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Xingli Li, Jilei Zhang, Qi Liu, Liyuan Zhang, Shiyang Zhao, Hao Zhao, Rui Zhao, Yuanyuan Jing & Lisheng Yu

Mismatch negativity-like responses in nitroglycerin-elicited migraine model

Authors: Xingli Li[1+], Jilei Zhang[1+], Qi Liu[1+], Liyuan Zhang[1,2], Shiyang Zhao[1], Hao Zhao[1], Rui Zhao[1], Yuanyuan Jing*, Lisheng Yu*

[1] Department of Otorhinolaryngology-Head and Neck Surgery, Peking University People's Hospital, Beijing, 100044, China.

[2] Department of Otorhinolaryngology & Hearing International Jiangsu Ear and Hearing Center, The First Affiliated Hospital with Nanjing Medical University, Nanjing, 210029, China.

* laugh_jing@163.com; yulish68@126.com.

+these authors contributed equally to this work.

Abstract

Mismatch negativity (MMN) is an endogenous event-related potential that reflects automatic information processing of the brain and exhibits alterations in latency and amplitude across various pathological conditions. The investigation of MMN-like responses in animal models has provided significant insights into the neural underpinnings of aberrant change-detection mechanisms. This study aims to investigate MMN-like responses in rat models of nitroglycerin (NTG)-induced migraine, to determine whether these responses resemble MMN alterations observed in migraine patients, and to explore novel tools for assessing cortical function in animal models of migraine. Male Wistar rats were assigned to two groups: an NTG-treated group, which received intermittent intraperitoneal injections of nitroglycerin to induce a migraine-like state, and a control group, which received equal volumes of saline. The classical oddball paradigm was employed as the stimulation protocol, and electroencephalography (EEG) signals were recorded concurrently. The latency and amplitude of MMN-like responses were compared between the NTG and control groups. During modeling, the mechanical threshold of rats in the NTG group gradually decreased over time, indicating the development of nociceptive hypersensitivity. Moreover, the

mechanical threshold was significantly different from that of the control group on days 3, 5, 7, and 9 of drug administration. The latency in the NTG group exhibited an overall trend toward shortening compared with the control group, with significant differences observed between the second and third assays. The amplitude showed an overall upward trend compared with the control group, with significant differences detected in the third assay. Rats in the NTG group exhibited accelerated information processing and heightened cortical excitability during auditory stimulation, a finding consistent with observations in migraine patients.

Keywords: Mismatch negativity; Migraine; Rat model; Cortical excitability;

Introduction

Migraine is a chronic neurological disorder characterized by attacks of moderate or severe headache and reversible neurological and systemic symptoms, and it is categorized into two types: episodic migraine and chronic migraine [1, 2]. Some individuals with migraine experience hearing impairment, often accompanied by auditory hypersensitivity [3], which primarily manifests as phonophobia [4, 5]. The incidence of hearing loss rises with advancing age, prolonged attack duration, and heightened attack frequency. Previous studies have suggested that auditory hypersensitivity reflects sensitization in brain regions involved in auditory information processing [6]. Current research on auditory and cortical functional changes in migraine remains controversial.

Intraperitoneal injection of nitroglycerin to induce migraine attacks is a well-established animal model of migraine. However, research using migraine animal models to explore the relationship between migraine and the inner ear remains limited. Arakaki et al. reported that the latencies of waves IV, V, and VI of the 8-kHz acoustic auditory brainstem response (ABR) were significantly prolonged in a rat model of nitroglycerin-induced migraine 2 h after administration [3]. Previous experiments by our group found

that ABR latency was prolonged in a rat model of chronic migraine induced by intraperitoneal nitroglycerin injection, whereas no significant change in threshold was observed [7]. These findings suggest that no damage occurs in the peripheral auditory system in the intraperitoneal nitroglycerin (NTG) injection model of migraine, but alterations may be present in the central auditory system. Notably, while ABR assesses basic auditory conduction and pathway integrity, it cannot evaluate advanced cortical auditory processing or account for migraine-related auditory phenotypes. This limits its utility in elucidating the cortical mechanisms underlying migraine-related auditory dysfunction and necessitates complementary higher-order central auditory indicators.

Event-related potentials (ERPs) are bioelectrical responses recorded following the application of specific stimuli to the nervous system. They reflect cortical activity originating from higher-order brain regions in response to these stimuli. Common auditory ERPs include mismatch negativity (MMN), the P1-N1-P2 complex, and the P300 component. MMN is a negative deflection in human auditory evoked potentials elicited by cognitively discriminable changes [8]. MMN is typically derived as a difference wave by subtracting the ERP elicited by standard stimuli from that elicited by deviant stimuli, with deviants interspersed among standards. MMN is commonly quantified by its amplitude and latency, with the peak occurring approximately 150-250 ms after stimulus onset [9]. The largest negative deflection is typically observed over frontal electrode sites. Latency shortens as the magnitude of stimulus deviation increases [10]. In the oddball paradigm, alterations in the frequency or intensity of auditory stimuli elicit MMN responses independently of attention or conscious awareness. In contrast to ABR, MMN reflects higher-order auditory cognitive processing, thereby addressing a critical limitation of ABR in assessing cortical function.

Migraine patients exhibit shortened latencies, increased amplitudes, and heightened overall auditory sensitivity during

MMN testing compared with healthy controls, indicating accelerated auditory information processing [11]. Individuals with migraine also display enhanced attentional responses to auditory stimuli [12]. Neuroimaging studies suggest that these alterations may arise from reduced cortical responsiveness to stimulus variations, leading to inefficient processing of diverse auditory inputs in migraine patients [13]. MMN-like responses have been extensively investigated in animal models. Electrophysiological, pharmacological, and other lines of evidence indicate that MMN-like responses are homologous to human MMN responses [14]. However, findings from MMN studies in rodent models remain inconsistent owing to substantial inter-study variability, which may reflect differences in reference electrode placement or the anesthetic agents used [15].

In this study, a chronic migraine model was established in rats using repeated intermittent administration of nitroglycerin. A series of auditory stimuli were employed to record MMN-like responses, and alterations in the latency and amplitude of these responses served as indices of cortical function. We hypothesized that NTG-treated migraine model rats may exhibit altered MMN-like responses similar to those observed in migraine patients, reflecting changes in cortical hyperexcitability and auditory information processing. This approach may provide insight into potential central mechanisms linking migraine to auditory dysfunction.

Materials and methods

Grouping and establishment of experimental animals

Male Wistar rats weighing 250-300 g were obtained from Beijing HFK Biological Technology Co., Ltd. (License No. SCXK(Jing)2024-0003). All animals exhibited sensitive auricular reflexes and no known hearing or balance disorders. Animals were housed under specific pathogen-free (SPF) conditions at a room

temperature of 19-22 °C and humidity of 54-64%, with a 12:12 h light/dark cycle and ad libitum access to water and food. This study was approved by the Animal Ethics Committee of Peking University People's Hospital (Approval No. 2023PHE025). All animal care procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals established by the China Association for Laboratory Animal Care. All experiments were conducted in compliance with the ARRIVE guidelines.

All rats were randomly assigned to experimental groups using a computer-based random number generator. Each individual rat was considered an experimental unit.

The NTG group (n = 14) received intraperitoneal injections of nitroglycerin (10 mg/kg) every other day on days 1, 3, 5, 7, and 9. The nitroglycerin solution was diluted with saline at a ratio of 1:4 to a final concentration of 1 mg/mL. The control group (n = 11) received an equivalent volume of 0.9% saline (10 mL/kg) intraperitoneally on the same days.

The nitroglycerin stock solution (5.0 mg/mL; Beijing Yimin, China) contained 30% ethanol, 30% propylene glycol, and water. Prior to each experiment, the stock solution was diluted with sterile 0.9% saline (Otsuka, China) to a working concentration of 1.0 mg/mL [7, 16]. After dilution, the final concentrations of ethanol and propylene glycol in the working solution were below 1%, a level at which effects on rat behavior and inflammatory responses are considered negligible. Previous studies have demonstrated no significant difference in mechanically evoked pain thresholds between measurements obtained with 0.9% saline and those obtained with 6% propylene glycol or 6% ethanol [17].

Behavioral observation and measurement of mechanical allodynia

Behavioral observation: Thirty minutes prior to and 2 h after each administration, rats were placed in clear acrylic cages for observation of spontaneous activity.

Plantar mechanical allodynia measurement: The von Frey

filament test kit consists of 20 filaments capable of applying forces ranging from 0.008 to 300 g. According to the Up-Down Reader software instructions [18], tactile sensitivity in the rat forelimb was determined using the von Frey filament and the up-down method prior to each administration and 2 h after administration by an experimenter blinded to group allocation. The daily testing order was randomized. The up-down method is a widely used technique for determining paw withdrawal thresholds (PWT) in animal models [19, 20]. Mechanical allodynia assessment served only as a supplementary indicator for validating the successful establishment of migraine models. Mechanical allodynia testing on the plantar surface of the rat forelimb is straightforward, yields stable results, and is minimally invasive.

The testing procedure was as follows: a. Rats were placed in the testing apparatus and allowed to acclimate for 15 min. b. While the rat was stationary, the von Frey filament was applied perpendicularly to the central plantar surface of the forelimb, with sustained pressure applied until the filament bent into a ‘C’ or ‘S’ shape for approximately 5 s. c. Paw-withdrawal responses were observed. Paw lifting or licking during the stimulation period was considered a positive response. d. Testing was initiated using a 4 g filament. If a positive response was observed, the central plantar surface was stimulated again after 2 min with filaments of progressively lower force (2 g, 1.4 g, 1 g, 0.6 g) until the first negative response was obtained. This procedure followed the up-down method, in which a filament of one higher force level is applied after a negative response and one lower force level after a positive response [21].

Paw withdrawal thresholds were determined from the response patterns using Up-Down Reader software (https://sourceforge.net/projects/updownreader/). The plantar mechanical threshold measured with von Frey filaments was calculated for each rat before and after each administration. The mean of the left and right paw withdrawal thresholds was

calculated and recorded as the PWT for each time point.

Surgery

Auditory event-related potentials were recorded in rats exhibiting stable mechanical allodynia. Six rats (three per group) underwent surgery under isoflurane anesthesia. A standardized isoflurane anesthesia protocol was consistently applied during surgery and electrophysiological recordings to ensure neural stability and experimental consistency. Anesthesia was induced in a sealed chamber with 4.0% isoflurane (1.0 L/min O_2) for 5 min, until loss of righting reflex. During surgery, isoflurane was maintained at 1.5-2.0% (1.0 L/min O_2) to maintain a surgical plane of anesthesia. During recordings, a calibrated vaporizer and flowmeter delivered isoflurane at 1.0-1.2% (1.0 L/min O_2), maintaining a stable, light anesthetic plane that preserved mild behavioral responses but prevented voluntary movement.

After shaving the scalp with an electric razor, the rat was placed in a stereotaxic frame, and the eyes were lubricated with ophthalmic gel to prevent corneal desiccation. During surgery, body temperature was maintained at 37 °C using a heating pad. Prior to incision, a local anesthetic was injected subcutaneously along the sagittal midline of the scalp. The skull was then exposed, and burr holes were drilled for electrode placement at the following stereotaxic coordinates: recording electrode (frontal lobe): AP 1.0 mm, ML 1.0 mm; reference electrode (frontal lobe): AP 8.0 mm, ML -1.0 mm; and ground electrode (frontal lobe): AP 2.3 mm, ML 2.7 mm. The electrodes were inserted into a miniature six-channel connector and secured with dental cement [22, 23]. All surgeries were performed by the same surgeon. Animals were excluded if premature death prevented behavioral data collection, or if surgical complications or severe stress unrelated to the experimental protocol occurred.

Stimulation sequence

Each rat underwent three recording sessions, with a 1 min interval between sessions. The auditory oddball paradigm comprised two tones: a frequent non-target stimulus (S1) at 1000 Hz (75% of trials) and a rare target stimulus (S2) at 2000 Hz (25% of trials). At least three S1 stimuli occurred between consecutive S2 stimuli. Sound intensity was set to 90 dB, stimulus duration was 100 ms, and a total of 900 target stimuli were presented [24]. Rats were maintained under light anesthesia and positioned prone on a flat surface. Continuous monitoring of anesthetic depth using combined physiological and behavioral indices ensured consistency within and between groups. Physiological indices included respiratory rate, heart rate, and pink, moist mucous membranes. Behavioral indices comprised absence of voluntary movement or righting reflex, mild withdrawal to toe pinch, and a weak but consistent acoustic startle response. Physiological and behavioral indices were recorded every 5 min; isoflurane concentration was adjusted in increments of ± 0.1% if deviations occurred.

The loudspeakers were positioned 1 cm from the external auditory meatus. The recording room was maintained quiet and at a constant temperature. Recordings commenced when rats exhibited a mild withdrawal reflex to toe pinch and a startle response to an abrupt auditory stimulus. Each recording session was repeated 2-3 times per animal. Only waveforms with high reproducibility were retained for analysis. At the end of the experiment, rats were euthanized by carbon dioxide (CO_2) inhalation. The procedure was performed on conscious animals using compressed CO_2 gas delivered at a flow rate sufficient to displace 20-30% of the cage volume per minute. Animals were maintained in the CO_2-enriched atmosphere for a minimum of 10 minutes after respiratory arrest to ensure irreversible death. Death was confirmed by respiratory arrest, absence of heartbeat, and loss of reflexes. All procedures were conducted in accordance with the Chinese national standard GB/T 39760-2021 (Laboratory Animal -

Guidelines for Euthanasia).

MMN-like responses data processing

Signal processing was performed using custom MATLAB scripts. Continuous EEG was segmented into epochs time-locked to each auditory stimulus [25]. The recorded signals were low-pass filtered at 300 Hz and digitized at a sampling rate of 1000 Hz [26]. For analysis, the signals were further low-pass filtered at 50 Hz to remove high-frequency noise. Segments containing amplitudes exceeding ± 300 μV were discarded to eliminate large artefacts caused by body movement or electrode displacement. Baseline correction was performed by subtracting the mean voltage of the pre-stimulus baseline period (-100 to 0 ms relative to S1) from each data point across the entire epoch. ERP waveforms from frontal electrode sites were selected for analysis, as this region exhibits a pronounced auditory evoked potential [27]. Only epochs meeting the artefact rejection criterion (retention rate ≥ 80%) were included in subsequent analyses.

The ERP waveform for each rat was obtained by averaging the signal from 100 ms before S1 onset to 500 ms after S2 onset. Trials with an S1-S2 interval of 2000 ms were excluded from ERP analysis because the response to S2 occurred outside the analyzable time window. Signals from each electrode were analyzed separately. Mean ERPs were calculated separately for deviant stimuli, for standard stimuli immediately preceding or following a deviant, and for all standard stimuli [28]. MMN reflects stimulus deviance and is expected to be elicited when a stimulus deviates from the preceding regular pattern. Deviant-stimulus ERPs were then subtracted from standard-stimulus ERPs within the oddball block to obtain the difference waveform, defined as the MMN-like response [24]. MMN-like responses were identified by the presence of a stable negative deflection in the difference waveform. Amplitude was defined as the mean voltage between the onset and offset of this

negative deflection, and latency as the interval from stimulus onset to the peak of the deflection. MMN-like response data processing and analysis were conducted by an experimenter blinded to group allocation.

Statistical analysis

Descriptive data are presented as mean ± standard deviation (SD). Normality and homogeneity of variances were assessed using the Shapiro-Wilk test and Levene's test, respectively. An independent-samples t-test was used to compare PWT between the two groups. Mean latencies and amplitudes of ERPs were compared between groups using two-way ANOVA followed by Šidák's multiple comparisons test. All statistical analyses were performed with SPSS (version 27.0, IBM, USA) and GraphPad Prism (version 10.1, GraphPad, USA). Statistical significance was set at $p < 0.05$.

Results

Establishment of the chronic migraine rat model

General conditions

Rats in the NTG group received intraperitoneal injections of nitroglycerin on days 1, 3, 5, 7, and 9. During modeling, rats in the NTG group exhibited hypophagia, reduced food intake, and delayed weight gain (Fig. 1A), consistent with previous clinical observations. Each intraperitoneal injection of nitroglycerin elicited acute nociception-related behaviors in rats of the NTG group, including restlessness, excessive grooming (Fig. 1B), and frequent, sustained episodes of head scratching (Fig. 1C). Thirty minutes after each injection, rats in the NTG group displayed fatigue, lethargy, reduced locomotor activity, and a pain-like facial expression characterized by orbital tightening and flattened cheeks (Fig. 1D), in stark contrast to rats in the control group (Fig. 1E).

No significant difference in baseline body weight was observed between the two groups prior to the first administration (NTG:

276.71 ± 10.28 g vs. control: 275.36 ± 10.20 g). However, on day 7 (before the fourth injection), body weight was significantly lower in the NTG group (294.29 ± 13.21 g) than in the control group (312.55 ± 13.92 g; ** $p = 0.003$, $t(23) = -3.351$). This difference persisted on day 9 (before the fifth injection), with the NTG group weighing 297.21 ± 13.31 g compared with 324.27 ± 16.32 g in the control group (***$p < 0.001$, $t(23) = -4.570$).

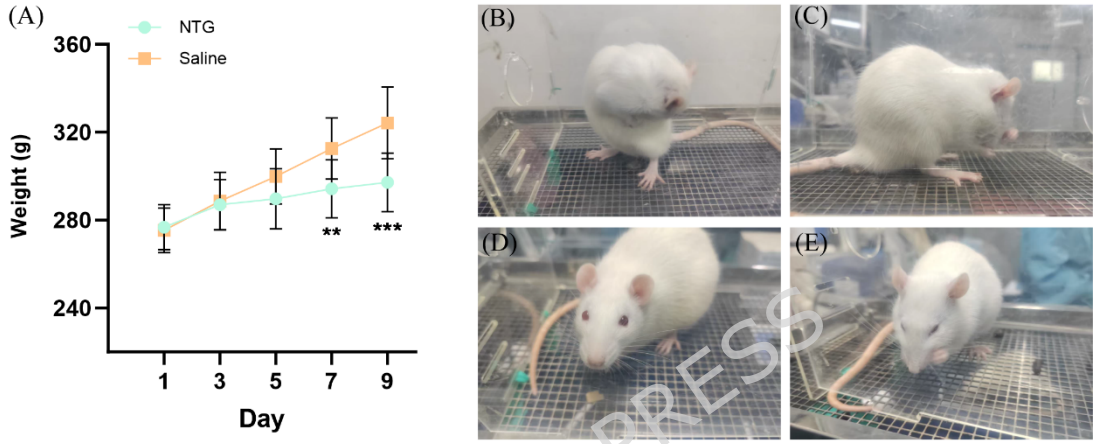

Figure 1. Repeated intermittent administration of nitroglycerin induces delayed weight gain, nociceptive behaviors, and pain-like facial expressions in rats.
(A) The NTG group exhibited delayed weight gain throughout the modeling period. Body weights of the NTG group were significantly lower than those of the control group on day 7 (294.29 ± 13.21 g vs. 312.55 ± 13.92 g; unpaired t-test: ** $p = 0.003$, $t(23) = -3.351$) and on day 9 (297.21 ± 13.31 g vs. 324.27 ± 16.32 g; ***$p < 0.001$, $t(23) = -4.570$). (NTG, $n = 14$; control, $n = 11$). Results are presented as mean ± SD. Significance levels: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (B) Representative image showing excessive grooming behavior in a rat from the NTG group following nitroglycerin administration. (C) Representative image showing frequent head-scratching behavior in a rat from the NTG group following nitroglycerin administration. (D) Representative facial expression of a rat from the control group following 0.9% saline injection. (E) Representative pain-like facial expression of a rat from the NTG group following nitroglycerin injection, characterized by orbital tightening and flattened cheeks.

Mechanical allodynia assessment

PWT was determined using von Frey filaments for both groups before and 2 h after each administration. Baseline PWT measured

prior to each NTG injection progressively declined over the course of the modeling period, indicating the development of mechanical allodynia in the NTG group. By days 3, 5, 7, and 9, PWT in the NTG group was significantly lower than that in the control group (***$p <$ 0.001, Fig. 2A, Table 1). Post-administration PWT in the NTG group showed a mild decrease after the first injection, which did not reach statistical significance compared with the control group. However, on days 3, 5, 7, and 9, PWT in the NTG group was significantly lower than that in the control group (***$p < 0.001$, Fig. 2B, Table 2). To further evaluate the effect of a single dose, baseline PWT was compared with post-administration PWT within the NTG group. A trend toward decreased PWT was observed 2 h after administration, although this difference did not reach statistical significance (Fig. 2C). The control group exhibited no discernible changes in PWT. No animals were excluded from the analysis due to failure to meet the inclusion criteria. These findings indicate that a single administration of nitroglycerin was accompanied by a mild reduction in mechanical threshold, whereas repeated intermittent administration resulted in sustained mechanical hyperalgesia, thereby supporting the successful establishment of the chronic migraine model.

Table 1. Comparison of baseline mechanical allodynia between the Nitroglycerin and control groups (mean ± SD).

DAY	Nitroglycerin (g)	Control (g)	*t*	*df*	*p value*
1	7.91 ± 1.56	7.42 ± 0.88	0.936	23	0.359
3	3.62 ± 1.35	6.72 ± 1.79	-4.951	23	<0.001
5	3.28 ± 1.49	7.26 ± 0.85	-8.416	23	<0.001
7	2.24 ± 1.35	7.11 ± 0.78	-10.606	23	<0.001
9	2.93 ± 1.34	7.57 ±	-9.925	23	<0.001

	0.88

Nitroglycerin group (n = 14): nitroglycerin injections; control group (n = 11): 0.9% saline injections.

Table 2. Comparison of mechanical allodynia between the Nitroglycerin and control groups after administration (mean ± SD).

DAY	Nitroglycerin (g)	Control (g)	*t*	*df*	*p value*
1	6.88 ± 1.13	7.26 ± 0.85	-0.928	23	0.363
3	2.61 ± 1.31	6.95 ± 1.28	-8.296	23	<0.001
5	2.48 ± 1.15	7.11 ± 0.78	-11.384	23	<0.001
7	1.97 ± 0.95	6.96 ± 0.68	-14.710	23	<0.001
9	2.24 ± 0.86	7.42 ± 0.88	-14.844	23	<0.001

Nitroglycerin group (n = 14): nitroglycerin injections; control group (n = 11): 0.9% saline injections.

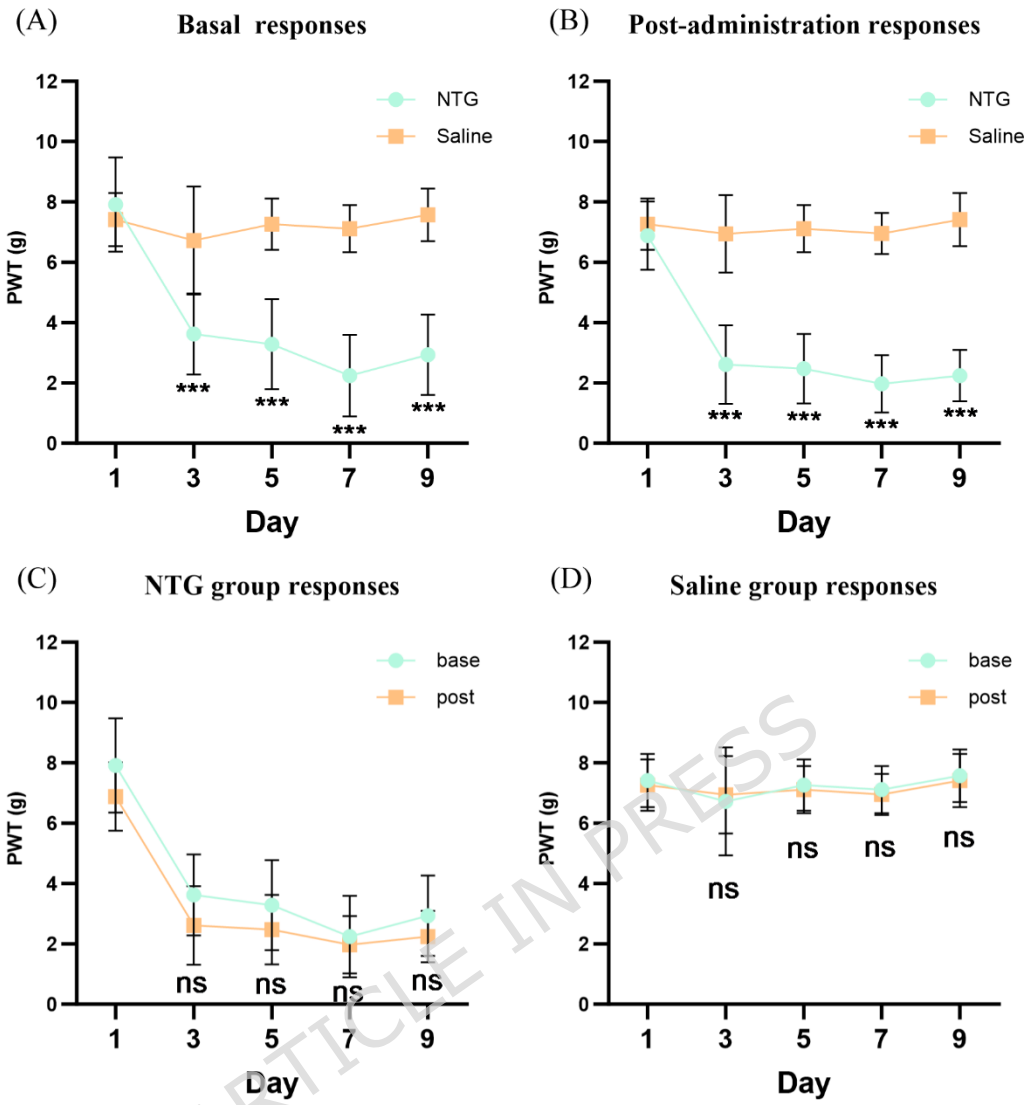

Figure 2. Repeated intermittent administration of nitroglycerin induces mechanical allodynia in rats.

(A) Baseline paw withdrawal thresholds (PWT) in the NTG group progressively decreased over the treatment period. PWT in the NTG group was significantly lower than that in the control group on days 3, 5, 7, and 9 (independent-samples t-test, ***$p < 0.001$). (B) Post-administration PWT in the NTG group was significantly lower than that in the control group on days 3, 5, 7, and 9 (independent-samples t-test, ***$p < 0.001$). (C) PWT measured 2 h after administration exhibited a trend toward decrease in the NTG group, although this difference did not reach statistical significance. (D) Post-administration PWT in the control group exhibited no discernible changes. (NTG, $n = 14$; control, $n = 11$). Results are presented as mean ± SD. ns, not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Measurement of ERP

After completion of the mechanical allodynia assessments, three rats from the NTG group and three rats from the control group were randomly selected for ERP recordings. Each rat underwent three consecutive recording sessions, with a 1 min interval between sessions. ERPs elicited by standard and deviant stimuli were recorded, and the difference waveform (MMN-like response) was obtained by subtracting the standard-stimulus ERP from the deviant-stimulus ERP. Consistent and reproducible negative deflections, characteristic of rodent MMN-like responses, were observed in the difference waveforms across all recording sessions and animals (Fig. 3A-F). Signals were filtered and processed as described in the data processing section.

A two-way repeated-measures ANOVA was conducted to evaluate the effects of group (NTG vs. control) and recording session (first, second, third) on MMN-like latency and amplitude. No significant group-by-session interaction was observed, nor was there a significant main effect of session on either latency or amplitude. However, a significant main effect of group was found for latency ($df = 1$, $F(1,12) = 21.63$, *** $p < 0.001$), with the NTG group exhibiting shorter latencies overall compared with the control group (Fig. 3G). Post-hoc comparisons using Šidák's correction revealed that latency in the NTG group was significantly shorter than that in the control group during the second (270.67 ± 28.04 ms, $p = 0.003$) and third (276.67 ± 25.11 ms, $p = 0.029$) recording sessions. A significant main effect of group was also observed for amplitude ($df = 1$, $F(1,12) = 16.54$, ** $p = 0.002$), with the NTG group displaying larger amplitudes than the control group (Fig. 3H). Post-hoc analysis indicated a significant between-group difference in the third recording session (0.005 ± 0.004 μV, $p = 0.031$).

Separate one-way repeated-measures ANOVAs were performed for each group to assess changes in latency and amplitude across the three recording sessions. In the control group, latency showed

a progressive prolongation over sessions, although this trend did not reach statistical significance. In the NTG group, latency remained stable across sessions, with no significant differences detected. Amplitude in the control group declined numerically in the second and third sessions relative to the first, whereas amplitude in the NTG group increased progressively across sessions. However, none of these within-group changes reached statistical significance (Fig. 3I-J).

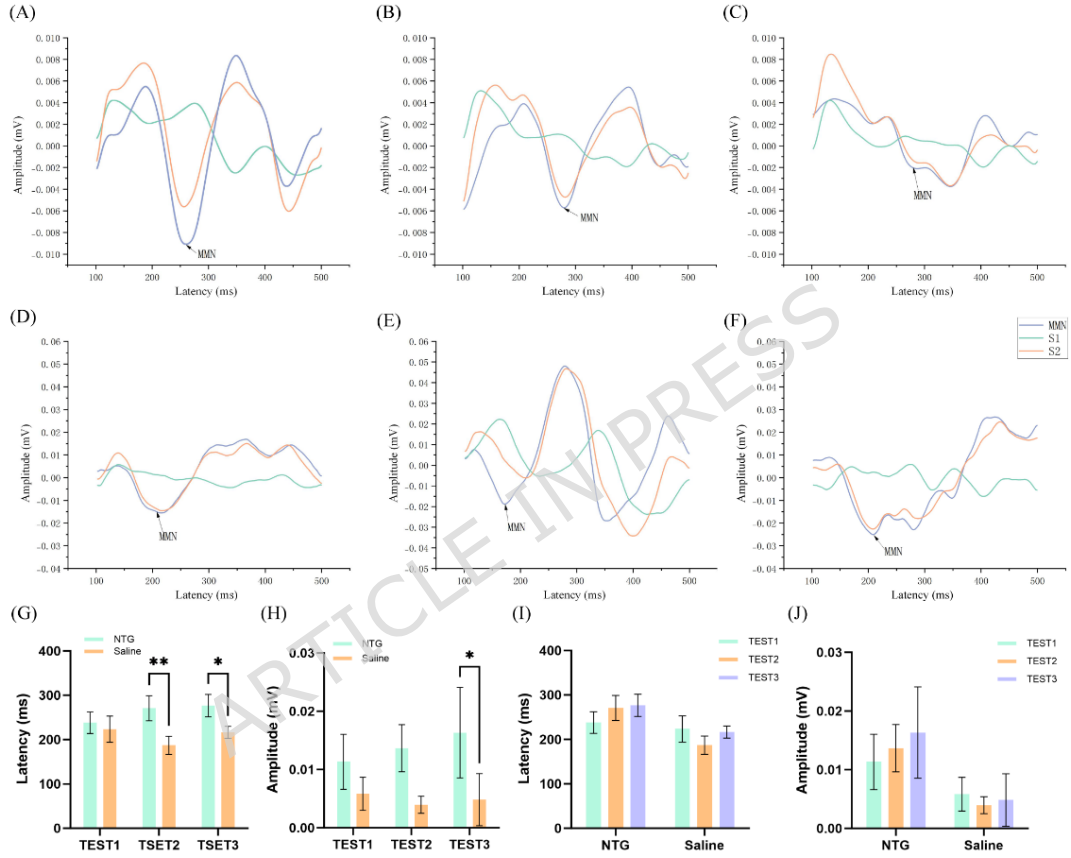

Figure 3. Rats in the NTG and control groups exhibited stable MMN-like waveforms, with significant differences in latency and amplitude between groups (n = 3 per group).

(A) Waveforms recorded during the first recording session in the control group. (B) Waveforms recorded during the second recording session in the control group. (C) Waveforms recorded during the third recording session in the control group. (D) Waveforms recorded during the first recording session in the NTG group. (E) Waveforms recorded during the second recording session in the NTG group. (F) Waveforms recorded during the third recording session in the NTG group. (G) Latency across the three recording sessions in the NTG and control groups. Two-way repeated-measures ANOVA revealed a significant main effect of group (df = 1, F(1,12) = 21.63, ***p < 0.001), but

no significant effect of session (df = 2, F (2,12) = 0.97, p = 0.407) and no group-by-session interaction (df = 2, F(2,12) = 3.22, p = 0.076). (H) Amplitude across the three recording sessions in the NTG and control groups. Two-way repeated-measures ANOVA showed a significant main effect of group (df = 2, F (1,12) = 16.54, **p = 0.002), with no significant effect of session (df = 2, F (2,12) = 0.33, p = 0.723) and no interaction (df = 2, F(2,12) = 0.66, p = 0.535). (I) Within-group comparison of latency across the three recording sessions for the NTG and control groups. (J) Within-group comparison of amplitude across the three recording sessions for the NTG and control groups.

Discussion

Migraine patients frequently exhibit symptoms of altered auditory function, notably auditory hypersensitivity. This hypersensitivity is thought to reflect altered central sensory processing, potentially involving both cortical hyperexcitability and central sensitization—a state of heightened neuronal responsiveness within the central nervous system. A study of interictal migraine patients demonstrated that they exhibit a reduced psychophysical discomfort threshold for sound stimuli and that lower sound intensities are sufficient to elicit brainstem auditory evoked potential waves IV-V [29] . Our preliminary findings indicate that migraine patients score significantly higher on auditory hypersensitivity scales [11]. Several studies have suggested that auditory hypersensitivity in migraine arises from abnormal cortical activity in the auditory pathway, and evidence implicates the brainstem as a potential locus of neural hyperexcitability [29]. Dysfunction of the medial olivocochlear complex within the brainstem may contribute to the phonophobia associated with migraine [30]. Yalin et al. suggested that recurrent migraine attacks increase excitability of the trigeminal pathway and brainstem, thereby contributing to the persistence of pain and associated symptoms [31]. Bhola et al. employed single-pulse transcranial magnetic stimulation to modulate specific cortical areas as a novel acute treatment for migraine. During the 3-month treatment period, in addition to reduced migraine pain, 53% of subjects reported significant relief from auditory hypersensitivity [4] . Although the efficacy of this treatment requires further

investigation, these findings suggest that cortical hyperexcitability contributes, at least in part, to the dysfunction of the pain and auditory systems in patients with chronic migraine.

MMN has long been a key focus of research into central nervous system function. The primary generators of MMN are located in the auditory and frontal cortices. These areas receive direct sensory input and are highly sensitive to behaviorally relevant stimuli and attentional shifts. Importantly, MMN can be elicited by complex stimuli, including categorical changes in chord resonances and grammatical errors [32], phenomena that cannot be accounted for solely by stimulus-specific adaptation. Therefore, MMN is thought to reflect a predictive coding mechanism, in which a mismatch occurs between sensory input and the internal model of the brain related to the auditory environment [33, 34]. Furthermore, MMN is modulated by learning and experience, implicating higher-order brain functions in its generation [35]. A key advantage of MMN is that it can be elicited independently of attention and consciousness. This property has significantly promoted the investigation of MMN-like responses, the homologs of human MMN, in animal models.

Employing well-established recording techniques [36], investigations of MMN-like responses in animals have provided important insights into the neural substrates of aberrant change detection and the neural mechanisms of schizophrenia. Considerable debate remains regarding the homology between rodent MMN-like responses and human MMN, particularly concerning their underlying neural mechanisms. To date, animal studies have addressed these questions by examining morphological, pharmacological, and functional properties [37]. The morphological and pharmacological criteria are relatively easy to test. Morphologically, longer latencies compared to middle-latency responses satisfy this criterion. Pharmacologically, sensitivity to N-methyl-D-aspartic acid receptor (NMDA) receptor antagonists confirms dependence on NMDA receptor activation [38]. In addition,

because MMN-like responses originate from higher-order sensory cortex, their relevance to higher brain functions can be examined. Stimulus-specific adaptation can also be tested to distinguish MMN from lower-level adaptation effects. Based on accumulating evidence of homology, researchers now consider animal models suitable for investigating the functional role of MMN [14] .

We previously reported shortened MMN latencies and increased amplitudes in migraine patients, suggesting that individuals with migraine process auditory stimuli more rapidly, exhibit heightened cortical excitability, and show accelerated information processing [11]. In the present study, we assessed cortical function using an MMN-like paradigm in an NTG-induced rat model that exhibits migraine-associated features. The NTG group exhibited shorter latencies of auditory-related potentials compared with the control group across multiple recording sessions, with significant differences observed in the second and third sessions. Amplitudes also tended to be larger in the NTG group, and a significant difference was found in the third session. These electrophysiological findings, recorded from frontal electrodes, indicate altered cortical responsiveness within auditory-related frontotemporal networks, suggesting heightened cortical excitability. Of note, these recordings were obtained under light anesthesia, and the small sample size precludes strong statistical inference. Therefore, the results should be regarded as preliminary and hypothesis-generating. Nevertheless, the observed direction of change, specifically shorter latency and increased amplitude, is consistent with MMN alterations in patients with migraine, suggesting parallel auditory processing changes.

A common framework for understanding sensory hypersensitivity in migraine involves maladaptive neuroplasticity that leads to central sensitization. In the context of pain, central sensitization refers to increased synaptic efficacy and reduced inhibition in central nociceptive pathways, resulting in heightened pain sensitivity such as allodynia and hyperalgesia. This state may

not be confined to nociceptive circuits and may also facilitate cross-modal hyperexcitability in other sensory systems, such as the auditory pathway [39]. Peripheral nociceptive sensitization is hypothesized to promote hyperexcitability of spinal dorsal horn neurons and enhance central neurotransmission in the brainstem, midbrain, and cerebral cortex. This sensitization may also spread beyond primary nociceptive pathways to other regions of the nervous system. Auditory hypersensitivity reflects sensitization of brain areas involved in auditory processing.

In this study, in addition to shortened latencies and increased amplitudes on ERP testing, rats in the NTG group showed a gradual decrease in paw withdrawal threshold, consistent with the development of extracephalic mechanical hypersensitivity, a behavioral correlate often associated with central sensitization in rodent migraine models. However, due to limitations in testing conditions, we did not assess cognitive functions such as attention and memory in the migraine model rats to further examine cognitive alterations and their relationship with MMN-like responses. A further key limitation is the absence of direct mechanistic evidence linking MMN-like response changes specifically to the spinal or supraspinal processes classically defined as central sensitization. Therefore, although our data suggest that MMN-like responses reflect a state of generalized sensory hyperexcitability, we cannot conclude that they are a specific biomarker for central sensitization itself. Future studies that integrate multimodal assessments are required to clarify this relationship.

In summary, we recorded ERPs using an auditory oddball paradigm in a rat model that exhibits migraine-associated features. The NTG-treated group exhibited alterations in MMN-like response parameters together with behavioral signs of mechanical hypersensitivity, findings that parallel neurophysiological features observed in patients with migraine. Taken together, these preliminary findings suggest that MMN-like responses may serve

as a sensitive tool for assessing cortical hyperexcitability in preclinical migraine models. Building on these results, future research should prioritize larger sample sizes, evaluate the effects of established migraine therapeutics on these responses, and examine correlations with other biomarkers. The sample size for MMN recordings in the present study was small. Therefore, our findings are preliminary and hypothesis-generating. Further validation in larger animal cohorts is required. Such work is necessary to determine the potential of MMN and MMN-like responses as translational biomarkers for altered sensory processing in migraine.

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Data availability
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare no competing interests.