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TITLE: Therapeutic Function of Glucagon-Like Peptide-1 (GLP-1) for Hearing Restoration after Blast Exposure or Traumatic Brain Injury (TBI)

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14. ABSTRACT

The objective of this project is to determine the therapeutic function of Liraglutide (glucagon-like peptide-1 receptor (GLP-1R)) to mitigate the auditory injury after blast exposure in animal model of chinchilla. There are two aims: **Aim 1.** Identify the therapeutics of liraglutide in ameliorating auditory function injuries in both pre- and post-treatments in relation to the blast overpressure (BOP) level or traumatic brain injury (TBI) severity over different time courses. **Aim 2.** Investigate the beneficial effects of liraglutide on the mitigation of the central auditory damage following repetitive exposures to the low BOP or mild TBI. Accomplishments in Year 1 include: 1) therapeutic efficacy of liraglutide (drug) was tested in 4 experimental groups of chinchillas: pre- and post-blast drug treatment animals with both ears open or protected by hearing protection devices (e.g. earplugs) under BOP level of 3-5 psi for 6 blasts; 2) hearing function tests were conducted on Day 1 pre- and post-blast and on Days 4, 7, 14, or 28; 3) immunofluorescence imaging and analysis of chinchilla brain tissues from 4 drug-treated groups and two blast control groups (open and protected) were performed and compared. Results demonstrate that the effect of liraglutide on mitigation of hearing damage in ears with and without protection is different, and particularly the liraglutide affects hearing recovery process after blast exposure.

15. SUBJECT TERMS
Hearing restoration, blast overpressure, auditory dysfunction, therapeutics, liraglutide, hearing function test, hearing protection devices, traumatic brain injury

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1. INTRODUCTION

Hearing damage caused by blast exposure is a frequent and common injury for Service members even at relatively low or mild blast overpressure. To date, there is no therapeutic treatment for blast-induced progressive hearing impairment. The current clinical research on military personnel indicates an increasingly strong correlation between the traumatic brain injuries (TBI) and sensorineural hearing loss, and the blast-induced hearing damage shares similar mechanisms with the TBI-induced memory deficits such as the loss of neurons but in auditory cortex and spiral ganglion. These research findings raise a crucial question: whether neurotrophic drugs offer therapeutic benefits against blast-induced auditory damage that causes cognitive deficits, decreases synaptic plasticity, and leads to neurodegeneration.

The **objective** of this project is to determine the therapeutic function of Liraglutide, the long-acting glucagon-like peptide-1 receptor (GLP-1R), to mitigate the auditory injury after blast exposure in animal model of chinchilla. Liraglutide's neurotrophic and protective activity in cellular and animal models of stroke and TBI has been reported, but the function of the GLP-1R agonist to reduce the blast-induced hearing damage and restore hearing has not been studied. Our proposed studies on Liraglutide's function to restore hearing in chinchillas will provide the first promising candidate therapeutics that promote auditory neural proliferation and reduce cell apoptosis caused by blast overpressure.

To reach the objective and long-term goal of the therapeutic treatment for blast-induced progressive hearing damage, we have a series of tasks under two specific aims to test our **general hypothesis**: hearing damage induced by repeated blast exposures at a low (below mild TBI) or mild TBI level will involve both peripheral and central auditory pathways in chinchillas, and liraglutide treatment will mitigate these abnormalities and restore hearing function.

2. KEYWORDS

Blast overpressure, auditory dysfunction, hearing restoration, therapeutics, liraglutide, hearing protection devices, traumatic brain injury

3. ACCOMPLISHMENTS

● What were the major goals of the project?

The project has two specific aims with 7 tasks over 3 years of funding period.

Aim 1: Identify the therapeutics of liraglutide in ameliorating auditory function injuries in both pre- and post-treatments in relation to the blast overpressure (BOP) level or TBI severity over different time courses.

Task 1-1. To determine therapeutic efficacy of liraglutide in chinchillas repetitively exposed to low BOP (**G1)** level with hearing protection.

Task 1-2. To determine therapeutic efficacy of liraglutide in chinchillas repetitively exposed to low BOP (**G1**) level without hearing protection.

Task 1-3. To determine therapeutic efficacy of liraglutide in chinchillas repetitively exposed to high BOP (**G2**) level causing mTBI with hearing protection.

Aim 2: Investigate the beneficial effects of liraglutide on the mitigation of the central auditory damage following repetitive exposures to the low BOP or mild TBI pressure levels.
Task 2-1. To determine the effect of liraglutide on glutamate and GABA neurotransmitter receptors in the central auditory system.
Task 2-2. To determine the effect of liraglutide on synaptic plasticity changes.
Task 2-3. To examine the efficacy of liraglutide to prevent oxidative stress-induced neuronal loss.
Task 2-4. To investigate mechanisms by which liraglutide offers neuroprotection and neuro-regeneration.

- **What was accomplished under these goals?**

Key Research Accomplishments:

Aim 1:
♦ Blast tests were conducted in 6 groups of chinchillas (liraglutide treated and control) with both ear open and protected by hearing protection devices (HPDs, e.g., earplugs). The animals were exposed to BOP level: 3-5 psi or 21-35 kPa for 6 consecutive blasts.
♦ Hearing function tests including the auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE) and middle latency response (MLR) were performed on Day 1 (pre- and post-blast) and Days 4, 7, 14, and 28, respectively, to determine the effect of liraglutide on mitigation of hearing damage over recovery time length and to identify whether the liraglutide affects the hearing restoration differently between the ears open and protected.

Aim 2:
♦ Chinchilla brain tissue samples from 6 groups of animals were prepared and conducted for immunofluorescence imaging and analysis.
♦ The effect of liraglutide on blast-induced changes in excitatory (glutamate-NMDA) and inhibitory (GABAA) neurotransmitter receptor densities in vulnerable central auditory regions were identified when animals were exposed to repeated low-level blast injury with and without ear protection.

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(1) In this 1st year of the project, the major activities under Aim 1 include:

1) Performing blast tests in two control groups of chinchillas (e.g., without liraglutide treatment) with both ears open (one group) or protected with earplugs (another group) at BOP of 3-5 psi (named as G1 BOP level) for 6 consecutive blasts and conducting hearing function tests on Day 1 (pre- and post-blast) and Days 4, 7, and 14.
2) Performing blast tests in two liraglutide (drug) treatment groups of chinchillas (pre-drug treatment and post-drug treatment) with both ears open or protected by earplugs (i.e., a total of 4 groups) at BOP G1 level for 6 consecutive blasts and conducting hearing function tests over the time course on Day 1 (pre- and post-blast) and on Days 4, 7, 14, and 28.

3) Preparing brain tissues harvested from chinchillas after the function tests on Day 14 or Day 28 and shipping to Dr. Chandra's lab at New Jersey Institute of Technology for histology studies (Aim 2).

The **specific objectives** are: 1) to determine the therapeutic function of liraglutide in mitigation of auditory injury in chinchillas repetitively exposed to low BOP (**G1)** level in the ears with and without hearing protection; 2) to identify if there is a different effect of liraglutide on mitigation of hearing damage in the pre-liraglutide (pre-drug) treatment animals and the post-drug treatment animals over 14 or 28 days of recovery time; 3) to prepare chinchilla brain tissue samples from animals exposed to blasts in two control groups and four drug-treatment groups.

(1-1) Performing blast tests with liraglutide in chinchillas and complete hearing function tests

The possible therapeutic function of glucagon-like peptide-1 (GLP-1) agonist (Liraglutide) to the acute and progressive hearing damage over 14 or 28 days was investigated in animal model of chinchilla repeatedly exposed to BOP (**G1)** level blasts. Young adult chinchillas (weighing 600-800 g) were assigned to four liraglutide (drug) treatment groups: pre-drug treatment to blast injury with and without HPDs (e.g., earplugs) and post-drug treatment to blast injury with and without HPDs. For pre-drug treatment, liraglutide was delivered to animals with subcutaneous injection at 2 days prior to blast on Day 1 and in the consecutive 7 days; for post-drug treatment, liraglutide was injected to animals on Day 1 at 2 hours after the blast and in each day thereafter for 7 days.

Figure 1 shows the time frame and experimental procedure for animals involved in pre-drug treatment and post-drug treatment groups**.** On Day 1, animals received 6 consecutive blasts with 5-minute intervals between exposures at low BOP of 3-5 psi or 21-35 kPa, named as G1 BOP level. Hearing function tests, including the auditory brainstem response (ABR), distortion product otoacoustic emission (DPOAE), and middle latency responses (MLRs), were performed on Day 1 (pre- and post-blast) and on Days 4, 7, 14, and 28, respectively. Animals were euthanized after the function tests on Day 14 or 28. The MLRs and ABR were measured to reflect the cortex and subcortical hearing function, respectively. DPOAE was measured to detect the damage of outer hair cells.

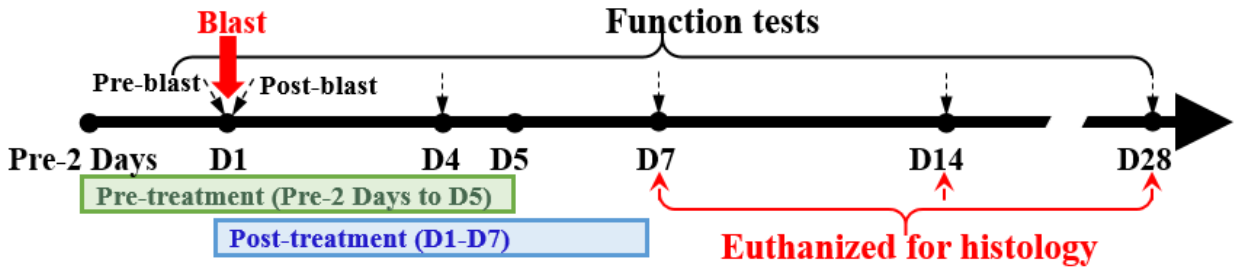

Fig. 1. Diagram showing the time frame and experimental procedure for animals involved in pre-drug treatment, post-drug treatment, and blast-control groups in Aim 1. Note that each animal has pre-blast and post-blast on Day 1 (D1). The time points for function tests are denoted by dashed lines with arrows. Three subgroup animals will be euthanized for histology studies on Days 7, 14, and 28, respectively.

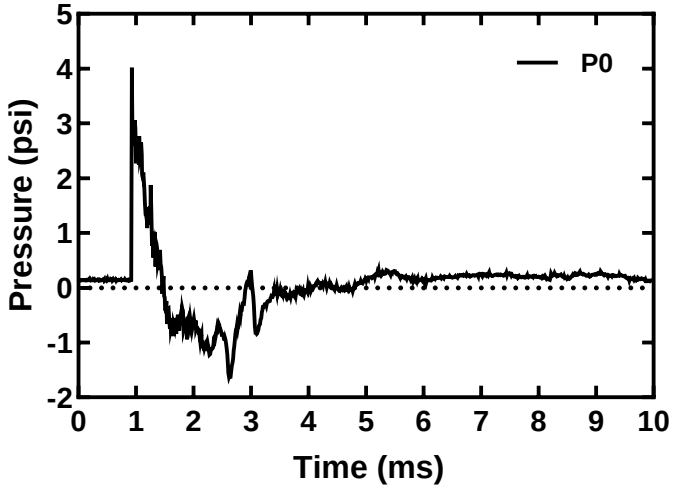

Fig. 2. A recorded BOP waveform at the entrance of the ear canal from an animal test with a peak pressure of 4.0 psi.

● ABR threshold changes observed over 28 days

The ABR threshold shift or elevation was derived by subtracting the pre-blast value from the post-blast on Day 1 and that on Days 4, 7, 14, and 28. Figure 3A shows the ABR threshold shifts or elevations after blasts in open ears without earplug (w/o EP, N=8), and Fig. 3B shows the ABR threshold shift in ears with EP (w/ EP, N=10). The ABR threshold shifts measured from post-drug treatment groups are displayed in Fig. 4. Figure 4A shows the results from open ears (w/o EP, N=8) and Fig. 4B shows the results from protected ears (w/ EP, N=9).

In pre-drug treatment group (Fig. 3), when ears were open without protection (Fig. 3A), the ABR threshold shift or elevation after blast decreased from 33-44 dB to below10 dB at frequencies 1-8 kHz from Day 1 to Day 28 and the maximum reduction happened on Day 4. We noticed that there was a high shift value (44 dB) at 8 kHz on Day 1, but the shift value on Day 28 was 5 dB. When ears were protected (Fig. 3B), the ABR threshold shift was all under 15 dB (except 8 kHz) and without obvious decrease on Day 4. A high shift value of 21 dB on Day 1 at 8 kHz was also observed.

Between the open and protected ears of the pre-blast drug treatment group, we found a difference in the effect of recovery time on progressive hearing damage. There was a major difference in threshold shift between Days 1 and 4 in open ears, but it was not seen in protected ears. This indicates a fast rate of recovery during the first 4 days in open ears but not in protected ears. Furthermore, the threshold shift range was larger in open ears than that in protected ears, corresponding to the greater hearing damage in the open ears.

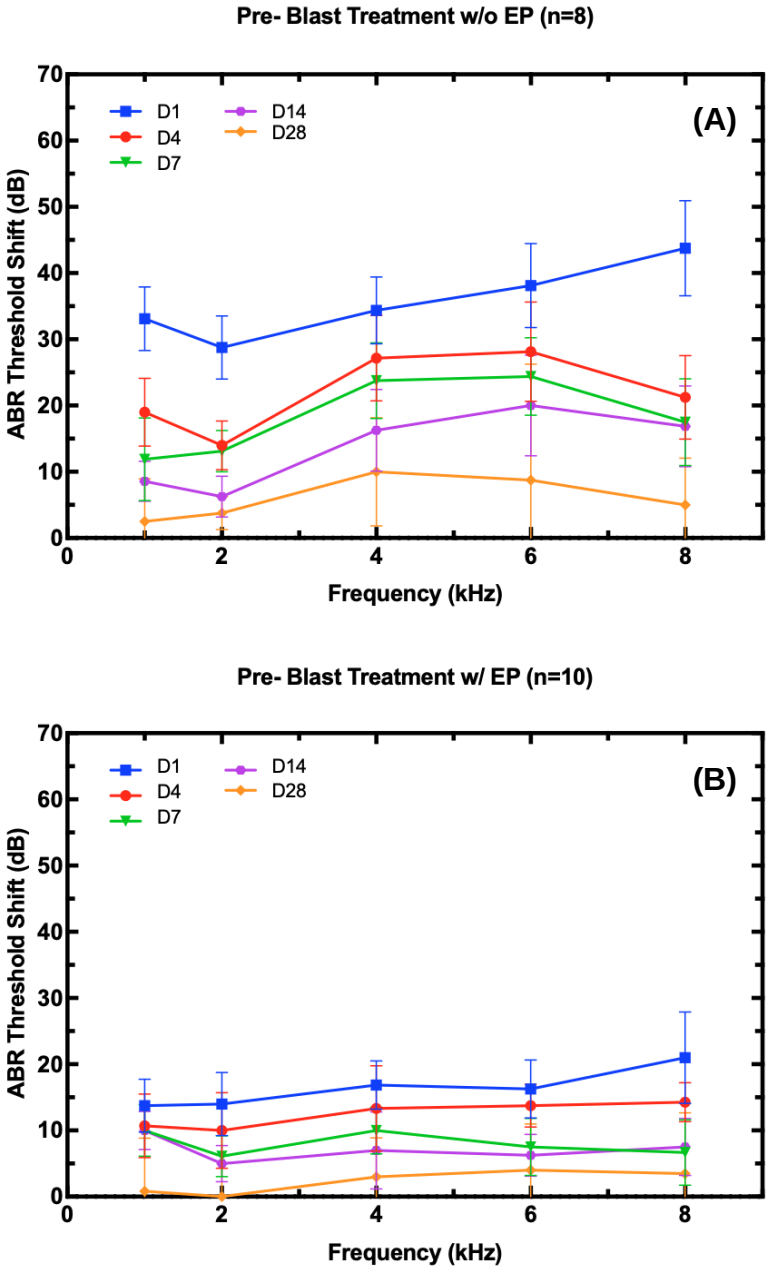

Fig. 3. (A) ABR threshold shift (mean ± SEM, n=8) of open ears measured in pre-drug treatment animals after 6 blasts on Day 1 and observed through Days 4, 7, 14, and 28. (B) ABR threshold shift (mean ± SEM, n=10) of protected ears measured in pre-drug treatment animals after 6 blasts on Day 1 and observed through Days 4, 7, 14, and 28.

In post-drug treatment group (Fig. 4), when ears were open, the ABR threshold shift was 28–40 dB across the frequencies on Day 1 with a maximum ABR threshold shift (40 dB) at 4 kHz, not like that in pre-drug treatment group with the maximum shift at 8 kHz on Day 1. However, the maximum reduction of the ABR threshold shift happened on Day 4, similar to the pre-drug group. When ears were protected, the ABR threshold shift was 17–25 dB on Day 1 and decreased to below 5 dB on Day 28 with a large reduction on Day 4.

Between the open and protected ears of the post-blast drug treatment group (Figs. 4A and 4B), there was a commonly large decrease of ABR threshold shift from Day 1 to Day 4, which indicates a fast rate of recovery during the first 4 days in both open and plugged ears. It is also found that the open ears had a larger range of threshold shift than the plugged ears, corresponding to the greater hearing damage occurred in open ears.

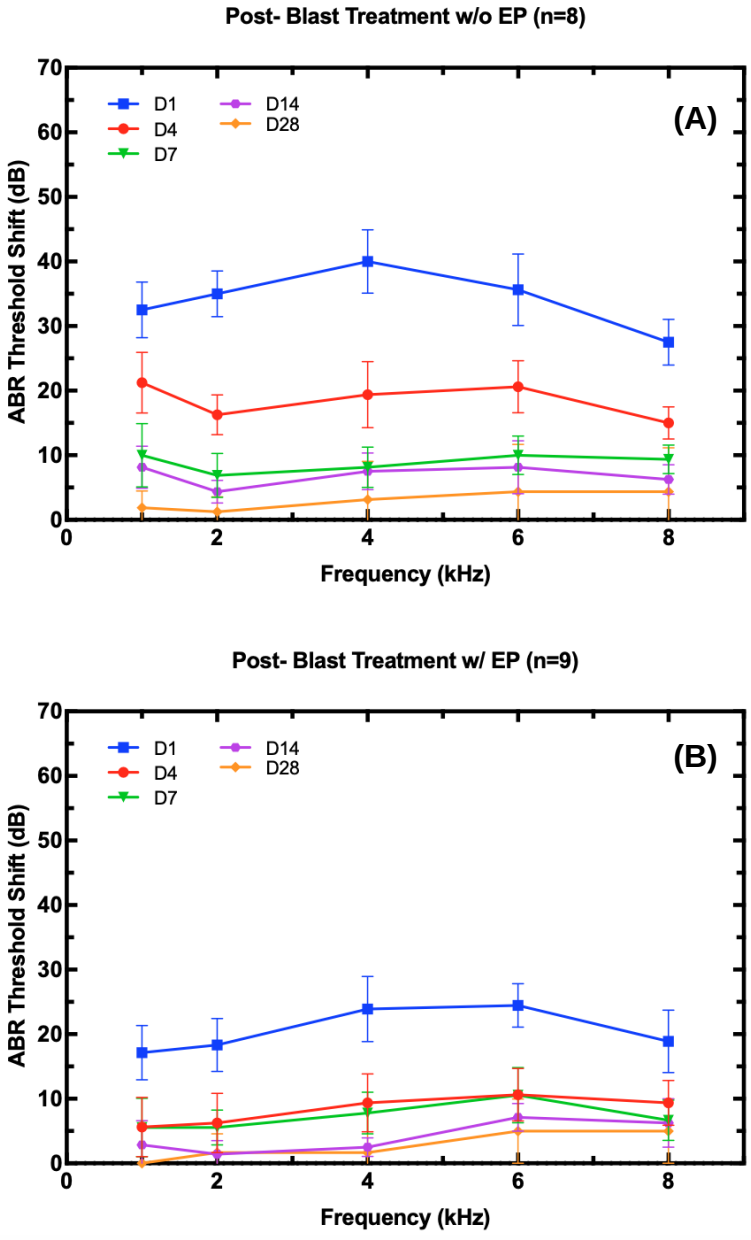

Fig. 4. (A) ABR threshold shift (mean ± SEM, n=8) of open ears measured in post-drug treatment animals after 6 blasts on Day 1 and observed through Days 4, 7, 14, and 28. (B) ABR threshold shift (mean ± SEM, n=9) of protected ears measured in post-drug treatment animals after 6 blasts on Day 1 and observed through Days 4, 7, 14, and 28.

● ABR wave I amplitude and latency changes observed over 28 days

ABR wave I amplitude and latency in response to stimulus level from 80 to 100 dB SPL at frequencies of 0.5-8 kHz were measured from 4 experiment groups of animals: pre-drug treatment to blast injury with and without HPDs (e.g., earplugs) and post-drug treatment to blast injury with and without HPDs. The ABR wave 1 suprathreshold amplitude and latency measured at 8 kHz showed clear changes between the treatment groups and the results are displayed in Figs. 5-8 at pre-blast and post-blast on Day 1 and Days 14 and 28. Figure 5 shows the wave I amplitude measured from pre-drug treatment groups of open ears without EP (Fig. 5A) and protected ears with EP (Fig. 5B). Figure 6 shows the wave I amplitude measured from post-drug treatment groups of open ears without EP (Fig. 6A) and protected ears with EP (Fig. 6B). Figure 7 shows the wave I latency measured from pre-drug treatment groups of open ears without EP (Fig. 7A) and protected ears with EP (Fig. 7B). Figure 8 shows the wave I latency measured from post-drug treatment groups of open ears without EP (Fig. 8A) and protected ears with EP (Fig. 8B).

In pre-drug treatment group (Fig. 5), when ears were open (Fig. 5A), there was a large decrease of wave I amplitude after blast on Day 1. Then, the amplitude increased on Days 14 and 28 and reached the pre-blast level. When ears were protected (Fig. 5B), the wave I amplitude was much less reduced on Day 1 than that in open ears and gradually increased on Days 14 and 28 to reach the pre-blast level.

In post-drug treatment group (Fig. 6), when ears were open (Fig. 6A), after the initial decrease on Day 1, the amplitude increased to close to the pre-blast values on Days 14 and 28. When ears were protected (Fig. 6B), the wave I amplitude was less reduced on Day 1 than that in open ears and the amplitude on Day 28 was close to that of pre-blast. However, the wave I amplitude at both pre-blast and Day 28 slightly reduced than that in open ears. Note that the experimental data on Day 14 were not included.

There are more variations in wave I latency data as shown in Figs. 7 and 8, particularly for the open ears. Different from what was observed from wave I amplitude, the latencies did not return to the pre-blast level on D14 or D28. In all four treatment groups, the latency was elevated after the blast and did not return to normal with time. The wave I latency data showed an obvious increase of latency after blast in both open and protected ears, but there was no significant variation between the pre- and post-drug treatment groups.

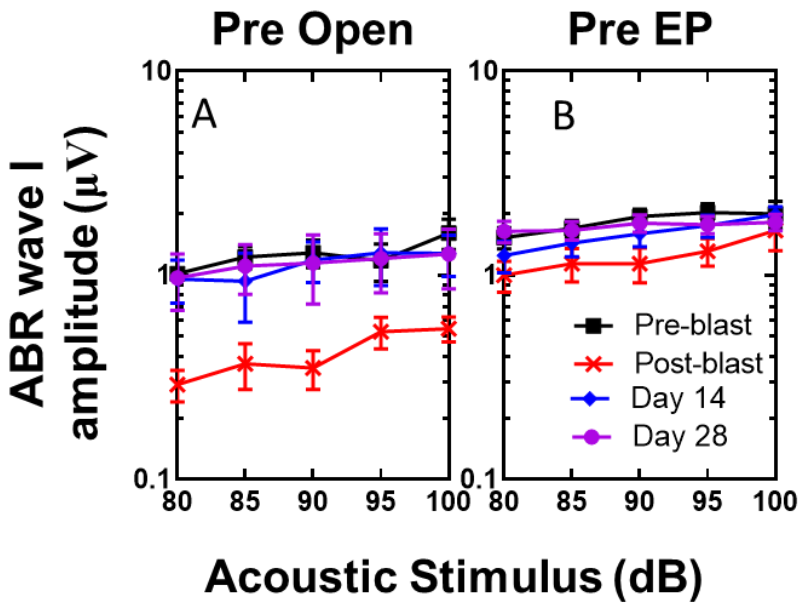

Fig. 5. ABR wave I suprathreshold amplitude measured from pre-drug treatment animals at 8 kHz. (A) open ears without EP (N=4); (B) protected ears with EP (N=10). Post-blast (red) and Pre-blast (black) represent the measurements on Day 1. The measurements on Day 14 and Day 28 are represented by blue and purple lines, respectively.

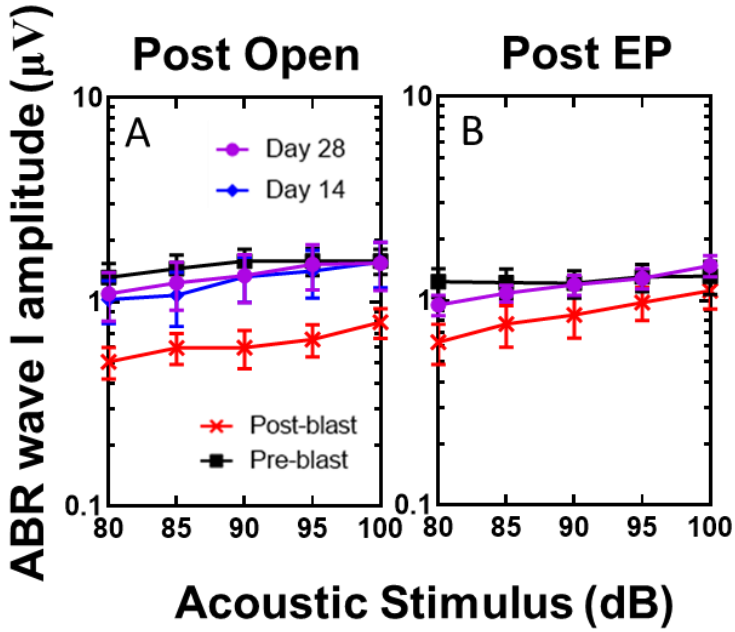

Fig. 6. ABR wave I suprathreshold amplitude measured from post-drug treatment animals at 8 kHz. (A) open ears without EP (N=8); (B) protected ears with EP (N=7). Post-blast (red) and Pre-blast (black) represent the measurements on Day 1. The measurements on Day 14 and Day 28 are represented by blue and purple lines, respectively.

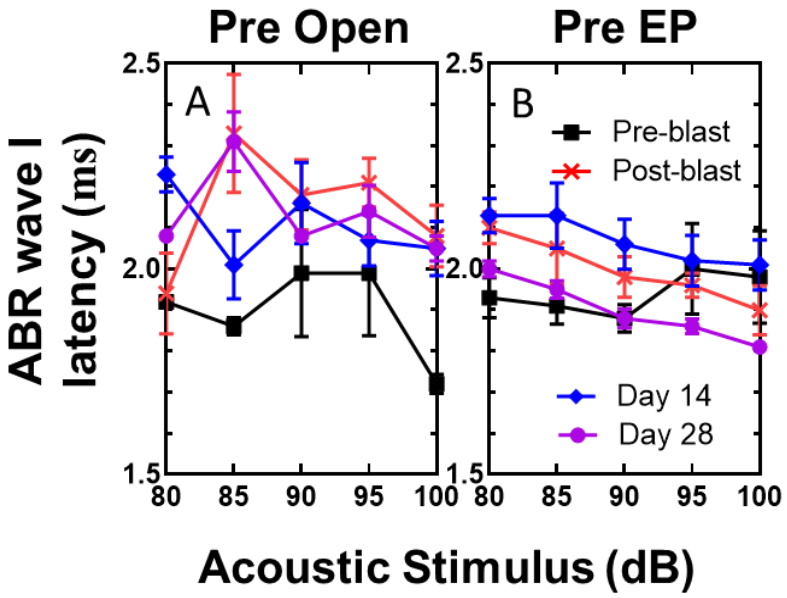

Fig. 7. ABR wave I latency measured from pre-drug treatment animals at 8 kHz. (A) open ears without EP (N=4); (B) protected ears with EP (N=10). Post-blast (red) and Pre-blast (black) represent the measurements on Day 1. The measurements on Day 14 and Day 28 are represented by blue and purple lines, respectively.

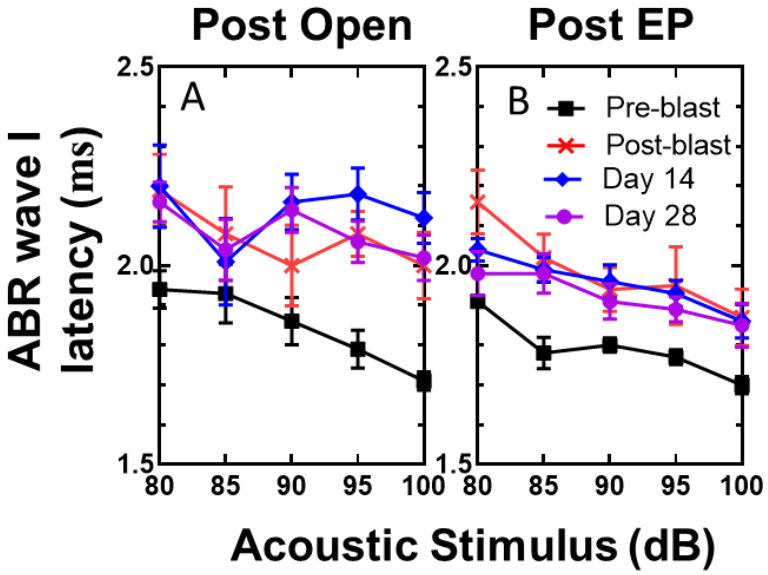

Fig. 8. ABR wave I latency measured from post-drug treatment animals at 8 kHz. (A) open ears without EP (N=8); (B) protected ears with EP (N=7). Post-blast (red) and Pre-blast (black) represent the measurements on Day 1. The measurements on Day 14 and Day 28 are represented by blue and purple lines, respectively.

In conclusion, due to the limited sample size, especially in pre-drug treatment with open ear group, the standard error was relatively high, and the shape of the curves was irregular. We plan to conduct more studies on auditory function tests in animals in the next year to provide statistical significance on the measurement data.

● MLR measurements over 28 days

For the measured MLR waveforms, two major components were used to describe the effect of BOP waves on the central nervous system: the peak-to-peak amplitude and the latency of the negative voltage waves (Na) and/or the positive voltage waves (Pa). The amplitude and latency of Na and Pa reflect neural conduction velocity from the peripheral auditory nerve to the central auditory nervous system.

Figure 9 shows the MLR Na-Pa peak-to-peak amplitudes measured from 4 drug-treatment groups and Fig. 10 displays the Na latency data from 4 drug-treatment groups in response to the stimulus of 80 dB at 500 Hz. The x-axis represents the time point of measurement from pre-blast and post-blast on Day 1 to Days 4, 7, 14, and 28. The MLR amplitude is positively related to the function of the central auditory system (i.e. amplitude increase means function increase) while the latency is negatively related to the function of the central auditory system (i.e. latency increase means function decrease).

In pre-drug treatment group (Figs. 9A and 9B), when ears were open (w/o EP, Fig. 9A), the MLR amplitude decreased over the time period of 28 days from 0.7 μV at post-blast to 0.25 μV on Day 28. When ears were protected (with EP, Fig. 9B), there was a slight increase of the MLR amplitude on Day 1 after blast (1.75 to 2 μV) followed by a large decrease to 1.0 μV on Day 4. From Day 4 to Day 28, a stable decrease from 1.0 to 0.75 μV was observed which is the same trend as shown in open ears but with the higher amplitude value in protected ears.

In post-drug treatment group (Figs. 9C and 9D), when ears were open (w/o EP, Fig. 9C), the MLR amplitude did not change much after blast on Day 1 (1.7 μV), then decreased to Day 4 (0.75 μV) and slightly increased to Day 7 and Day 28 (1.1 μV). When ears were protected (with EP, Fig. 9D), the MLR amplitude showed similar change induced by blast on Day 1 as that of the pre-treatment ears, and followed by large decrease to Day 4, and relatively stable from Day 4 to Day 28 (0.75 – 0.6 μV). The slight increase of amplitude in open ears was not observed in protected ears on Day 28.

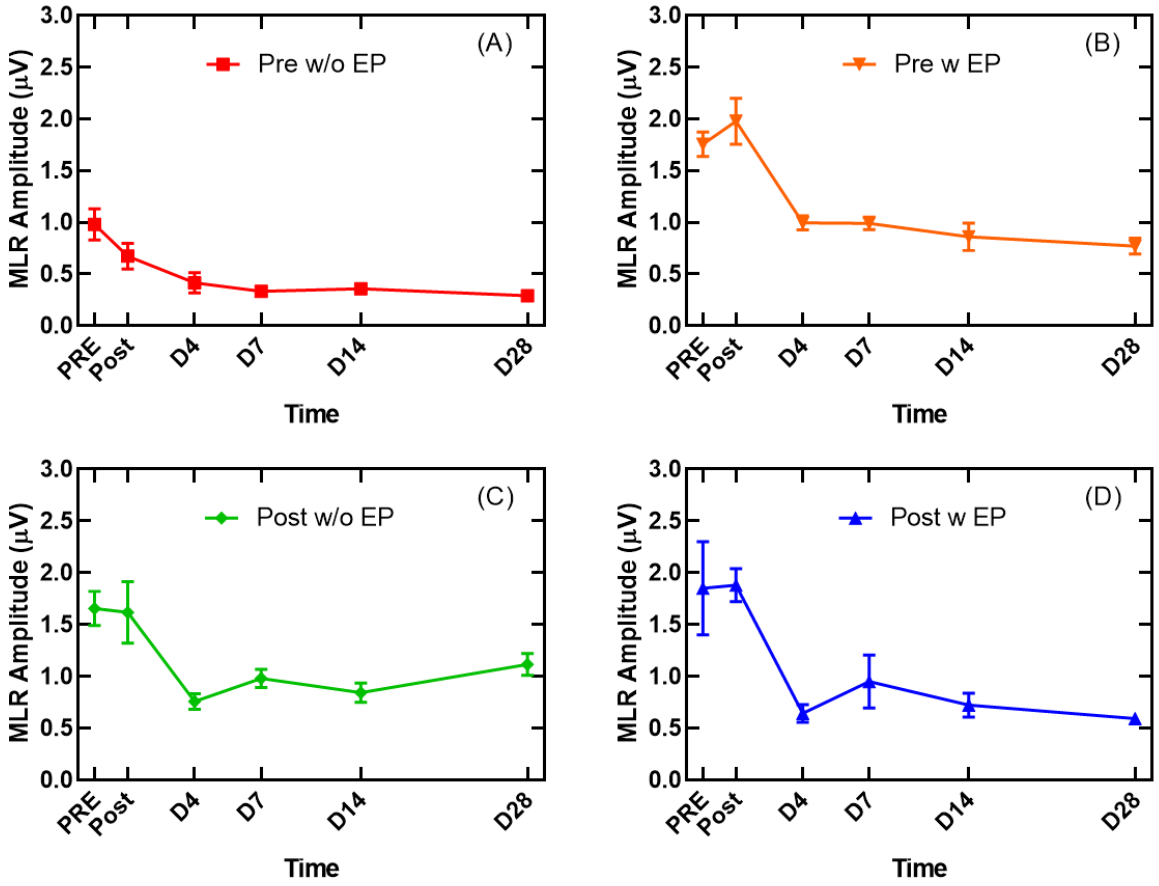

Fig. 9. MLR peak-to-peak amplitudes (mean ± SEM) in response to the stimulus of 80 dB measured at 500 Hz from chinchillas of 4 drug-treatment groups: (A) Pre-treatment open ears without earplug (w/o EP, N=8); (B) Pre-treatment ears with EP (N=14); (C) Post-treatment open ears (N=12); (D) Post-treatment with EP (N=6).

The results of Na latencies measured from 4 treatment groups are shown in Fig. 10. In pre-drug treatment group (Figs. 10A and 10B), when ears were open (w/o EP, Fig. 10A), there was an increase of the Na latency on Day 1 after blast (15.6 to 17.5 ms) followed by a continually decrease to 15 ms on Day 28. When ears were protected (Fig. 10B), a slight increase after blast on Day 1, followed by a relatively stable to Day 28 (15.8 ms).

In post-drug treatment group (Figs. 10C and 10D), the trends of MLR latency changes from pre- to post-blast on Day 1 and to Days 4, 7, 14, and 28 in the open and protected ears were similar, no significant difference.

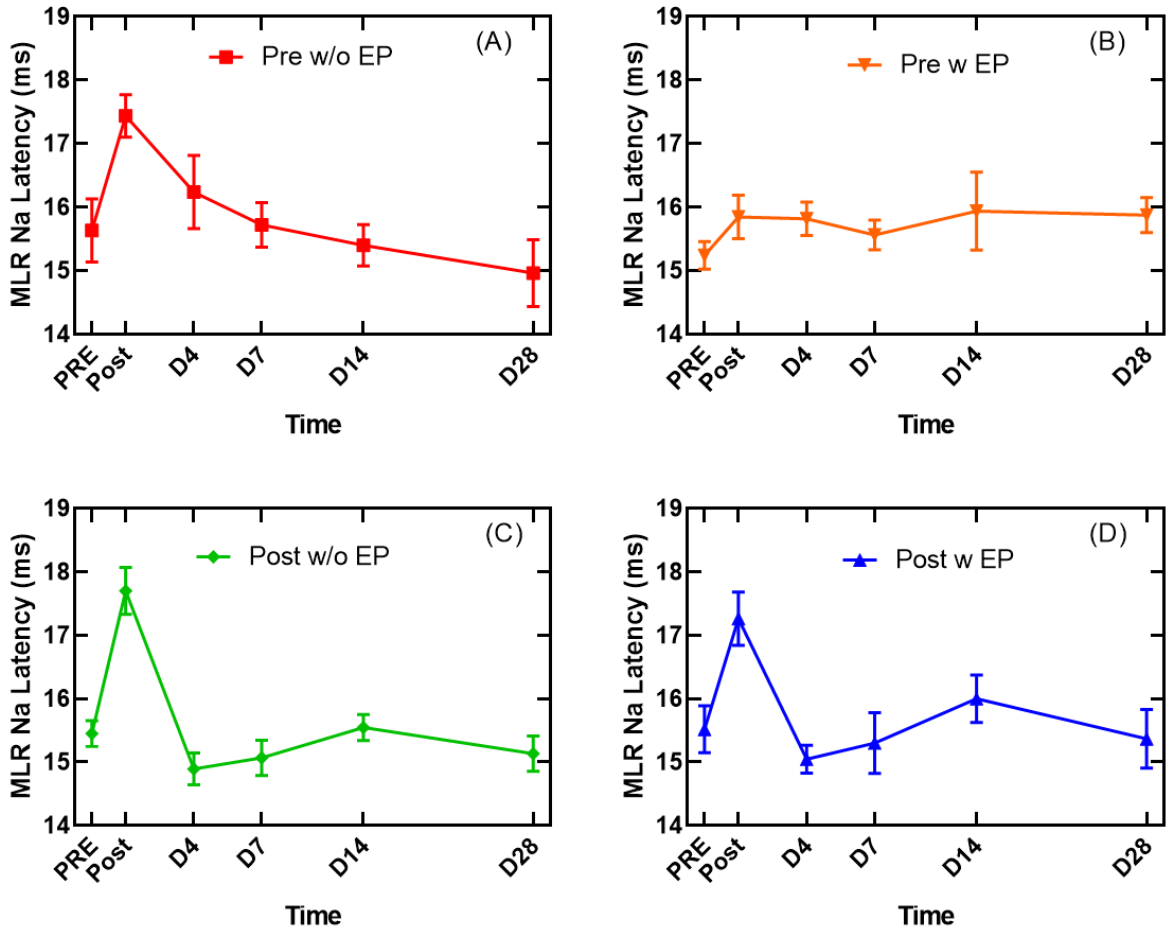

Fig. 10. MLR Na latency (mean ± SEM) in response to the stimulus of 80 dB measured at 500 Hz from chinchillas of four drug-treatment groups: (A) Pre-treatment open ears without earplug (w/o EP, N=8); (B) Pre-treatment ears with EP (N=14); (C) Post-treatment open ears (N=12); (D) Post-treatment with EP (N=6).

In conclusion, although there were individual differences, the MLR results showed a similar trend over the time among 4 drug treatment groups. However, the current MLR data are still the preliminary results and we need further investigation with larger animal sample size, especially in pre-drug without EP and post-drug with EP groups.

● DPOAE measurements over 28 days

The DPOAE results are shown in Fig. 11 for the protected ears. As demonstrated by the DPOAE level shown in Fig. 11, the blast-induced damage in the pre-drug blast group was most severe immediately after the blast on Day 1, which reflects the acute damage of the cochlear outer hair cells. The damage was largely recovered by Day 4. From the Day 4 to Day 28, the DOPAE level gradually recovered to the normal level. However, the data obtained from the post-drug blast group was randomly distributed which need further analysis.

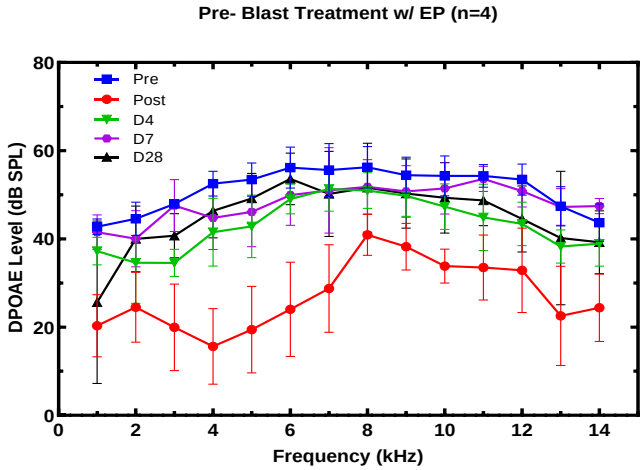

Fig. 11. DPOAE level (mean ± SEM) measured in protected and pre-drug treated ears on pre- and post-blast of Day 1 and on Days 4, 7, and 28. Day 14 data is not measured in this group of animals.

(1-2) Performing blast tests in chinchillas (blast control) and complete hearing function tests

14 chinchillas were involved in blast control study and divided into two groups (N=7 each): with and without HPDs (e.g. standard foam earplugs) and exposed to BOP level of 3-5 psi or 21-35 kPa for 6 consecutive blasts on Day 1. Hearing function tests, including ABR, DPOAE, and MLR, were conducted on Day 1 (pre- and post-blast) and on Days 4, 7, and 14 to assess progressive hearing damage after blasts. No liraglutide was used in this blast control study.

● <u>ABR threshold changes observed over 14 days</u>

The mean and SEM of the ABR threshold shifts or elevations measured from two groups of chinchillas after blast exposure on Days 1, 4, and 14 are shown in Fig. 12. Figure 12A displays ABR threshold shifts measured from protected ears with earplugs (N=14 plugged ears) and Fig. 12B shows the threshold shifts from the open ears without earplugs (N=14 open ears) on Days 1, 4, and 14. As shown in Fig. 12A, the protected ears experienced the greatest threshold shift on Day 1, with a shift increasing from approximately 32 dB at 1 kHz to around 48 dB at 8 kHz. The ABR threshold shift and its dependency on frequency decreased over time from Day 1 to Day 14. The ABR threshold shift was around 20 dB on Day 4 and 10 dB on Day 14 at all frequencies. Like the protected ears, the ABR threshold shifts of the open ears were the largest on Day 1 and increased from approximately 36 dB at 1 kHz to roughly 48 dB at 8 kHz as shown in Fig. 12B. This indicated that the acute damage occurred in both protected and open ears are similar after blast exposure. However, the ABR threshold shifts of the open ears were greater than those of the plugged ears on Days 4 and 14, especially shown in Fig. 12 C when comparing the threshold shift for both plugged and open ears over Days 1, 4, and 14. Notably, by Day 14 the threshold shift of open ears reduced to 15 dB at 1 kHz and 30 dB at 8 kHz while the threshold shift of plugged ears decreased to about 10 dB across all frequencies.

In conclusion, the blast control study in chinchillas indicated that 1) the acute damage occurred in both protected and open ears was similar, but earplug protection affected the hearing recovery after 4 days and 14 days of post blast. Note that the TM rupture in open ears may affect their recovery on Days 4 and 14. 2) Statistical results indicated that the significant difference of ABR threshold shifts between the open and protected ears occurred at $f \geq 4$ kHz on Day 14. Overall, the results suggest that permanent hearing damage occurred in both unprotected and protected ears, but to a greater extent in open ears, especially at $f > 2$ kHz.

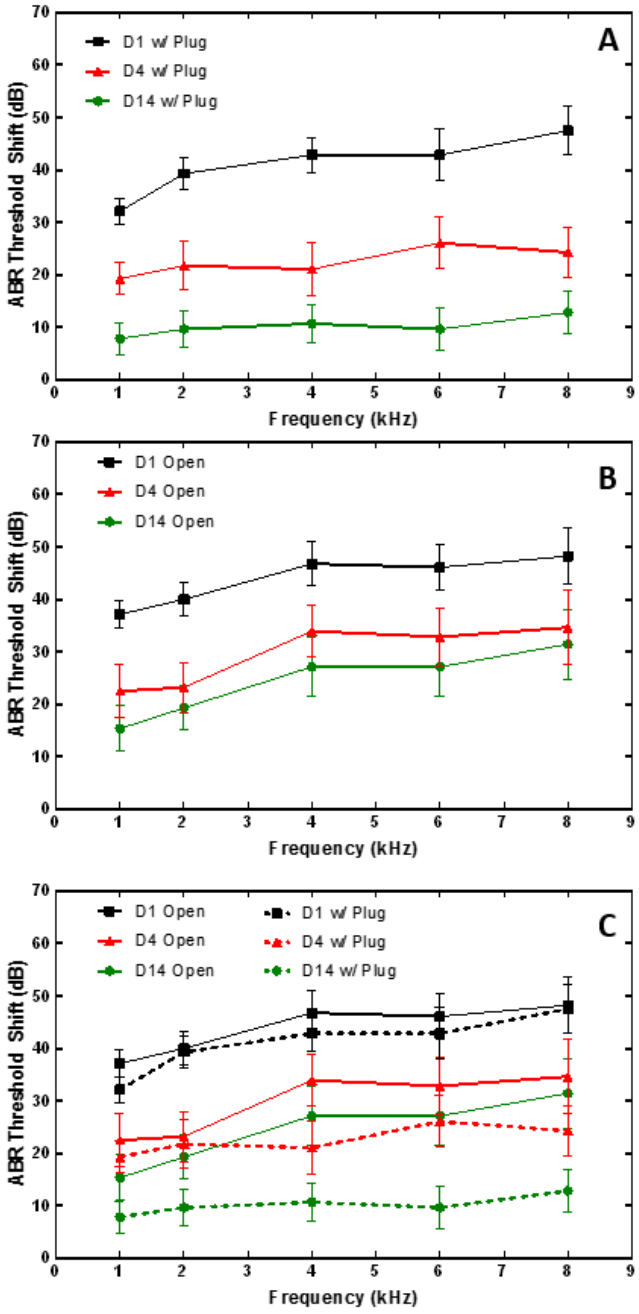

Fig. 12. (A) ABR threshold shifts (mean ± SEM, n = 14 ears) measured in protected ears after 6 blasts on Days 1, 4, and 14. (B) ABR threshold shifts (mean ± SEM, n = 14 ears) measured in open ears after 6 blasts on Days 1, 4, and 14. (C) Comparison of ABR threshold shifts in plugged and open ears on Days 1, 4, and 14.

● DPOAE level changes observed over 14 days

The mean and SEM of DPOAE level shifts (reductions) measured from animals with earplugs on Days 1 and Day 14 at frequencies of 1-14 kHz are shown in Fig. 13. The mean values of the DPOAE level shifts increased from about 7 dB at 1 kHz to a peak of 33 dB at 11 kHz on Day 1. The shift decreased substantially on Day 14 to around zero dB at 1 kHz and below 10 dB over the rest of the frequency range. There was a statistically significant difference between the DPOAE level shift measured on Day 1 and that measured on Day14 over the entire frequency range. The results obtained from plugged ears indicate that the protection of earplugs may have facilitated the recovery and prevented the permanent damage of the outer hair cells in the cochlea.

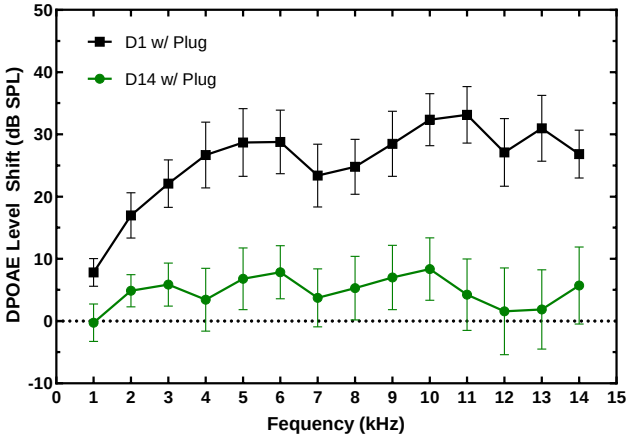

Fig. 13. DPOAE level shifts (mean ± SEM, n = 14 ears) measured from protected ears on Days 1 and 14.

● MLR amplitude and latency changes observed over 14 days

The MLR peak-to-peak amplitude of Na to Pa, Na latency, and Pa latency for the protected and unprotected ears are plotted against the time point of measurement in Fig. 14. Figure 14A shows the Na-Pa amplitude (mean) for protected (w/plug) and unprotected (w/o plug) ears at pre-blast was 2.71 and 2.29 μV, respectively. After blast exposure, the amplitude in protected ears had decreased to 2.09 μV. Likewise, the amplitude in unprotected ears had also decreased to 1.85 μV post-blast. The amplitudes for protected and unprotected ears continued to decrease to Day 14 with a value recorded as 1.43 and 1.53 μV, respectively. The continued reduction of the amplitude in both groups of animals might indicate that there was some progressive damage to the central auditory system that persisted over 14 days.

Figure 14B shows the average Na peak latency in protected and open ears. Before blast exposure, the Na peak latencies in protected and open ears were 15.70 and 16.33 ms, respectively. After the blast, the Na latencies for both plugged and open ears increased to 18.50 and 18.17 ms, respectively. The Na latencies for both plugged ears and open ears then decreased to 16.3 ms on Day 4 and finally reached 17.1 ms on Day 14.

The Pa peak latency in open and protected ears over 14 days is displayed in Fig. 14C, which shows a trend similar to Na latency. Prior to exposure to repeated blasts, the Pa latencies for open and protected ears were measured to be 21.71 and 21.14 ms, respectively. After the blast, the latency of the Pa peak increased to 22.80 ms for both protected and unprotected ears. The Pa latency then decreased on Day 4, fluctuated slightly from Day 4 to Day 14, and finally returned to pre-blast levels on Day 14. Collectively, the Na and Pa latency data for the protected and open ears showed a sharp peak on the post-blast time point, which indicated that there were acute changes of the acoustic-evoked responses in the central auditory system after blast exposures. It should be noted that there were no statistically significant differences between MLR measurements of the protected and open ears on both amplitudes and latencies.

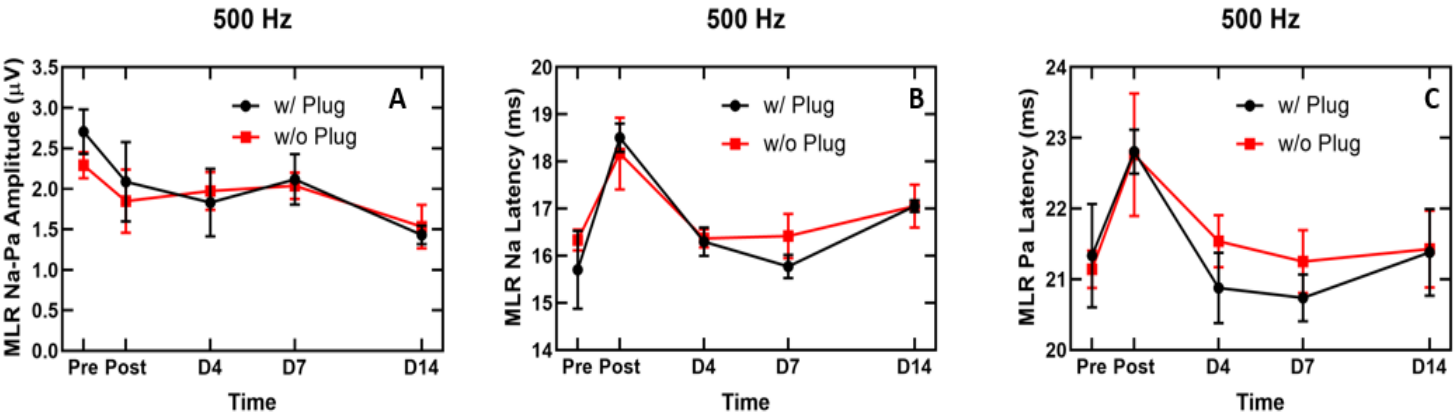

Fig. 14. MLR amplitude (peak-to-peak) from Na to Pa and the latency of Na and Pa obtained at 500 Hz with 80 dB SPL stimulus in open ears (mean ± SEM, n=6) and protected ears (mean ± SEM, n=4) over the time point of measurement. (A) Peak-to-peak amplitudes, (B) Na latencies, and (C) Pa latencies.

(1-3) Preparing chinchilla brain tissue samples for histology study after completion of hearing function tests

Upon the completion of the hearing function tests at the time points shown in Fig. 1, chinchillas were perfused transcardially with 0.9% saline solution, followed by 4% paraformaldehyde in 0.1M PBS. The brain was excised from the cranial vault and the bullae were harvested. The brain sample for each animal was fixed in 4% paraformaldehyde in 0.1 M PBS for 48 hours. Then, the chinchilla brains were immersed in 30% sucrose solution before shipping to the laboratory of Dr. Namas Chandra at New Jersey Institute of Technology (NJIT) – subcontractor for this project. However, all the brain tissues collected in this quarter are still in our lab because the NJIT lab was closed due to COVID-19 Pandemic until July.

(2) In this 1st year of the project, the major activities under Aim 2 are performed in Dr. Chandra's lab at NJIT to investigate the beneficial effects of liraglutide on the mitigation of the central auditory damage following repetitive exposure to the low BOP or mild TBI pressure levels, named G1 and G2 BOP level, respectively. **Task 1**: To determine the impact of ear protection on liraglutide-mediated effects on glutamate and GABA neurotransmitter receptors in the central auditory system. Techniques include performing frozen sectioning of brain tissues, incubating sections with respective antibodies, conducting immunostaining of brain sections and fluorescence imaging, and performing data analysis

The **specific objective** is to examine whether ear protection (and subsequent peripheral auditory damage) affects the central auditory response regarding excitatory and inhibitory neurotransmitter receptor changes. We have also examined whether liraglutide displays any differential response with and without ear protection on central auditory changes following blast.

(2-1) Ear protection displays a differential response in excitatory and inhibitory neurotransmitter receptor densities in vulnerable central auditory regions: Effect of Liraglutide.

● ***Methodology***:

Following blast injury, animals were sacrificed by transcardial perfusion with PBS followed by 4% paraformaldehyde. Brain were cryoprotected and ultra-thin sections (20 μm) of different brain regions including auditory cortex, inferior colliculus and geniculate body were prepared using vibratome (Leica Instruments). Sections were immunostained for glutamate and GABAa receptors (receptors for excitatory and inhibitor neurotransmitter respectively) using specific antibodies NMDA-R1(glutamate receptor) and GABAa (GABA receptor). Slides were digitized using Leica Aperio scanner attached with fluorescent microscope and fluorescent intensities were quantified using AreaQuant software. Sections were also double-stained with neuronal marker (Neu-N) so as to specifically quantitate changes in these receptors on neuronal synapses.

● ***NMDA receptor densities with and without ear protection: Effect of Liraglutide***:

In the previous progress report (1st quarter), we discussed the data that immunofluorescence analysis and subsequent quantification of the levels of NMDA-R1receptor in auditory cortex showed an increase at post-blast of 14 days compared to controls. *Noteworthy* that treatment of animals with liraglutide showed a strong trend towards reduction in the NMDA receptor levels 14 days after blast suggesting protective effect of liraglutide.

In contrast, ear protection showed a sharp difference in NMDA receptor densities. Unlike, without ear protection, protecting both ears (hence protecting peripheral auditory damage), we observed that there was no significant difference in NMDA receptor densities between control and blast groups, which interestingly, suggest that there is a good correlation between peripheral vs central auditory damage. In other words, peripheral auditory damage precedes the central auditory damage response since ear protection did not display changes in excitatory neurotransmission (as shown by lack changes in NMDA receptor densities between control and blast groups). Additionally, liraglutide (LG) also showed a differential response between ear protection and without ear protection. These data strongly suggest that: 1). Ear protection plays a significant role in central auditory damage. 2). LG displays a differential response between ear protection vs. without ear protection. However, further studies are required to substantiate this concept.

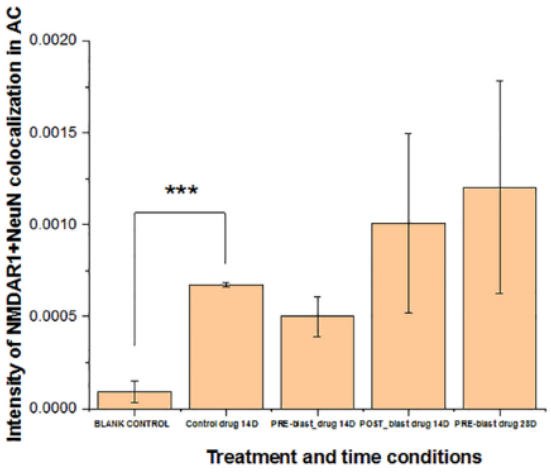

Fig. 15. Quantification of fluorescence intensities of NMDA-R1 in auditory cortex in animal groups without ear protection showing a significant increase in NMDA receptor densities in blast groups compared to control. A pre-treatment with liraglutide (Pre-blast drug 14D) showed a strong trend in decreasing the NMDA receptor level suggesting the property of liraglutide to reduce excitatory neurotransmission.

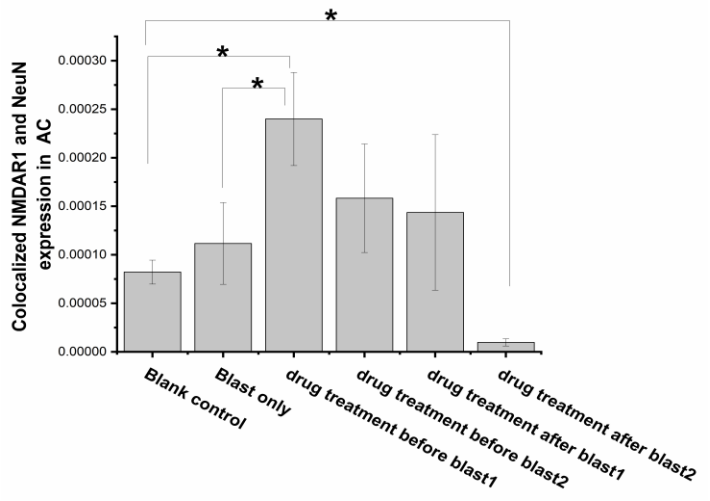

Fig. 16. Quantification of fluorescence intensities of NMDA-R1 in auditory cortex in animal groups with ear protection showing no change in NMDA receptor densities in blast groups compared to control. However, a pre-treatment with liraglutide (Pre-blast drug 14D) showed a significant increase in the NMDA receptor levels suggesting that liraglutide increases excitatory neurotransmission under conditions of no central auditory damage.

- ***GABAA receptor densities with and without ear protection: Effect of Liraglutide**:*

GABA receptors. In the previous progress report (1st quarter) we showed that immunofluorescence analysis and subsequent quantification of the levels of GABA receptor in auditory cortex showed a decrease in its levels in post-blast at 14 days (control for Drug 14 D) compared to controls. Interestingly, liraglutide treatment again showed a strong tendency to increase the GABA receptor level.

Similar to the results without ear protection, in animal groups with ear protection, we observed a reduction the levels of GABA receptor densities in blast groups compared to control. Likewise, LG treatment significantly increased the levels of GABA receptors in both pre-and-post treatment groups with the exception of post-drug treatment day 14. These data are consistent with the above results and suggest that irrespective of ear protection, LG has a property of increasing GABA neurotransmission, which likely helps in counteracting increased excitatory neurotransmission following blast.

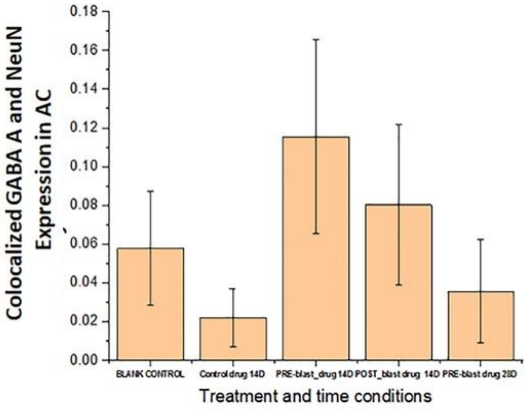

Fig. 17. Quantification of fluorescence intensities of GABA receptor density in auditory cortex without ear protection showing a strong tendency of decrease in GABAA receptor levels. A pre-treatment with liraglutide (LG) (Pre-blast drug 14D) showed an increase in its levels showing a property of LG to augment GABA neurotransmission.

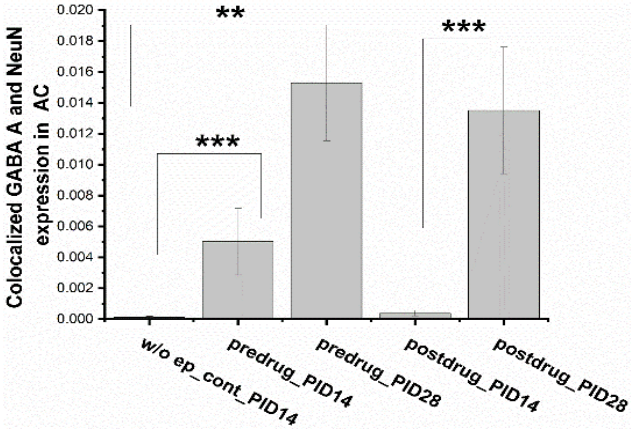

Fig. 18. Quantification of fluorescence intensities of GABAA receptor densities in auditory cortex in animal groups with ear protection showing a significant decrease in blast groups compared to control. However, a pre-treatment with liraglutide (Pre-blast drug 14 & 28 DD) showed a significant increase in the receptor levels suggesting that liraglutide has natural tendency to increase inhibitory neurotransmission of peripheral auditory damage.

The above Figs. 15-18 were reported in the 3rd quarter progress report as the preliminary data (n=2 in each group) on the effect of external ear protection on the central neurotransmitter receptors and the effect of liraglutide on blast-induced changes in excitatory (glutamate-NMDA) and inhibitory neurotransmitter receptor densities in animals exposed to repeated low-level blast injury without ear protection. We examined temporal changes in receptor changes (14 D and 28D after blast). In the 4th quarter, *extended these studies using additional number of animals in each group (n=4) as well as including other auditory region namely inferior colliculus in the experimental setting of both the ears were protected from blast-induced noise with ear plugs and these changes were compared with animals without ear protection* .

NMDA receptor densities with ear protection: Effect of Liraglutide:. In the previous progress report (3rd quarter) we discussed the data that immunofluorescence analysis and subsequent quantification of the levels of NMDA-R1 receptor in auditory cortex showed an increase at post-blast at 14 days (control for Drug 14D) compared to controls. We continued to observe a similar effect with increase in number of animals (n=4). *Noteworthy* that *post-treatment* of animals with liraglutide showed a significance towards reduction in the NMDA receptor levels 14 days post-treatment suggesting protective effect of liraglutide. We also observed a similar trend in inferior colliculus regions.

NMDA receptor densities without ear protection: Effect of Liraglutide:. In this experimental paradigm anesthetized animals' external ear were not protected and the animals were exposed to blast trauma. Sham animals' external ears were also not protected. In sharp contrast to ear protection, we observed a highly significant reduction in NMDA-R1 receptor density in auditory cortex and post-treatment with liraglutide significantly restored the NMDA receptor levels beyond

control values. These puzzling data will have to be further analyzed carefully. However, in inferior colliculus, irrespective of ear protection or not, blast continues to show increase in NMDA receptor level while post-treatment with liraglutide significantly reduced such increase. The data in inferior colliculus suggests that external ear protection has not significant impact on the NMDA receptor level following blast and post-treatment with liraglutide is effective to maintain normal levels of NMDA receptor.

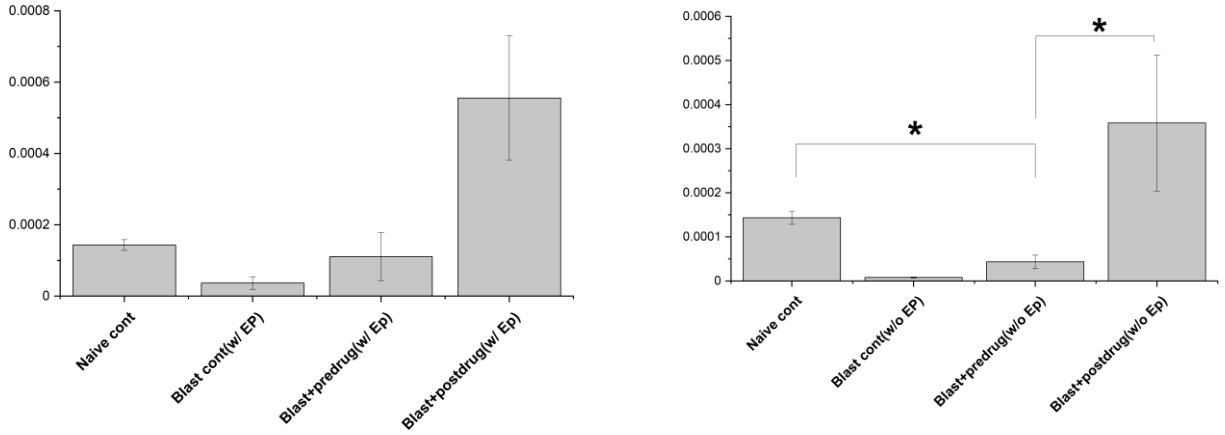

Fig. 19. Quantification of fluorescence intensities of NMDA-R1 in auditory cortex in animal groups with and without ear protection showing a significant decrease in NMDA receptor densities in blast groups compared to control. A post-treatment with liraglutide significantly restored the receptor levels to that of control suggesting the property of liraglutide to maintain normal levels of excitatory neurotransmission.

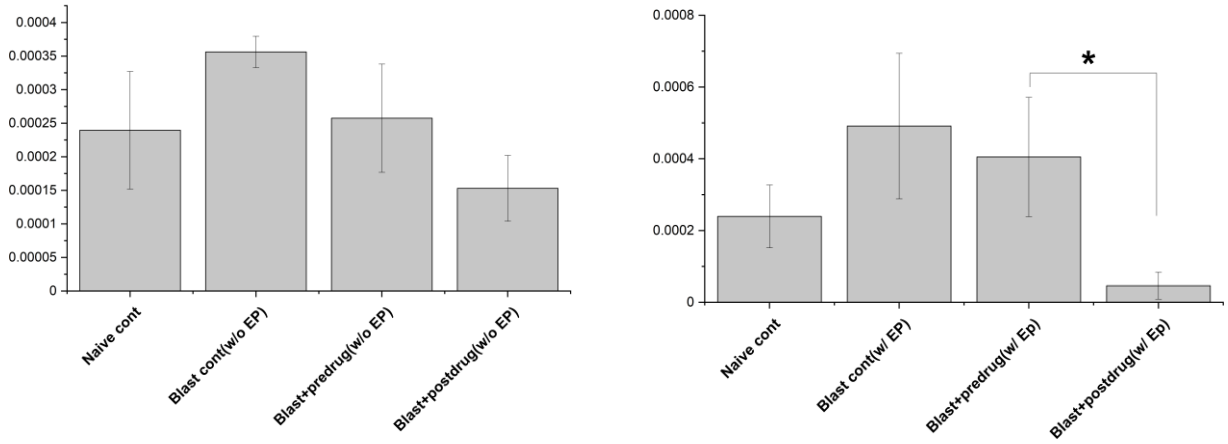

Fig. 20. Quantification of fluorescence intensities of NMDA-R1 in inferior colliculus in animal groups with and without ear protection showing a significant increase in NMDA receptor densities in blast groups compared to control. A post-treatment with liraglutide significantly restored the receptor levels to that of control suggesting the property of liraglutide to maintain normal levels of excitatory neurotransmission.

GABAA receptor densities with ear protection: Effect of Liraglutide:. In the previous progress report (3rd quarter) we discussed the data that immunofluorescence analysis and subsequent quantification of the levels of GABAA receptor in auditory cortex showed an increase at post-blast

at 14 days (control for Drug 14 D) compared to controls when it was co-localized with NeuN, a neuronal marker. However, when these experiments were repeated with more appropriate and suitable synaptic marker, PSD95 we still observed a strong tendency of increase in GABAA receptor in auditory cortex, while liraglutide either with a pre-or-post-treatment had no effect. However, in inferior colliculus, we continued to find a significant reduction in the receptor levels, whereas liraglutide restored the loss of the receptor.

GABAA receptor densities without ear protection: Effect of Liraglutide:

It is noteworthy that in sharp contrast to ear protection strategy, we find a highly significant decrease in GABA receptor level in auditory cortex and liraglutide significantly restored the levels to that of control, whereas in inferior colliculus, there was a significant reduction in the receptor level was observed and liraglutide had no effect. These data have to be carefully reevaluated further.

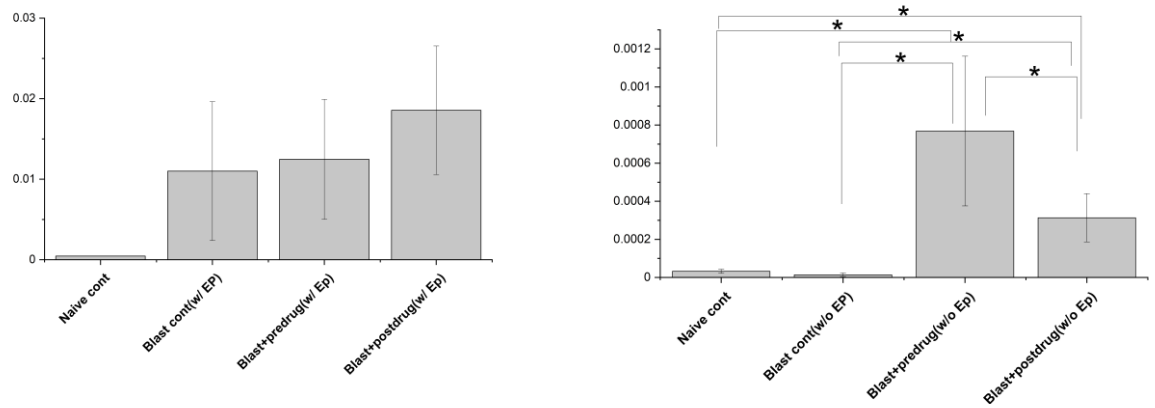

Fig. 21. Quantification of fluorescence intensities of GABAA receptor in auditory cortex in animal groups with and without ear protection showing a differential effect between ear protection and ear non-protection in blast groups compared to control. However, both pre-and-post treatment with liraglutide significantly restored the receptor levels to that of control suggesting the property of liraglutide to maintain normal levels of inhibitory neurotransmission.

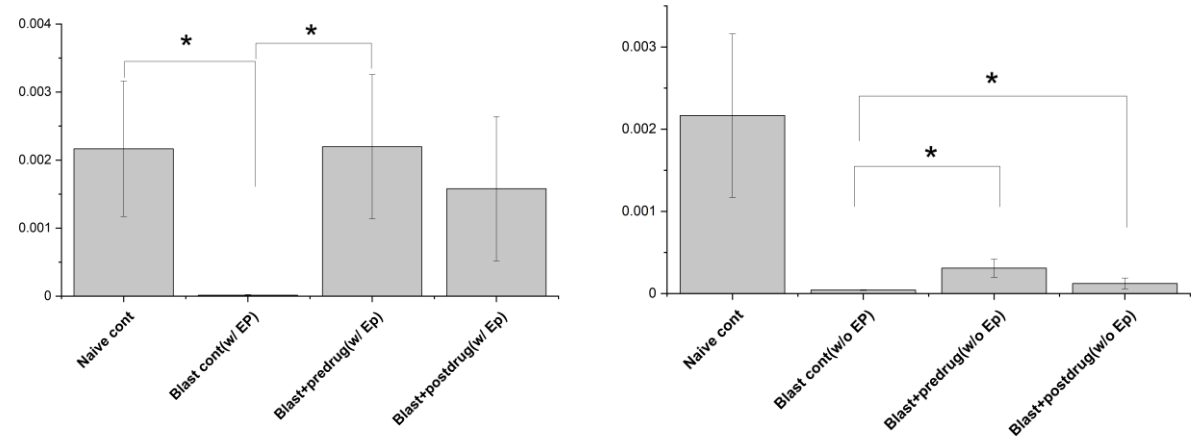

Fig. 22. Quantification of fluorescence intensities of GABAA receptor in inferior colliculus in animal groups with and without ear protection showing a significant reduction in receptor levels in both conditions. However, both pre-and-post treatment with liraglutide significantly restored the receptor levels to that of control only in animal groups with ear protection suggesting that r liraglutide to maintain normal levels of inhibitory neurotransmission.

(2-2) Summary/conclusion

1. Several lines of evidence point out that alterations in various neurotransmitter receptors on the neuronal synapses in different central auditory brain regions contribute to hearing loss. Here we show changes in these receptors in vulnerable brain regions particularly in auditory cortex. The present study observed a differential degree of changes in the glutamate, GABA receptor densities with or without ear protection, i.e., protecting peripheral auditory damage vs not protecting peripheral auditory damage. Noteworthy that the effect of liraglutide also showed a differential protective effect in these receptor density levels.

2. N-methyl-D-aspartate receptor (NMDAR) is a glutamate receptor and ion channel protein found in glutamatergic neurons. It is activated when glutamate and glycine (or D-serine) bind to it, and when activated it allows positively charged ions to flow through the cell membrane. The NMDA receptor is very important for controlling synaptic plasticity and memory function. The activity of the NMDA receptor is affected by many psychoactive drugs and free radicals. In our study, LG treatment showed a significant increase in NMDA receptor levels with preserving peripheral auditory function (PAS, with ear protection) which strongly suggest that LG has likely improves synaptic plasticity properties following blast injury.

3. The $GABA_A$ receptor is an ionotropic receptor and a ligand-gated ion channel. Its endogenous ligand is γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system. Upon activation, these receptors selectively open Cl^- channel, resulting in hyperpolarization of the neurons, which diminishes the post-synaptic potential. The present study showed a generalized reduction in $GABA_A$ receptor levels in AC in groups without ear protection suggesting that PAS damage contributes to reduced GABA neurotransmission in central auditory cortex, whereas, ear protection preserves GABA neurotransmission. The effect of LG on increasing GABA receptors in both the conditions suggest that increasing GABA neurotransmission may have a beneficial effect in recovering central auditory damage.

4. While the above studies show that LG has some beneficial effects in mitigating central auditory damage, it is still premature to arrive at any conclusion since the mechanisms (cAMP pathway, CREB pathway) potentially responsive to LG need to be further evaluated.

- **What opportunities for training and professional development has the project provided?**

Nothing to Report

- **How were the results disseminated to communities of interest?**

Nothing to Report

- **What do you plan to do during the next reporting period to accomplish the goals?**

Under Aim 1, the effects of liraglutide on mitigation of hearing damage in 4 experimental groups will be further analyzed based on the existing experimental data. The additional animal studies will be conducted to complete Tasks 1-1 and 1-2.

We will prepare 2 manuscripts on therapeutic function of liraglutide in the ears without protection vs the ears with earplugs and on the effectivity of liraglutide treatment in pre-blast vs post-blast under repetitive blast exposure at low BOP (G1) level.

Under Aim 2, the effects of liraglutide on repeated blasts under low level (G1) condition are nearing completion. Hence, we will prepare a manuscript comprising all the data on peripheral and central auditory changes before next reporting period.

Initiate studies on the mechanisms responsible for auditory damage including evaluation of cAMP, CREB phosphorylation and examine the effect of liraglutide.

4. IMPACT

- **What was the impact on the development of the principal discipline(s) of the project?**

The accomplishments in the 1th year have great impact to understanding the therapeutic efficacy of liraglutide in animal model of chinchillas repetitively exposed to low BOP (**G1)** level (3-5 psi) with and without hearing protection (earplugs). The hearing function tests have been conducted over the entire time course on Days 1, 4, 7, 14, and 28.

The impact of ear protection on the beneficial effects of liraglutide in mitigating the central auditory damage following repetitive exposure to the low BOP or mild TBI pressure levels were investigated in: 1) the hearing function recovery after blast through ABR threshold, ABR wave 1 amplitude and latency, DPOAE, and MLR amplitude and latency measurements, 2) the response of excitatory and inhibitory neurotransmitter receptor densities in the central auditory regions.

- **What was the impact on other disciplines?**

Nothing to Report

● **What was the impact on technology transfer?**

Nothing to Report

● **What was the impact on society beyond science and technology?**

Nothing to Report

5. CHANGES/PROBLEMS

● **Changes in approach and reasons for change**

No significant changes in approach.

● **Actual or anticipated problems or delays and actions or plans to resolve them**

No significant problems and delays.

● **Changes that had a significant impact on expenditures**

No changes in expenditures.

● **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

No significant changes in use and care of chinchillas. A new Attending veterinarian Dr. Wendy Williams on OU Norman campus was appointed on September 1, 2020

The USAMRMC ACURO site visit to the University of Oklahoma (Norman, Oklahoma) was conducted on November 14, 2019. LTC Joseph Royal, Executive Officer, and Ms. Nina Cisar, Manager, ACURO, USAMRDC, conducted the site visit.

6. PRODUCTS

• publications, conference papers, and presentations;
• website(s) or other Internet site(s);
• technologies or techniques;
• inventions, patent applications, and/or licenses; and
• other products.

● **Publications, conference papers, and presentations**

Publications – Journal papers:

1. Jiang, S., Welch, P., Smith, K., Jiang, S. and **Gan, R. Z.** Auditory dysfunction induced by repeated low intensity blast exposures in chinchilla model. *Hearing Research*, 2020 (In Submission)

2. Shao, N., Jiang, S., Younger D., Cheng, J. T., Brown M., Rama Rao, K., Skotak, M., **Gan, R. Z.** and Chandra, N. Central and Peripheral Auditory Abnormalities in Chinchilla Animal Model of Blast-Injury. *Hearing Research*, 2020 (Under Review).

3. Welch, P. Measurement and 3D Finite Element Modeling of Blast Wave Transmission through Chinchilla Ear. Thesis for M.S. degree in Biomedical Engineering at the University of Oklahoma, May 2020.

Publications – Conference papers:

1. Jiang, S., Smith, K. Liang, J., Wang, X., Gannon, A., Brown, M., and **Gan, R. Z.** A novel chinchilla model of blast-induced auditory injury for hearing damage prediction and prevention using 3D printed "helmet" and earplug. *Association for Research in Otolaryngology (ARO) - Midwinter Meeting*, San Jose, CA, January 25-29, 2020.

2. **Gan, R. Z.**, Welch, P., Sanders, S., and Jiang, S., Hearing function restoration with liraglutide treatment after repeated low-intensity blast exposures in an animal model of chinchilla. *DoD 2020 Military Health System Research Symposium (MHSRS)*. (Meeting was canceled due to COVID-19 pandemic, but the abstract was posted)

3. **Gan, R. Z.**, Welch, P., Sanders, S., and Jiang, S. Therapeutic function of liraglutide for mitigation of hearing damage after blast exposures in animal model of chinchilla with or without hearing protection devices. *Biomedical Engineering Society 2020 Annual Meeting*, San Diego, CA, October 14-17, 2020. (Virtual Meeting due to COVID-19 pandemic)

Seminar Presentations:

1. **Gan, R. Z.,** Invited Speaker at Columbia University BME Breaks/Virtual Seminar Series on July 24, 2020. Title of the seminar: "Measurement and Modeling of Blast-Induced Auditory Injury in Animal Model of Chinchilla".

2. **Gan, R. Z.,** Invited Speaker at The City College of New York – Department of Biomedical Engineering on October 28, 2020. Title of the seminar: "Biomechanical Measurement and Modeling of Blast Wave Transmission through the Ear".

Books or other non-periodical, one-time publications:

N/A

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

- **What individuals have worked on the project?**

Provide the name and identify the role the person played in the project. Indicate the nearest whole person month (Calendar, Academic, Summer) that the individual worked on the project. Show the most senior role in which the person worked on the project for any significant length of time. For example, if an undergraduate student graduated, entered graduate school, and continued to work on the project, show that person as a graduate student, preferably explaining the change in involvement.
Describe how this person contributed to the project and with what funding support. If information is unchanged from a previous submission, provide the name only and indicate "no change".

Name: Rong Gan, Ph.D.
Project Role: PI
Researcher Identifier (OU ID): 112129499
Nearest person month worked: 3
Contribution to Project: Dr. Gan has involved in all research activities for the project and coordinated with NJIT on animal brain tissue immunostaining imaging.

Name: Shangyuan Jiang, Ph.D.
Project Role: Postdoc/Research Associate
Researcher Identifier (OU ID): 112979369
Nearest person month worked: 3
Contribution to Project: Dr. Jiang has been involved in all animal experiments in PI's lab at the University of Oklahoma including blast tests, hearing function tests, and chinchilla brain sample preparation.

Name: Paige Welch
Project Role: M.S. Student
Researcher Identifier (OU ID): 113287784
Nearest person month worked: 2
Contribution to Project: Paige Welch has participated in all animal studies in PI's lab including blast tests, hearing function tests, and data analysis.

Name: Sarah Sanders
Project Role: Undergraduate Student
Researcher Identifier (OU ID): 113438109
Nearest person month worked: 1
Contribution to Project: Sarah Sanders has participated in animal experiments and analysis of the data measured from animals in PI's lab.

Name: Chenkai Dai, M.D., Ph.D.
Project Role: Associate Professor
Researcher Identifier (OU ID): 112148756
Nearest person month worked: 0.2
Contribution to Project: Dr. Dai has assisted animal experiments in PI's lab and helped in understanding brain tissue imaging results.

Name: Ningning Sao
Project Role: Ph.D. Student
Researcher Identifier (NJIT ID): N/A
Nearest person month worked: 3
Contribution to Project: Ms. Shao has performed studies central neurotransmitter receptor expression in different brain regions.

Name: Namas Chandra
Project Role: Professor
Researcher Identifier (NJIT ID): N/A
Nearest person month worked: 0.5
Contribution to Project: Dr. Chandra has overseen the research activities in his lab at NJIT and participated in manuscript preparation.

Name: Rama Kakulavarapu
Project Role: Research Assistant Professor
Researcher Identifier (NJIT ID): N/A
Nearest person month worked: 1
Contribution to Project: Dr. Kakulavarapu has been in charge of animal brain tissue immunostaining imaging and analysis and participated in manuscript preparation

● **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to Report.

● **What other organizations were involved as partners?**

Nothing to Report.

7. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: The Quad Chart (available on https://www.usamraa.army.mil) shall be updated and submitted as an appendix.

A Quad Chart is submitted as an appendix.

8. APPENDICES

● **Quad Chart**

●

Therapeutic Function of Glucagon-Like Peptide-1 (GLP-1) for Hearing Restoration after Blast Exposure or Traumatic Brain Injury (TBI)

ERMS# RH180040 Award Number: W81XWH-19-1-0469

PI: Rong Z. Gan, Ph.D. **Org: University of Oklahoma** **Award Amount:** $1,290,428

Specific Aims: 1) Identify the therapeutics of GLP-1R (Liraglutide) in ameliorating auditory function injuries in pre- and post-treatments in relation to blast overpressure (BOP) level or TBI severity over the time course. **2)** Investigate the beneficial effects of liraglutide on the mitigation of the central auditory damage following repetitive exposures to the low BOP or mild TBI (mTBI) pressure levels.

Hypothesis: Liraglutide treatment will reduce hearing damage severity in blast animal model of chinchilla by modulating the excitatory and inhibitory neurotransmitter responses in the central auditory system, improving synaptic integrity, and preventing oxidative stress-induced neuronal loss.

Objectives: To investigate the potential therapeutic function of GLP-1R agonists, Liraglutide, to mitigate the auditory injury after blast exposure in animal model of chinchilla. The ability of liraglutide to offer protection, stabilization, and regeneration of neurons and synapses located along the entire auditory pathway will be investigated in chinchillas exposed to blast injury.

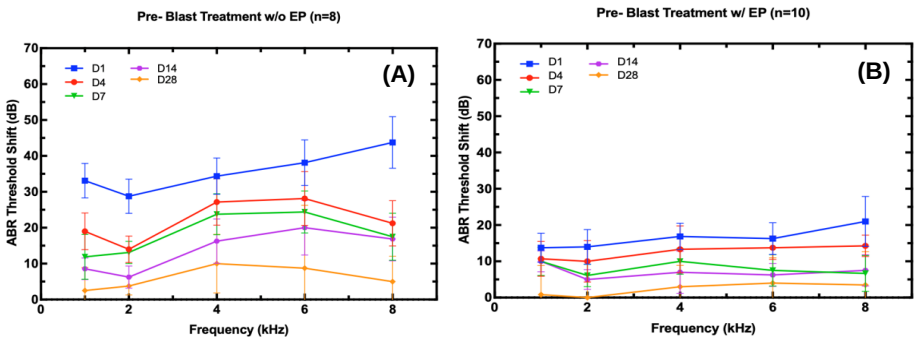

Fig. (A) shows the mean and SE of the ABR threshold shifts from open ears (w/o earplug or EP, n=8) and **Fig. (B)** shows protected ears with earplugs (w/ EP, n=10) in chinchillas with pre-treatment of liraglutide (2 days before blast). Animals received 6 blast exposures on Day 1 at BOP level of 3-5 psi or 21-35 kPa. The measurements were conducted on Day 1 pre- and post-blast and Days 4, 7,14, and 28 after blast.

Timeline and Cost

Activities / CY	19	20	21	22
Tasks 1-1 and 1-2 (low BOP level)				
Task 1-3 (high BOP level or mTBI)				
Tasks 2-1/2-2 (neurotransmitter)				
Tasks 2-3/2-4 (signal pathways)				
Estimated Direct Cost ($K)	**$333**	**$333**	**$334**	

Updated: October 30, 2020

Goals/Milestones

CY19 Goal – Aim 1:Task 1-1 and Aim 2: Tasks 2-1 and 2-2

☑ Hearing function tests and brain section imaging

CY20 Goal – Aim 1:Task 1-2 and Aim 2: Tasks 2-1 and 2-2

☐Investigate the effect of hearing protection on liraglutide's efficacy

☐Complete the tests at low BOP level

CY21 Goal – Aim 1: Task 1-3 and Aim 2: Tasks 2-1 and 2-2

☐ Hearing function tests and brain section imaging to demonstrate the effect of high BOP or mTBI on liraglutide's efficacy

CY22 Goal – Aim 2: Tasks 2-3 and 2-4

☐ Mechanisms of liraglutide's neuroprotection and neuro-regeneration functions

Approach: ● Conducting blast tests in chinchillas with drug treatment at two BOP levels with or without hearing protection and performing hearing function tests over the time course to determine liraglutide's therapeutics; ● Immunostaining of brain sections for fluorescence imaging to determine the effect of liraglutide on synaptic protein changes, neuroprotection, and neuro-regeneration.