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Altered brainstem auditory evoked potentials in a rat central sensitization model are similar to those in migraine

Xianghong Arakaki[1], **Gary Galbraith**[2], **Victor Pikov**[3], **Alfred N. Fonteh**[1], and **Michael G. Harrington**[1]

[1]Molecular Neurology Program, Huntington Medical Research Institutes, 99 North El Molino Avenue, Pasadena, CA 91101

[2]Mental Retardation Research Center, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California at Los Angeles, USA

[3]Neural Engineering Program, Huntington Medical Research Institutes, 734 Fair mount Avenue, Pasadena, CA 91101

Abstract

Migraine symptoms often include auditory discomfort. Nitroglycerin (NTG)-triggered central sensitization (CS) provides a rodent model of migraine, but auditory brainstem pathways have not yet been studied in this example. Our objective was to examine brainstem auditory evoked potentials (BAEPs) in rat CS as a measure of possible auditory abnormalities. We used four subdermal electrodes to record horizontal (h) and vertical (v) dipole channel BAEPs before and after injection of NTG or saline. We measured the peak latencies (PLs), interpeak latencies (IPLs), and amplitudes for detectable waveforms evoked by 8, 16, or 32 KHz auditory stimulation. At 8 KHz stimulation, vertical channel positive PLs of waves 4, 5, and 6 (vP4, vP5, and vP6), and related IPLs from earlier negative or positive peaks (vN1-vP4, vN1-vP5, vN1-vP6; vP3-vP4, vP3-vP6) increased significantly 2 hours after NTG injection compared to the saline group. However, BAEP peak amplitudes at all frequencies, PLs and IPLs from the horizontal channel at all frequencies, and the vertical channel stimulated at 16 and 32 KHz showed no significant/consistent change. For the first time in the rat CS model, we show that BAEP PLs and IPLs ranging from putative bilateral medial superior olivary nuclei (P4) to the more rostral structures such as the medial geniculate body (P6) were prolonged 2 hours after NTG administration. These BAEP alterations could reflect changes in neurotransmitters and/or hypoperfusion in the midbrain. The similarity of our results with previous human studies further validates the rodent CS model for future migraine research.

Corresponding authors: Xianghong Arakaki and Michael G. Harrington, Molecular Neurology Program, Huntington Medical Research Institutes, 99 North El Molino Avenue, Pasadena, CA 91101. xianghong@hmri.org, mghworks@hmri.org,. Phone 626 795 4343; fax 626 795 5774.

1. Introduction

Migraine affects more than 30 million people in the United States who suffer from episodes lasting hours to days (Mueller, 2007). The symptoms include: severe headache, nausea, cognitive impairment, and discomfort from normal light, sound, and smells (Buse et al., 2012;Charles and Brennan, 2010;Goadsby et al., 2009;Noseda and Burstein, 2013;Woodhouse and Drummond, 1993). The migraine pathophysiology is not fully elucidated, though several brain circuits have been implicated (Goadsby et al., 2009;Noseda and Burstein, 2013). Auditory symptoms are prominent in migraine and are the focus of this study; they include fluctuating low-frequency hearing loss, sudden deafness, auditory hallucinations, tinnitus, and phonophobia. Significant phonophobia is often found in interictal migraine and worsened during ictal migraine compared to controls (Ashkenazi et al., 2009;Vingen et al., 1998).

Approaches to investigate auditory function include basic audiological evaluation such as initial otoscopic examination, standard pure tone audiometry, speech audiometry, tympanometry, distortion product otoacoustic emission, and others (Hamed et al., 2012). It is hard to measure the auditory function behaviorally in the rat, but it can be readily measured by evoked potentials (EPs) *in vivo*. Auditory EPs include the following three categories based on their response latencies (Quian, 2006), all of which have been found changed in migraine:

Early EPs include the electrocochleogram and brainstem auditory evoked potentials (BAEPs). The electrocochleogram has latencies of less than 2.5 ms and reflects responses from the auditory nerve and cochlea. BAEPs reflect responses from the brainstem with latencies less than 12 ms (Quian, 2006). Studies by Dash et al suggest that BAEP abnormality may be the earliest auditory indication in migraine since all migraineurs with auditory symptoms have prolonged peak latencies (PLs) and/or interpeak latencies (IPLs) in BAEP recordings (Dash et al., 2008). Studies in children with headache, however, did not find significant BAEP changes between migraineurs and controls (Unay et al., 2008).

Middle latency EPs have latencies between 12 and 50 ms. Middle latency auditory evoked potentials lacked auditory sensory gating in migraine patients, results that were considered to stem from a hypofunction of monoaminergic subcortico-cortical connections (Ambrosini et al., 2001).

Late, or cortical, EPs have latencies between 50 and 250 ms. Auditory evoked cortical potentials show potentiation or lack of habituation in interictal migraineurs instead of the habituation found in healthy controls, and the intensity dependence of auditory evoked cortical potentials is higher in migraineurs (Afra et al., 2000;Ambrosini et al., 2003). The lack of habituation of auditory evoked potentials is thought to be due to a decreased pre-activation level of the sensory cortex (Afra et al., 2000;Coppola et al., 2009;Wang and Schoenen, 1998).

Nitroglycerin (NTG)-induced central sensitization (CS) in rodents (Harrington et al., 2011;Tassorelli et al., 1996;Tassorelli et al., 2005) is a widely accepted migraine model. Besides triggering migraine in humans (Olesen, 2010), NTG causes hyperalgesia and

allodynia (Harrington et al., 2011;Oshinsky and Luo, 2006), photophobia and meningeal dilatation in mice (Markovics et al., 2012); cFos expression in the trigeminal pathway and brain sodium elevation after NTG injection (Harrington et al., 2011) in rats. Many of the symptoms were reversed by sumatriptan (Bates et al., 2010;Ramachandran et al., 2012;Read et al., 1999). The overall aim of our study is to determine whether BAEPs in this CS model have similar changes to those in migraine, and whether they add objective and functional information to help in understanding the early sensory changes in this migraine model. For this purpose, we measured the BAEP peaks, PLs, IPLs, and amplitudes in rats that were given an intraperitoneal (ip) injection of either saline or NTG.

The rodent auditory system is more dominant than the visual system, and is easier to test (Martin et al., 2006;Yang et al., 2008;Yang and Zador, 2012). Rodent BAEPs, similar to those in humans, have a series of waves that reflect the synchronous short-latency synaptic activity along the brainstem auditory pathway. These 5~6 positive peaks are thought to represent the activity in the following auditory processing centers: auditory nerve (P1), posterior anteroventral cochlear nucleus (AVCN) (P2), anterior AVCN and cells of the contralateral medial nucleus of the trapezoid body (P3), bilateral medial superior olivary nuclei (P4), lateral lemniscus and/or inferior colliculus (P5), and the more rostral structures such as the medial geniculate body (P6) (Henry, 1979;Parham K. et al., 2001;Shaw, 1988).

2. Results

Examples of horizontal and vertical channel BAEPs at 8, 16, and 32 KHz stimulation frequencies are shown in Figure 1. The BAEP measurements were similar in shape and amplitude to those recorded by other laboratories (Galbraith et al., 2006;Jirka et al., 1985;Liu et al., 2011;Rice et al., 2011). As shown in Figure 1, BAEP horizontal channel P1 and P2 from all frequencies were clear. However, BAEP vertical channel peaks P5 and P6 at 16 KHz stimulation and vertical peaks P3–P6 at 32 KHz stimulation were missing. Thus only a subset of BAEP peaks was discernible and analyzed. At 8 KHz stimulation, horizontal channel P1 and P2 and vertical channel N1, P2~6 were studied (Fig.1A). At 16 KHz stimulation, horizontal channel P1 and P2 and vertical channel N1, P2~4 were measured (Fig.1B). At 32 KHz stimulation, horizontal channel P1 and P2 and vertical channel N1 and P2 were studied (Fig.1C).

The PLs and IPLs from NTG and saline groups at different frequency stimulations were compared in detail in the following paragraphs. Briefly, later PLs and related IPLs significantly increased in the NTG group compared with the saline group, but only at 8 KHz in the vertical channel. The NTG or saline treatment/time interaction was significant ($p = 0.001$); therefore, the drug effect was tested at each time individually.

In saline-treated rats at 8 KHz stimulation, horizontal channel hP1-hP2 latencies and vertical channel vN1 and vP2-vP6 latencies were slightly increased with time, but were not significantly different from the baseline (Fig. 2A, Fig. 3, Table 1). The hPL2 in the NTG group was decreased 1 hour after injection as well as before the NTG injection ($p < 0.05$) (Table 1). In the vertical channel, vPL2 was decreased in the NTG group compared with that in the saline group (Fig. 3A). Later peaks in the NTG group, specifically peak absolute

latencies of vP4-6 were increased ($p < 0.05$ or $p<0.01$) at different time points after injection (mostly 2 hours after injection) compared to that in the saline group (Fig. 2, Fig. 3B, 3C, 3D).

At 8 KHz stimulation, we also calculated the IPLs from the PLs in the NTG and saline groups. The IPLs of the NTG group from vN1 to vP4, vP5, and vP6 were increased 2 hours after injection compared to that in the saline group ($p < 0.05$, Fig. 4A, 4C, 4E). The IPLs in the NTG group from vP3 to vP4 and vP6 were also prolonged significantly (Fig. 4B, 4F).

At 16 KHz auditory stimulation (Table 2), horizontal channel hP1-2 latencies and vertical channel vN1 and vP2-vP4 latencies were slightly increased with time in the saline group, but not significantly. The hPL2 in the NTG group was decreased at 2 hours after injection ($p < 0.01$) compared to that in the saline group. In the vertical channel, vN1 and vP2-4 absolute latencies in the NTG group were not significantly different from that in the saline group.

At 32 KHz stimulation (Table 3), horizontal channel hP1-hP2 latencies and vertical channel vN1 and vP2 latencies were slightly increased with time for the saline group. The hPL2 in the NTG group was increased at 2 hours after injection ($p < 0.05$) compared to that in the saline group. In the vertical channel, neither PLs nor IPLs were significantly different between the NTG and saline groups.

In addition, when comparing the more reliable (larger amplitude) 8 and 16 KHz-responses, our data also confirm that some BAEP peak latencies evoked by lower frequency (8 KHz) auditory stimulation (activating more apical hair cells in the cochlear) are slightly longer than responses evoked by higher frequency (16 KHz) stimulation (activating more basal hair cells) (Tables 1 and 2). For example, the baseline horizontal PL1 (hPL1) in the saline group was 0.78 ± 0.09 at 8 KHz stimulation and 0.69 ± 0.05 at 16 KHz stimulation ($p < 0.01$). The baseline hPL2 of the saline group was 1.68 ± 0.06 at 8 KHz stimulation and 1.56 ± 0.06 at 16 KHz ($p < 0.01$). However, the longer-latency peaks did not follow the same pattern. For example, the baseline vPL4 in the saline group was 3.19 ± 0.16 at 8 KHz stimulation and 3.22 ± 0.15 at 16 KHz ($p = 0.95$).

In horizontal channels, hPL2 changes were not consistent across different frequency auditory stimulations. During 8 KHz stimulation, hPL2 decreased before and at 1 hour after NTG injection ($p < 0.05$) (Table 1); during 16 KHz stimulation, hPL2 increased at 2 hours after NTG injection ($p < 0.05$) (Table 2); and during 32 KHz stimulation, hPL2 decreased at 2 hours after NTG injection ($p < 0.01$) (Table 3).

3. Discussion

BAEPs are a non-invasive and sensitive measure of central nervous system functions that have been used to identify changes in different physiologies and in a wide variety of disorders. A role for estrogen is suggested since female BAEP P1 and P3 amplitudes, wave 5 latency and IPL 3–5 decrease across the menstrual cycle (Tasman et al., 1999). Probably from a smaller head size, females have slightly shorter latencies of later BAEP waves and greater amplitude than males (Stone et al., 2009). To exclude both factors (estrogen and head size), only male rats were used in these studies. The click BAEP waves P1, P3, and P5

absolute latencies, but not IPLs (P1–3, 3–5, or 1–5), are significantly increased with age, indicating a peripheral rather than brainstem origin of the absolute latency delay (Stone et al., 2009). On the other hand, BAEPs are sensitive to brainstem lesions from tumor, metabolic disorders, and vascular dysfunction (Davis et al., 1985;Drake, Jr. et al., 1990;Sand et al., 2008;Stone et al., 2012). BAEPs are also used for intraoperative monitoring during surgery in the brainstem area (Acioly et al., 2010), and can indicate coma prognosis (Balogh et al., 2001). Recordings in Down syndrome were found to have significantly shorter latencies of P3 and P5 and IPL 3–5 and 1–5, which indicated an input modulation defect at the brainstem level (Seidl et al., 1997). Similar findings to our results were found in people with vertebrobasilar transient ischemic attacks (Drake, Jr. et al., 1990), notable since hypoperfusion occurs in migraine (Sanchez del Rio et al., 2000).

Auditory discomfort is often found in interictal migraine and worsened during ictal migraine. The interictal sound aversion threshold for 1000, 4000, and 8000 Hz stimulation was significantly lower than that of controls and decreased further during ictal migraine (Ashkenazi et al., 2009). BAEPs are altered in migraine, especially the later PL5 and IPL P1 to P5 (Bussone et al., 1985;Firat et al., 2006;Kochar et al., 2002). Clinical studies of short and long-latency auditory EPs indicate that phonophobia may be more dependent on brainstem than cortical mechanisms: results suggested a subcortical pontomesencephalic involvement as indicated by the later BAEP wave changes in migraine (Sand and Vingen, 2000).

To our knowledge, the BAEPs have not been evaluated in the rat NTG-triggered migraine model. This study provides evidence that the later BAEP peak latencies increase in a rat CS model upon auditory stimulation, changes consistent with findings in migraine (Bussone et al., 1985;Firat et al., 2006;Kochar et al., 2002). At 8 KHz stimulation, the later PLs (vPL4-6) and IPLs (IPLs from vN1 to vP4-6 or IPLs of vP3 to vP4, vP3 to vP6) increase 2–4 hours after NTG injection, corresponding with the time of the allodynia from von -Frey hair testing, increased cFos expression in the TCC, and cranial sodium elevation in this rat CS model (Harrington et al., 2011;Tassorelli et al., 1996;Tassorelli et al., 2005). Our study provides the first data to support this rodent CS model for future studies of auditory neuronal excitability analogous to the auditory discomfort in human migraine.

BAEP changes can reflect changes in neurotransmitter levels. In rats, high doses of carbachol (1 ~ 5 mg/kg) significantly increased the BAEP wave P3 and P4 peak amplitudes and latencies, while lower doses (0.1 ~ 0.5 mg/kg) decreased both latencies and amplitudes; these observations indicate cholinergic activity is involved in the BAEPs (Bhargava et al., 1978). BAEP wave 3, 4, and 5 latencies were also prolonged with reserpine in rat, suggesting a monoamine involvement including norepinephrine and serotonin (Morales-Martinez et al., 2002), and P3 and P4 amplitudes were decreased with serotonin (5-HT) activation (Bhargava and McKean, 1977).

The changes we found in the 8 KHz later peak latencies compare well with the data from the human midbrain, where the PL5 and IPL 3–5 increase in migraine (Sand et al., 2008). Our findings were consistent with brainstem monoamine and cholinergic dysfunction or related hypoperfusion, supported by the following studies from human and animals. In migraineurs,

reserpine significantly prolonged the latency of BAEP wave 5, which indicates involvement of monoamine signaling in rostral brainstem dysfunction (Bank, 1991). In our study we observed a similar increase in the wave 5 latency, suggesting the involvement of monoamines. This hypothesis is further supported by the reported changes in 5-HT metabolism (Sand et al., 2008). The brainstem 5-HT regulates glutamatergic presynaptic thalamocortical neurons, thus the 5-HT disorder might dysregulate these thalamocortical neurons and affect cortical excitability (Scruggs et al., 2000;Torres-Escalante et al., 2004). Studies also suggest that abnormal monoamines including serotonin and norepinephrine in the brainstem contribute to increased cortical excitability or cortical arousal in migraine (Bjork and Sand, 2008;Kropp and Gerber, 1993). Besides monoamines, the ascending pontine and mesencephalic cholinergic system also contribute to cortical arousal: reduced cholinergic activity in the brainstem and basal forebrain projection could cause thalamocortical disconnection and increase θ and β electroencephalographic activity in wakefulness (Bjork and Sand, 2008;Llinas and Steriade, 2006). Thus, the “thalamo-cortical dysrhythmia” resulting from abnormal monoamine and cholinergic transmission observed in studies of spontaneous EEG, visual, and somatosensory evoked potentials could cause the lack of habituation reported in interictal migraine and contribute to increased cortical arousal or cortical hyperexcitability in migraine (Bjork and Sand, 2008;Coppola et al., 2005;Coppola et al., 2007;Restuccia et al., 2013). The cortical hyperexcitability in migraine could also be caused by decreased intracortical lateral inhibition as shown by visual EP studies (Coppola et al., 2007;Coppola et al., 2013). Besides monoamines, our results could also reflect hypoperfusion similar to the BAEP changes in ischemia (Drake, Jr. et al., 1990).

Consistently, the rat model has early elevation of norepinephrine (1 h) in the pons, which could result from the autonomic response to NTG, followed by a late decrease of 5-HT (4 h) in the pons and medulla (Tassorelli et al., 2002). In the rat model, both *in vivo* and *in vitro* proton MR spectroscopy demonstrate an elevation of choline containing compounds in the thalamus (Ma et al., 2008). Brainstem choline levels were not studied in this model, probably because of the resolution limit. Since either monoamine or cholinergic changes can cause the aforementioned abnormal BAEP latencies of later waves, our results could reflect any combination of monoamine and/or cholinergic variations in the brainstem affecting neurotransmission or by causing ischemia in this rat CS model (Bjork and Sand, 2008). At this stage, we do not have direct evidence of the connection between our data and neurotransmitter dysfunction and/or hypoperfusion in the midbrain. Pharmacological studies and vascular flow monitoring will be of interest in the future to elucidate the connection.

In addition, our recent studies suggest the Na^+-K^+-ATPase at the blood-brain barrier may be more labile in migraineurs (Arakaki et al., 2013;Harrington et al., 2010), and this transporter is modulated by neurotransmitters such as serotonin. Brain alpha2 type Na^+-K^+-ATPase activity was significantly increased by 5-HT (Antonelli et al., 1998), thus an additional role for Na^+-K^+-ATPase involvement in the rat model requires further study.

It is interesting that many of the changes in peak or interpeak latencies in the NTG group were observed 2 hours post-administration. This is in line with the finding that, in humans, migraine induced by NTG occurs hours after the vasodilatory effects have worn off (Christiansen et al., 1999), and that hyperalgesia in rodents hits its peak 2–4 hours post-NTG

(Tassorelli et al., 2003). It will be of interest to explore whether the BAEP changes could be reversed or alleviated by anti-migraine drugs, such as sumatriptan.

In addition to altered BAEPs, brainstem dysfunction in migraine has been implicated by recent PET studies (Afridi et al., 2005;Demarquay et al., 2011;Weiller et al., 1995): even though the dorsal pontine activation was ipsilateral to the pain location, the phonophobia was bilateral (Afridi et al., 2005), perhaps as a result of the multiple connections crossing in the brainstem above the cochlear nucleus (Vingen et al., 1998).

The BAEP latency and amplitude are affected by temperature changes as small as 1 °C (Janssen et al., 1991). In our experiment, this was not a significant confounding factor, since the rectal temperature was kept at 36.9 ~ 37 °C. The BAEP latency and amplitude are also affected by injectable anesthetics, such as pentobarbital and ketamine (Church and Gritzke, 1987;Shapiro et al., 1984), and inhalant anesthesia such as isoflurane (Santarelli et al., 2003). We avoided the confounding effect from isoflurane anesthesia by maintaining the same concentration for both saline and NTG groups.

When comparing the larger amplitude 8 and 16 KHz responses, the early peak latencies were longer for higher frequency stimulation based on the hair cell locations, while later peak latencies do not follow the same rule (results and Table 1 and 2). The reason for these results might be that there are more synaptic activities involved in the auditory transduction after the cochlea nucleus, and these might normalize the later peak latencies from different frequency stimulations.

As shown in the horizontal channels, hPL2 changes were not consistent across different frequency auditory stimulations. There might be several explanations for the observed inconsistencies: the rats were perhaps not well matched for this particular parameter, since the hPL2 was significantly different before the injection. In addition, early BAEP peaks contributed by the auditory nerve and cochlear nucleus could exhibit more variability than later peaks.

We recorded the lowest auditory stimulation intensity that could evoke a visible EP as the BAEP threshold. BAEP thresholds do not demonstrate any significant differences between saline and NTG-treated groups (data not shown) in contrast to observations in migraine. The reason could be either our rats are under anesthesia, or the discomfort threshold is more accurate from the cortical level, or both. BAEP amplitudes also do not demonstrate any significant differences between saline and NTG groups (data not shown). This, at least partially, could be due to high variability in BAEP amplitudes among animals, since they are more affected by background noise and are thus less reliable than PLs (Stone et al., 2009).

One limit of this study is that BAEPs are related to the brainstem auditory structures rather than the cortical or subcortical circuits. To further investigate the involvement of brain circuits in migraine, it will be necessary to study mid-latency (P13) or cortical auditory evoked potentials (Miyazato et al., 2000;Stienen et al., 2006). Detection of P5 and P6 at 32 KHz stimulation is difficult using subdermal recording, thus epidural or subdural implanted electrodes may be necessary for future studies. Multiple testing such as was done in this analysis increases the likelihood of a type 1 error, therefore it is likely that some of the

associations we report are spurious. However, this was an exploratory study undertaken to develop hypotheses for more rigorous work; thus, we are less concerned with false findings than we would be if this was a confirmatory study (Bender and Lange, 2001). If the very conservative Bonferroni adjustment is applied to this analysis, each comparison would have been tested at the 0.0083 level (0.05/6).

4. Materials and Methods

4.1. Animal preparation

Male Sprague Dawley rats (n = 18, 170~250 g) from Harlan were used in these studies and all procedures were approved by the Institutional Animal Care and Use Committees at Huntington Medical Research Institutes (HMRI) in Pasadena, CA. Rats were anesthetized under 2% isoflurane for less than 2 minutes, then maintained at 1~2% isoflurane. Body temperature was maintained throughout at 37 ± 0.1°C with a homeothermal blanket and a rectal probe (Harvard Apparatus, Holliston, MA). The respiratory signal was provided by a respiration sensor taped to the animal's chest, connected to a high sensitivity differential pressure transducer TSD160A, and monitored by MP150 via a DA100C amplifier (both from Biopac System Inc., Goleta, CA). Breathing rate was kept within 20% from the baseline by adjusting the isoflurane level.

4.2. Study design

BAEPs were measured as previously described (Galbraith et al., 2006). Briefly, four subdermal needle electrodes were used. Two were positioned at the mastoid behind and at the base of each pinna (horizontal channel), and two on the scalp midline located symmetrically on either side of the inter-aural line (vertical channel). A ground electrode was inserted in the left front paw. After baseline measurements for 30–60 minutes, either nitroglycerin (NTG) (10 mg/kg) or the same volume of saline were administered by intraperitoneal (i.p.) injection. BAEPs were recorded immediately after, and 30, 60, 90, 120, 180, 240 minutes after injection. Stimulation frequencies were 16, 32, and 8 KHz, in that order, and each BAEP series was collected with a gap of at least 5 minutes between the recordings. Data collection and analysis were performed while blinded to the drug treatment.

4.3. Data collection and averaging of BAEPs

The acoustic stimulus consisted of a 16 KHz, 32 KHz, or 8 KHz tone pip, 1 ms duration, with 0.5 ms linear rise/fall amplitude programmed by an arbitrary waveform generator (Model 630, BNC, San Rafael, CA). Stimulus repetition rate was 20 s^{-1}. Sound from a high frequency transducer (Intelligent Hearing Systems, Miami, FL) was delivered monaurally via a 22 cm air tube placed into the left ear canal. The acoustic delay in the air tube was 1 ms, which guaranteed that sound arrived at the ear canal exactly at the termination of the stimulus electrical artifact. Peak stimulus intensity was 80 dB. The rat BAEPs were triggered by a TTL stimulus from an MP 150. Signals of BAEPs from two channels were collected by two P511 amplifiers (Grass Technologies, Warwick, RI) with a gain of 200 k, and band pass filtered at 100–10,000 Hz. Data were digitized at a rate of 50 kHz by MP150.

Horizontal and vertical channel BAEP signals were averaged by the MP150 in real-time from 1000 recordings each time. Averaged BAEP waveforms were saved for offline analysis.

4.4. Offline data analysis and statistics

Because the 8 KHz stimulation fell within the amplifier bandpass, while the other two frequency stimulation (16 KHz and 32 KHz) were outside, the BAEP data at 8 KHz stimulation were low-pass filtered by performing fast Fourier transform (FFT), removing the electromagnetic noise above 7 kHz and then performing inverse FFT. The low-pass filtering was done using a custom program written in Visual Basic 6.0 (Microsoft).

The BAEP peak latencies and amplitudes were measured in IGOR (Wavemetrics, Lake Oswego, OR). The IPLs were calculated in Excel (Microsoft Corporation, Redmond, WA). The BAEP PLs, IPLs, and amplitudes of discernable peaks in Figure 1 were measured. Each latency was measured from the end of the stimulus (time zero when the auditory stimulus arrived at the ear) to the time of the corresponding peak. Each amplitude was measured as the voltage difference between the baseline and the corresponding peak. Data were calculated as mean ± SD (standard deviation). For each time point the saline and NTG groups were compared using a student t-test in Excel™ and Prism (GraphPad Software Inc., La Jolla, CA).

The interaction between NTG/saline injection and time of BAEP measures was tested by including injection and time as main effects along with the injection/time interaction effect in a mixed linear model to account for repeated measurements from the same animal. Comparisons at individual time points were tested using t tests. There were no adjustments for multiple testing.

5. Conclusion

Two hours after i.p. injection of NTG, the rats exhibited longer latencies at the putative bilateral medial superior olivary nuclei (P4), the lateral lemniscus and/or inferior colliculus (P5), and more rostral structures such as the medial geniculate body (P6) at 8 KHz auditory stimulation. From previous animal and human studies involving the same locations and approach as our BAEP studies, these results could reflect changes in neurotransmitters and/or hypoperfusion in the brainstem during NTG-triggered CS.

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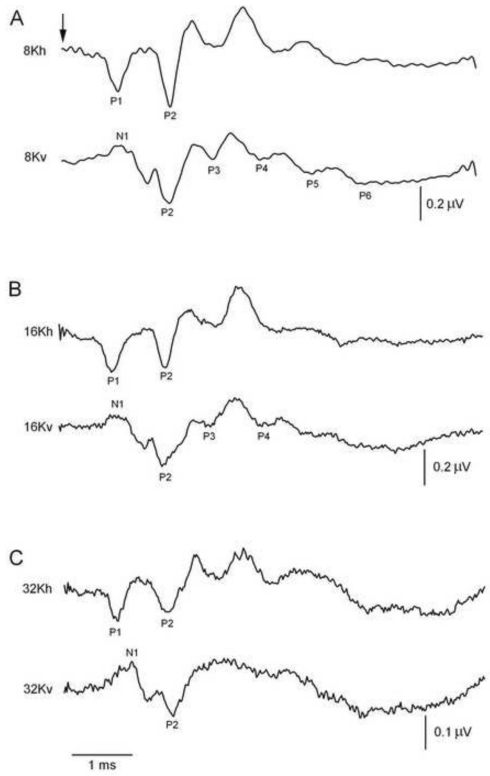

Fig. 1.

Representative horizontal and vertical BAEPs from a single rat resulting from different auditory stimulations. A, sample BAEPs from horizontal (8Kh) and vertical (8Kv) channel with 8 KHz stimulation. B, sample BAEPs from horizontal (16Kh) and vertical (16Kv) channel with 16 KHz stimulation. C, sample BAEPs from horizontal (32Kh) and vertical (32Kv) channel with 32 KHz stimulation. Auditory stimulation arrived at the ear at the beginning of each recording indicated by the arrow (the initial 1ms of stimulus electrical artifact is deleted from the graphs). Recordings from each frequency stimulation have their own vertical scale bar on their bottom right, while all recordings share the same horizontal scale on the bottom.

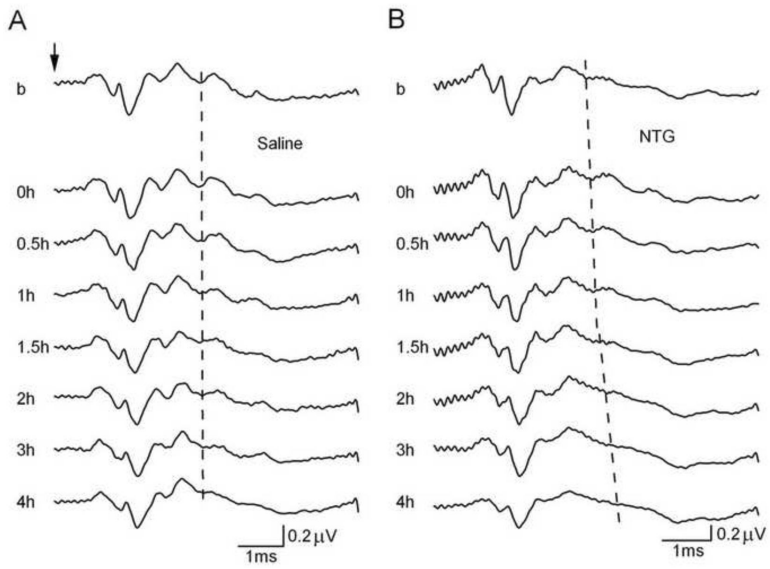

Fig. 2.

The BAEPs from saline or NTG injected rats. A. BAEPs from a single representative rat at baseline (b) and at different time points after injection of saline. B. BAEPs from a single representative rat at baseline (b) and at different time points after injection of NTG. Dotted lines indicate P4. Scale bars are shown on bottom right of each panel.

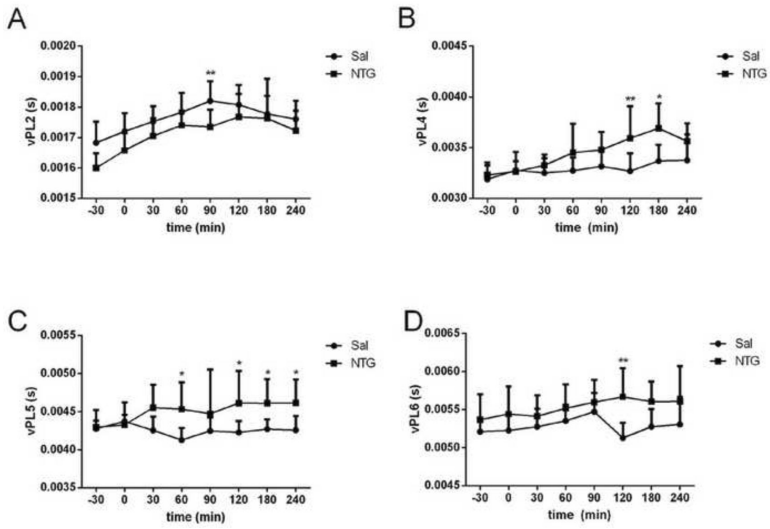

Fig. 3.

Group BAEP peak latency (PL) comparisons between saline and NTG treated rats. The BAEP peak latencies induced by 8 KHz auditory stimulation were contrasted between NTG (filled squares) and saline (filled circles) injection. The vP2 latency (A) decreased in NTG rats 90 minutes after injection ($p < 0.01$). The vP4 (B), vP5 (C), and vP6 (D) latencies increased significantly in NTG rats at different time points after injection. *, $p < 0.05$, **, $p < 0.01$, $n = 8$ per group.

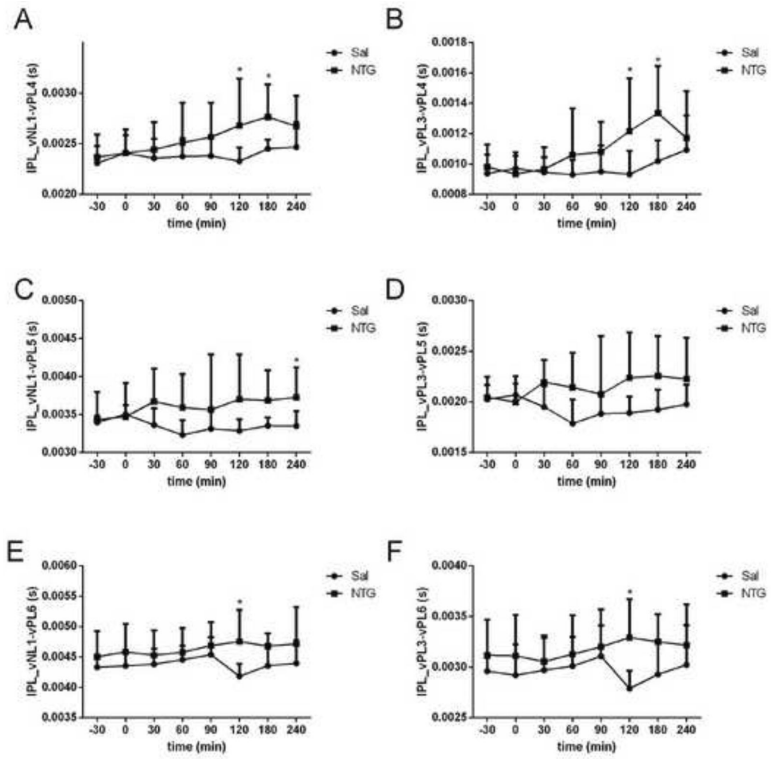

Fig. 4.

Group BAEP inter-peak latency (IPL) comparisons between saline and NTG treated rats. The BAEP vertical channel inter-peak latencies (vIPLs) induced by 8 KHz auditory stimulation were contrasted between NTG (filled squares) and saline (filled circles) injection. The IPLs from vNL1 to vPL4 (A), to vPL5 (C), and to vPL6 (E), as well as the IPLs from vPL3 to vPL4 (B), to vPL5 (D), and to vPL6 (F) increased significantly in NTG rats at different time points after injection. *, $p < 0.05$, $n = 8$ per group.

Table 1

hv PL/IPL at 8 KHz stimulation

	time after injection (min)							
	baseline	**0**	**30**	**60**	**90**	**120**	**180**	**240**
Sal Rats								
hPL1	0.78 ± 0.09	0.81 ± 0.09	0.84 ± 0.10	0.87 ± 0.06	0.88 ± 0.05	0.89 ± 0.06	0.88 ± 0.02	0.88 ± 0.07
hPL2	1.68 ± 0.06	1.71 ± 0.07	1.74 ± 0.06	1.76 ± 0.06	1.76 ± 0.05	1.77 ± 0.04	1.75 ± 0.03	1.76 ± 0.06
hPL1-2	0.89 ± 0.05	0.90 ± 0.04	0.90 ± 0.06	0.89 ± 0.03	0.89 ± 0.02	0.88 ± 0.04	0.88 ± 0.03	0.88 ± 0.06
vNL1	0.88 ± 0.08	0.87 ± 0.08	0.89 ± 0.10	0.90 ± 0.07	0.94 ± 0.08	0.94 ± 0.07	0.92 ± 0.10	0.91 ± 0.08
vPL2	1.68 ± 0.07	1.72 ± 0.06	1.75 ± 0.05	1.78 ± 0.06	1.82 ± 0.07	1.81 ± 0.07	1.78 ± 0.06	1.76 ± 0.06
vPL3	2.25 ± 0.14	2.31 ± 0.12	2.31 ± 0.12	2.34 ± 0.12	2.37 ± 0.14	2.34 ± 0.15	2.35 ± 0.15	2.28 ± 0.11
vPL4	3.19 ± 0.16	3.28 ± 0.18	3.25 ± 0.15	3.27 ± 0.13	3.32 ± 0.16	3.27 ± 0.17	3.37 ± 0.16	3.38 ± 0.25
vPL5	4.28 ± 0.11	4.37 ± 0.09	4.26 ± 0.18	4.13 ± 0.16	4.25 ± 0.18	4.23 ± 0.15	4.27 ± 0.13	4.26 ± 0.19
vPL6	5.21 ± 0.15	5.23 ± 0.24	5.28 ± 0.24	5.35 ± 0.21	5.47 ± 0.24	5.13 ± 0.20	5.28 ± 0.23	5.31 ± 0.36
vNL1-vPL2	0.81 ± 0.13	0.85 ± 0.12	0.86 ± 0.13	0.89 ± 0.12	0.89 ± 0.11	0.87 ± 0.12	0.86 ± 0.12	0.85 ± 0.11
vNL1-vPL3	1.37 ± 0.17	1.44 ± 0.15	1.41 ± 0.15	1.45 ± 0.16	1.43 ± 0.15	1.40 ± 0.16	1.43 ± 0.13	1.38 ± 0.13
vNL1-vPL4	2.31 ± 0.17	2.41 ± 0.18	2.36 ± 0.19	2.38 ± 0.18	2.38 ± 0.17	2.33 ± 0.14	2.45 ± 0.09	2.47 ± 0.25
vNL1-vPL5	3.40 ± 0.09	3.50 ± 0.12	3.36 ± 0.22	3.23 ± 0.19	3.31 ± 0.23	3.29 ± 0.15	3.35 ± 0.11	3.35 ± 0.20
vNL1-vPL6	4.33 ± 0.20	4.36 ± 0.24	4.38 ± 0.25	4.46 ± 0.23	4.54 ± 0.29	4.19 ± 0.20	4.36 ± 0.29	4.40 ± 0.38
vPL3-4	0.94 ± 0.12	0.97 ± 0.11	0.95 ± 0.10	0.93 ± 0.09	0.95 ± 0.17	0.93 ± 0.15	1.02 ± 0.14	1.09 ± 0.23
vPL3-5	2.03 ± 0.14	2.07 ± 0.11	1.95 ± 0.21	1.79 ± 0.24	1.88 ± 0.21	1.89 ± 0.16	1.92 ± 0.20	1.98 ± 0.20
vPL3-6	2.96 ± 0.13	2.92 ± 0.31	2.97 ± 0.32	3.01 ± 0.29	3.11 ± 0.30	2.79 ± 0.17	2.93 ± 0.32	3.02 ± 0.39
NTG Rats								
hPL1	0.78 ± 0.18	0.84 ± 0.18	0.86 ± 0.18	0.90 ± 0.16	0.91 ± 0.15	0.94 ± 0.15	0.91 ± 0.17	0.95 ± 0.18
hPL2	**1.61 ± 0.04** *	1.67 ± 0.04	1.70 ± 0.04	**1.70 ± 0.03** *	1.72 ± 0.02	1.75 ± 0.04	1.73 ± 0.10	1.72 ± 0.08
hPL1-2	0.82 ± 0.17	0.83 ± 0.17	0.84 ± 0.17	0.81 ± 0.15	0.81 ± 0.15	0.81 ± 0.15	0.82 ± 0.15	0.77 ± 0.14
vNL1	0.86 ± 0.16	0.86 ± 0.18	0.88 ± 0.18	0.94 ± 0.14	0.91 ± 0.19	0.91 ± 0.18	0.93 ± 0.11	0.89 ± 0.17
vPL2	1.60 ± 0.05	1.66 ± 0.06	1.71 ± 0.06	1.74 ± 0.05	**1.74 ± 0.06** **	1.77 ± 0.08	1.76 ± 0.13	1.72 ± 0.07
vPL3	2.25 ± 0.13	2.33 ± 0.09	2.36 ± 0.11	2.39 ± 0.10	2.40 ± 0.13	2.38 ± 0.12	2.36 ± 0.15	2.39 ± 0.16
vPL4	3.23 ± 0.10	3.27 ± 0.10	3.33 ± 0.10	3.45 ± 0.29	3.48 ± 0.18	**3.59 ± 0.31** **	**3.69 ± 0.24** *	3.56 ± 0.18
vPL5	4.30 ± 0.23	4.33 ± 0.29	4.55 ± 0.30	**4.53 ± 0.35** *	4.47 ± 0.58	**4.61 ± 0.43** *	**4.61 ± 0.32** *	**4.62 ± 0.31** *
vPL6	5.37 ± 0.33	5.44 ± 0.36	5.41 ± 0.27	5.52 ± 0.31	5.60 ± 0.29	**5.67 ± 0.38** **	5.61 ± 0.26	5.61 ± 0.46
vNL1-vPL2	0.74 ± 0.17	0.80 ± 0.20	0.82 ± 0.21	0.80 ± 0.15	0.83 ± 0.21	0.86 ± 0.23	0.84 ± 0.18	0.83 ± 0.19
vNL1-vPL3	1.39 ± 0.22	1.47 ± 0.21	1.48 ± 0.24	1.45 ± 0.13	1.49 ± 0.21	1.46 ± 0.23	1.43 ± 0.12	1.50 ± 0.24
vNL1-vPL4	2.37 ± 0.23	2.41 ± 0.23	2.44 ± 0.27	2.51 ± 0.40	2.57 ± 0.34	**2.68 ± 0.46** *	**2.77 ± 0.32** *	2.67 ± 0.30
vNL1-vPL5	3.43 ± 0.37	3.47 ± 0.45	3.67 ± 0.44	3.59 ± 0.44	3.56 ± 0.73	3.70 ± 0.59	3.69 ± 0.40	**3.73 ± 0.40** *
vNL1-vPL6	4.51 ± 0.42	4.59 ± 0.46	4.53 ± 0.41	4.58 ± 0.40	4.69 ± 0.38	**4.76 ± 0.53** *	4.68 ± 0.21	4.72 ± 0.61
vPL3-4	0.98 ± 0.15	0.94 ± 0.12	0.97 ± 0.14	1.06 ± 0.31	1.08 ± 0.20	**1.22 ± 0.35** *	**1.34 ± 0.31** *	1.17 ± 0.31
vPL3-5	2.05 ± 0.20	2.00 ± 0.26	2.20 ± 0.22	2.14 ± 0.34	2.08 ± 0.58	2.24 ± 0.45	2.26 ± 0.40	2.23 ± 0.41
vPL3-6	3.12 ± 0.35	3.11 ± 0.41	3.06 ± 0.26	3.13 ± 0.39	3.20 ± 0.37	**3.29 ± 0.38** *	3.25 ± 0.27	3.22 ± 0.40

Horizontal and vertical channel BAEP peak latencies (PLs) and inter-peak latencies (IPLs) at 8 KHz auditory stimulation (mean ± SD) (n = 8 each group).

hPL1: horizontal channel P1 latency; hPL2: horizontal channel P2 latency; hPL1-2: horizontal channel IPL from hP1 to hP2; vNL1: vertical channel N1 latency; vPL(2 to 6): vertical channel P(2 to 6) latency; vNL1-vPL (2 to 6): vertical channel IPL from vN1 to vP(2 to 6); vPL3-(4 to 6): vertical channel IPL from vP3 to vP(4–6).

* $p < 0.05$,

** $p < 0.01$.

Table 2

hv PL/IPL at 16 KHz stimulation

	time after injection (min)							
	baseline	**0**	**30**	**60**	**90**	**120**	**180**	**240**
Sal Rats								
hPL1	0.69 ± 0.05	0.72 ± 0.06	0.73 ± 0.06	0.76 ± 0.04	0.77 ± 0.05	0.78 ± 0.03	0.79 ± 0.03	0.77 ± 0.07
hPL2	1.56 ± 0.06	1.60 ± 0.06	1.62 ± 0.04	1.63 ± 0.04	1.63 ± 0.05	1.62 ± 0.04	1.65 ± 0.03	1.64 ± 0.06
hPL1-2	0.87 ± 0.05	0.88 ± 0.04	0.88 ± 0.06	0.87 ± 0.05	0.86 ± 0.06	0.84 ± 0.05	0.86 ± 0.03	0.87 ± 0.07
vNL1	0.82 ± 0.12	0.82 ± 0.10	0.85 ± 0.11	0.87 ± 0.11	0.88 ± 0.11	0.84 ± 0.15	0.85 ± 0.10	0.86 ± 0.09
vPL2	1.57 ± 0.11	1.62 ± 0.10	1.68 ± 0.08	1.66 ± 0.07	1.68 ± 0.10	1.68 ± 0.11	1.67 ± 0.07	1.64 ± 0.07
vPL3	2.12 ± 0.15	2.19 ± 0.13	2.22 ± 0.11	2.24 ± 0.12	2.28 ± 0.08	2.24 ± 0.09	2.17 ± 0.11	2.16 ± 0.10
vPL4	3.22 ± 0.15	3.19 ± 0.14	3.24 ± 0.15	3.26 ± 0.15	3.25 ± 0.22	3.38 ± 0.18	3.41 ± 0.32	3.36 ± 0.17
vNL1-vPL2	0.75 ± 0.20	0.80 ± 0.17	0.83 ± 0.17	0.80 ± 0.14	0.81 ± 0.12	0.84 ± 0.21	0.82 ± 0.12	0.78 ± 0.12
vNL1-vPL3	1.30 ± 0.22	1.37 ± 0.16	1.37 ± 0.15	1.38 ± 0.15	1.40 ± 0.13	1.40 ± 0.18	1.33 ± 0.13	1.30 ± 0.13
vNL1-vPL4	2.40 ± 0.15	2.37 ± 0.13	2.39 ± 0.12	2.39 ± 0.13	2.38 ± 0.23	2.54 ± 0.17	2.56 ± 0.27	2.50 ± 0.13
vPL3-4	1.10 ± 0.19	1.00 ± 0.12	1.02 ± 0.11	1.02 ± 0.09	0.98 ± 0.23	1.14 ± 0.14	1.24 ± 0.31	1.20 ± 0.18
NTG Rats								
hPL1	0.71 ± 0.18	0.73 ± 0.18	0.78 ± 0.17	0.82 ± 0.15	0.84 ± 0.15	0.88 ± 0.18	0.84 ± 0.15	0.83 ± 0.17
hPL2	1.52 ± 0.03	1.56 ± 0.04	1.60 ± 0.05	1.62 ± 0.05	1.65 ± 0.05	**1.68 ± 0.07** *	1.67 ± 0.05	1.64 ± 0.07
hPL1-2	0.81 ± 0.17	0.83 ± 0.18	0.83 ± 0.15	0.80 ± 0.13	0.81 ± 0.15	0.81 ± 0.21	0.83 ± 0.15	0.81 ± 0.13
vNL1	0.80 ± 0.14	0.81 ± 0.14	0.86 ± 0.13	0.86 ± 0.16	0.87 ± 0.17	0.88 ± 0.14	0.86 ± 0.15	0.84 ± 0.09
vPL2	1.52 ± 0.05	1.54 ± 0.04	1.62 ± 0.08	1.64 ± 0.08	1.63 ± 0.07	1.69 ± 0.08	1.66 ± 0.07	1.66 ± 0.11
vPL3	2.15 ± 0.11	2.14 ± 0.13	2.22 ± 0.14	2.27 ± 0.15	2.33 ± 0.14	2.35 ± 0.18	2.23 ± 0.11	2.29 ± 0.25
vPL4	3.22 ± 0.11	3.21 ± 0.11	3.35 ± 0.11	3.41 ± 0.19	3.43 ± 0.25	3.43 ± 0.20	3.59 ± 0.40	3.47 ± 0.31
vNL1-vPL2	0.72 ± 0.13	0.74 ± 0.17	0.77 ± 0.14	0.78 ± 0.20	0.77 ± 0.18	0.81 ± 0.18	0.80 ± 0.20	0.82 ± 0.19
vNL1-vPL3	1.35 ± 0.22	1.33 ± 0.20	1.37 ± 0.21	1.41 ± 0.25	1.46 ± 0.20	1.48 ± 0.19	1.37 ± 0.19	1.45 ± 0.30
vNL1-vPL4	2.42 ± 0.14	2.41 ± 0.21	2.50 ± 0.22	2.55 ± 0.31	2.56 ± 0.38	2.55 ± 0.30	2.74 ± 0.47	2.64 ± 0.35
vPL3-4	1.08 ± 0.17	1.08 ± 0.14	1.13 ± 0.16	1.14 ± 0.20	1.10 ± 0.27	1.08 ± 0.27	1.36 ± 0.32	1.18 ± 0.40

Horizontal and vertical channel BAEP PLs and IPLs at 16 KHz auditory stimulation (mean ± SD) (n = 9 each group).

All abbreviations are the same as in Table 1

* $p < 0.05$.

Table 3

hv PL/IPL at 32 KHz stimulation

	time after injection (min)							
	baseline	**0**	**30**	**60**	**90**	**120**	**180**	**240**
Sal Rats								
hPL1	0.72 ± 0.12	0.79 ± 0.15	0.81 ± 0.09	0.86 ± 0.11	0.89 ± 0.13	0.85 ± 0.18	0.84 ± 0.14	0.88 ± 0.24
hPL2	1.61 ± 0.25	1.65 ± 0.18	1.68 ± 0.13	1.70 ± 0.07	1.74 ± 0.18	1.65 ± 0.12	1.67 ± 0.14	1.72 ± 0.11
hPL1-2	0.89 ± 0.15	0.85 ± 0.06	0.87 ± 0.05	0.84 ± 0.05	0.85 ± 0.14	0.80 ± 0.28	0.83 ± 0.06	0.84 ± 0.16
vNL1	0.79 ± 0.13	0.87 ± 0.16	0.87 ± 0.12	0.92 ± 0.10	0.95 ± 0.15	0.82 ± 0.19	0.91 ± 0.19	0.94 ± 0.24
vPL2	1.55 ± 0.08	1.60 ± 0.07	1.66 ± 0.16	1.63 ± 0.10	1.74 ± 0.21	1.63 ± 0.11	1.55 ± 0.09	1.65 ± 0.18
vNL1-vPL2	0.76 ± 0.12	0.74 ± 0.19	0.78 ± 0.20	0.71 ± 0.12	0.80 ± 0.20	0.81 ± 0.18	0.64 ± 0.23	0.72 ± 0.37
NTG Rats								
hPL1	0.71 ± 0.18	0.78 ± 0.18	0.89 ± 0.17	0.87 ± 0.24	0.84 ± 0.19	0.83 ± 0.17	0.81 ± 0.23	0.83 ± 0.15
hPL2	1.55 ± 0.08	1.59 ± 0.07	1.68 ± 0.08	1.64 ± 0.07	1.64 ± 0.05	**1.56 ± 0.08** **	1.64 ± 0.09	1.65 ± 0.06
hPL1-2	0.84 ± 0.13	0.81 ± 0.19	0.79 ± 0.21	0.77 ± 0.22	0.80 ± 0.18	0.73 ± 0.14	0.83 ± 0.21	0.82 ± 0.12
vNL1	0.81 ± 0.19	0.88 ± 0.20	0.91 ± 0.21	0.98 ± 0.25	0.89 ± 0.22	0.86 ± 0.24	0.86 ± 0.25	0.88 ± 0.19
vPL2	1.58 ± 0.09	1.58 ± 0.08	1.63 ± 0.11	1.68 ± 0.14	1.68 ± 0.12	1.55 ± 0.18	1.65 ± 0.07	1.67 ± 0.06
vNL1-vPL2	0.78 ± 0.21	0.70 ± 0.18	0.73 ± 0.22	0.70 ± 0.23	0.79 ± 0.24	0.69 ± 0.30	0.79 ± 0.22	0.79 ± 0.24

Horizontal and vertical channel BAEP PLs and IPL at 32 KHz auditory stimulation (mean ± SD) (n = 8 each group).

All abbreviations are the same as in Table 1.

** $p < 0.01$.