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

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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.

Keywords

baclofen; eye movement; gamma-amino butyric acid (GABA); vestibulo-ocular reflex (VOR); off-vertical axis rotation (OVAR); velocity storage mechanism (VSM)

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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 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 is considered to represent the altered vestibular function under the influence of the drug.

2. Methods

2.1. Animals

The eye movements of eleven drug-naïve 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-hour light, 12-hour 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 to 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 deg 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 deg/s^2 to a constant angular velocity of 245 deg/s in the counter-clockwise direction, so that the resultant force of the centrifugal acceleration (radius = 0.45 m) and the gravity was tilted 40 deg relative to earth vertical. After initial transient eye movements through canal and otolith activities subsided to zero, about 10 seconds later, the eccentric axis was then rotated at a constant velocity of 72 deg/s in the clockwise direction, while the main axis was still rotating at a constant velocity. To minimize influence of canal stimulation, data of 30 seconds after the start of eccentric rotation were also removed. Eventually, a subset of traces in which eye movement amplitude was constant for the cycles of eccentric axis rotation, 5-7 cycles, were used for data analysis and data of 2-3 trials were averaged.

2.5. Canal stimulation

The sinusoidal stimulation was elicited by a single yaw axis rotation of turntable (Fig. 1). Five different frequencies of 0.05, 0.1, 0.2, 0.4 and 0.8 Hz were tested while peak velocity was kept constant at 60 deg/s , for the duration of 7-10 cycles. Gain of the eye movement was defined as the ratio of eye velocity to head velocity. While, phase of eye movement with respect to stimulus movement were derived from nonlinear least squares sinusoidal curve fits to the slow phase eye velocity and stimulus velocity, which reflects the time lag between peak eye velocity and peak head velocity. Zero degree phase was defined as perfectly compensatory ocular performance of aVOR and positive phase values indicated eye leading head. Data of 2-3 trials were averaged.

In addition, the quick phase of eye movements was examined at the end of the trials. During pseudo-OVAR, we found reduced nystagmus, a combination of quick and slow phases, with increase in drug dosage leaving slow velocity eye movements. However, because the higher doses of baclofen ($>10 \text{ mg/kg}$) could produce general muscle relaxation or ataxia [25, 38], there was a possibility that the suppressed quick movements might be attributed to peripheral oculomotor disturbances rather than central inactivation. Therefore, we explored whether the nystagmus could be driven at the dose of 15 mg/kg , when rotation about the main axis was decelerated at 60 deg/s/s while eccentric axis was rotating at 72 deg/s . At this

phase of the paradigm, both centripetal and tangential linear accelerations were present in addition to the angular deceleration.

3. Results

3.1. General characteristics during pseudo-OVAR (otolith stimulation)

Fig. 2 shows sample response profiles obtained from a single mouse 90 minutes after administration of saline (left panel) and baclofen at a dose of 15 mg/kg (right panel). Eye movements in the nasal-to-temporal and upward directions are positive. After saline injection, all studied mice exhibited basic features of eye movements during OVAR in various species, including primates [11, 14, 39], cat [12], rat [40] and mice [33]: horizontal eye movements showed cyclic modulation of the eye position onto which horizontal nystagmus was superimposed (Fig. 2A). After removal of quick phase of eye movements (Fig. 2C, faint gray lines), slow phase mean velocity was offset in the negative direction opposite to eccentric turntable rotation (Fig. 2C, dashed horizontal line). Vertical eye movements also changed periodically with maximal elevation and depression when the centrifugal force generated by the main axis rotation was aligned with interaural axis (Fig. 2B and D-F). The vertical component associated with the unidirectional nystagmus was negligible (Fig. 2B), thus vertical slow phase eye velocity modulated approximately around zero (Fig. 2D, solid horizontal line).

In each mouse, after administration of 15 mg/kg of baclofen, the horizontal eye position modulated in the same manner as that of saline, whereas horizontal nystagmus was severely attenuated (Fig. 2G), resulting in reduced slow phase mean velocity around zero (Fig. 2I, dashed horizontal line). The amplitudes of modulation in the vertical eye position and velocity showed similar values to those of saline (Fig. 2H and J). Inset figure (Fig. 2L) shows the horizontal eye velocity profile at the end of trial when the rotation of the main axis was decelerated while the eccentric axis was still rotating at a constant velocity. The evoked post-rotatory equivalent nystagmus through canal and otolith co-activation indicated that a capability of generating quick phase eye movements was being preserved at this dose.

3.2. Effects on modulatory eye movements

The modulation components arise predominantly through tilt vestibulo-ocular reflex (tVOR) orientation mechanisms [10-14, 41]. The effects of baclofen on amplitudes of horizontal and vertical modulations were further evaluated in eleven mice. The average results at 0 (saline), 5, 10 and 15 mg/kg of baclofen were shown in Fig. 3A (eye positions) and 3B (eye velocities). Statistical analysis (ANOVA) revealed no significant effect on the horizontal modulations, however there was a tendency of decreasing eye position with increasing dosage from 11.2 ± 2.3 deg (mean $\pm$ SD) at 0 mg/kg to 10.0 ± 1.8 deg at 15 mg/kg, as well as a decrease in velocity from 16.5 ± 3.4 deg/s at 0mg/kg to 13.2 ± 5.2 deg/s at 15mg/kg. On the other hand, there was a significant effect on the vertical modulatory eye movements. Changes in vertical position increased from 20.9 ± 2.6 deg to 27.1 ± 3.4 deg, and vertical velocity increased from 20.2 ± 3.7 deg/s to 24.3 ± 4.5 deg/s. Statistical analysis revealed that there was no significant difference between saline (0mg/kg) and 5 mg/kg baclofen, whereas, there was significant increase in the vertical position change at 10 and 15 mg/kg ($p < 0.01$).

3.3. Effects on horizontal nystagmus

The effects on horizontal nystagmus for which the VSM is responsible [10-14] were evaluated by measuring the slow phase mean eye velocity. To collect the data on slow phase eye movements, quick phase of the nystagmus (eye velocity > 200 deg/s) were removed leaving slow phase eye movements, then the mean eye velocity was calculated. The average results of eleven mice are shown in Fig. 3C at each dose. There was a close relationship between the use of baclofen and the evoked nystagmus. Consistent with the previous studies of primates during OVAR after baclofen [14, 39], the horizontal mean eye velocity decreased as a function of the dose from 38.6 ± 11.3 deg/s at 0 mg/kg to 13.1 ± 13.2 deg/s at 15 mg/kg. Statistically, no significant difference was found up to 10 mg/kg of administration, however there was a significant effect at 15 mg/kg of baclofen ($p < 0.001$). In most mice, overall activity levels were also reduced with increase in baclofen dose.

3.4. Characteristics of aVOR (canal stimulation)

Fig. 4 shows the average results of eleven mice of the aVOR gain and phase during sinusoidal rotation (0.05-0.8 Hz) after saline (0 mg/kg) or baclofen administration (5-15 mg/kg). Effects of the drug were larger on aVOR phases compared with aVOR gains. The aVOR gain after saline injection increased from 0.14 ± 0.10 at 0.05 Hz to 0.70 ± 0.16 at 0.8 Hz, while the phase decreased from 46.9 ± 12.4 deg at 0.05 Hz to 15.3 ± 3.4 deg at 0.8 Hz. After administration of baclofen, the aVOR gain decreased as the dosage increased, however the differences were relatively small, so that they were not significant at any of the frequencies ($p > 0.05$). On the other hand, clear phase-leading effects were obtained at each frequency in a dose dependent manner. There was no significant difference between saline and 5 mg/kg baclofen at all frequencies, whereas at 10 mg/kg, the phase increased ranging from 66.6 ± 10.7 deg at 0.05 Hz to 18.2 ± 3.5 deg at 0.8 Hz yielding significant differences at four frequencies (0.05 Hz, $p < 0.001$; 0.1, 0.2, and 0.4 Hz, $p < 0.01$). Thus, the effects on phase lead were more prominent at lower frequencies. After 15 mg/kg baclofen, the phase values were significantly increased at all tested frequencies ranging from 80.6 ± 7.8 deg at 0.05 Hz to 21.8 ± 2.7 deg at 0.8 Hz ($p < 0.001$).

4. Discussion

The effects of baclofen on vestibular oculomotor system were studied in mice. There was a clear distinction in the drug sensitivity showing different involvement of GABA transmission system in the oculomotor pathway. Effects of the drug were larger on the responses that require higher-order neural processes in the brain, than those through direct pathways. The data corresponded to the previous observations in nodulectomized mice [32, 33]. These findings were consistent with the studies in primates [14, 21, 22, 39, 42], suggesting a well-conserved nature of GABAergic mechanism in the vestibular oculomotor pathway across lateral-eyed and frontal-eyed animals.

4.1. Potential action in the vestibular oculomotor system

In this study, intraperitoneal injection was chosen on the basis of its well-established route of administration for mouse behavioral research. In rats, effects of baclofen on the aVOR have been explored applying two different routes i.e. intramuscular and

intracerebroventricular administration [43]. Both routes showed similar qualitative effects on ocular performance with increase in dosage suggesting that the drug was distributed to the same action site in the central vestibular oculomotor system. In addition, blocking effects of the GABA_B antagonist suggested that actions of baclofen were specifically related to the GABA_B receptors. In our study, we found that the responses related to the direct pathways were robust, showing that afferent activities from the peripheral vestibular hair cells were functional, while those through the VSM were substantially reduced after baclofen administration. In primates, reciprocal connections between the vestibular nuclei and cerebellar nodulus/uvula are crucial to shape the neural network that underlies the VSM [44, 45]. There is immunochemical evidence that baclofen sensitive GABA_B binding sites are located in the medial vestibular nucleus, which are innervated by contra-lateral vestibular neurons and Purkinje cells in the nodulus/uvula [23]. In rat, occurrence of GABA_B receptor activities and baclofen sensitive binding sites are also elucidated in the medial vestibular nucleus neurons [23, 24]. Accordingly, the actions of the drug have been considered to be mediated through the interaction between GABAergic Purkinje cell terminals from nodulus/uvula and GABA_B receptor bearing dendrites from medial vestibular nuclei [21-23, 39]. Consistent with these findings, we showed here that baclofen caused a significant reduction of the otolith-driven horizontal nystagmus during pseudo-OVAR (Fig. 2G, I and Fig. 3C). Given that lesions of the nodulus/uvula produce similar effects in mice during OVAR [33], it was suggested that baclofen caused the VSM suppression through a central GABA_B receptor inhibition between the vestibular nucleus and the nodulus/uvula in mice as well. On the other hand, the VSM is also inactivated by a peripheral manipulation, such as the lateral semicircular canal nerve section, or canal plugging in primates [46]. In human studies, because GABA_B receptors are also present on the primary labyrinthine afferents, several authors suggest that baclofen affects vestibular oculomotor system both centrally and peripherally [22, 42]. Although the precise effect of baclofen crossing the blood-labyrinth barrier is still unknown in mice, these findings raise the possibility that the inhibitory action of baclofen on the peripheral vestibular inputs may contribute in part towards the inactivation of the VSM presented here. Furthermore, although evoked eye movements through the current stimuli were derived from brainstem and cerebellar levels, we cannot totally rule out potential interplays from other eye movement related brain areas. Further studies should be conducted to identify the location of drug activation in detail.

4.2. Drug-insensitive oculomotor pathway

The vestibular ocular pathways from the canal and otolith signals to the resultant eye movements are composed of various parallel pathways. Three-neuron arcs are the most direct pathways where the primary afferents project secondary neurons in the vestibular nuclei, which in turn project to the ocular motoneurons. The three-neuron arcs have been well demonstrated in canal-driven aVOR. The latency of the aVOR is quite short (5 ms in primates), which effectively stabilizes the visual images on retina when the head moves [47, 48]. A direct disynaptic pathway also exists between the otolith afferents and the oculomotor neurons, however, a longer response time (>10 ms) of the tVOR implies that more polysynaptic connections are involved in the tVOR pathway [49-51]. The modulation components during otolith stimulation, which have head-position related properties, are induced through the tVOR pathways [10-14, 41]. In our study, together with aVOR gain,

they were robust over the range of doses tested (Fig. 3A, B). There was a tendency for the amplitudes of horizontal movements to be slightly decreased or unaltered with increase in dose, while for vertical responses, the amplitudes significantly increased at higher doses (>10 mg/kg). This difference may indicate that different GABAergic mechanisms are at work in each directional oculomotor pathway.

Meanwhile, to address the mechanism of brain plasticity, the effects of baclofen on ocular motility after unilateral vestibular lesion have been well studied in rodents, which regulates aVOR gain [52, 53]. Brain slice experiments have revealed that a down-regulation of GABA_B receptor sensitivity in the ipsi-lesional vestibular nucleus is important reflecting the behavioral results [54]. Whereas here, we found that a neural pathway which underlies the aVOR gain was less sensitive to the drug. The data may represent the distinct effects of baclofen in healthy subjects highlighting the dynamic GABAergic plasticity in the brain.

4.3. Drug-sensitive oculomotor pathway

We have shown that the direct pathways were less sensitive to the drug, suggesting less involvement of GABA_B receptor activation for the three-neuron arcs, while larger effects were shown in the horizontal nystagmus during pseudo-OVAR and the phase of aVOR. Thus far, along with the otolith-driven horizontal nystagmus, post-rotatory nystagmus, or the time constant of aVOR, has been widely investigated to verify the underlying physiology of the VSM. After whole body rotation at a constant rotational velocity, the prolonged vestibular time constant is obtained in many species, which represents the fundamental property of central VSM. In contrast, the time constant of the mouse aVOR is remarkably short reflecting almost just the peripheral cupular time constant without any prolongation [8, 9]. These findings raise the question whether mice possess VSM, and thus very little is known about the mouse VSM so far. Recently, we characterized the mouse VSM by using similar rotation paradigm as used in this study [17]. This counter rotation centrifugation can generate a larger magnitude rotational vector than can be achieved using conventional OVAR (pseudo-OVAR) [15, 16]. Therefore, detailed analysis with a wide range of shear force was available by generating the robust VSM related response in this small species. The functional implication of the VSM under natural condition still remains controversial, though it has been generally accepted that it prolongs peripheral information and integrates multimodal and multisensory neural processes for self-motion perception and spatial orientation [2, 7, 55]. In humans, eye movements through the VSM have been thoroughly investigated for understanding of high-order vestibular functions like motion sickness or anxiety [1, 3, 18-20]. Whereas, more recent studies suggest that the thalamocortical networks are also important for the higher-level functions in the vestibular system [56].

Primarily, we were interested in the underlying neurotransmitter system in the mouse VSM for further corroborating human data. In primates, there is evidence to suggest that GABA is a major neurotransmitter involved in the VSM [14, 21-23, 39, 42]. To verify the involvement of GABA transmission in the mouse VSM, we shed light on the profile of horizontal nystagmus during pseudo-OVAR after baclofen administration. In primates, the horizontal nystagmus of OVAR and the prolonged time constant of the aVOR both disappear without affecting the direct aVOR pathways when the VSM is inactivated by

midline section between the vestibular nuclei or systemic administration of baclofen [14, 57-60]. In addition, ablation of nodulus/uvula is also known to decrease the horizontal nystagmus during OVAR [44]. Based on these findings, it is suggested that nodulus Purkinje cells projecting to the vestibular nuclei play a significant role to form the VSM [22, 23]. Here, we have extended previous findings in primates to mice showing the similar effects of baclofen in the mouse VSM.

We found increased lead of the eyes with respect to the head after baclofen administration (Fig. 4), suggesting larger involvement of GABA_B receptors in the phase of aVOR than gain control. The phase values significantly increased in a dose dependent manner, and predominantly at lower frequencies. In primates, the phase leading effect of baclofen is suggested to be a consequence of inhibitory action of the drug on the velocity storage activity [22, 42], which is generally prominent at low frequency sinusoidal stimuli (less than 0.1 Hz). The findings in mice after nodulectomy are in line with this hypothesis presenting a prolonged phase lead property and suppressed nystagmus during OVAR [32, 33]. However, the velocity storage component of the canal-related aVOR is still unclear in mice, while in primates, ablation of nodulus/uvula is known to increase the aVOR time constant [45]. On the other hand, a large phase lead at low frequencies is reported in P/Q calcium channel mutant, *rocker* and *tottering*, model mice of vestibulo-cerebellar dysfunction [9, 61]. The shift of phase is suggested to result from the inability to maintain eccentric eye position rather than an impaired VSM. In the rat, systemic administration of baclofen produced dysfunction of holding eye position [43]. Given that GABAergic neurons have been identified in prepositus hypoglossi in brainstem and cerebellar flocculus in mice [62, 63] which are crucial for correct gaze holding or adequate aVOR [64, 65], GABA_B inactivation of neural integrators in these areas might lead to the increase in aVOR phase under baclofen.

4.4. Vestibular interaction in animal behaviors under baclofen

Effects of baclofen on behavioral performance have been extensively studied in mice to understand the underlying GABAergic mechanisms of various brain functions [25-30]. In parallel, there is strong evidence that GABA transmission modulates the mouse vestibular system, which may contribute to other resultant behaviors under the drug's influence [66-69]. Generally, in the behavioral studies, vestibular evaluations have been carried out through observation of whole body activities, such as righting reflexes, locomotion in open field or a rotarod, and performance on an unstable platform [70, 71]. However, because GABA_B receptors are widely distributed throughout the brain, it is challenging to identify the source of modulated behaviors separating out any vestibular interactions. For instance, an animal may huddle in corners due to spatial disorientation rather than anxiety or fall off a rotarod due to gaze holding abnormalities rather than memory or motor learning impairment. Indeed, although not quantified, the suppressed animal activities were clearly visible with increase in dose in our mice. Unlike general motor activities, an advantage of analyzing eye movements is that they are relatively simple. Our second purpose was to examine the vestibular function under baclofen based on the eye movement measurements. Both sinusoidal rotation and OVAR are well-established tests to detect vestibular impairments in human. Obtained data is suggested to reflect inherent vestibular dysfunction after administration of baclofen. Throughout the current vestibular examinations, statistically

similar response properties were obtained after saline and 5 mg/kg of baclofen. Whereas at higher doses, the eye movements were gradually affected (Fig. 3C and Fig. 4). In other words, when conducting behavioral evaluations at higher doses, care should be taken for the potential involvement of altered vestibular function.

Currently, we have carried out a thorough investigation of the mouse vestibular function under baclofen based on the eye movement analysis. However, potential effects on visual-oculomotor system or vestibulo-spinal reflexes or somatosensory reflexes which are also important for maintaining posture and gait, remains unsolved. Furthermore, the vestibular neuronal projections are widely distributed throughout the brain areas such as thalamus, hippocampus and cortex including the amygdaloid nucleus [72-77]. Therefore, these profound vestibular neural processes may partially contribute to the drug-induced animal performance mediating general motor activities, body temperature, memory, learning or anxiety without affecting eye movements. In addition, recent electrophysiological studies in both primates and mice have revealed that on-going behavior adjusts the coding of multiple vestibular inputs at the first central stage of processing [56]. Such data may provide important insights into the multimodal nature of the vestibular processes, particularly under natural condition. To understand the more detailed behavioral effects of baclofen, these inherent complexities and diverse aspects of vestibular system should be taken into consideration.

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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

A comparison of the single and dual axis rotation

Fig. 1.

A comparison of the sinusoidal rotation for canal stimulation and counter rotation and off-vertical axis rotation (OVAR) for otolith stimulation. During pseudo-OVAR, the main axis velocity and radius determine the magnitude of the resultant gravito-inertial force (GIF), while the velocity of the eccentric axis determines the frequency of the linear force modulation. The magnitude of the resultant GIF during pseudo-OVAR is greater than 1g. θ indicates an angle of resultant GIF from the earth vertical, which is equivalent to the tilt angle of rotation axis during OVAR.

Fig. 2.

Eye movements from one mouse during pseudo-OVAR after saline (left panel) or baclofen administration (right panel). Eye movements in the nasal-to-temporal and upward directions are positive. The faint gray lines in C, D, I and J show the velocities of the quick phase that have been removed. (A, C and G, I) Horizontal eye trace after baclofen shows that eye position and velocity modulated in the same manner as that of control, while the continuous nystagmus was substantially attenuated. Note that the horizontal mean slow phase velocity (dashed line in C) decreased to around zero due to suppressed nystagmus after administration of baclofen (I). (B, D and H, J) Vertical eye movements were not altered after baclofen. The solid lines in D and J indicate zero velocity. (E, K) The positions of the mouse in the eccentric table at various points are shown in the inset (nose-out, left side out, nose-in and right side out). (F) Egocentric frame of reference. Arrows indicate the direction of centrifugal shear force generated by main axis rotation at each point. (L) Inset figure L shows the generated horizontal nystagmus when main axis was decelerated while eccentric axis was rotating. The horizontal bar indicates the time scale.

Fig. 3.

Effects of baclofen on amplitudes of modulations in the horizontal and vertical positions (A), slow phase velocities (B) and horizontal nystagmus (C) during pseudo-OVAR. Average results of eleven mice are shown at each dose. Statistically similar responses were observed after saline (0 mg/kg) and 5 mg/kg baclofen administration. Amplitude of vertical modulation significantly increased in position at higher doses (A) (* $p < 0.01$). At 15 mg/kg, mean slow phase velocity significantly decreased (C) (** $p < 0.001$). There was a tendency for horizontal modulations to be lower or unaltered while vertical modulations were higher with increase in dosage (A, B). Error bars indicate 1 SD.

Fig. 4.

Effects of baclofen on gain and phase of angular vestibulo-ocular reflex (aVOR) induced by sinusoidal rotation. Average results of eleven mice are shown at each dose. Statistically similar responses were obtained after saline (0 mg/kg) and 5 mg/kg baclofen. With increase in dose, the aVOR phase significantly increased (* $p < 0.01$, ** $p < 0.001$), whereas there was no significant difference in the aVOR gain at all frequencies over the range of doses tested ($p > 0.05$). Error bars indicate 1 SD.