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Pentobarbital anesthesia alters neural responses in the precedence effect

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ABSTRACT

The precedence effect (PE) is thought to be beneficial for proper localization and perception of sounds. The majority of recent physiological studies focus on the neural discharges correlated with PE in the inferior colliculus (IC). Pentobarbital anesthesia is widely used in physiological studies. However, little is known of the effect of pentobarbital on the discharge of neurons in PE. Neuronal responses in the IC from 23 male SD rats were recorded by standard extracellular recording techniques following presentation of 4 ms white noise bursts, presented from either or both of two loud speakers, at different interstimulus delays (ISDs). The neural responses were recorded for off-line analysis before or after intraperitoneal administration of pentobarbital at a loading or maintenance dose. Data were assessed by one-way repeated measures analysis of variance and pairwise comparisons. When the ipsilateral stimuli were leading, pentobarbital at a loading dose significantly increased normalized response to lagging stimuli during recovery from anesthesia. However, it was not the case when the contralateral stimuli were leading. At a maintenance dose, the normalized response to lagging stimuli were significantly reduced, independent of whether contralateral or ipsilateral stimuli were leading. These data show that pentobarbital have no effect on the normalized response of leading stimuli but can prolong the recovery time of lagging stimuli to paired sources produced PE illusions, which was gradually attenuated during recovery from anesthesia. Thus, extracellular recording immediately after administration of pentobarbital should be avoided in physiological studies of neural correlates of PE.

In a reverberant environment, accurate localization of an initial sound source is a basic challenge to our auditory system as the sound propagates to the ear directly, but is followed by sound reflections from nearby surfaces that compete with the initial sound for perception and localization. The ability to normally localize the original sound correctly without influence from the reflected sound is termed the precedence effect (PE), which is thought to be responsible for the ability to localize and perceive sounds accurately and for accurate speech perception. Although the psychoacoustics associated with echo suppression has been well described, the underlying neural mechanisms, especially the neural circuits responsible for mediating the suppressive effects, are poorly understood. The neural correlates of the PE and the echo threshold have been studied at virtually all levels of the auditory system, including the auditory nerve [13], the cochlear nucleus [14,18], superior olivary complex, inferior colliculus (IC) [2,9,10], and auditory cortex [11,15]. In animals, echo thresholds have also been measured behaviorally in cats, rats and barn owls [6,12,17].

The IC occupies a critical relay station in both ascending and descending auditory pathways, and is widely examined in studies on physiological correlates of PE. A number of authors have calculated neural echo thresholds using the half-maximal ISDs, a measure at which neurons reach a responses rate of 50% recovery to the lag stimulus [9,12,19], and the half-maximal ISDs is widely used in electrophysiological studies of PE. At the level of the IC, the half-maximal ISDs of neurons approximates behaviorally-measured echo thresholds [12,17], making it possible to explain the PE by neural correlates in the IC [9,10,17]. However, Tollin et al. found that barbiturate anesthetic may prolong the recovery times of IC neurons to a paired source stimuli that produce the PE illusions [17]; in that study the neural responses in the IC to lagging stimuli recovered with ISDs were virtually identical to that in unanesthetized rabbits, and much faster than their previous anesthetized studies. They postulated that pentobarbital anesthesia was responsible for the prolonged recovery times of lagging stimuli in IC. In addition, Fitzpatrick et al. suggested that pentobarbital anesthesia might affect the responses of IC neurons to paired stimuli in

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non-behaving rabbits [4]. The effects of anesthesia are important to understand in studies connecting behavioral phenomenon of the PE with neural responses in the IC using the half-maximal ISDs.

In the present study, to minimize the effect of sampling bias which can not be avoided in prior studies, we directly compared the neural responses to lagging stimuli before and after administration of pentobarbital at a loading or maintenance dose in the same neuron. We determined whether pentobarbital anesthesia has an effect on the recovery time of lagging stimuli by comparing of neural responses at different times and ISDs before and after administration.

Successful experiments were performed on a total of 23 male Sprague Dawley rats (220–250 g). The Animal Research Committee of Capital University of Medical Science approved the care and use of animals, and all procedures in the present study complied with the National Institutes of Health guidelines for animal use.

All rats were initially anesthetized by intraperitoneal (i.p.) administration of pentobarbital sodium at a loading dose of 50 mg/kg and maintenance dose of 20 mg/kg. Experiments were conducted in a dimly illuminated, double-walled, electrically shielded, sound-attenuating chamber (0.9 m × 0.75 m × 0.8 m) which was placed on a vibration isolation table. The rat was fixed in a stereotaxic frame. The ear bars were replaced by zygomatic arch bars designed especially for this study (Patent application number: 201020600962.8). The zygomatic arch bar fixed the head of rat firmly into the stereotaxic frame, leaving the pinna and external acoustic meatus of untouched. A craniotomy was performed and the dura was removed to expose the cortical surface over the inferior colliculus. After electrode insertion, the exposed cortex was covered with a 2% agar solution. At the end of each experiment, the recording site was stained via iontophoretic ejection of pontamine sky blue from the tip of recording electrode. The animal was given a lethal dose of sodium pentobarbitone and then perfused through the left ventricle with a wash solution followed by cold 4% paraformaldehyde in a 0.1 M phosphate buffer (pH 7.4). The brain coronal sections were counterstained with Nissl stain for identification of the recording marker and the cytoarchitectural borders of IC.

Stimulus generation and data acquisition were both computer-controlled. Acoustic stimuli were 4 ms white noise bursts (no rise-fall time), referred to as clicks, that were digitally generated at a sampling rate of 192 kHz by Adobe Audition 3.0 (build 7283, Adobe Systems Inc., USA). Two electrostatic speakers (EC-1 electrostatic speakers; Tucker-Davis Tech., USA) were held constant at ±18° on the horizontal plane and were located along an arc (25 cm radius measured from the center of the rats' head) in the horizontal and the median sagittal plane in the chamber. The stimuli were presented from either of the two loudspeakers (single source), or from both of two loudspeakers (paired source) with equal levels, coherent in phase, at an ISD between the onsets.

Standard extracellular recording techniques were used to record single neuron discharges. The glass micropipettes were filled with 2% pontamine sky blue dissolved in 5 M sodium acetate (impedance 1–4 MΩ). The electrodes were positioned above the IC by means of a precision (1 μm) digital micromanipulator. Vertical advancement of the electrodes was made by a precision piezoelectric microdriver (PF5-1, Narishige Scientific Instrument Lab, Japan). Extracellular spikes from the recording electrode were amplified (2400A; Dagan Co., USA) and passed through a bandpass filter (500–10 kHz) and an analog-to-digital converter (PowerLab 4/30; ADInstruments Pty Ltd., Australia). The output was finally digitized at 20 kHz and then stored on a computer hard drive using LabChart 7.1 software (ADInstruments Pty Ltd., Australia).

The neural responses to paired stimuli trials with different ISDs (2, 4, 10, 50 ms) and configurations (contralateral stimulus leading or ipsilateral stimulus leading) were recorded after administration of pentobarbital sodium at both loading and maintenance dose. The neural responses to a single stimulus in isolation (contralateral or ipsilateral) were collected prior to each paired-stimulus trial. The neural responses to lagging (leading) stimulus were normalized by the responses to a single stimulus in isolation from the lagging (leading) location. A normalized response of 1.0 indicates the responses to the leading stimulus had no effect on the responses to lagging stimulus, whereas values <1.0 indicate a reduction in the lagging responses. The alteration of normalized response to both leading and lagging stimuli also indicates the effect of pentobarbital anesthesia on neural normalized response in this paper. For some smaller ISDs, such as 2 and 4 ms, the stimuli overlapped and the responses to lagging stimulus were estimated by subtracting the single-stimulus responses measured at the location of leading stimulus from the paired-stimulus responses [9].

Spike sorting is crucial for extracellular recordings in electrophysiological studies. The collected spike units must be sequences of spikes generated by the same physiological entity, such as a neuron or a group of neurons. In the present study the spike units were detected by the spike height and width in 64 repeat stimuli which also match with two clicks in an approximate interval, termed time locking. In addition, autocorrelation function of spike trains were used to strengthen our assumption that a particular cluster of spikes arose from a single neuron rather than an aggregate of spikes from two or more neurons.

Results were based on 23 neurons from the IC of 23 rats, which were analyzed using SPSS 17.0 (SPSS China Inc., Shanghai, China). One-way repeated measures analysis of variance was performed to assess the effect of pentobarbital anesthesia on normalized response to both leading and lagging stimuli, and the Greenhouse–Geisser corrected value was used if Mauchly's test was significant ($P < 0.05$). Pairwise comparisons were followed to assess at which time points the responses differed. The level of significance was set at a P value of 0.05.

Fig. 1 shows post-stimulus time histogram (PSTH) of responses from one neuron under paired source conditions (4 ms and 10 ms ISDs) before and after intraperitoneal administration of pentobarbital at a maintenance dose. For the 10 ms ISD, there are two peaks separated by more than 10 ms and evoked by leading and lagging stimuli respectively, while at the 4 ms ISD, peak responses to lagging stimuli were weak, especially when the contralateral stimuli were leading. The neural responses to the lagging stimuli were visibly weaker 10 min after the injection, but gradually recovered.

The normalized response of leading stimuli show no significant alteration in both configurations after administration of pentobarbital at loading or maintenance dose (Fig. 2A–D: $F_{(3, 66)} = 0.697$, $P = 0.557 > 0.05$; $F_{(1.98, 43.5)} = 1.221$, $P = 0.304 > 0.05$; $F_{(2.35, 51.6)} = 0.179$, $P = 0.868 > 0.05$; $F_{(3, 66)} = 1.63$, $P = 0.191 > 0.05$), which illustrate that pentobarbital anesthesia have no effect on the normalized response of leading stimuli.

However, whether pentobarbital anesthesia affects normalized response of lagging stimuli or not is determined by the configurations and administration of pentobarbital at loading or maintenance dose. After administration of pentobarbital at a loading dose, the normalized response to the contralateral lagging stimuli at different ISDs gradually increased during recovery from anesthesia when the ipsilateral source was leading (Fig. 3A; $F_{(3, 66)} = 19.76$, $P < 0.01$). The half-maximal ISDs decreased gradually, along with a left shift of the normalized response curves (Fig. 3A). However, when the contralateral sources were leading, the normalized response to ipsilateral lagging stimuli showed no statistically significant changes after administration at a loading dose (Fig. 3B; $F_{(1.12, 24.73)} = 2.06$, $P = 0.16 > 0.05$).

After administration of pentobarbital at a maintenance dose, the normalized response to the lagging stimuli at different ISDs decreased, independent of whether the leading stimuli were