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'Ecstasy' Enhances Noise-Induced Hearing Loss

Michael W. Church, Ph.D.^{a,b}, Jinsheng S. Zhang, Ph.D.^{a,b,*}, Megan M. Langford, B.A.^c, and Shane A. Perrine, Ph.D.^c

^aDepartment of Otolaryngology & Head Neck Surgery, Wayne State University School of Medicine, Detroit, Michigan 48201, USA

^bDepartment of Communication Sciences & Disorders, Wayne State University College of Liberal Arts & Sciences, Detroit, Michigan 48202, USA

^cDepartment of Psychiatry & Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, Michigan 48201, USA

Abstract

'Ecstasy' or 3,4-methylenedioxy-N-methamphetamine (MDMA) is an amphetamine abused for its euphoric, empathogenic, hallucinatory, and stimulant effects. It is also used to treat certain psychiatric disorders. Common settings for Ecstasy use are nightclubs and "rave" parties where participants consume MDMA and dance to loud music. One concern with the club setting is that exposure to loud sounds can cause permanent sensorineural hearing loss. Another concern is that consumption of MDMA may enhance such hearing loss. Whereas this latter possibility has not been investigated, this study tested the hypothesis that MDMA enhances noise-induced hearing loss (NIHL) by exposing rats to either MDMA, noise trauma, both MDMA and noise, or neither treatment. MDMA was given in a binge pattern of 5 mg/kg per intraperitoneal injections every 2 h for a total of four injections to animals in the two MDMA-treated groups (MDMA-only and Noise +MDMA). Saline injections were given to the animals in the two non-MDMA groups (Control and Noise-only). Following the final injection, noise trauma was induced by a 10 kHz tone at 120 dB SPL for 1 h to animals in the two noise trauma-treated groups (Noise-only and Noise +MDMA). Hearing loss was assessed by the auditory brainstem response (ABR) and cochlear histology. Results showed that MDMA enhanced NIHL compared to Noise-only and that MDMA alone caused no hearing loss. This implies that "clubbers" and "rave-goers" are exacerbating the amount of NIHL when they consume MDMA and listen to loud sounds. In contrast to earlier reports, the present study found that MDMA by itself caused no changes in the click-evoked ABR's wave latencies or amplitudes.

Keywords

Auditory brainstem response; Ecstasy; MDMA; Noise-induced hearing loss; Rat; Rave party

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*Please Address Correspondence to: Michael W. Church, PhD, Dept Otolaryngology Head Neck Surgery, C.S. Mott Center, 275 East Hancock, Wayne State University School of Medicine, Detroit, MI 48201 (USA), TEL: +01 313 577-1184, mchurch@med.wayne.edu.

Disclosure Statement

There are no conflicts of interest to report.

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1. Introduction

Ecstasy (which contains the psychoactive drug $\pm$ 3,4-methylenedioxy-N-methamphetamine; MDMA) is an amphetamine derivative that is abused for its euphoric, empathogenic, hallucinatory, and stimulant effects. MDMA was developed to treat depression and anxiety disorders and is useful in treating patients with post-traumatic stress disorder (PTSD) (Mithoefer et al., 2013). First used recreationally in the 1970's, its abuse escalated in the late 1980's and continues to be a world-wide problem (Weir, 2000). Recent reports show that MDMA and other amphetamine abuse continue to increase, particularly in the adolescent and young adult populations (Johnston et al., 2011). Common settings for Ecstasy usage are nightclubs and "raves", which are dance parties where participants often consume large amounts of MDMA and dance to loud electronic music for lengthy periods of time. "Clubbers" and "rave-goers" report that consuming MDMA enhances the music experience (Weir, 2000).

People attending such venues are typically exposed to loud music for a period of 4–5 h at a time (Weir, 2000; Williams et al., 2010) with average sound levels ranging from 100 to 124 dB(A) (Gunderson et al., 1997; Sathra et al., 2002; Serra et al., 2005; Williams et al., 2010). This is a major concern because it is well established that exposures to loud sounds for prolonged periods of time or on repeated occasions cause permanent noise-induced hearing loss (NIHL) (Gunderson et al., 1997; Sathra et al., 2002; Serra et al., 2005; Williams et al., 2010).

An added concern is that MDMA consumption may enhance the NIHL among clubbers, rave-goers, and psychiatric patients. For example, MDMA can deplete the brain neurotransmitters serotonin and dopamine (Perrine et al., 2010; Sarkar & Schmued, 2010). Both of these neurotransmitters are believed to play a protective role against acoustic trauma (Lendvai et al., 2011; Papesh & Hurley, 2012; Tong et al., 2005). Second, both high-dose MDMA administration (Sarkar & Schmued, 2010) and noise trauma (Le Prell et al., 2007) induce neurotoxicity, often by similar mechanisms as described later (see Discussion). Third, an emerging body of literature reports that loud sound and MDMA can interact whereby loud sound enhances MDMA's myocardial (Gesi et al., 2002) and neural damage (Feduccia & Duvauchelle, 2008; Gesi et al., 2004; Morton et al., 2001), and MDMA-induced stereotypy and seizures (Morton et al., 2001). Thus far, no studies have addressed the converse possibility that MDMA enhances the toxicity of loud sound exposure. This is an important health issue because of MDMA's widespread consumption and the debilitating effects of hearing loss.

To address this issue, we hypothesized that MDMA enhances NIHL and tested this hypothesis by exposing laboratory rats to high-dose MDMA, noise trauma, both MDMA and noise, or neither treatment. Hearing loss was assessed by the auditory brainstem response (ABR). The cumulative levels of noise trauma and MDMA consumption were intended to model amounts experienced by clubbers or rave-goers.

In addition, there is an interest in MDMA's effects on brain electrophysiology. For example, studies have reported that MDMA alters electroencephalographic (Dafters et al., 1999; Gamma et al., 2000; Obrocki et al., 1999) and other measures of brain function such as the ABR (Taffe et al., 2003; Taffe et al., 2001). Regarding the ABR, a repeated high-dose MDMA regimen in the rhesus monkey caused shortening of P3 and P4 wave latencies and prolongation of P5 wave latency that lasted up to 13 wk post-treatment (Taffe et al., 2003; Taffe et al., 2001).

Accordingly, a second interest was to use the MDMA-only and Control rats to determine if MDMA by itself caused changes in ABR latencies such as those described in the recent

monkey studies (Taffe et al., 2003; Taffe et al., 2001), but using an animal that is lower on the phylogenetic scale and a more moderate dosing regimen. These are important issues, because the United States Animal Welfare Act requires investigators to consider the use of less traumatic procedures and animal models that are lower on the phylogenetic scale (i.e., the principles of “replacement” and “reduction”). Relatedly, the dosing regimen in the two monkey studies was relative high (4 d, 10 mg/kg IM, twice daily) (Taffe et al., 2003; Taffe et al., 2001) compared to the standard human dose of 1 mg/kg per pill (Green et al., 2012) where a pill might be taken in a binge pattern of 2–4 times during the course of a rave party (Green et al., 2012; Johnston et al., 2011; Weir, 2000). Thus, the dosing regimen of our experiments were more relevant to the human situation while adjusting for the different metabolic rates between the rat and human (Green et al., 2012). We also sought to extend previous findings by examining both ABR amplitudes and latencies, as well as the interactive effects of MDMA administration with an auditory stressor condition (viz., rapid stimulus repetition rates). This would provide information about the best animal models, dosing regimens, stimulus parameters and general electrophysiology procedures for future studies.

2. Methods

2.1. Experiment #1 methods

2.1.1. Experimental design and subjects—All animal procedures were approved by the Wayne State University Institutional Animal Care and Use Committee and were in compliance with the National Institutes of Health and National Research Council’s “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources (U.S.). Committee on Care and Use of Laboratory Animals., 2011). The Division of Laboratory Animal Resources maintains animal facilities accredited by the Association for Assessment and Accreditation for Laboratory Animal Care (Frederick, MD 21703–2879 USA), and animals were cared for in accordance with the Animal Welfare Act.

Male Sprague-Dawley rats (Charles River Laboratories, Portage, MI), aged ~60 d at the onset of the study, were divided into four experimental groups: (1) no MDMA and no noise trauma (Control), (2) Noise-only, (3) MDMA-only, and (4) Noise+MDMA. Each group had $n=9$ rats, except the Control group which had $n=10$. Rats were handled and weighed on days of treatment and ABR measurements. Otherwise, rats were left undisturbed in pair-housed conditions with ad libitum normal lab rat chow (5001 Rodent Chow; PMI Nutrition International LLC, Brentwood, MO) and water, 12 h light/dark cycle with lights on at 7 am, and standard room temperature (~22–24°C) and humidity (~35%).

2.1.2. MDMA administration and body temperature measurements—MDMA (5 mg/kg at a solution volume of 1 ml/kg) was intraperitoneally (IP) injected in a binge pattern with MDMA administered once every 2 h for a total of four injections into the rats of the MDMA-only and Noise+MDMA groups. This paradigm of MDMA administration was chosen, because binge-pattern administration results in cumulatively high doses that consistently show serotonin depletion, behavioral effects, hyperthermia (Johnson & Yamamoto, 2010; Perrine et al., 2009; Perrine et al., 2010) and serotonin-mediated neurotoxicity and neurodegeneration, rather than neuroplasticity (Biezonski & Meyer, 2011). Normal saline (0.9% NaCl; 1 ml/kg) was IP injected every 2 h for a total of four injections into rats of the Control and Noise-only groups. The injection site was varied from side to side to minimize discomfort and potential tissue damage that may result from multiple injections, and injections were done in the home cage environment. The MDMA-treated rats (i.e., MDMA-only and Noise+MDMA) were pair-housed together, and the saline-treated rats (i.e., Control and Noise-only) were pair-housed together. Rectal body temperature was measured between MDMA or saline injections to monitor hyperthermia, a

hallmark effect of amphetamines (Sarkar & Schmued, 2010). Baseline temperatures were measured 30 min before the first MDMA (or saline) injection. Temperatures were also taken 30 min before the last (4th) injection (i.e., just before the start of noise trauma) and just after the noise trauma, using a small animal rectal thermometer (Pivia Rectal Temp; Pavia Sales Group, Inc., Plymouth, MN) coated with a lubricating jelly.

2.1.3. Noise trauma procedure—Immediately following the last (4th) MDMA or saline injection, one animal from the Noise-only and one from the Noise+MDMA condition were placed together in a 20 × 45 × 24 cm polycarbonate cage with a polycarbonate lid and filter. The caged animals were then placed inside a double-walled sound-attenuation booth (Industrial Acoustics Co., Bronx, NY). Animals were free to move about the cage. Some noise trauma studies used fully anesthetized animals during the noise trauma procedure (Cody & Robertson, 1983; Zhang et al., 2006), while others used alert and freely moving animals (Gourevitch et al., 2009; Manzoor et al., 2012; Wang et al., 2002). The anesthesia method was rejected because this would not mimic the Rave party scenario and would introduce a possible confounding effect from the co-administration of the anesthetics and the MDMA. Following procedures used by others (Gourevitch et al., 2009; Manzoor et al., 2012; Wang et al., 2002), an open field 10 kHz tone at 120 dB SPL was generated by commercial hardware [RX6 MultiFunction Processor, Tucker Davis Technologies (TDT), Alachua, FL) and software (OpenEx Suite, TDT), amplified (RA300, Alesis, Cumberland, RI) and delivered to a loud speaker (TW67, Pyramid, Brooklyn, NY) located on top of the cage and pointed downward into the cage for 1 h of continuous sound exposure. This procedure was repeated for each of the remaining pairs of Noise-only and Noise+MDMA animals. Having the animals exposed in pairs ensured that the animals from these two groups received the exact same noise exposure. Rats that did not receive essentially the same noise trauma (i.e., Control and MDMA-only) were left in their home cages outside of the sound attenuation booth. The sound level of 120 dB SPL was calibrated at the level of the animal's ear using a sound level meter (BZ 7100, Bruel & Kjar, Norcross, GA) immediately before and after each session to ensure that sound exposure varied by no more than ±1 dB. Animals were monitored visually by one or more technicians through a one-way window in the acoustic chamber. As noted by others, the animals remained calm, engaging in normal exploratory behavior without any apparent distress during the noise exposure (Gourevitch et al., 2009; Manzoor et al., 2012). No animal was observed to be reducing its sound exposure by burying its head beneath the body of its cage mate and there was no preferred orientation of the animal in relation to the speaker.

The noise trauma parameters of a 10 kHz tone at 120 dB SPL for 1 h are commonly used in rodent studies and are sufficient to cause moderate sensorineural hearing loss (Cody & Robertson, 1983; Gourevitch et al., 2009; Wang et al., 2002; Zhang et al., 2006) where a moderate hearing loss is clinically defined as being between 40 and 69 dB (Tannahill & Smoski, 1982). The sound exposure of 1 h at 120 dB SPL resulted in a cumulative exposure level of 400 Pa² h which is equivalent to 10 h of exposure at 110 dB or 32 h of exposure at 105 dB (Williams et al., 2010). These exposure levels are well within the range experienced by patrons who repeatedly attend nightclubs and raves (Gunderson et al., 1997; Sadhra et al., 2002; Serra et al., 2005; Williams et al., 2010).

2.1.4. General ABR procedures—Four rats, one rat from each treatment group, were ABR tested on any single testing day. Rats were first given a pre-treatment (baseline) testing session on Day -7, allowed 7 d to recover, and then given MDMA and/or noise trauma treatments as described above. Three wks later (Day 21), the animals were again tested with ABRs. This time interval allowed for the dissipation of any temporary ABR threshold shifts and provided confidence that only permanent ABR threshold shifts were being measured (Cody & Robertson, 1983; Gourevitch et al., 2009; Manzoor et al., 2012). ABR recordings

were conducted on all animals in all four treatment groups on Days 1 and 8 to monitor and verify the dissipation of such temporary threshold shifts in the two noise-exposed groups. A substantial dissipation of the temporary threshold shifts were observed from Day 1 to Day 8, but very little change in the ABR thresholds between Days 8 and 21. For the sake of brevity, only the pre-treatment baseline (Day -7) and final post-treatment ABR data (Day 21) will be presented.

ABR recordings followed standard procedures (Church et al., 2004; Dehmel et al., 2012). Prior to ABR recording, each animal was given 100 mg/kg ketamine plus 20 mg/kg xylazine (IP). Body temperature was carefully regulated in a narrow normothermic range of 37.6 ± 0.5 °C, because body temperature changes can influence ABR results (Rossi & Britt, 1984). A small animal rectal probe, coated with a lubricating jelly and connected to a temperature monitor (Model 43TD, Yellow Springs Instruments Co., Yellow Springs, OH), was inserted ~2.5 cm into the animal's rectum and secured to the tail with surgical tape. A water-circulating heating pad was used to regulate and maintain normothermia by raising or lowering the temperature of the circulating water (Model TP500, Gaymar Industries, Orchard Park, NY). Ophthalmic ointment was applied to both eyes to prevent corneal drying during anesthesia.

The ABR was differentially recorded between two subcutaneous platinum needle electrodes (E-2, Grass Instrument Division, Astro-Med, Inc., West Warwick, RI). The active electrode was inserted at the vertex, the reference electrode below the left ear, and the ground electrode below the right ear. Evoked potentials were collected by a commercial instrument and amplified 300,000 times with a digital bandpass of 300–3000 Hz (Bio-logic Corp, Mundelein, IL). Electrode impedances ranged from 0–9 k Ω . Recordings were made in an electrically shielded, double-walled sound attenuation chamber (Allotech, Inc., Raleigh, NC). Binaural 'open field' tone pips in the ascending order of 2, 4, 8, 16 and 32 kHz were delivered through either a headphone (TDH-39P, Telephonics Corporation, Farmingdale, NY) for the 2–8 kHz tone pips or a tweeter speaker (Super Tweeter, Tandy Corporation, Fort Worth, TX) for the 16 and 32 kHz tone pips that were placed in front of the animal (rise/fall time = 0.5 ms, plateau = 10 ms, polarity = alternating, repetition rate = 19/s). These tone pip frequencies cover the range of greatest hearing sensitivity for a rat and are comparable to the range tested in other recent rat ABR or mouse auditory neurophysiology studies, permitting an assessment of frequency-dependent effects (Chen & Henderson, 2009; Church et al., 2004; Church et al., 2012; Henry, 2003; Tanaka et al., 2003).

2.1.5. ABR threshold determination—ABR threshold shifts (elevations) were used to assess hearing loss (Church et al., 2004; Dehmel et al., 2012; Hood, 1998). ABR thresholds were determined by the method of limits (Church et al., 2004; Dehmel et al., 2012; Hood, 1998). Serial ABRs were gathered to a range of stimulus intensities starting at 100 dB ppeSPL, and then descending to 80, 60, 50, 40, 35, 30, 25, 20 and 15 dB ppeSPL as the ABR threshold was reached and passed. To establish ABR threshold more precisely, 2 and 3 dB changes in stimulus intensity levels were tested around the ABR's threshold (as determined by visual detection) and two to five ABR traces were collected at each near-threshold intensity level. Each ABR trace was comprised of 256 stimulus-evoked responses. Threshold was defined as the lowest intensity to elicit a reliably scored ABR component (Church et al., 1984; Church et al., 2004; Dehmel et al., 2012; Hood, 1998) which was either the P2 wave or the P2-N2-P4 complex in the rat, because these waveforms were the last to disappear at the ABR's threshold (Alvarado et al., 2012; Church et al., 1984; Church et al., 2004). Thresholds were first blind scored by one researcher, then separately checked for agreement by a second researcher (agreement >98%). The amounts of ABR threshold shifts were calculated by subtracting the baseline (pre-treatment) ABR thresholds from the post-treatment thresholds. For example, if the baseline ABR threshold for the 16 kHz tone pip

condition was 20 dB ppeSPL and the post-treatment threshold was 80 dB ppeSPL, then the amount of threshold shift would be $80-20=60$ dB.

2.1.6. Cochlear histologies and cytochleograms—To provide anatomical verification that the noise trauma caused permanent inner ear damage, the right and left cochleae were harvested from one representative animal in each of the four experimental groups. Animals were euthanized 1 wk after the last ABR recording by rapid decapitation. The temporal bones were rapidly removed and cochleae exposed, followed by intrascalar perfusion of fixative containing 4% paraformaldehyde in phosphate-buffered saline (PBS, pH 7.4) and immersion in this fixative at 4°C. Cochleae were then transported in this fixative to the Histology Core of the Hearing, Balance and Chemical Senses Core Center at the Kresge Hearing Research Institute, University of Michigan, for histological processing and assessment. The right and left cochleae from each animal were rinsed in PBS and decalcified in 5% ethylenediaminetetraacetate (EDTA) at pH=7.4 for 72 h at room temperature. Following decalcification, the lateral wall (spiral ligament and stria vascularis), and Reissner's and tectorial membranes were removed. The remaining tissue, still attached to the modiolus, was permeabilized (0.3% Triton X) in PBS for 30 min and stained with a fluorescent dye (Alexa-Fluor 568 phalloidin, Life Technologies) diluted 1 to 100 in PBS for 60 min at room temperature. After three rinses, the organ of Corti was dissected from the modiolus in four to five segments. The segments were mounted on slides using an aqueous mounting medium (Fluoromount, Diagnostic Biosystems Inc., Pleasanton, CA).

Slides were assessed on a microscope equipped for epifluorescence, using a 50x oil immersion objective and a scale for measuring a portion of the microscope field (Leitz Wetzlar, Wetzlar, Germany). The scale was calibrated to measure a slide field of 0.19 mm. The rows of IHCs and OHCs were oriented longitudinally within each 0.19 mm field. The first two scale lengths were not counted. The apical turn was counted first; the other turns were followed by moving down the cochlear spiral. Each successive 0.19 mm field was evaluated for the presence or absence of hair cells. Manual count data were entered into a computer program (KHRI Cytochleogram, version 3.0.7) and compared to a database of normal Sprague Dawley rat cochleae. The percentage of missing hair cells was calculated for each row and plotted as a function of distance (in mm) from the apical end of the surface preparation. To view the thick organ of Corti at different focal planes, representative images were taken with a confocal microscope (LSM 510, Zeiss, Jena, Germany) at 1 μ m increments and at 15 to 25 different focal planes. Z-stacks of 15 to 25 images were then combined to make 3-D projections. The 3-D projections were visualized using real-time interactive image-analysis software (Imaris version 7.4, Bitplane Inc., South Windsor, CT, USA). The Histology Core staff members were "blind" as to each rat's treatment group and the experiment's nature.

2.1.7. Data analyses—To examine hearing loss, the primary outcome variable was the ABR threshold shift (i.e., threshold elevation). ABR threshold shifts were calculated by subtracting each animal's pre-treatment (baseline) ABR thresholds from their post-treatment thresholds. The resulting ABR threshold shifts were then analyzed by a 2-way analysis of variance (ANOVA) for treatment group (Control, Noise-only, MDMA-only, and Noise +MDMA) and tone pip frequency (2, 4, 8 and 16 kHz). Although ABR thresholds were tested in response to 32 kHz tone pips, four (44.4%) of the Noise-only and five (55.6%) of the Noise+MDMA animals had ABR thresholds that were beyond the testing limits of our equipment (i.e., > 100 dB ppeSPL at 32 kHz). Thus, the true threshold shifts of these two groups could not be determined, because of so much missing data. Therefore, the 32 kHz condition was dropped from the ANOVA. Treatment group was a between-subject measure and Frequency was a within-subject measure. Whereas body temperature can influence some ABR parameters, the same 2-way ANOVA design assessed possible group

temperature differences recorded at the time of the four ABR threshold determinations. A 2-way ANOVA (group x day) assessed possible treatment effects on body weight; where weight was a within-subject measure recorded on the pre-treatment baseline ABR day (Day -7), the noise/MDMA treatment day (Day 0) as well as 1, 8 and 21 d post-treatment (Day 21 = the post-treatment ABR day). To assess possible treatment-induced body temperature effects at the time of drug and noise exposure, temperatures were analyzed by a 2-way ANOVA for the between-subject measure of the four treatment Groups and the within-subject measure of three time points (pre-treatment, prior to the last (4th) injection but just before noise trauma, and post-noise trauma). The Greenhouse-Geisser corrections for the degrees of freedom were used for all within-subject variables (Greenhouse & Geisser, 1959). When any 2-way ANOVA indicated a significant treatment group main effect or interaction, simple effects tests (1-way ANOVAs) and Student-Newman-Keuls or paired t-tests were used to make post hoc pairwise comparisons between individual treatment groups. Whereas hyperthermia can enhance NIHL (Chen et al., 2007) and MDMA can induce hyperthermia (Johnson & Yamamoto, 2010), the correlation (Pearson r) was calculated between the body temperatures recorded just prior to the noise trauma and the subsequent NIHL using data from the two groups that were exposed to the noise trauma. This comparison determined if there was a relationship between the amount of NIHL and an animal's body temperature at the time of noise trauma. Statistics were done by Statistical Package for the Social Sciences software (SPSS 21.0 for Microsoft Windows, SPSS Inc., Chicago, IL, USA), and the criterion for statistical significance was a 2-tailed probability value of $p = 0.05$.

2.2. Experiment #2 methods

2.2.1. Treatment groups—Experiment #2 used the same MDMA-only and Control groups described in Experiment #1. The data for both experiments were collected simultaneously.

2.2.2. Click-evoked ABRs—Binaural click stimuli (generated by a 0.1 ms square wave pulse), presented at repetition rates of 13, 50 and 100 clicks/s and at a stimulus intensity of 80 dB ppeSPL. Presenting stimuli at fast repetition rates constitutes an auditory stressor test that can reveal neurological abnormalities (e.g., poor neural synchrony) that may not be present when stimuli are presented at a slow repetition rate (Church et al., 2012; Santos et al., 2004; Starr et al., 2001). Thus, Experiment #2 used this stressor test to see if MDMA treatment interacted with the stimulus repetition rate.

The rat and mouse ABR typically consists of 4–5 positive-going waveforms labeled as P1, P2, P3, P4 and P5 (Alvarado et al., 2012; Church et al., 1984; Church et al., 2004). The neurogenerators for these waves are uncertain, but it is believed that the major neurogenerators in the rat or mouse are the auditory nerve (P1), cochlear nucleus (P2), superior olivary complex (P3), lateral lemniscus and/or inferior colliculus (P4), and the inferior colliculus, medial geniculate body and/or primary auditory cortex (P5) (Chen & Chen, 1991; Henry, 1979). In comparison, the human ABR is labeled with Roman numerals with the major neurogenerators believed to be the distal auditory nerve (I), proximal auditory nerve (II), cochlear nucleus (III), superior olivary complex (IV), and the termination of the contralateral lateral lemniscus in the inferior colliculus (V) (Moller et al., 1995). The latency of each wave is measured from the stimulus onset to the peak of the wave, usually including an acoustic travel time between the speaker or earphone diaphragm and the animal's ear (Church et al., 1984; Church et al., 2004; Church et al., 2012). In our lab, we measure the amplitudes as the height between the wave's positive peak and the following negative trough (i.e., P1-N1, P2-N2, P3-N3, P4-N4 and P5-N5).

2.2.3. ABR threshold determination—ABR threshold determinations were the same as described for Experiment #1 with the exception that Experiment #2 was able to report the 32 kHz tone pip results.

2.2.4. Data analyses—For the click-evoked ABRs, the latencies and amplitudes for each ABR waveform were assessed separately by 3-way ANOVAs with a between-subjects factor for group (MDMA and Control), and within-subject factors for day (the pre-treatment day and post-treatment Day 1, Day 8, and Day 21) and stimulus repetition rate (13, 50 and 100 clicks/s). The Greenhouse-Geisser corrections for the degrees of freedom were used for all within-subject variables. When an ANOVA indicated a significant treatment group difference, the Student-Newman-Keuls test was used for post hoc pairwise comparisons between individual treatment groups ($p < 0.05$, two-tailed). For the ABR thresholds, the data were assessed as previously described in Experiment #1.

3. Results

3.1. Experiment #1 results

3.1.1. ABR thresholds—The ANOVA on the post-treatment ABR threshold shifts indicated a significant main effect for treatment group: $F(3, 33) = 37.47$, $p < 0.001$. The respective threshold shift marginal means ($\pm$ SEM) for the Control, MDMA-only, Noise-only and Noise+MDMA groups were 0.5 ± 0.5 , 1.2 ± 0.6 , 40.3 ± 4.4 and 53.8 ± 4.7 dB. Post hoc tests indicated the Noise+MDMA group had a significantly greater mean ABR threshold shift than the Noise-only group which had a significantly greater mean threshold shift than the Control and MDMA-only groups ($p < 0.05$). The Control and MDMA-only groups did not differ significantly from each other. A significant interaction was found between the group and tone pip frequency factors, indicating that treatment group differences varied as a function of tone pip frequency: $F(9, 99) = 28.85$, $p < 0.001$. There was also a significant main effect for tone pip frequency, reflecting the known fact that ABR thresholds vary as a function of tonal frequency: $F(3, 99) = 95.35$, $p < 0.001$.

Figure 1 shows the mean ABR threshold shifts for each treatment group at each tone pip frequency as well as the results of post hoc pairwise comparisons. This figure illustrates that the Noise+MDMA group had the greatest threshold shifts in comparison with all other groups. Specifically, the Noise+MDMA group's threshold shifts were significantly greater than those of the Noise-only group at the tone pip frequencies of 2 and 4 kHz, and significantly greater than those of the Control and MDMA-only groups at all four tone pip frequencies. The Noise+MDMA group also had greater threshold shifts than the Noise-only group at 8 and 16 kHz, but not significantly. The Noise-only group's threshold shifts were significantly greater than those of the Control and MDMA-only groups at 4, 8 and 16 kHz, but not at 2 kHz.

Figure 2 shows representative serial ABRs used in the threshold determinations for animals in the Control, Noise-only and Noise+MDMA groups. These ABRs were from animals that were representative of their group's mean performance. This figure shows that the Control animal had an ABR threshold at the stimulus intensity of 20 dB ppeSPL in response to the 4 kHz tone pip stimulus condition. In contrast, the Noise-only and Noise+MDMA animals had respective ABR thresholds of 53 and 73 dB ppeSPL, indicating threshold elevations of 33 and 53 dB relative to the Control animal. Serial ABRs from the MDMA-only animals (not shown) were essentially identical to those from the Control animals.

3.1.2. Body temperatures during ABR recordings—The mean ($\pm$ SEM) body temperatures recorded at the time of each ABR threshold determination for the 2 through 16

kHz conditions ranged from 37.6 ± 0.1 , 37.8 ± 0.1 °C and showed no significant treatment group differences.

3.1.3. Cochlear histology—Figure 3 shows representative images from the basal turn of the cochlear tissue surface preparations from one representative animal in each of the four treatment groups. The animals from the two noise trauma groups (i.e., Noise-only and Noise+MDMA) showed extensive outer hair cell (OHC) loss in the basal region. Each of these animals had ABR threshold shifts that closely matched their group's mean performances. For example, the chosen Noise-only rat (rat N9) had respective ABR threshold shifts of 10, 27, 57 and 63 dB ppeSPL at 2, 4, 8, and 16 kHz. The chosen Noise+MDMA rat (rat N+M9) had respective ABR threshold shifts of 20, 55, 66 and 66 dB ppeSPL. The chosen Control and MDMA-only rats (rats C10 and M9) had essentially no ABR threshold shifts. All these ABR threshold values compared favorably with their respective group's average ABR threshold shifts (see Fig. 1). None of the animals had OHC or IHC damage in the apical region.

The assessments of hair cell loss are presented as cytochleograms in Figure 4. There were numerous gaps in the cytochleograms from the Noise-only and Noise+MDMA animals. These gaps were most likely due to mechanical shearing from the noise trauma itself, rather than fixation or dissection artifact (personal communication on 12 January 2012 with Dr. Josef Miller, Kresge Hearing Research Institute, University of Michigan, Ann Arbor, Michigan). These cytochleograms indicated that the Noise+MDMA animal had more OHC and IHC damage than the Noise-only animal. In the Noise-only animal, the damage was confined to the basal region starting at 53% of the distance from the apex, whereas the Noise+MDMA animal had damage starting at 43% of the distance from the apex. The rat's cochlea is approximately 10 mm in length and 10 kHz is mapped (processed) at approximately 40% of the distance from the apex (Muller, 1991). Cochlear damage following noise trauma typically maps to frequency regions extending above the region associated with trauma-inducing stimulus (Chen & Henderson, 2009; Cody & Robertson, 1983; Zhang et al., 2006). In contrast to the two noise exposed groups, the animals from the two groups that were not exposed to the noise trauma (i.e. the Control and MDMA-only animals) had essentially no OHC or IHC loss.

3.1.4. Body temperatures and weights associated with MDMA administration

—Body temperatures recorded before, during, and after administering the treatment conditions showed a significant effect for the treatment Groups, Time, and the Group-by-Time interaction: $F(3,33)=5.01$, $p=0.006$; $F(2,66)=17.39$, $p<0.001$; and $F(6, 66)=5.41$, $p<0.001$, respectively. Post hoc tests (simple effects ANOVAs followed by Student-Newman-Keuls pairwise comparisons at $\alpha=0.05$) indicated that pre-treatment body temperatures showed no treatment group differences (mean $\pm$ SEM ranged from 36.4 ± 0.3 to 36.7 ± 0.3 °C). Prior to the last (4th) injection but just before the noise trauma, both MDMA groups had significantly higher body temperatures than both Saline groups (mean $\pm$ SEM for Control= 36.5 ± 0.2 , Noise-only= 36.5 ± 0.2 , MDMA-only= 37.6 ± 0.2 and Noise+MDMA= 38.3 ± 0.4 °C), where $F(3, 33)=11.29$, $p=0.001$. Following the noise trauma procedure (~1.5 h after the last MDMA or Saline injection), both MDMA groups still had significantly higher body temperatures than both Saline groups (mean $\pm$ SEM for Control= 36.9 ± 0.2 , Noise-only= 36.6 ± 0.3 , MDMA-only= 37.8 ± 0.2 and Noise+MDMA= 37.8 ± 0.2 °C), where $F(3, 33)=7.36$, $p=0.001$.

There were no significant correlations between an animal's body temperature immediately prior to noise trauma and the animal's subsequent mean ABR threshold shift for either the Noise+MDMA group, Noise-only group or for these two groups combined (all p -values 0.426).

In regards to the body weights measured before (Days -7 and 0) and after treatment (Days 1, 8 and 21). There was a significant effect for day, $F(2,5)=462.93$, $p<0.001$, but not for treatment group or the group-by-day interaction. Paired t-tests indicated that both MDMA-treated groups showed significant body weight decreases the day following their MDMA treatments (from Day 0 to Day1), but the other two groups did not. Specifically, the MDMA-only group showed a weight decrease from 336 ± 8 to 330 ± 9 g and the Noise +MDMA group ($n=9$) showed a weight decrease from 337 ± 8 to 321 ± 9 g (Student's paired t-test, $p<0.05$).

3.2. Experiment #2 results

3.2.1. Click-evoked ABRs—The ANOVA results indicated no significant differences between the MDMA and Control groups for the latencies of waves P1, P2, P3, P4 or P5, and there were no significant effects for the treatment day factor. There were significant effects for the stimulus repetition rate, reflecting the fact that all ABR wave latencies prolonged significantly in response to progressively faster stimulus repetition rates: all $F(2, 34)$ values > 208.726 , all p values < 0.001 (Church et al., 2012). There were no significant interactions of any kind.

The ANOVA results also indicated no significant differences between the MDMA and Control groups for the amplitudes of waves P1, P2, P3, or P4, and there were no significant effects for the treatment day factor. There were significant effects for the stimulus repetition rate, reflecting the fact that all ABR wave amplitudes became significantly smaller in response to progressively faster stimulus repetition rates: all $F(2, 34)$ values > 55.545 and all p values < 0.001 , except for P3 amplitude where $F(2, 34)=1.986$ and $p=0.173$ (Church et al., 2012). There were no significant interactions of any kind.

Body temperatures during the click stimulus conditions showed no significant treatment group, day, or group-by-day differences. The respective body temperature marginal means $\pm$ SD for the Control and MDMA groups were 37.2 ± 0.6 and 37.1 ± 0.5 °C.

3.2.2. ABR thresholds—The ANOVA indicated no significant treatment group differences. There was a significant effect for the stimulus frequency factor, indicating that the ABR thresholds varied across the different tone pip frequencies: $F(4, 121)=860.925$, $p=0.001$. There were no significant differences for the day factor. The only significant interaction was for day-by-frequency. Here, both groups had slight improvements (2 dB decreases) in their mean ABR thresholds in response to the 32 kHz tone pips on Day 21 which was likely due to a modest maturational effect: $F(12, 121)=2.368$, $p=0.026$.

4. Discussion

Experiment #1 is the first study to observe that MDMA (or any abused drug) enhanced NIHL. Figure 1 suggests that the effect was possibly synergistic because the Noise+MDMA group had greater ABR threshold shifts than the linear addition of the threshold shifts caused by the Noise-only and the MDMA-only conditions. This finding suggests that persons attending the loud noise environments of nightclubs and rave parties are greatly increasing the amount of NIHL whenever they take MDMA. The present findings add to the small but growing literature showing combined toxicities from high-dose MDMA and loud noise (Feduccia & Duvauchelle, 2008; Gesi et al., 2002; Gesi et al., 2004; Morton et al., 2001). The present study is unique from these publications in that they studied loud sound's enhancement of MDMA toxicity, whereas the current study did the converse by studying MDMA's enhancement of loud noise toxicity. In addition, prior studies evaluated neural, myocardial and behavioral toxicities; whereas the current study investigated hearing toxicity.

Hearing loss in association with MDMA consumption has been reported in just one case study (Sharma, 2001). It showed that a 19-year-old polydrug-abusing male patient, who was found unconscious following MDMA use and subsequently complained of a ringing in his ears (tinnitus). Audiological examinations revealed that the patient had permanent bilateral sensorineural hearing loss of 45–55 dB at 4 to 8 kHz. Because the patient denied listening to loud music during the onset of tinnitus, the author concluded that the tinnitus and hearing loss were both caused by MDMA alone. Unfortunately, the amount of pre-existing hearing loss and other drug use in this patient were not documented. Thus, the cause of his hearing loss is actually uncertain. In contrast to this case report, our MDMA-only group had normal hearing, strongly suggesting that MDMA by itself would not cause hearing loss unless there was a catastrophic event such a hemorrhage in the inner ear.

Cochlear histology confirmed that the hearing losses in the Noise-only and Noise+MDMA animals were permanent SNHLs caused by OHC and IHC damage. The Noise+MDMA rat (rat N+M9) showed more extensive OHC and IHC damage than the Noise-only rat (rat N9). Although these rats were typical of their respective group's average ABR threshold shifts, it is uncertain if their cochlear histological results were equally typical of group performance. That is, ABR threshold shifts and OHC losses do not always correlate strongly, because hearing loss can involve damage other than just OHC loss (e.g., functional damage without OHC death) (Cody & Robertson, 1983). Although cochlear histology revealed permanent OHC and IHC damage at the peripheral level, further damage and related maladaptive plasticity may have extended beyond this region by involving neurodegeneration and such morbidities as tinnitus, hyperacusis, and hearing difficulties in noisy environments (Kujawa & Liberman, 2009).

The mechanisms underlying MDMA's enhancement of NIHL are unknown but are likely related to MDMA and noise trauma sharing similar mechanisms of neural toxicity (Kujawa & Liberman, 2009). For example, a high-dose binge-pattern administration of MDMA causes neural toxicity from reductions in various neuroprotective mechanisms such as antagonism of adenosine receptor activity (Vanattou-Saifoudine et al., 2010; Vlajkovic et al., 2009), inhibition of heteromeric nicotinic acetylcholine receptor activation (Garcia-Rates et al., 2010), reduced glucocorticoid receptor activity (Johnson et al., 2002; Yau et al., 1994), and depletion of the brain neurotransmitters serotonin and dopamine (Perrine et al., 2010; Sarkar & Schmued, 2010). High doses of MDMA also cause neurotoxicity through increased glutamate levels (Sarkar & Schmued, 2010), oxidative stress (Fiaschi & Cerretani, 2010; Sarkar & Schmued, 2010), and ischemia/vasoconstriction (Baker et al., 2007; Bingham et al., 1998). Similarly, NIHL is associated with neural toxicity from reduced adenosine levels (Vlajkovic et al., 2009), reduced nicotinic acetylcholinergic activity (Elgoyhen et al., 2009), reduced glucocorticoid receptor activity (Jin et al., 2009), increased glutamate release (Le Prell et al., 2007), oxidative stress (Le Prell et al., 2007), ischemia in the stria vascularis (Le Prell et al., 2007), and serotonin and dopamine depletion (Lendvai et al., 2011; Papesh & Hurley, 2012; Tong et al., 2005).

The toxicity of MDMA is often dependent on its hyperthermic effects (Johnson & Yamamoto, 2010) and hyperthermia can enhance NIHL (Chen et al., 2007). However, in the present study, there was no correlation between these parameters in the Noise+MDMA group, the Noise-only group, or these two groups combined. Thus, the idea that MDMA-induced hyperthermia played a role in the enhancement of NIHL in the Noise+MDMA group was not supported. One interpretation is that the MDMA-induced increases in body temperatures had no effect, because the body temperatures were still within the normothermic range for the rat's rectal temperature rather than hyperthermic per se. For example, hyperthermia at 40 °C rectal temperature for 20 min was found to enhance NIHL in the mouse (Henry, 2003), but our Noise+MDMA rats had mean rectal temperatures of

only 38.3 and 37.8 °C just before and after their exposure to the noise trauma. It is also possible that the present study did not have enough statistical power to adequately test this hypothesis and that brain or tympanic temperature would have been a more appropriate outcome measure. Thus, the temperature results should not be considered conclusive.

Even though the finding that MDMA enhanced NIHL is novel, there are precedents for other toxic agents enhancing NIHL. These include the organic solvents (Chen & Henderson, 2009; Hoet & Lison, 2008), cisplatin (Gratton et al., 1990), antibiotic aminoglycosides (Li & Steyger, 2009), and opiates (Rawool & Dluhy, 2011). One difference between MDMA and the above toxic agents is that the latter have some ototoxicity when used alone, whereas MDMA alone caused no ototoxicity.

One aim of preclinical studies is to evaluate toxicity so as to predict a drug's adverse effects on clinical populations and recreational users. An inherent problem with an animal model is that drug metabolism, and therefore dosing, will be different from the human experience. Typically, the rat requires more of a drug than a human, because of its faster metabolic rate. A recent article pointed out that the rat metabolizes MDMA 10-times faster than a human. Thus, the rat needs a higher and more sustained dosing regimen to achieve the same level of toxicity experienced by humans. Our MDMA dosing regimen was based on prior rat models of serotonin depletion, behavioral, electrophysiological, and hyperthermic effects. Also, the present study used a dosing regimen that was substantially more moderate than several recent rat (Darvesh et al., 2005; Hemmerle et al., 2012; Kay et al., 2011; Piper et al., 2008) and monkey studies (Taffe et al., 2003; Taffe et al., 2001) that used 2- to 4-times the dose we used. Our dosing was well within the acceptable limits of dosing as well as physiologically and allometrically relevant to the rat, whereas some studies seemed to exceed these boundaries (Green et al., 2012).

The present findings may have broad clinical implications. Listening to loud sounds are major causes of hearing loss in teenagers and adults (Le Prell et al., 2007; Williams et al., 2010). The enhancement of NIHL by MDMA poses an additional health risk and increases the need for intervention. As a primary intervention, public awareness that MDMA enhances NIHL may deter potential victims from using the drug recreationally and also benefit patients being treated with MDMA for anxiety, depression and PTSD. As a secondary intervention, anti-oxidant therapies used to treat NIHL may ameliorate the amount of hearing loss (Le Prell et al., 2007). Finally, the observation that an abused or psychotherapeutic drug can enhance NIHL opens a new field of research with important clinical implications.

The current study's Experiment #2 found no effects of MDMA administration on the click-evoked ABR. This is contrary to two previous studies reporting that MDMA administration shortened P3 and P4's latencies and prolonged P5's latency for a period lasting up to 13 wk following the final MDMA administration in monkeys (Taffe et al., 2003; Taffe et al., 2001). Experiment #2 went beyond the scope of these two previous studies by examining ABR wave amplitudes in addition to wave latencies and by using an auditory stress test of rapid stimulus repetition rates in rats. Despite these increased efforts, Experiment #2 found no MDMA-induced effects on the latencies nor amplitudes of any ABR wave. In addition, the both Experiments #1 and #2 found no effects of MDMA on the ABR thresholds over a broad range of tone pip frequencies. Thus, we conclude that MDMA, by itself and at a standard psycho-active serotonin-depleting dosing regimen for the rat model, had no effects on the rat ABR.

The question is why the two previous monkey studies observed MDMA-induced effects on the ABR and the present study did not? The previous studies used rhesus monkeys, a

repeated dosing regimen of 10 mg/kg MDMA (IM) twice daily at 12 h intervals for 4 d, click stimulus parameters of 70 dB and 10 clicks/s, collected ABRs at 2 to 21 wk post-treatment, and did not report monitoring and regulating the subjects' body temperatures. In contrast, the present study used rats, a binge dosing regimen of 5 mg/kg MDMA (IP) administered once every 2 h for a total of four injections, click stimulus parameters of 80 dB and 13 clicks/s and faster, collected ABRs at ~16 h to 21 d post-treatment, and monitored and regulated the subjects' body temperatures.

Some of these methodological differences may explain the differing findings, whereas others do not: (A) It has been noted that the CNS effects of MDMA in primates are more profound than in rats (Fischer et al., 1995). (B) The dosing regimen used by the previous monkey studies resulted in a higher cumulative dose and possibly higher peak MDMA blood levels than the present rat study. (C) The differences in stimulus parameters for the click-evoked ABRs were minor. The present study used 80 dB ppeSPL click stimuli, because wave P5 in the rat is often small or absent in response to 70 dB ppeSPL clicks. Indeed, four of the 18 rats did not have a wave P5 even in response to the 80 dB ppeSPL clicks (one rat in the MDMA group and three rats in the Control group). The present study also used a stimulus repetition rate of 13 clicks/s instead of 10 click/s, because using a stimulus repetition rate that is a harmonic of 60 Hz power line radiation will time-lock 60 Hz artifact into the sensory evoked potential (Hyde, 1993). (D) Anesthetics used during animal ABR collection will cause body temperatures to decrease and consequently prolong ABR wave latencies and alter wave amplitudes in a biphasic manner, particularly the later waves (Rossi & Britt, 1984). Our experience is that a mere 1 °C decrease in body temperature will significantly prolong the rat's P4 latency and that a rat's body temperature will decrease by 4–5 °C if not thermoregulated. There was no documentation that such precautions were taken in the monkey studies. A decrease in body temperature could explain some or all of the prolongation of the P5 wave in the monkey studies; but it probably does not explain the simultaneous shortening of the P3 and P4 waves, unless ABR wave latencies have a biphasic response to a decrease in body temperature as seen with wave amplitudes (Rossi & Britt, 1984).

Considering these issues, the differences in animal species (monkey versus rat) and dosing regimen are the most likely explanations for the different findings between the current study and the previous monkey studies. The present study used a dosing regimen that has created profound effects in the rat, including enhancing NIHL, temporary weight loss, increased body temperatures, and causing serotonin depletion (Perrine et al., 2010), as well as electrophysiological and behavioral changes (Biezonski & Meyer, 2011; Feduccia & Duvauchelle, 2008; Perrine et al., 2009; Perrine et al., 2010; Sarkar & Schmued, 2010). Thus, the present study used a standard psycho-active dosing regimen that was physiologically and allometrically relevant, and therefore was not under-dosing the rats (Green et al., 2012). Furthermore, differences in time windows to assess MDMA's effects as well as stimulus parameters, ABR scoring and recording differences probably were not major factors in the differing results. The importance is that various factors and procedural issues should be considered when justifying or selecting one's animal model, MDMA dosing regimens, auditory stimulus parameters, post-treatment assessment time frames, and body temperature regulation for future ABR, sensory evoked potential, and other types of CNS neurophysiology studies.

It has been speculated that ABRs and other sensory evoked potential can gain insight into MDMA's CNS and sensory effects, providing a sensitive non-invasive diagnostic tool for early identification of functional impairment in MDMA users (Taffe et al., 2003; Taffe et al., 2001), and provide insight into the harmful interactive effects of sensory trauma and MDMA use. The results of the current study indicate that a moderate MDMA dosing

regimen by itself has no effects on the rat ABR. The present study, however, found that the MDMA dosing regimen did enhance NIHL in rats. Thus, using MDMA in combination with loud noise or other sensory trauma is likely to be a fruitful and insightful line of investigation, perhaps more so than studying the effects of MDMA alone. This is particularly true for studying MDMA's depletion of serotonin and dopamine, because these neurotransmitters have inhibitory and possibly neural protective roles in the auditory system (Hurley & Hall, 2011; Papesh & Hurley, 2012; Tong et al., 2005) [however, see (Shah & Salamy, 1984)]. Thus, MDMA-induced depletion of these neurotransmitters should have adverse effects on auditory information processing and possibly sensory receptor and neural damage when combined with sensory trauma.

5. Conclusions

The current study demonstrated that 'Ecstasy' (MDMA) enhanced NIHL but that MDMA alone caused no hearing loss. This is a major health concern because MDMA is a psychotherapeutic drug with increasing patient applications. Also, it is a widely abused recreational drug that is commonly used in conjunction with listening to loud music, and the resulting hearing loss has debilitating intellectual, functional and social effects. Thus, persons consuming MDMA while listening to loud music or other loud sounds are likely to increase their risk for permanent hearing loss and other harm such as neural (Feduccia & Duvauchelle, 2008; Gesi et al., 2004; Morton et al., 2001) and myocardial damage (Gesi et al., 2002). These results also suggest that MDMA might be a useful drug for studying the roles of serotonin and dopamine in auditory function, and suggest that other psychotherapeutic and abused drugs should be investigated for potential enhancement of NIHL.

The current study also demonstrated that 'Ecstasy' alone had no significant effect on ABR latencies or amplitudes even when using MDMA in combination with an auditory stress test of rapid stimulus click repetition rates. Both experiments found that MDMA by itself had no effect on ABR thresholds in response to a broad range of stimulus frequencies. These results do not support previous findings in monkeys. Differences in dosing regimens, animal models, and possibly other factors could explain the discrepancies. These observations will help guide future studies in terms of selecting animal models, dosing regimens, stimulus parameters, noise and other forms of sensory trauma, and body temperature regulation.

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Abbreviations

ABR	auditory brainstem response
ANOVA	analysis of variances
d	day
dB ppeSPL	decibels peak-to-peak equivalent Sound Pressure Level
EDTA	ethylenediaminetetraacetate

g	grams
h	hour
IHC	inner hair cell
IM	intramuscular
IP	intraperitoneal
MDMA	±3,4-methylenedioxy-N-methamphetamine
min	minute
NIHL	noise-induced hearing loss
OHC	outer hair cell
p	probability level
Pa	Pascal energy units
PBS	phosphate-buffered saline
PTSD	Post-traumatic stress disorder
SD	standard deviation
SEM	standard error of the mean
SNHL	sensorineural hearing loss
wk	week

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Highlights

- Ecstasy (MDMA) enhanced noise-induced hearing loss (NIHL) in rats
- MDMA alone caused no hearing loss
- Rave-goers and certain psychiatric patients may be exacerbating their NIHL by taking MDMA

Fig. 1.

Average ($\pm$ SEM) 3 wks post-treatment ABR threshold shifts (elevations) as functions of treatment group and tone pip frequency showed that MDMA enhanced noise-induced hearing loss. The Control (n=10) and MDMA-only (n=9) groups showed essentially no ABR threshold shifts. In contrast, the Noise-only and Noise+MDMA groups (n=9 each) showed dramatic ABR threshold shifts, with the Noise+MDMA group showing the greatest degree of hearing loss. Post hoc tests indicated the following group differences: *Noise +MDMA > Noise-only = MDMA-only = Control; †Noise+MDMA > Noise-only > MDMA-only = Control; and ‡Noise+MDMA = Noise-only > MDMA-only = Control.

Fig. 2.

Representative serial ABRs used for determining thresholds in Control (rat C5), Noise-only (rat N4) and Noise+MDMA (rat N+M4) animals typical of group performance 3 wks post-treatment (stimuli 4 kHz tone pips). Control rat had an ABR threshold of 20 dB ppeSPL. In contrast, the Noise-only and Noise+MDMA rats had respective ABR thresholds of 53 and 73 dB, indicating threshold elevations of 33 and 53 dB relative to the Control rat. Multiple ABR traces are taken at the lower stimulus intensities to aid wave identification and to verify reproducibility. Serial ABRs from the MDMA-only animals (not shown) were essentially identical to those from the Control animals. The ABR latency scale includes a 0.3 ms acoustic transit time between the speaker and the rat's ears.

Fig. 3.

Confocal images of cochlear tissue surface preparations show damage to cochlear hair cells in the basal region following Noise-only and Noise+MDMA exposure (N9 and N+M9). In the base region, the animals in the Control and MDMA-only (rats C10 and M9) had normal patterns of OHC and IHC. In contrast, the Noise-only and Noise+MDMA animals had numerous missing OHC. In addition, the Noise+MDMA animal had noticeably more OHC damage than the Noise-only animal (Magnification=63x).

Fig. 4.

Right ear cytochleograms from the same animals depicted in Fig. 3 and representative of the ABR threshold shifts seen in the Control (A), MDMA-only (B), Noise-only (C) and Noise+MDMA (D) groups (one animal from each group). There were essentially no cell loss in the one row of inner hair cells (IHC) or the three rows of outer hair cells (OHC-1, OHC-2 and OHC-3) for the animals in the Control and MDMA-only groups. There was extensive OHC damage and some minor IHC damage in the Noise-only animal starting at about 53% of the distance from the apex. There was even more extensive OHC and IHC damage in the Noise+MDMA animal starting at about 43% of the distance from the apex. Gaps in the cytochleograms reflect tissue damage from fragmentation that was too severe to accurately calculate missing IHC and OHC. The tonal frequency scale was adapted from the Wistar (Muller, 1991) and Long-Evans rats (Chen & Henderson, 2009).