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Research report

The role of $GABA_B$ receptors in the vestibular oculomotor system in mice

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HIGHLIGHTS

- We studied effects of systemic administration of baclofen on mice eye movements.
- Large effects were shown on the responses through higher-order vestibular system.
- Obtained findings were in good agreement with the findings of nodulectomized mice.
- Well-conserved GABAergic system in oculomotor pathway across mammals was suggested.
- For study of GABA transmission, diversity of vestibular system should be considered.

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ABSTRACT

Systemic administration of a gamma-amino butyric acid type B ($GABA_B$) receptor agonist, baclofen, affects various physiological and psychological processes. To date, the effects on oculomotor system have been well characterized in primates, however those in mice have not been explored. In this study, we investigated the effects of baclofen focusing on vestibular-related eye movements. Two rotational paradigms, i.e. sinusoidal rotation and counter rotation were employed to stimulate semicircular canals and otolith organs in the inner ear. Experimental conditions (dosage, routes and onset of recording) were determined based on the prior studies exploring the behavioral effects of baclofen in mice. With an increase in dosage, both canal and otolith induced ocular responses were gradually affected. There was a clear distinction in the drug sensitivity showing that eye movements derived from direct vestibulo-ocular reflex pathways were relatively unaltered, while the responses through higher-order neural networks in the vestibular system were substantially decreased. These findings were consistent with those observed in primates suggesting a well-conserved role of $GABA_B$ receptors in the oculomotor system across frontal-eyed and lateral-eyed animals. We showed here a previously unrecognized effect of baclofen on the vestibular oculomotor function in mice. When interpreting general animal performance under the drug, the potential contribution of altered balance system should be taken into consideration.

1. Introduction

Vestibular evoked eye movements are highly-conserved across mammals being regulated by several parallel neural pathways in the brain. They are largely divided into two components: those composed of simple and direct pathways represented by three-neuron arcs and those shaping the high-order neural networks in the vestibular system. The later indirect and multimodal central processes play an important role in self-motion perception and spatial orientation [1–3] integrating semicircular canal and otolith information, together with visual and proprioceptive inputs. In the

Abbreviations: GABA, gamma-amino butyric acid; VSM, velocity storage mechanism; OVAR, off-vertical axis rotation; aVOR, angular vestibulo-ocular response; tVOR, tilt vestibulo-ocular response; GIF, gravito-inertial force.

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past, for better understanding of the high-order vestibular neural processes, mathematical modeling approaches have been well utilized. The model of Raphan and Cohen is most widely recognized defining this central integrator as the velocity storage mechanism (VSM) [4,5]. Although the characteristics of the VSM have been thoroughly investigated across a wide range of species [6,7], very little is known about the mouse VSM, because the mouse lacks typical eye movements through the VSM, such as a prolonged time constant of the angular vestibulo-ocular reflex (aVOR), under commonly used canal stimulation [8,9]. Alternatively, a constant velocity rotation around a tilted axis produces a continuous unidirectional nystagmus derived from the VSM by activating otolith organs [10–14]. This paradigm is called off-vertical axis rotation (OVAR) and has been used in the studies of the VSM. We recently found that a similar type of otolith stimulation, with a larger magnitude rotational force (pseudo-OVAR; [15,16]) can generate robust response though the VSM in mice and showed its well-conserved response properties across mammals [17]. In this study, we first addressed the neurotransmitter mechanism underlying the mouse VSM to compare with prior human data. Clinical and basic research in humans revealed that the vestibular-related eye movements are closely related to anxiety and motion sickness [1,3,18–20]. It is further known that systemic administration of baclofen specifically suppressed the VSM along with the level of motion sickness susceptibility [21,22]. Given the findings in primates and rats [23,24], GABAergic synaptic activities are likely to play a key role in the mouse VSM as well. To test this hypothesis, we examined the VSM related response evoked during pseudo-OVAR after intraperitoneal injection of baclofen.

On the other hand, systemic administration of baclofen is known to modulate numerous behavioral and physiological functions including motor activity, hypothermia, learning, alcohol consumption and anxiety-like behavior in mice [25–30]. In these studies, general activities such as distance of locomotion or limb coordination are often quantified as an effective parameter, for which normal balance system is required. Nevertheless, evaluation of vestibular function is mostly limited to observation of their overall performance. Thus far, study of ocular motility under baclofen has not been conducted, although its potential influences may affect the resultant animal behaviors. A second purpose of this study was to determine how baclofen modulates vestibular function based on eye movement analysis. We have designed the experimental condition following the prior behavioral studies in mice [25–29,31] to allow a comparison of co-occurring other behaviors under similar condition and help estimate their correlation. In addition to the pseudo-OVAR for otolith stimulation, a traditional sinusoidal rotation testing canal function was also employed for thorough investigation of the vestibular-related ocular performance. We found characteristic effects of baclofen with increase in dose, which corresponds to the findings after cerebellar nodulus/uvula lesions in mice [32,33]. Here we report the characterization of the baclofen-induced eye movements in mice. Obtained data are considered to represent the altered vestibular function under the influence of the drug.

2. Methods

2.1. Animals

The eye movements of eleven drug-naive male C57BL/6J mice (Jackson Laboratory, ME, USA; wt. 24–29), which are often used in the behavioral research, were studied. All mice were housed on a 12-h light, 12-h dark cycle with free access to food and water except during testing and experiments, which took place in the morning. All of the experiments and procedures were approved by the Institutional Animal Care and Use Committee at University of Texas Medical Branch and with the guidelines laid by the NIH regarding the care and use of animals for experimental procedures.

2.2. Baclofen

Baclofen was purchased from Sigma–Aldrich (St. Louis, MO, USA) and dissolved in sterile 0.9% saline solution. Mice were injected intraperitoneally with either saline or baclofen (5, 10 or 15 mg/kg) 90 min before recording eye movements. Animals were free to move around in the cage until the recording. Each mouse received a single injection of each dose. All compounds were injected in a volume of 0.14–0.3 ml. A minimum of six days separated successive trials. The doses of baclofen and route of administration were chosen based on previously published studies [25–29,31].

2.3. Experimental procedures and data analysis

The recordings were performed in complete darkness to avoid generating a contribution from visually-driven eye movements. The experimental setup, apparatus, procedures for recording and data analysis were identical to those described in the previous studies in our laboratory [17,34,35]. Briefly, ten minutes before the start of recordings, animals were treated with pilocarpine (4%) eye drops to limit pupil dilation in darkness. The body was wrapped in a plastic cone that fit snug around the mouse and the head was stabilized by clamping the maxilla firmly to a bite bar attached on the turntable, so that lateral canals were tilted upwards ~30° from the earth-horizontal position during recording [36]. This allowed us to keep alert mice immobile during the recording typically around twenty minutes. Two different whole-body rotation stimuli were employed to evaluate vestibular-induced eye movements. For activation of otolith organs, a dual yaw axis rotation was first tested (otolith stimulation). Once otolith testing was completed, aVOR through canal activation was then examined by sinusoidal rotation around earth vertical axis (canal stimulation). These two sets of vestibular organs are highly conserved in the vertebrate inner ear and detect linear acceleration/head tilt or angular acceleration/head rotation. The image of right eye on a mirror 4 cm in front of the animal was monitored by an infrared camera fixed on a turntable 2 cm to the right of the body [16]. Horizontal and vertical positions of the pupil center were recorded by a custom pupil tracking system at 60-Hz sampling rate [37]. Data were stored on a computer along with stimulation parameters, including velocity and position profiles of motion control axes and analyzed with a custom interactive script (MATLAB, The MathWork Inc., Natic, MA, USA). Eye position signals were calibrated off-line using obtained calibration scale factors based on the ocular image verified by rotating a camera from the perspective of the optic axis [34]. Pupil position was differentiated and desaccaded using velocity thresholds to obtain slow phase eye velocity. Nonlinear least squares sinusoidal curve fits were used to describe the modulation of eye position and slow phase eye velocity as well as characterize the angular and linear stimuli parameters. Amplitude (gain) and phase were analyzed separately using one-way repeated measures analysis of variance (ANOVA), and Tukey test was used for post-hoc analysis. A test static of $p < 0.05$ was considered significantly different.

2.4. Otolith stimulation

A similar rotating vector as OVAR but with a larger resultant gravito-inertial force (i.e. resultant of gravity and centrifugal force) was elicited by a counter rotation centrifugation (pseudo-OVAR; Fig. 1). First, the main axis was accelerated at 60°/s^2 to a constant angular velocity of 245°/s in the counter-clockwise direc-