral change in AN response; the side lesioned showed enhancement while the contralateral side showed suppression of the same magnitude as the lesioned sides' enhancement (Darrow et al., 2006a). The authors argue that if this is present in lesioned ears, normal hearing ears utilize the LOC for interaural balancing of intensity differences, aiding in accurate localization of sound (Darrow et al., 2006a).

LOC and Protection from Acoustic Trauma. Liberman and Gao (1995) evaluated the OCB bundle as a whole and how its presence impacts permanent threshold shift (PTS) associated with over-exposure to high levels of noise. The researchers cut the OCB in guinea pigs and split them into three groups: control, 109 dB SPL noise exposure, or 112 dB SPL noise exposure. For the 112 dB SPL noise exposed group, researchers found a small but significant increase in PTS in the group with the ablated OCB when compared to the control group with the intact OCB; they also found a greater degree of PTS at high frequencies compared to low frequencies (Liberman and Gao, 1995). Interestingly, the pattern of OHC loss did not coincide with the frequencies where the greatest amount of PTS was found; peak OHC loss occurred between 13k and 17 kHz where peak PTS shift occurred closer to 10 kHz (Liberman and Gao, 1995). While this study did not specifically differentiate the MOC from the LOC and their involvement in protection from acoustic trauma, it does provide reasonable evidence that the OCB plays a small but significant role in protection of the afferent auditory system (Liberman and Gao, 1995).

Another study found evidence of the LOC specifically being involved in the protection of the cochlea from acoustic trauma in mice (Darrow et al., 2007). The researchers successfully sectioned the LOC in mice; this was shown by no change in ABR at threshold and DPOAE

responses, with a 50% lower amount of olivocochlear synapses on the IHCs with no change in OHC density of the ipsilateral ear and no change in hair cell density of the contralateral ear (Darrow et al., 2007). Researchers did see an enhancement of ipsilateral ABR response in lesioned ears at suprathreshold levels, and this enhancement coincided with the amount of intensity increase, but did not vary across frequencies (Darrow et al., 2007). This enhancement only seen ipsilaterally coincides with what is known about the anatomy of the LOC pathway and provides evidence of the LOC being directly involved with suppressing high intensity sounds and therefore protecting the afferent auditory system from acoustic trauma (Darrow et al., 2007).

Activation and Measurement of the OCB

Many studies have been conducted on how to stimulate and measure the response of the OCB neurons. Several studies show the MOC activation effect on different auditory evoked potentials in response to sound in humans (Najem, Ferraro, and Chertoff, 2016; Jamos et al., 2020) and cats (Liberman, 1988). Wiederhold and Kiang (1970) found electrical stimulation of the contralateral OCB in cats reduces auditory nerve activity at all levels, but best at moderate levels. Additionally, after the removal of the electric shocks, the auditory nerve becomes hyper-active and results in a response that "overshoots" the baseline response. These results are derived from stimulating the OCB as a whole. In a study conducted by Liberman (1988) researchers found that efferent fibers within the cat cochlea have similar characteristic frequencies as well as tuning curves compared to their afferent counterparts. These researchers also found that binaural acoustic stimuli will decrease threshold and increase the discharge rate of the efferent nerve fibers within the cat cochlea (Liberman, 1988). Additionally, monaural acoustic stimulation will only activate around 10% of efferent fibers regardless of the side being stimulated, and efferent

fibers have more binaural inputs than previously believed – as many as 60% (Liberman, 1988). Multiple studies have demonstrated a suppression in otoacoustic emission (OAE) response with activation of the MOC reflex (Sun, 2008; Abdala, Mishra, and Williams, 2009).). However, a more accurate, robust suppressive response can be found when assessing the CAP response following MOC activation (Puria et al., 1996)

Groff and Liberman (2003) have investigated the effect of stimulating the LOC fibers electrically on the auditory nerve responses. Groff and Liberman (2003) conducted a study where they were able to differentiate the MOC and LOC when stimulating the inferior colliculus (IC) as well as stimulate the LSO directly. When stimulating the IC, researchers found evidence of both MOC and LOC suppressive effects; the LOC effect was separated from the MOC effect through a suppressive CAP effect that was present after crossed OCB sectioning and through no change in DPOAE response. The LOC neural pathway was identified through CAP suppression and/or enhancement being present in the ipsilateral ear only after electrically stimulating the LSO. This finding is consistent with the anatomical distribution of the LOC neural pathway; additionally, enhancement of the CAP response was present when stimulating the LSO, which disagrees with studies of suppressive MOC effect, furthering the argument that the enhancement maybe associated with the LOC. Groff and Liberman (2003) also found the responses which they attributed to the LOC to show long-lasting, or "slow," suppression or enhancement. The authors argue this slow effect from the LOC is due to the various neurotransmitters released at the synaptic sites, with neurotransmitters such as dopamine having an opposite effect of ACh and CGRP and causing a complex reaction at the IHCs and AN (Groff and Liberman, 2003). The results found by those researchers provide evidence the LOC is responsible for some of the observed slow changes in CAP response rather than the MOC.

Electrocochleography

ECochG is a clinical tool used to evaluate the inner ear and the auditory nerve in humans (Ferraro, 2010). This can be broken down into the cochlear microphonic (CM), which originates from the OHCs; the summating potential (SP), which is thought to originate from the IHCs and a portion of the distal portion of the auditory nerve; and the CAP, which comes from the distal portion of the auditory nerve (Ferraro, 2003). ECochG traditionally is recorded using either click or tone burst stimuli present at a slow presentation rate (e.g. 7.1/sec) to allow for a synchronous response (Ferraro, 2003). More recently, a new paradigm called continuous loop averaging deconvolution (CLAD) has been used to evaluate ECochG and ABR at high presentation rate (up to 500/sec) (Delgado and Ozdamar, 2004; Kaf et al., 2017; Kennedy et al., 2017). Essentially, CLAD acquires data continuously and obtains recordings at specific periods in time; the software will deconvolve this continuous recording at the end, leaving you with a smoother waveform compared to standard averaging techniques (Delgado and Ozdamar, 2004). Furthermore, Kennedy et al. (2017) found evidence of the origin of the SP being stimulus dependent, with a hair cell origin from high-rate long duration stimuli and a neural origin from short duration stimuli. Kaf et al. (2017) found a decrease in amplitude with an increase in latency of the ECochG CAP while the SP remained stable as rate increased from 7.1 to 507.81 clicks/second when using the CLAD paradigm. The SP is a direct current potential, and it is theorized this potential depends on both the movement of the inner hair cells, much like the OHCs to the CM, as well as the mechanical movement of the basilar membrane and the impulse from the auditory nerve firing to create the positive shift from baseline seen in ECochG recordings (Hallowell, et al., 1958). These rate effects are consistent with the effect of presenting stimulus at high rate on neural and pre-neural response, as it shows how AN firing falls apart as rate increases leading to

decreasing of the CAP response (Delgado and Ozdamar, 2004; Kaf et al., 2017). However, the study conducted by Kaf et al. (2017) provides evidence of CLAD utility for high rate ECochG and ABR due to the maintenance of good waveform morphology. As rate increases, the SP amplitude remained stable with a slightly larger SP amplitude when running ABR compared to ECochG; conversely, the AP amplitude decreased by nearly 1 uV and latency increased with increasing rate even while utilizing the CLAD paradigm (Kaf et al., 2017). This decrease in amplitude and increase in latency with increasing rate agrees with what is known about neural adaptation wherein the continuous stress on the auditory nerve makes it more difficult for the nerve to fire at full capacity (Kaf et al., 2017). However, the utilization of the CLAD paradigm allowed the researchers to test and accurately mark much higher rates compared to standard ECochG or ABR averaging (Kaf et al., 2017).

Clinically, ECochG is primarily known for diagnosing the presence of Meniere's disease, but recently, studies have been investigating the utility in evaluating the efferent system. Najem et al. (2016) have found variable CAP suppression response of the efferent system that is dependent on both stimulus and suppressor frequency and intensity. These researchers looked at pure tones, tone-pips, and click stimuli with contralateral pure-tone suppression. They found maximal onset (amplitude measured from beginning of response to first negative peak or N1) suppression of 1 and 4 kHz tone-pip stimuli to 1 and 8 kHz contralateral pure tones at moderate intensities, respectively (Najem et al., 2016). Conversely, maximal suppression of click stimuli was found at the offset (N1 to first positive peak, or P1) when using 8 kHz pure tone at a moderate level contralaterally (Najem et al., 2016). Interestingly, maximal suppression of the tone-pip occurred with a contralateral suppressive stimulus of similar frequency, showing the frequency specificity and integration of the efferent system (Najem et al., 2016). Another study

showed that contralateral broadband noise (CBBN) showed similar suppression of the N1-P1 response (Dragicevic et al., 2015). Additionally, Jamos et al. (2020) found enhancement of the CM in humans when stimulating the contralateral ear auditorily, providing evidence of yet another way the MOC can be evaluated.

Though we have only seen direct afferent effects when the LOC is stimulated electrically, it is believed that the LOC responds to auditory stimuli given its innervation within the cochlea and its origin within the LSO (Guinan, 2018). Interestingly, LePrell et al. (2003) found that post-lesioning of the LSO in the guinea pig, the CAP amplitude was reduced to acoustic stimuli at all intensity levels, providing evidence that the LOC plays a part in the response of the AN to acoustic stimuli.

OBJECTIVES

Research has provided evidence that LOC neurons modulate the afferent auditory response through electrical stimulation in guinea pigs (Groff and Liberman, 2003). Additionally, studies have shown that severing LOC neurons will alter afferent auditory responses in guinea pigs (LePrell et al., 2003) and mice (Darrow, et al., 2006). Furthermore, it is reasonable to infer that the LOC responds to auditory stimuli, demonstrated by the OCB's response to both monaural and binaural stimulation with BBN (Liberman, 1988) and numerous studies demonstrating the MOC's effect on afferent auditory response demonstrated by both DPOAEs and electrophysiological measurements (Puria et al., 1996).

The goal of this research study is to evaluate the LOC and its impact on the human afferent auditory system through acoustic stimulation. This study aims to inspect the modulation of afferent auditory activity via various subparts of participants' ECochG response – SP and CAP – measured to different presentation rates while stimulating the LOC reflex using CBBN. Furthermore, we will attempt to assess the time course of the LOC effect by testing in carefully timed blocks. The null hypothesis has three parts: 1) the SP response with CBBN will not be different from SP response without CBBN, 2) the CAP response with CBBN will not be different from the CAP response without CBBN, and 3) the CBBN on CAP response will not differ with variation in rate. The alternative hypothesis has three parts: 1) the SP response will differ between the with CBBN and without CBBN conditions, 2) the CAP response will differ between the with CBBN and without CBBN conditions, and 3) the effect of CBBN on CAP response will differ with variation in rate.

METHODS

Participants

Thirty young adult participants, with the age range of 18-27, were recruited from the Missouri State University campus. The participants group consisted of fourteen males and sixteen females. Prior to testing, the experiment was explained to all participants, their questions were answered, and they were given consent forms which they read and signed. Institutional Review Board approval was obtained on November 10, 2020 through Missouri State University (IRB-FY-2021-262, see Appendix). In order to qualify for this research, participants met the following requirements: 1) normal otoscopic examination, 2) normal Jerger Type A tympanometry, 3) normal pure-tone air conduction hearing sensitivity (≤ 20 dB HL) from 500 – 8000 Hz, 4) no significant otologic or audiological history, 5) no history of noise exposure, and 6) middle ear muscle (MEMR) thresholds >65 dB HL to CBBN. The presence of MEMR at a threshold >65 dB HL makes it unlikely for the MEMR to be activated during testing and ensures that the participant's MEMR is within the average MEMR threshold to BBN reported in the literature (i.e., between 70 and 75 dB HL) (Margolis, 1993). ECochG testing was only conducted on the right ear, as there is a right ear efferent advantage (Bidelman and Bhagat, 2015).

Equipment

A Welch-Allyn otoscope was used to evaluate the external ear canal and TM. Immittance measurements were conducted with the GSI Tympstar Middle Ear Analyzer. Pure-tone air conduction hearing sensitivity was evaluated under ER-3 insert earphones with a GSI AudioStar Pro audiometer (ANSI Spec: S3.6-2004). For participants that met all inclusion criteria, ECochG testing was conducted. ECochG testing was conducted utilizing Intelligent Hearing System

Smart-Evoked Potential equipment. ECochG testing was conducted using two different electrodes: Ambu Neuroline 720 disposable snap surface electrodes and homemade tympanic membrane electrodes –"tymptrodes" outlined by Ferraro (2010). To construct the tymptrode outlined by Ferraro (2010), the researcher used silver wire coated in teflon, housed within silastic laboratory tubing and intertwined with cotton at the end. Prior to testing, conductive gel was applied to the internal portion of the cotton of the tymptrode via a syringe, while the external portion of the cotton was soaked in conductive gel. An alligator clip was used to connect the tymptrode to the IHS equipment. Pediatric ER-3 insert earphones were also used to conduct ECochG recordings. All testing was conducted in a sound treated booth in the Missouri State University Auditory Research Lab.

Stimulus and Recording Parameters

A horizontal, one-channel montage was used to test the participants' right ear. The tymptrode acted as the inverting electrode (-), while the left mastoid snap electrode acted as the non-inverting electrode (+), and the mid-forehead (Fpz) electrode acted as the ground electrode. Impedances were kept below 7 kΩ for all electrode contacts. Three rates were utilized for ECochG recording: 11.1, 58.59, and 97.66 clicks/second. To better maintain the response morphology of the moderate and high-rate waveform (i.e., 58.59 and 97.66 clicks/second rate), ECochG testing was conducted at those rates using the CLAD paradigm. 100 μsec click stimuli was delivered to the right ear using an alternating polarity at 80 dB nHL. In total, 36 recordings were made for each participant: four baseline recordings at 80 dB nHL with no noise for each rate and two time-blocked recordings (Figure 1) consisting of four recordings each at 80 dB nHL with 50 dB SPL of CBBN for each rate. The baseline recordings followed the time-blocked

paradigm seen in the figure below to ensure no neural adaptation occurred without the presence of CBBN. The recording epoch was set at 7 ms for rate of 11.1 clicks/second and 50 ms for rates utilizing CLAD paradigm. The response gain was set to 100,000x amplification, filtered using a 30 – 3000 Hz band-pass filter, and each recording was the average of 1024 sweeps.

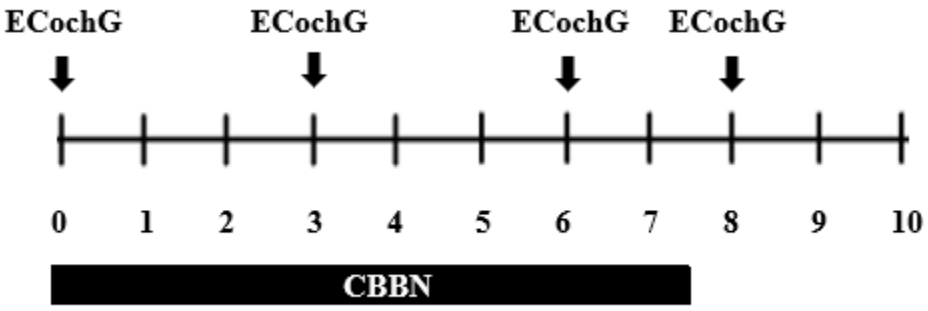

Figure 1. Time-block paradigm shown in minutes with "0" being onset of CBBN as well as the onset of the initial ECochG recording. CBBN was played continuously until the ECochG recording made at the 6-minute mark was complete. The CBBN would then be shut off until the end of the 10-minute time block and a final ECochG recording would be made at the 8-minute mark without the presence of CBBN.

Procedures

Prior to testing, otoscopy was completed to ensure a clear external ear canal and an intact TM. Following otoscopy, tympanometry was performed to ensure good middle ear status. MEMR threshold to BBN was found contralaterally for both left and right ears. Puretone hearing thresholds were obtained from 500 – 8000 Hz, bilaterally, to ensure normal hearing sensitivity. ECochG testing began by cleaning participants' forehead and left mastoid with an alcohol wipe and scrubbing with gauze and NuPrep skin preparation gel. Disposable snap electrodes were placed on the cleaned left mastoid (M_1) and forehead (F_{PZ}). The tymptrode was soaked in conductive gel for five minutes and carefully placed against the participants' right tympanic

membrane. The tymptrode was carefully taped to the participants' face and connected to an electrode lead via an alligator clip. ER-3 insert earphones were then carefully placed in both ears. Participants were seated in a reclining chair and asked to relax but not sleep. A baseline following the time-blocked paradigm was obtained for the 11.1, 58.59, and 97.66 clicks/second rates with no CBBN presented. To test the effect of CBBN, ECochG recordings were made at precise times within a 10-minute block; the onset of CBBN started the 10-minute time and remained on continuously for about 7.5 minutes. Recordings were made at the following time increments: 1) immediately following the onset of CBBN, 3 minutes following the onset of CBBN, and 6 minutes following the onset of CBBN. Following the end of the recording at minute 6, the CBBN was discontinued. A fourth recording was made at the 8-minute mark in the absence of CBBN. At minute 10, the timer was restarted, and the entire 10-minute block was repeated to ensure repeatability. The presentation of the various rate levels was randomized to prevent any effect caused by order. The two tracings for each minute marker (immediately after CBBN, 3-minute, 6-minute, and 8-minute) were averaged. At the conclusion of testing, the tymptrode was removed from the participants' ear and otoscopy was performed to ensure no irritations or abrasions were present in the external ear canal.

Data Analysis

Each tracing was averaged with its time-blocked pair, meaning the two 0-minute, 3-minute, 6-minute, and 8-minute tracings were averaged together; for rates utilizing CLAD paradigm (58.59 and 97.66 clicks/second), tracings were averaged prior to deconvolving. On the averaged tracing, the SP and AP were marked. The SP was marked as the first positive shift in amplitude immediately following stimulus onset on the shoulder of the AP (around the 1.0 ms

mark), and the AP was marked as the peak positive shift in amplitude immediately following the stimulus onset (around the 1.5 ms mark). The baseline tracings were compared to each of the averaged time-blocked recordings to determine if the presence or absence of CBBN modulates the response of the auditory nerve, specifically the SP and AP amplitude away from the baseline response, for the various rates and times of ECochG presentation. A one-way repeated measures ANOVA was run for each rate to evaluate the effect of both time and noise on the SP amplitude as well as the AP latency. Additionally, a three-way repeated measures ANOVA will be run to compare the interaction effects between the variables (rate x time x noise) for the AP amplitude.

RESULTS

All participants (n=30; 16 females and 14 males) evaluated in this study had within normal middle ear function. Furthermore, pure-tone audiometric data revealed hearing thresholds ≤ 20 dB HL for all participants with averages shown in Figure 2. Lastly, all participants had MEMR to CBBN present ≥ 70 dB HL in both right (M = 81 dB HL, SD = 7.234) and left (M = 81.5 dB HL, SD = 7.544) ears.

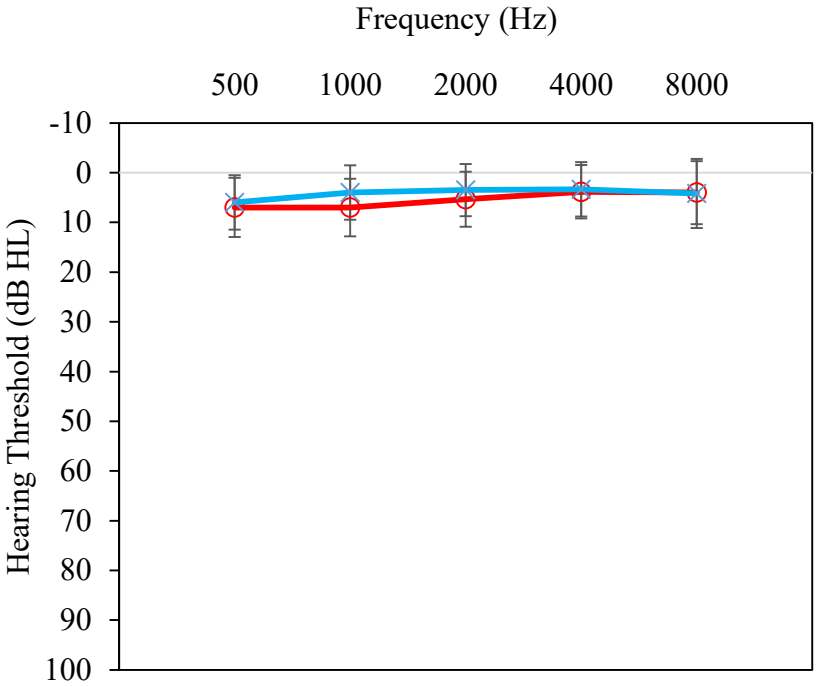

Figure 2. Mean pure-tone thresholds for all participants (n=30) from 500 – 8000 Hz for both the left and right ears. No response exceeding 20 dB HL. The error bars represent standard deviation (±1 SD).

Compound Action Potential Response Amplitude

The results of this study showed that an AP was successfully recorded in 16 females and 14 males (n=30) for all three rates that were utilized (11.1, 58.59, and 97.66 clicks/second).

Furthermore, the amplitude of the AP was modulated based on the presence of CBBN, the time of presentation, and the rate of presentation. Figure 3 shows recording blocks from one of the participants in the study (M09) showcasing the enhancement effect observed in the presence of CBBN and across presentation times for the three rates (11.1, 58.59, and 97.66 clicks/second). The AP response amplitude increased from 1.53 µV to 1.7 µV (11.1% enhancement) at onset, 1.55 µV to 1.73 µV (11.6% enhancement) at the 3-minute mark, 1.5 µV to 1.86 µV (24% enhancement) at the 6-minute mark, and 1.66 µV to 1.96 µV (18.1% enhancement) at the 8-minute mark for the 11.1 clicks/second rate. These tracings effectively showed enhancement in the presence of CBBN as well as a greater degree of enhancement when comparing the onset and 6-minute and the onset and 8-minute presentation time.

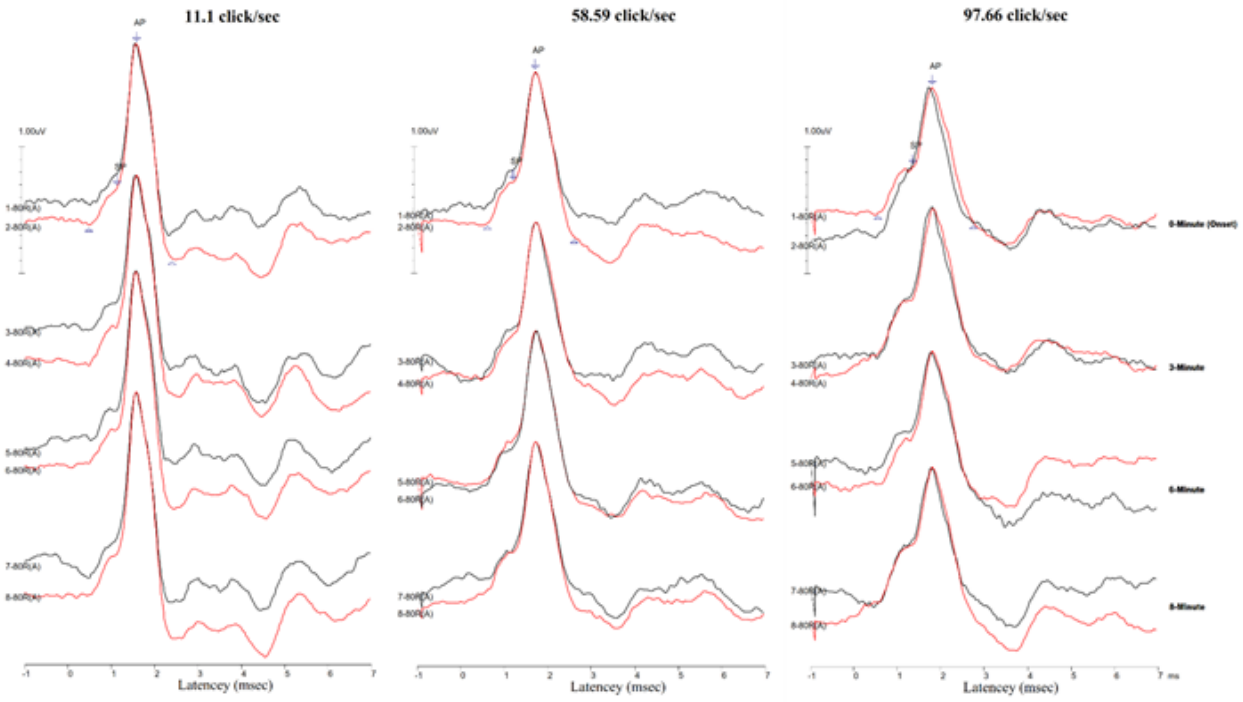

Figure 3. Tracings from participant M09 at 11.1, 58.59, and 97.66 clicks/second presentation rate from left to right, respectively. Tracings in black were recorded without the presence of CBBN, while tracings in red were recorded with CBBN present. The top two tracings for all rates indicate the "onset" recording with the 3-minute, 6-minute, and 8-minute time-blocked recordings, respectively, falling below.

Statistical analysis using a 2x4x3 repeated measures ANOVA was utilized to determine the effects of CBBN presence, time of presentation, and rate of presentation on the AP amplitude, as well as the interaction effects between the three. There was a significant difference in AP amplitude responses in the "No CBBN" condition (baseline) compared to the "With CBBN" condition [F (1,29) = 12.094, $p < 0.01$, $\eta^2 = 0.294$] when comparing all baselines to "With CBBN conditions" across rates and presentation times. Figure 4 showed enhancement from 1.278 µV AP amplitude in the without CBBN condition to 1.337 µV AP amplitude in the with CBBN condition.

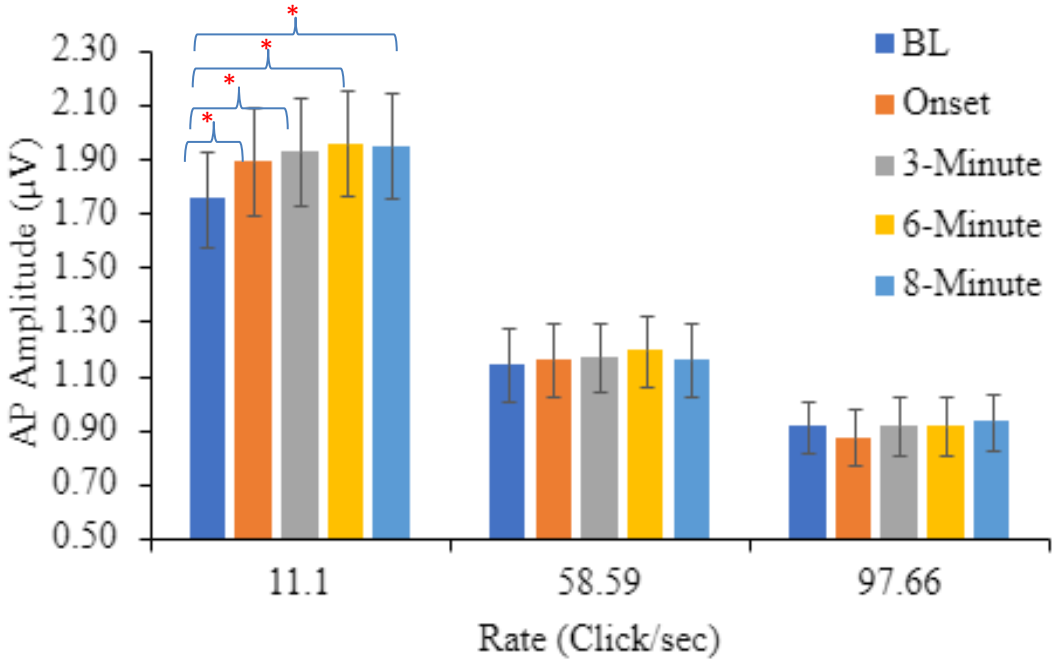

Figure 4. The effect of CBBN on AP amplitude for 11.1, 58.59, and 97.66 clicks/second rates for baseline (BL) and each subsequent CBBN presentation time: onset, 3-minute, 6-minute, and 8-minute. The error bars represent standard error [*p< 0.05; ±1 SE].

We used 2x4x3 repeated measures ANOVA to investigate the effect of rate on AP amplitude. Mauchly's test revealed that the assumption of sphericity was violated [χ^2= 30.298, p

< 0.01], so the Greenhouse-Geisser estimate (ε = 0.846) was used to correct the degrees of freedom. The results shown in Figure 4 demonstrate the significant effect of rate of presentation on the AP amplitude [F (1.204,34.916) = 69.711, $p < 0.01$, $\eta^2 = 0.706$]. As the presentation rate increased from 11.1, 58.59, and 97.66 clicks/second, AP amplitude decreased from 1.864 μV, 1.154 μV, and 0.904 μV, respectively. Post-hoc analysis using the Least Significant Difference (LSD) test showed a significant difference between the 11.1 and 58.59 clicks/second condition ($p < 0.01$), the 11.1 and 97.66 clicks/second condition ($p < 0.01$), and the 58.59 and 97.66 clicks/second condition ($p < 0.01$).

We also investigated the effect of CBBN over time. A 2x4x3 repeated measures ANOVA revealed a significant main effect of time of recording on the AP amplitude [F (3,87) = 3.672, $p = 0.015$, $\eta^2 = 0.112$]. Post-hoc analysis using LSD showed no significant difference when comparing the onset and 3-minute presentation time ($p = 0.137$), but there was a significant difference when comparing the onset and 6-minute ($p < 0.01$) and the onset and 8-minute presentation time ($p = 0.02$).

The 2x4x3 repeated measures ANOVA revealed a significant two-way interaction between the presence of CBBN and the rate of presentation [F (2,58) = 12.037, $p < 0.01$, $\eta^2 = 0.293$], shown in Figure 5. When comparing the effect of CBBN on average response amplitude, the 1.794 μv, 1.138 μv, and 0.901 μv AP amplitude responses increased to 1.933 μv, 1.170 μv, and 0.907 μv for the 11.1 clicks/second, 58.59 clicks/second, and 97.66 clicks/second rates, respectively. With an increase in presentation rate, there was a decrease in the amount of enhancement seen.

The 2x4x3 repeated measures ANOVA revealed no significant interaction between the presence of CBBN and the time of presentation [F (3,87) = 0.727, $p = 0.538$]. Though

enhancement was seen in the "with CBBN" condition compared to the "without CBBN" condition for all presentation times, the amount of AP amplitude enhancement with CBBN did not significantly change between the presentation times (Figure 6). Furthermore, the results revealed no significant interaction between the rate of presentation and the time of presentation [F (6,174) = 0.510, p = 0.510]. Figure 7 showed this significant difference between rates, but also shows no significant difference in the amplitude response between the presentation times for each presentation rate. Finally, the results revealed no significant three-way interaction [F (6,174) = 1.28, p = 0.269].

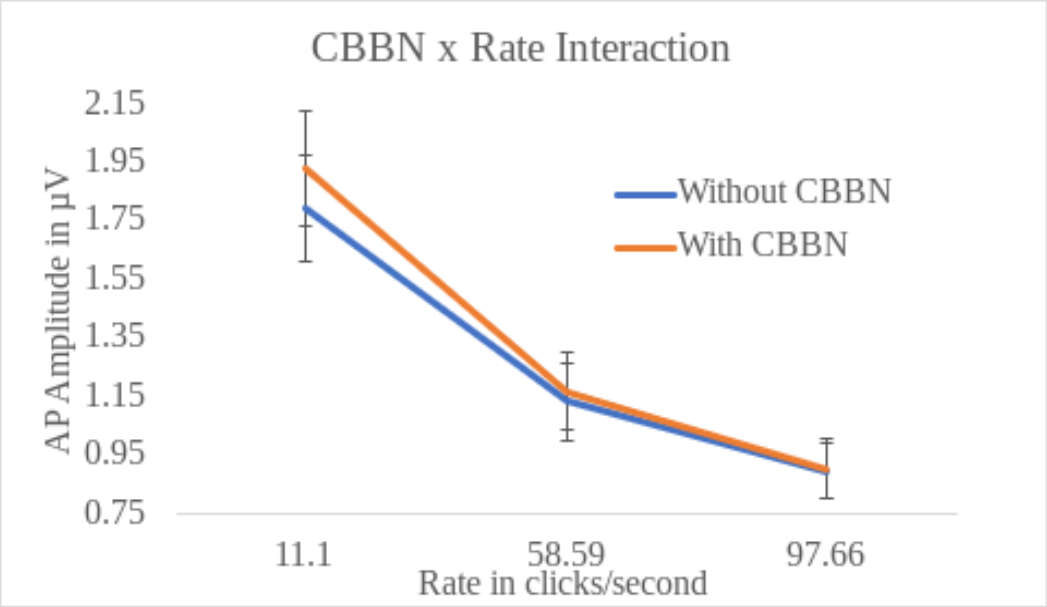

Figure 5. The effect of both rate and CBBN noise on AP amplitude response. This figure shows averaged AP amplitude with and without CBBN across three rate conditions. An enhancement effect can be seen in the "With CBBN" condition for all three rates, with the greatest effect seen at 11.1 clicks/second. The amount of enhancement decreases with increasing presentation rate, indicating a significant interaction effect between the presence of CBBN and the rate of stimulus presentation [$p < 0.01$]. The error bars represent standard error (±1 SE).

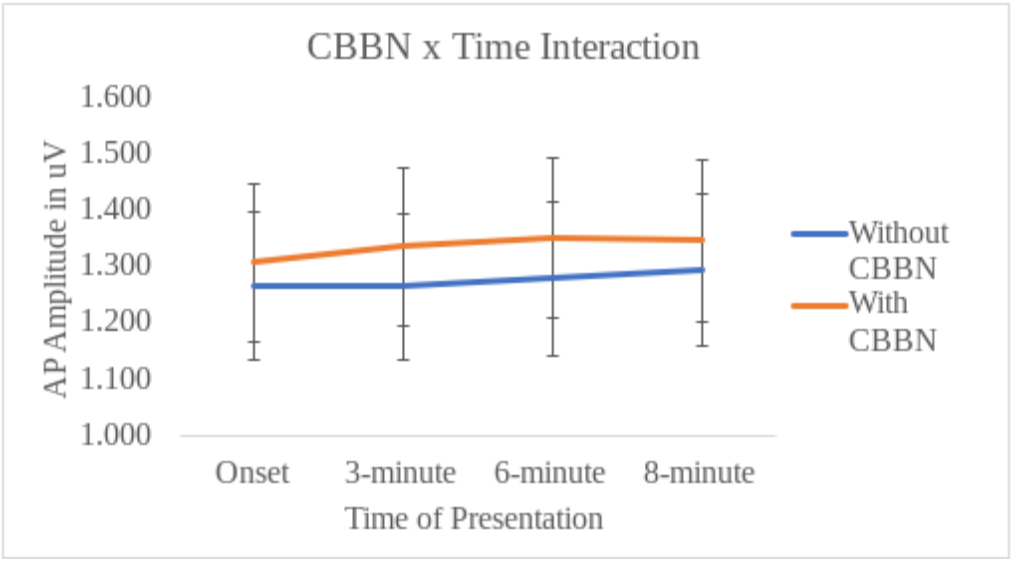

Figure 6. The effect of both the presence of CBBN and time of presentation on AP amplitude response. This figure shows enhancement of the AP amplitude in the "with CBBN" condition compared to the "without CBBN" condition across presentation times. Enhancement can be seen at all presentation times, but there is no statistically significant difference in the amount of enhancement between presentation times [p = 0.538]. The error bars represent standard error (±1 SE).

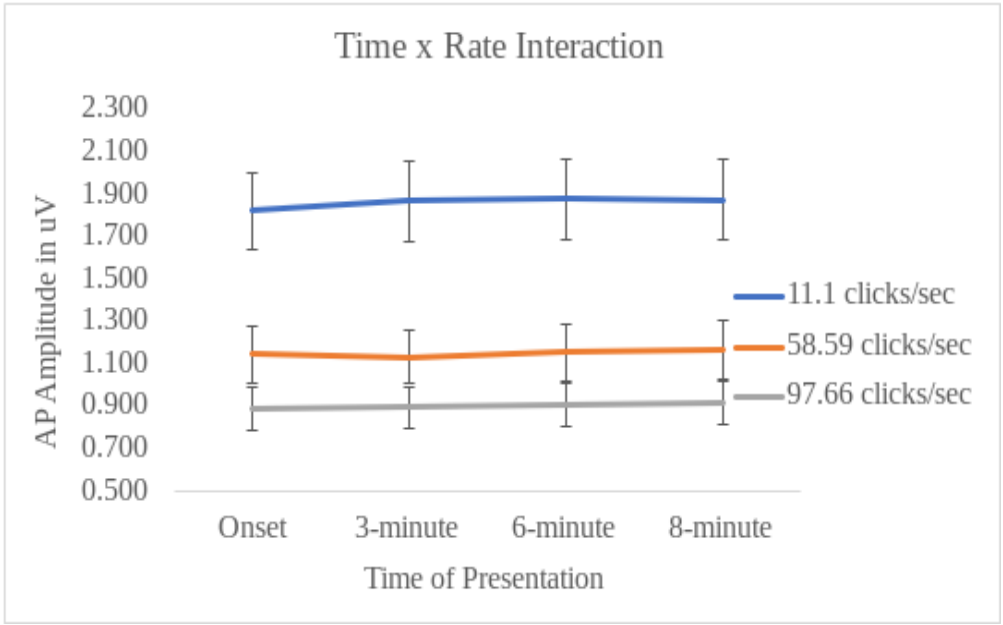

Figure 7. The effect of both rate and presentation time on AP amplitude. This figure shows a statistically significant decrease in AP amplitude with increasing presentation rate [p < 0.01]. There is no statistically significant difference in amplitude response between presentation times for each rate [*p* = 0.510]. The error bars represent standard error (±1 SE).

Compound Action Potential Response Latency

We investigated the effect of presenting CBBN over time on the AP latency using a 2x4 repeated measures ANOVA for each rate. At 11.1 clicks/second, the results revealed no significant main effect of presenting CBBN [F (1,29) = 0.970, p = 0.333], no significant main effect of time of CBBN presentation [F (3,87) = 0.756, p = 0.522], and there is no significant two-way interaction [F (3,87) = 0.632, p = 0.597]. Similarly, at 58.59 clicks/second, the results revealed no significant main effect of presenting CBBN [F (1,29) = 0.124, p = 0.727] and no significant main effect of time of CBBN presentation [F (3,87) = 2.549, p = 0.061]. Finally, the 97.66 clicks/second rate showed no significant main effect of presenting CBBN [F (1,29) = 2.719, p = 0.110] and no significant main effect of time of CBBN presentation [F (3,87) = 1.324, p = 0.272].

Summating Potential Response Amplitude

Statistical analysis using a one-way ANOVA was utilized to determine the effect of CBBN and timing of CBBN presentation on the SP amplitude for the slow presentation rate (11.1 clicks/seconds). There was a significant difference in SP amplitude in the "With CBBN" condition compared to the "Without CBBN" condition [F (1,29) = 20.498, $p < 0.01$, $\eta^2 = .414$] at all presentation times. Averaged SP amplitude was enhanced from 0.303 µV in the "without CBBN" condition to 0.354 µV in the "with CBBN" condition (Figure 8). Furthermore, the SP amplitudes increased from 0.279 µV, 0.310 µV, 0.312 µV, and 0.310 µV, to 0.357 µV, 0.358 µV, 0.355 µV, and 0.345 µV for the onset, 3-minute, 6-minute, and 8-minute time mark, respectively (Figure 8). However, there were no significant differences noted when comparing the SP amplitudes at the various presentation times [F (3,87) = 0.409, p = 0.747]. Additionally,

there was no interaction effect between the CBBN and time of presentation [F (3,87) = 0.703, p = 0.553].

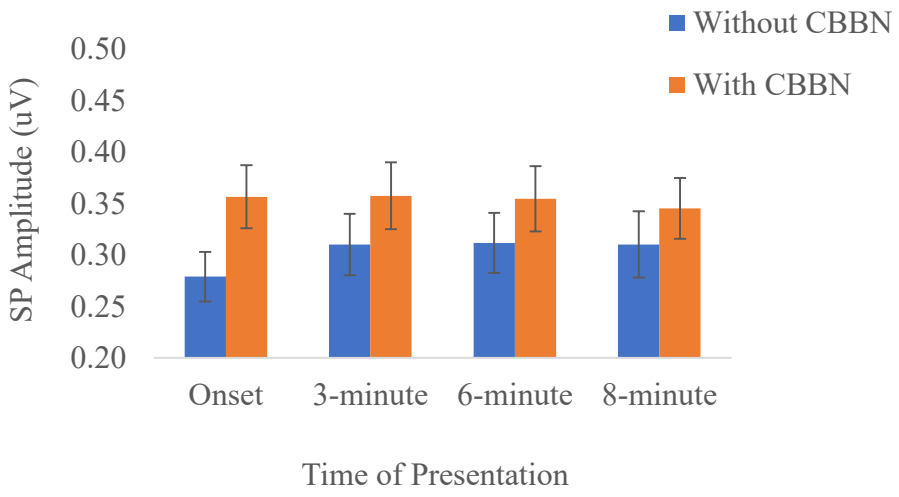

Figure 8. The effect of presentation time and presence of CBBN on SP amplitude response. While the presence of CBBN significantly enhances the SP amplitude [$p < 0.01$] at all presentation times, there is no significant modulation of SP amplitude response between the various presentation times regardless of the presence or absence of CBBN. The error bars represent standard error (±1 SE).

DISCUSSION

There are many known effects of the efferent system on cochlear afferent activity. However, the pattern and functional purpose of these modulatory effects is not well-understood. As demonstrated in Figure 4, this study showed the presence of CBBN functioned to enhance AP response in young adult participants with normal hearing.

CBBN Effect on AP Amplitude

The current study found consistent statistically significant enhancement rather than suppression. In 23 out of the 30 participants, enhancement was seen at each of the four time-blocked recordings at the 11.1 clicks/second rate. Previous studies have shown stimulation of the auditory efferent pathway can either suppress or enhance various cochlear responses. A study conducted by Najem et al. (2016) demonstrated a rather consistent suppression (with some enhancement) of tone-pip CAP response to moderate level (30 – 40 dB HL) contralateral stimulus; however, modulation of click-evoked CAP response was highly variable, which was attributed to the difference in contralateral stimuli. The current study used a moderate level CBBN stimulus while the study conducted by Najem et al. (2016) utilized various intensity levels of pure-tone contralateral stimuli to observe various modulatory responses. It is possible that the widened frequency band coverage of the CBBN played a part in showing the consistent enhancement effect while the specific, narrow, pure-tone contralateral stimuli showed variation in modulatory responses of click-evoked CAP. Another study by Lichtenhan et al. (2016) described a suppressive effect of CBBN on click-evoked AP measured at moderate stimulus levels (52-60 dB peSPL), which was attributed to the MOC effect. It must be noted that the click

level used in the current study is much higher level (80 dB nHL) compared to the level used in Lichtenhan et al. study (52-60 dB peSPL). This may suggest a difference in the efferent system effect on CAP measured to moderate vs high presentation level. Liberman et al. (1990) investigated the efferent innervation in the cat cochlea and were able to distinguish if the efferent fibers synapsed to the pillar or modiolar side of the IHCs. Liberman et al. found that in the apical region of the cochlea, there were nearly three times as many modiolar synapses compared to pillar synapses, and efferent innervation to the IHCs themselves were pillar-heavy in the apex and modiolar-heavy in the basal portion of the cochlea. It is worth noting that Liberman (1982) showed high spontaneous rate nerve fibers synapse to the pillar side of the inner hair cells while low spontaneous rate nerve fibers synapse the modiolar side of the hair cells. The heavy presence of modiolar innervation, known to have a low spontaneous firing rate, coupled with the known LOC innervation of various cochlear structures centered around the IHC, could indicate why there is a discrepancy in the results of the current study, which used a high-level click stimulus and Lichtenhan et al., (2016), which used a moderate level click stimulus. Results described by Lichtenhan et al. (2016) and Najem et al. (2016) were attributed to activation of the MOC reflex. It cannot be ruled out that the MOC reflex played a role in the enhancement effect seen in the current study. However, LOC fibers primarily innervate the type I afferent fibers responsible for the AP responses that were measured (Liberman and Liberman, 2019). Interestingly, Groff and Liberman (2003), showed that direct electrical stimulation of the LOC via the inferior colliculus produced CAP enhancement that lasted anywhere from 5 to 20 minutes following stimulation. The aforementioned study also showed that this long-lasting enhancement was not present after ablation of the olivocochlear bundle but could still be observed when ablating the MOC pathway (Groff and Liberman, 2003). This further suggests the LOC is likely the primary source of the

enhancement observed in the current study, given there was a greater degree of enhancement observed when comparing the onset and 6-minute and the onset and 8-minute time blocks (Figure 4). The greater degree of enhancement noted at the later recorded responses in the current study again supports the theory that the LOC, rather than the MOC, is responsible for the observed enhancement response. Groff and Liberman (2003) further elaborate that CGRP as well as acetylcholine are the likely neurotransmitters responsible for this enhancement.

However, one key theoretical finding not observed in the present study was the presence of the "slow" LOC modulation effect. As stated above, a modulation of the CAP response attributed to the LOC was noted in guinea pigs with enhancement present for anywhere from 5 – 20 minutes post-electrical stimulation and suppression present up to 5 minutes post-electrical stimulation (Groff and Liberman, 2003). However, Eyebalin (1993) theorizes the purpose of the dopaminergic LOC neurotransmitter sub-group is to interrupt the glutamate activity within synaptic clefts, therefore reducing the firing of the auditory nerve. Those dopaminergic LOC fibers are the smaller sub-group of LOC fibers, making up only 10-25% of all LOC fibers while prominently innervating the basal portion of the mouse cochlea (Darrow et al., 2006b). These fibers were also found to be slightly overlapped with the cholinergic sub-group of LOC fibers. Groff and Liberman (2003) theorized the "slow" effect was a later-seen combination of cholinergic and dopaminergic neurotransmitters causing a variation in response modulation post-stimulus. However, as previously mentioned, Groff and Liberman (2003) theorized the coexistence of CGRP and acetylcholine are responsible for the enhancement effect evoked by the LOC neurons; they further theorized the excitatory pathways and neurotransmitters may essentially over-ride the inhibitory pathways in a normal mammalian ear, leading to an essentially enhanced response. Nonetheless, given that the research paradigm of this particular

study only evaluated AP responses 8 minutes post-CBBN activation, and modulation of CAP responses in guinea pigs were seen up to 20 minutes post-stimulation, it is possible that the full scope of the LOC modulation effect was not seen.

CBBN with Different Stimulus Rates

It is well documented that increasing the rate of the click-evoked stimulus will decrease the amplitude and increase the latency of the AP response (Kaf et al., 2017; Lake and Stuart, 2019). Slower stimulation rates allow for a higher degree of neural synchrony, meaning auditory nerve fibers can fire together more effectively. Increasing the rate of stimulation allows us to see the neural adaptation present in the auditory nerve; meaning, the increased firing rate, or "stress" on the auditory nerve does not allow it to fire to its full potential. While the current study showed significant enhancement with CBBN present that increased at the 6-minute and 8-minute time block, the amount of enhancement also decreased with increasing the presentation rate (Figure 5). The physiological or functional purpose behind this finding is unclear. However, Eyebalin (1993) theorized that the purpose of the cholinergic transmitters of the LOC function to increase the release of glutamate within the auditory nerve, therefore increasing the firing rate. It is possible that increasing the stimulus rate leads to significant increase in release of glutamate that puts a physiological limit on the LOC's cholinergic transmitters' ability to increase the release of glutamate; therefore, limiting the amount of enhancement seen with increasing rate. Furthermore, the neural adaptation of the auditory nerve that is known to occur with higher stimulus presentation rates could also be hindering the amount of enhancement seen; meaning, it cannot be ruled out that enhancement is limited by the stress induced by the increased firing rate of the auditory nerve rather than LOC neurotransmitter involvement.

CBBN Effect on SP Amplitude

A secondary finding was observed in the current study that is a worth noting; the effect of CBBN on SP amplitude. As seen in Figure 8, there was a significant enhancement of SP amplitude in the presence of CBBN compared to without CBBN for all time conditions. This finding agrees with studies performed on the cat cochlea, where an increase in the summating potential was found when electrically stimulating the contralateral OCB (Carlier and Pujol, 1976). It cannot be overlooked that the MOC could contribute to this increase in summating potential. However, the LOC is a possible source of the increased amplitude effects. Yet, there was no significant difference in SP amplitude between the various time conditions. A recent study by Pappa et al. (2019) showed the OHCs, IHCs, and auditory nerve contribute to the SP within the gerbil cochlea. This study found that the IHCs overall contributed to an essentially positive shift in SP polarity, the OHCs contributed to an essentially negative shift in SP polarity, and AN input was variable across intensity and frequencies (Pappa et al., 2019). Due to the positive shift in polarity seen from the IHCs in the gerbil cochlea, and the known innervation of the LOC in the human cochlea (Liberman and Liberman, 2019), it is possible the LOC could have played a role in the enhancement seen, as a significant amount of LOC fibers innervate the Type 1 auditory nerve fibers. However, due to the complex physiologic nature and somewhat mysterious origin of the human SP, a true conclusion regarding this finding cannot be made.

Study Limitations

This study yielded several interesting findings. However, due to the unknown nature of the efferent system, particularly the LOC, further investigation is warranted to grasp a more robust understanding of this subsect of human audition. The hallmark “slow” effect of the LOC

system was unable to be identified in the present study. However, given that previous research has seen LOC effects up to approximately 20 minutes in guinea pigs (Groff and Liberman, 2003), it is possible that the time-block paradigm used in the research design did not allow for the full extent of the LOC effect to be seen. A longer time-block paradigm could be utilized in future research design in an attempt to observe this effect. Furthermore, it would be interesting to expand upon the current study by using various contralateral stimuli, as some of the participants' responses did not enhance in the presence of CBBN. As noted earlier, a previous study showed significant suppression of CAP response to contralateral tone-pip stimuli (Najem et al., 2016). It would be interesting to utilize a time-block paradigm with various contralateral stimuli to see if this combination of factors would reveal significant findings. Finally, the SP is a relatively understudied, less understood subsect of electrocochleography responses. Focusing on the SP in future studies could open many doors when it comes to further understanding the human efferent system.

CONCLUSION

While the true physiological function of the LOC in humans is unknown, it is theorized to assist with protection from acoustic trauma, as well as to assist in the localization of sound. The purpose of this study was to attempt to observe the effect of the LOC on the human afferent auditory system through acoustic stimulation. Additionally, this study attempted to isolate the "fast" vs. "slow" effect of the LOC that previous studies observed via electrical stimulation (Groff and Liberman, 2003) by using a 10-minute time-blocked paradigm in the presence of CBBN. This study showed statistically significant enhancement of the CAP response when comparing the onset and 6-minute and onset and 8-minute time-block. Additionally, this study found that the amount of CAP enhancement decreased with increasing stimulus rate, and it also showed a significant enhancement of the SP response in the presence of CBBN in all time conditions. Though the hallmark "slow" effect was not observed in this study, the enhancement effect of the CAP seen when comparing the onset vs. the 6-minute and 8-minute time-blocks, as well as the decrease in enhancement with increasing rate can likely be attributed to the LOC rather than the MOC. Further research is recommended utilizing a longer time-block to attempt to evoke the slow-effect of the LOC using acoustic stimulation. However, it does appear that while MOC effect cannot be completely ruled out, measurement of CAP response in the presence of CBBN can be utilized to observe the LOC effect on the human auditory system.

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APPENDIX: IRB APPROVAL CERTIFICATE

Date: 4-13-2022

IRB #: IRB-FY2021-262
Title: LOC and Electrocochleography
Creation Date: 10-26-2020
End Date:
Status: Approved
Principal Investigator: Abdullah Jamos
Review Board: MSU
Sponsor:

Study History

Submission Type	Review Type	Decision
Initial	Expedited	Approved

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