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Influence of physical parameters of sound on the sensory gating effects of N40 in rats

Dongming Zhou [a,b], Yuanye Ma [b,*], Ning Liu [b], Lin Chen [a,**], Min He [c], Yingda Miao [d]

[a] State Key Laboratory of Brain and Cognitive Science, Graduate University, Chinese Academy of Sciences and Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, PR China
[b] Laboratory of Primate Neuroscience Research and Key Laboratory of Animal Models and Human Disease Mechanisms, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan 650223, PR China
[c] Department of Physiology, Kunming Medical College, Kunming, Yunnan, 650031, PR China
[d] Department of Science and Technology, People's Bank of China, Kunming Branch, Kunming, Yunnan, 650000, PR China

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Abstract

Sensory gating is the ability of the brain to modulate its sensitivity to incoming stimuli. The N40 component of the auditory evoked potential, evaluated with the paired click paradigm, was used to probe the gating effect in rats. The physical characteristics of the first and second sounds (S1 and S2), such as frequency, duration, and intensity, were altered in three experiments in this study. Changes in the physical characteristics of the paired click influenced the gating effect. If the two clicks remained identical, physical characteristics of the stimuli has minimal effects on gating, but if S1 was more salient than S2, gating was stronger and the opposite if S1 was fainter than S2. The greater the physical difference between S1 and S2, the more the gating effect was affected.

Keywords: Sensory gating; N40; Auditory evoked potential; Rat; Physical parameter; Frequency; Duration; Intensity

Sensory gating refers to the ability of the brain to inhibit irrelevant sensory input [7]. Impaired sensory gating leads to consequent sensory inundation and cognitive fragmentation [9].

The negative component at 40–60 ms following auditory stimulation (N40) was used to probe the sensory gating mechanism with paired click paradigm in rats [1,4,9,13]. The paired click consists of two identical acoustic stimuli (S1 and S2), which are presented with a short interstimulus interval (e.g., 500 ms). The amplitude (A2) of N40 induced by S2 is usually smaller than the amplitude (A1) induced by S1 under normal conditions. The inhibition ability of the brain is measured as the A2/A1 ratio and the A1–A2 difference. Lower ratios or larger differences are presumed to reflect better inhibition ability of the brain [12].

The broad concept of sensory gating includes the inhibition of irrelevant incoming stimuli (gating out) and the excitation of novel stimuli (gating in) [2]. Several researchers have measured these gating effects with the attenuation and augmentation aspects of sensory gating in both rat N40 and human P50 suppression. It was revealed that the deviant sound could elicit augmentation of the P50 amplitude [2,5,3]. Boutros et al. [6] found when the deviant sound was higher (1500 Hz) than the trained clicks (1000 Hz) in frequency, N40 suppression was attenuated, whereas when the deviant frequency was lower (500 Hz), N40 suppression was augmented. These research results support the notion that gating or habituation is sensitive to stimulus changes, which could modulate the gating in and gating out effects in the central nervous system.

The physical relationship between the paired stimuli is important in understanding the information processing procedure of sensory gating. In this study, we varied the physical characteristics of S1 and S2, their frequency, duration, or intensity, to produce a more complete plot of the N40 gating effect in rats.

Twenty-three adult male Sprague Dawley rats, each weighing 250–300 g, were obtained from the animal center of Kunming Medical College (84 West Ren Min Road, Kunming, Yunnan, China). The animals were housed individually on an alternating 12 h light:12 h dark cycle (lights on at 7:00 am), with access to

* Corresponding author.
** Corresponding author.
E-mail addresses: yuanma0716@vip.sina.com (Y. Ma), lchen@cogsci.ibp.ac.cn (L. Chen).

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food and water ad libitum. After 1 week, during which the rats were frequently handled, the electrodes were implanted. The animals were treated in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996 by the National Academy of Sciences).

The rats were anesthetized with pentobarbital sodium (20 mg/kg) given intraperitoneally. The electrodes were implanted at a depth of 0.5 mm in the skull. The active and reference electrodes were made of stainless steel rods, 0.5 mm in diameter. The active electrode was placed 3 mm posterior to the bregma and 1 mm right lateral to the midline. The reference electrode was 2 mm anterior to the bregma and 1 mm right lateral to the midline. The ground electrode was a stainless steel skewer fixed 3 mm posterior to the bregma and 1 mm left lateral to the midline. Following surgery, the rats were placed in their own cages for 1 week to recover.

The auditory stimuli were delivered through a speaker to the freely mobile animals in their own cages in a sound- and electrically isolated chamber. The rat cage was a cylindrical barrel with a height of 0.5 m and a bottom diameter of 0.3 m. The speaker was set 1.0 m above the barrel bottom. No apparent loudness turbulence of the sound was observed at the periphery of the cage. The two clicks, S1 and S2, were delivered 500 ms apart. The intertrial interval was 10–15 s, varied randomly by the computer. Before the test began, the rats were exposed to the experimental stimuli for half an hour for adaptation.

The electrodes were connected to electroencephalogram (EEG) amplifiers with a head plug. The EEG signals were amplified 2500 times and were sampled at 1000 Hz with a 16 bit analog-to-digital converter. An online analogue filter setting of 0.1–100 Hz was used. The data were recorded continuously from awake animals and stored for offline analysis with EEGLab 4.5 [8] in MATLAB. Continuous data were extracted into epochs of 200 ms before and 1300 ms after stimulus onset. The data were band-pass filtered between 1 and 30 Hz, and the epochs with amplitudes greater than 300 μV were rejected.

N40 was identified as the most prominent negative peak between 30 and 60 ms after stimulus onset. The voltage, from the negative peak to the positive peak just before it, was used to calculate the amplitudes of N40 [6]. The N40 amplitudes for S1 and S2 were recorded as A1 and A2, respectively. The sensory gating effect was measured as the A2/A1 ratio and A1–A2 difference [12].

Three experiments were performed. S1 and S2 were varied in frequency (experiment 1), duration (experiment 2), or intensity (experiment 3). The experimental conditions, A2/A1 ratios, and A1–A2 differences are listed in Table 1.

Separate repeated-measures analysis of variance (r-ANOVA) was performed for the A2/A1 ratios, A1–A2 differences, and the latencies of A1 and A2 in each experiment, with two factors (in the first experiment, the frequencies of S1 and S2, SF1 and SF2, respectively; in the second experiment, the durations of S1 and S2, SD1 and SD2, respectively; in the third experiment, the intensities of S1 and S2, SI1 and SI2, respectively). There were three levels for each factor in the first two experiments (in the first, 1000, 1500, and 2000 Hz; in the second, 5, 10, and 20 ms), and two levels in the third experiment (65 and 85 dB sound

Table 1
Means and standard deviations (S.D.) of A2/A1 ratios and A1–A2 differences, and the parameters of S1 and S2 under the conditions of all three experiments

Experiment 1 S1 and S2 were in 5 ms, 85 dB SPL				Experiment 2 S1 and S2 were in 1000 Hz, 85 dB SPL				Experiment 3 S1 and S2 were in 5 ms, 1000 Hz			
Frequency (Hz)		A2/A1	A1–A2 (μV)	Duration (ms)		A2/A1	A1–A2 (μV)	Intensity (dB SPL)		A2/A1	A1 – A2 (μV)
SF1	SF2			SD1	SD2			SI1	SI2		
1000	1000	0.44 ± 0.13	30.1 ± 17.7	5	5	0.44 ± 0.21	27.8 ± 15.2	65	65	0.55 ± 0.16	31.5 ± 15.3
1000	1500	0.62 ± 0.05	19.6 ± 8.1	5	10	0.55 ± 0.18	23.2 ± 14.7	65	85	0.78 ± 0.14	44.0 ± 17.7
1000	2000	0.90 ± 0.28	6.1 ± 17.8	5	20	0.59 ± 0.18	20.9 ± 15.9	85	65	0.34 ± 0.11	8.7 ± 5.2
1500	1000	0.32 ± 0.06	42.7 ± 17.5	10	5	0.46 ± 0.19	29.6 ± 16.6	85	85	0.47 ± 0.14	17.3 ± 6.6
1500	1500	0.44 ± 0.08	35.7 ± 15.9	10	10	0.46 ± 0.20	30.9 ± 17.1				
1500	2000	0.65 ± 0.23	24.4 ± 21.8	10	20	0.44 ± 0.19	30.3 ± 16.7				
2000	1000	0.22 ± 0.06	59.7 ± 23.7	20	5	0.34 ± 0.12	35.4 ± 15.0				
2000	1500	0.36 ± 0.14	54.0 ± 28.9	20	10	0.38 ± 0.17	35.6 ± 16.9				
2000	2000	0.46 ± 0.10	42.4 ± 22.2	20	20	0.47 ± 0.50	30.3 ± 17.3				

pressure level [SPL]). One-way ANOVA and the least significant difference (LSD) post hoc test were used to compare differences between each two conditions in each experiment. The SPSS 10.0 Statistical Package was used to conduct the statistical analyses. Statistical significance was set at the 0.05 level.

To further understand our data, we compared the A2/A1 ratios and A1–A2 differences in each experiment based on the relationship between S1 and S2. First, S1 and S2 were identical (S1 = S2); second, S1 was equal to or more salient (either in higher frequency, longer duration, or greater intensity) than S2 (S1 ≥ S2); third, S1 was equal to or weaker than S2 (S1 ≤ S2). One-way ANOVA and the LSD post hoc test were used to test the data.

In the first experiment, nine sets of conditions were applied to eight rats when S1 and S2 were varied across three frequencies (SF1, SF2: 1000, 1500, and 2000 Hz) while their durations were maintained at 5 ms and their intensities at 85 dB SPL (see Table 1).

With r-ANOVA, the main significant effects of SF1 and SF2 on the A2/A1 ratios and A1–A2 differences were identified. There were significant differences in the A2/A1 ratios with changes in SF1 ($F(2, 14) = 23.58$, $P < 0.001$) and SF2 ($F(2, 14) = 15.72$, $P < 0.001$), and no significant interaction between SF1 and SF2 ($F(4, 28) = 2.68$, $P = 0.052 > 0.05$). The corresponding means revealed that the A2/A1 ratios decreased with an increase in SF1, and increased with an increase in SF2. There was a significant difference in the A1–A2 differences with changes in SF1 ($F(2, 14) = 16.63$, $P < 0.001$) and SF2 ($F(2, 14) = 12.02$, $P < 0.001$), but no significant interaction between SF1 and SF2 ($F(4, 28) = 0.709$, $P = 0.592 > 0.05$). The corresponding means revealed that the A1–A2 differences increased with an increase in SF1, and decreased with an increase in SF2. The means and differences are illustrated in Fig. 1(A) for the A2/A1 ratios and in Fig. 1(B) for the A1–A2 differences. Neither the A1 and A2 latencies were influenced by SF1 ($P > 0.05$) or SF2 ($P > 0.05$).

When S1 = S2, i.e., 1000–1000 Hz, 1500–1500 Hz, or 2000–2000 Hz, no significant differences were observed in the A2/A1 ratios ($F(2, 21) = 0.11$, $P = 0.90 > 0.05$) or A1–A2 differences ($F(2, 21) = 0.85$, $P = 0.44 > 0.05$). When S1 ≥ S2, we tested the cases where SF1–SF2 were 1000–1000 Hz, 1500–1000 Hz, and 2000–1000 Hz, respectively. Significant differences were observed in the A2/A1 ratios ($F(2, 21) = 9.71$, $P = 0.001 < 0.01$) and A1–A2 differences ($F(2, 21) = 4.54$, $P = 0.023 < 0.05$). The post hoc test and corresponding means revealed that when S1 was higher than S2 in frequency, the A2/A1 ratios were lower and the A1–A2 differences were higher than when S1 was equal to S2, indicating that the gating effect was stronger. For S1 ≤ S2, we tested frequencies of 1000–2000 Hz, 1500–2000 Hz, and 2000–2000 Hz, respectively. Significant differences were observed in the A2/A1 ratios ($F(2, 21) = 7.00$, $P = 0.005 < 0.01$) and A1–A2 differences ($F(2, 21) = 6.15$, $P = 0.008 < 0.01$). The post hoc test and corresponding means revealed that when S1 was lower in frequency than S2, the A2/A1 ratios were higher and the A1–A2 differences were lower than when S1 was equal to S2, indicating that the gating effect was weaker.

In the second experiment, nine sets of conditions were applied to eleven rats when S1 and S2 were varied across three durations (SD1, SD2: 5, 10, or 20 ms), while the intensity was maintained at 85 dB SPL and the frequency at 1000 Hz (see Table 1).

The main significant effects of SD1 and SD2 on the A2/A1 ratios and A1–A2 differences were identified with r-ANOVA. There were significant differences in the A2/A2 ratios with changes in SD1 ($F(2, 20) = 20.81$, $P < 0.001$) and SD2 ($F(2, 20) = 13.21$, $P < 0.001$), and a significant interaction between SD1 and SD2 ($F(4, 40) = 3.57$, $P = 0.041 < 0.05$). When SD1 = 5 ms, A2/A1 increased with an increase in SD2 ($F(2, 20) = 8.69$, $P = 0.002 < 0.01$). A similar effect was observed when SD1 = 20 ms ($F(2, 20) = 6.83$, $P = 0.005 < 0.01$). The corresponding means revealed that the A2/A1 ratio decreased with an increase in SD1, and increased with an increase in SD2. There was a significant difference between the A1–A2 differences with changes in SD1 ($F(2, 20) = 17.33$, $P < 0.001$) and SD2 ($F(2, 20) = 7.08$, $P = 0.005 < 0.01$) and no significant interaction between SD1 and SD2 ($F(4, 40) = 2.30$, $P = 0.075 > 0.05$). The corresponding means revealed that the A1–A2 difference increased with an increase in SD1, and decreased with an increase in SD2. Neither A1 nor A2 latencies were influenced by SD1 ($P > 0.05$) or SD2 ($P > 0.05$).

When S1 = S2, i.e., 5–5 ms, 10–10 ms, or 20–20 ms, no significant differences were observed in the A2/A1 ratios ($F(2, 30) = 0.05$, $P = 0.95 > 0.05$) or A1–A2 differences ($F(2, 30) = 0.11$, $P = 0.90 > 0.05$). When S1 ≥ S2, we tested sound durations of 5–5 ms, 10–5 ms, and 20–5 ms. No significant difference was observed in the A2/A1 ratios ($F(2, 30) = 1.44$, $P = 0.25 > 0.05$) or the A1–A2 differences ($F(2, 30) = 0.70$, $P = 0.50 > 0.05$). When S1 ≤ S2, we tested sound durations of 5–20 ms, 10–20 ms, and 20–20 ms. No significant difference was observed in the A2/A1 ratios ($F(2, 30) = 2.04$, $P = 0.15 > 0.05$) or the A1–A2 differences ($F(2, 30) = 1.17$, $P = 0.33$).

In the third experiment, four sets of conditions were applied to four rats when S1 and S2 varied across two levels of intensity (SI1, SI2: 65 and 85 dB SPL), while the duration was maintained at 5 ms and the frequency at 1000 Hz (see Table 1).

With r-ANOVA, SI1 and SI2 had significant effects on the A2/A1 ratios and A1–A2 differences. There were significant differences in the A2/A1 ratios with changes in SI1 ($F(1, 3) = 28.630$, $P = 0.013 < 0.05$) and in SI2 ($F(1, 3) = 40.536$, $P < 0.001$), but no significant interaction between SI1 and SI2 ($F(1, 3) = 0.783$, $P = 0.441 > 0.05$). The corresponding means revealed that the A2/A1 ratios decreased with an increase in SI1, and increased with an increase in SI2. There was no significant difference in the A1–A2 differences with changes in SI1 ($F(1, 3) = 8.46$, $P = 0.062 > 0.05$), but there was a significant difference with changes in SI2 ($F(1, 3) = 17.77$, $P = 0.024 < 0.05$) and no significant interaction between SI1 and SI2 ($F(1, 3) = 0.237$, $P = 0.660 > 0.05$). The corresponding means revealed that the A1–A2 differences increased with an increase in SI1, and decreased with an increase in SI2. Neither the A1 nor A2 latencies were influenced by SI1 ($P > 0.05$) or SI2 ($P > 0.05$).

When S1 = S2, i.e., 65–65 dB SPL or 85–85 dB SPL, there was no significant difference within the A2/A1 ratios ($F(1, 6) = 0.50$, $P = 0.51 > 0.05$) or the A1–A2 differences ($F(1,$

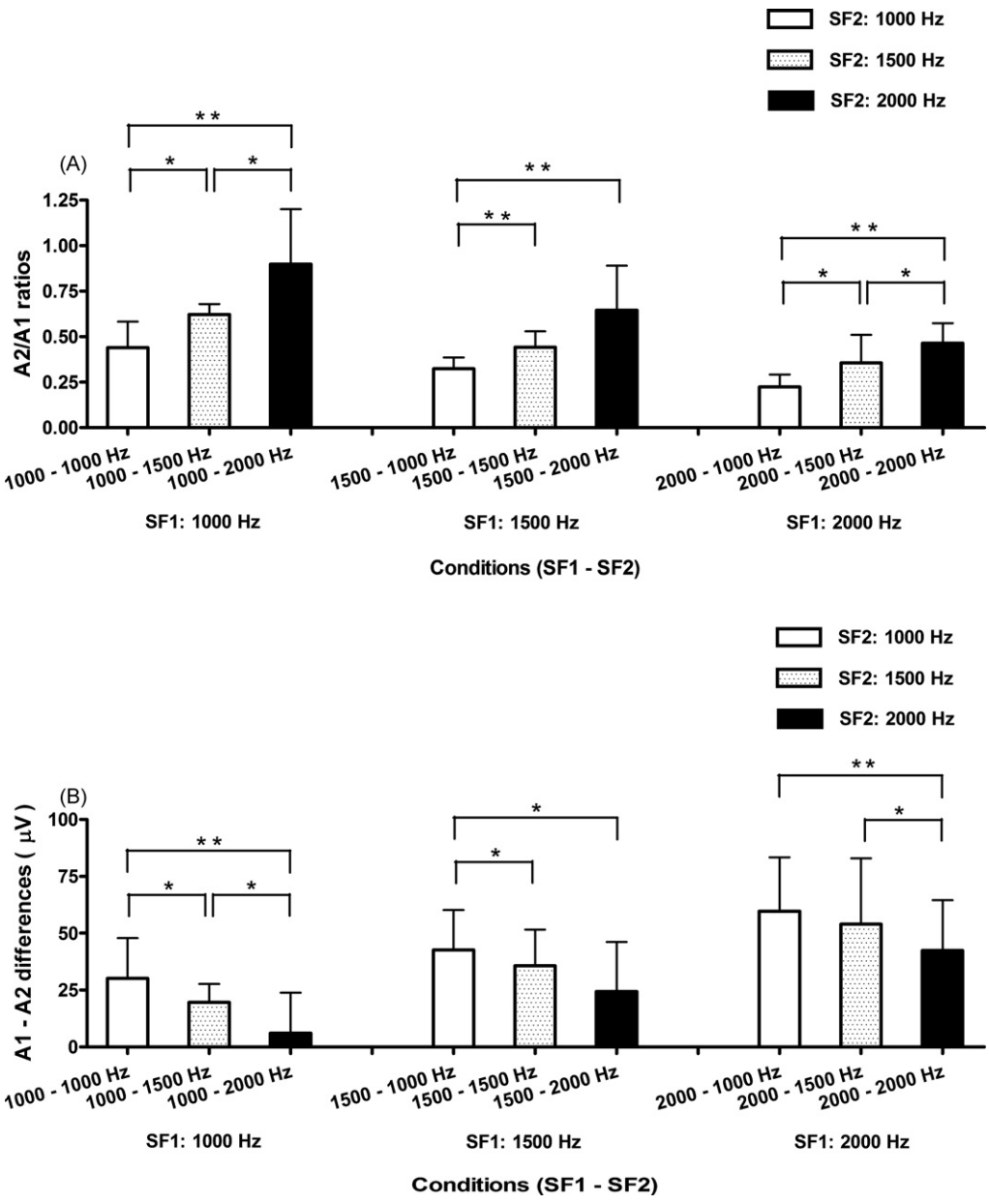

Fig. 1. (A) A2/A1 ratios and (B) A1–A2 differences in eight rats in the first experiment. S1 and S2 were varied across frequencies of 1000, 1500, and 2000 Hz while their durations were maintained at 5 ms and their intensities at 85 dB SPL. The A2/A1 ratios decreased with increases in the frequency of S1 (SF1), and increased with increases in SF2. A1–A2 differences increased with increases in SF1, and decreased with increases in SF2. These results indicate that the sensory gating effect increased with an increase in SF1, and decreased with an increase in SF2. Data trends in the second and the third experiment were similar. Data are presented as mean ± S.D. $^{*}P < 0.05$; $^{**}P < 0.01$.

6) = 2.91, $P = 0.14 > 0.05$). For S1 ≥ S2, i.e., 65–65 dB SPL or 85–65 dB SPL, no significant difference was observed in the A2/A1 ratios ($F(1, 6) = 4.67$, $P = 0.074 > 0.05$) but there was a significant difference in the A1–A2 differences ($F(1, 6) = 7.98$, $P = 0.030 < 0.05$). The corresponding means revealed that when S1 was more intense than S2, the A2/A1 ratios were lower and the A1–A2 differences were higher than those when S1 was equal to S2, indicating that the gating effect was stronger. When S1 ≤ S2, i.e., 65–85 dB SPL or 85–85 dB SPL, significant differences were observed within the A2/A1 ratios ($F(1, 6) = 8.55$, $P = 0.027 < 0.05$) and the A1–A2 differences ($F(1, 6) = 7.92$, $P = 0.031 < 0.05$). The corresponding means revealed that when S1 was weaker in intensity than S2, the A2/A1 ratios were higher and the A1–A2 differences were lower than those when S1 was equal to S2, indicating that the gating effect was weaker.

It can be concluded from the above data that changes in the physical characteristics of S1 and S2 influence the N40 sensory gating effect.

First, if S1 and S2 remained identical, physical characteristics of the stimuli had minimal effects on sensory gating.

Second, the gating effect changed when S1 and S2 were not identical. If S2 was more salient than S1, the gating effect was weaker, whereas when S2 was fainter than S1, the gating effect was stronger. However, despite changes in S1 or S2 under all

the sets of conditions examined in this study, the values for the A2/A1 ratios did not exceed 1, indicating that sensory gating always occurred.

Third, the greater the physical difference between S1 and S2, the more the gating effect was affected. This conclusion can be predominantly deduced from the first and second experiments.

Fourth, the effects of the three physical factors (frequency, duration, and intensity) on N40 sensory gating were similar, but the extents of their influence might be different.

This study not only replicated the N40 gating effects in the rat that Boutros et al. found [6], but also expanded our understanding of the traits of sensory gating in and gating out. These can be modulated by stimulus changes, with greater physical parameter differences between S1 and S2 in the paired click paradigm. Evidence has shown that sensory gating is a multistage (early and late suppression) process that occurs in multiple neural substrates [10,11], both in rat N40 and human P50 gating. Further and more detailed research is required to investigate this cross-species topic.

As described above, some new details of N40 sensory gating in rats were revealed with paired click experiments in this study. Further research must be undertaken to validate these gating effects under different conditions and cross-species.

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