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Research Article

Whole Body Vibration Enhances Choline Acetyltransferase-Immunoreactivity in Cortex and Amygdala

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- Nucleus basalis magnocellularis
- Fiber density

Abstract

Whole body vibration (WBV) is a form of physical stimulation brought about by mechanical vibrations transmitted to a subject. WBV can increase attention in cognitive tasks in mice and men. However, little is known about the mechanisms that underlie this improved brain functioning. We examined whether WBV affects the cholinergic forebrain system of mice. Male C57Bl/6J mice (2 months of age) received WBV in a cage attached to a small vibrating platform (30 Hz with peak-to-peak displacements ranging from 14 to 75 μ m). WBV was applied five days a week for a period of five weeks with daily sessions of ten minutes. Control mice (pseudo-WBV) were treated similarly, but did not receive the actual vibration. Mice were sacrificed 24 hours after the last session and their brains were processed immuno cyto chemically for the acetylcholine-synthesizing enzyme cholineacetyl transferase (ChAT). ChAT-immunoreactivity was measured in the nucleus basalis magnocellularis (NBM), the somatosensory cortex, and the basolateral amygdala (where the cholinergic fibers arising from the NBM terminate). ChAT-immunoreactivity was significantly increased due to WBV in layer 5 of the somatosensory cortex (by 23%; $p < 0.01$) and amygdala (by 21%; $p < 0.05$), but not in the NBM as compared to pseudo-WBV. As increased ChAT-immunoreactivity indicates a higher cholinergic activity, these results reveal that the positive effects of WBV on attention are most likely (at least in part) mediated by an increased activity of the NBM cholinergic system. WBV could therefore be a suitable intervention strategy in conditions where a reduced cholinergic forebrain activity plays a role.

INTRODUCTION

The cholinergic forebrain system consists of several subnuclei providing the forebrain with dense and widespread cholinergic innervations. A major cholinergic subnucleus in rodents is the nucleus basalis magnocellularis (NBM), innervating primarily the cortex and amygdala [1,2]. Within these projection areas, layer 5 of the somatosensory cortex and the basolateral amygdala are particularly densely innervated [1]. The cholinergic system is considered to be crucial in learning and memory performance, and the cholinergic NBM cells play a specific role in the performance of tasks that require selected attention [3]. In general, the cholinergic system is activated in response to environmental stimuli. The cholinergic NBM cells are ideally located within the basal forebrain region to process sensory stimuli arising from the midbrain [2-4]. Especially highly salient sensory stimuli activate the NBM and hence modulate the activity in their projection areas. A specific type of sensory stimulation is whole body vibration (WBV). During WBV a vibration source (usually a platform) transfers mechanical vibrations to the body

of a subject standing on the platform. The platform provides a mechanical oscillation of specific amplitude of displacement and frequency, improving neuromuscular performance suitable for clinical purposes [5,6]. WBV with limited durations (2-10 minutes) was found to improve attention in humans measured shortly after the WBV sessions [7-9]. Five weeks of WBV treatment was able to improve performance in learning tasks in different mouse strains. For example, in C57Bl/6J mice, WBV-mice learned faster in a two-arm spatial discrimination task [10]. In ICR (CD1) mice, cognitive performance in the object recognition task, a test by which the mice have to discriminate between a familiar and a novel object improved significantly [11]. These effects can be explained by increased (selective) attention, which may imply a contribution of the cholinergic forebrain system. Therefore, in the current study we examined in C57Bl/6J mice whether WBV could enhance selective attention by way of increased cholinergic activity. The level of immunoreactivity for the acetylcholine-synthesizing enzyme choline acetyltransferase (ChAT) correlates positively with ChAT enzyme activity and hence

cholinergic activity [12]. We analyzed ChAT-immunoreactivity in the NBM, somatosensory cortex and basolateral amygdala in mice subjected to the five weeks WBV protocol as employed in the above mentioned mouse studies.

MATERIAL AND METHODS

Animals

Young C57Bl/6J mice were used (n = 14; males; 2 months of age at the start of the experiment; Harlan Netherlands BV, Horst, the Netherlands). All mice were housed individually, with food and water available *ad libitum*. They were kept under a 12 hr light / 12 hr dark cycle (lights on at 7:00 am). The air humidity in the room was kept at 40% and temperature was held constant at 21° Celsius. All procedures concerning animal care and treatment were in accordance with the regulations of the ethical committee for the use of experimental animals of the University of Groningen. These regulations are consistent with the guidelines for the care and use of laboratory animals as described by the U.S. National Institutes of Health. All experiments of this study were approved by the ethical committee of the University of Groningen, The Netherlands.

Whole body vibration procedure

The WBV apparatus was made of an oscillator (LEVELL R.C. Oscillator Type TG200DMP) and power amplifier (V406 Shaker Power Amplifier). A cage (44.5(length) x 28 (width) x 16 (height) cm) was attached to the oscillator at the center point of the cage (a circular contact plate with a diameter of 5 cm). The cage contained 12 removable compartments (6.5 (length) x 7.5 (width) x 20 (height) cm). Mice were subjected to low intensity sinusoidal vibrations with a frequency of 30 Hz and a peak to peak displacement in the following directions: X (left-right; 40-60 μm), Y (front-back; 29 to 75 μm), and Z (up-down; 14-54 μm). The lowest values were measured at the center of the cage and the highest levels at the corners of the cage. Mice were placed in the compartments following a rotation schedule. Control mice (pseudo-WBV mice) were similarly placed in the compartments, but did not receive the actual vibration. Animals were randomly assigned to either the WBV or pseudo-WBV group. Body weight of the mice was measured weekly.

Immunohistochemistry

Mice were placed under deep anesthesia and transcardially perfused with a solution containing 4% buffered paraformaldehyde. After perfusion, the brains were removed and post-fixed for 18-24 hours in the same solution. After dehydration in 30% buffered sucrose the brains were frozen in liquid nitrogen and coronal sections of 20 μm were made on a Cryostat. Next, brain sections were incubated with goat anti-ChAT IgG (diluted 1:333, Millipore, Billerica, MA, USA). Thereafter, brain sections were incubated with biotinylated rabbit-anti-goat IgG (Sigma, St. Louis, MO, USA, 1:400). Sections were thereafter rinsed in 0.01M TBS and incubated for 2 hours with ABC solution (1:500). Finally, 3, 3'-diaminobenzidine (Sigma, St. Louis, MO, USA) was used as chromogen.

Quantification of the ChAT-immunostaining was performed by a Leica Quantimed system, and the obtained optical density (OD) was then corrected for a specific background staining measured in the corpus callosum, resulting in the corrected OD. Regions of interest were the NBM, somatosensory cortex layer 5 and the basolateral amygdala. OD was measured in six sections per mouse, aiming at sections at 0.6 mm posterior to Bregma. In the NBM ChAT was separately measured in cell bodies and processes (axons and dendrites; software was used to discriminate cell bodies from other structures). The differentiation between cell bodies and processes was done because of the much higher staining intensity of ChAT in the cell bodies compared to the processes (Figure 1), by which WBV-induced differences in the staining intensity of the processes could be marginalized.

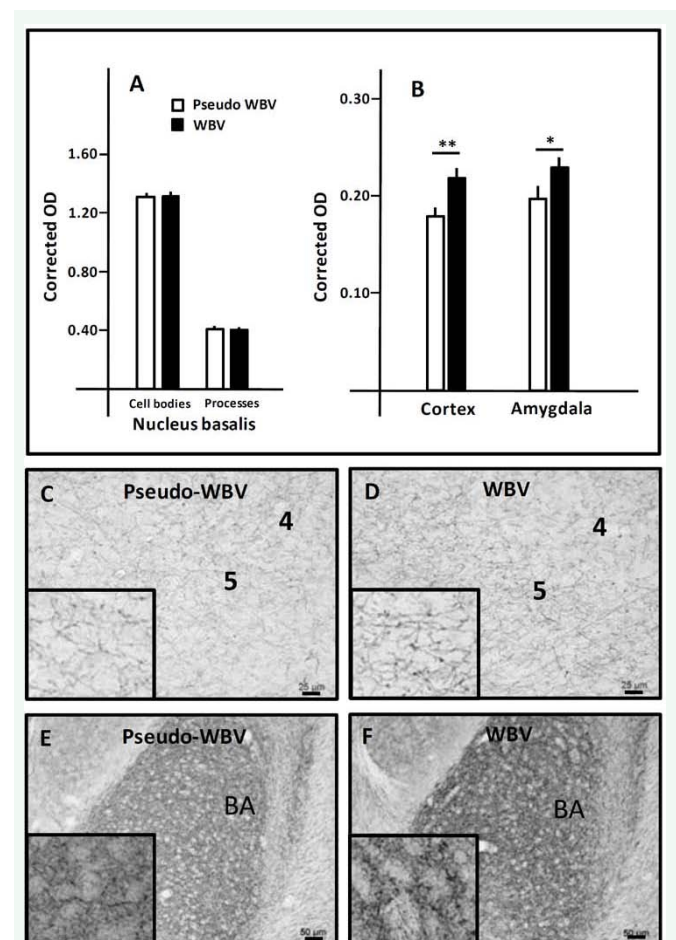


Figure 1 ChAT corrected OD (expressed in arbitrary units; mean ± SEM) in the NBM (panel A), and the somatosensory cortex and basolateral amygdala (panel B). WBV induced a significant increase in ChAT-immunostaining in the somatosensory cortex ($p < 0.01$) and the basolateral amygdala ($p < 0.05$). Representative pictures of ChAT-immunostaining show the somatosensory cortex (pseudo-WBV (panel C) and WBV (panel D); numbers indicate layer 4 and 5) and the basolateral amygdala (BA; pseudo-WBV (panel E) and WBV (panel F)). Inserts show a larger magnification of the center part of the images. Scale bars in panel C-D = 25 μm; in panel E-F = 50 μm.

Abbreviations: ADHD: Attention-Deficiency/Hyperactivity Disorder; ChAT: Choline Acetyltransferase; NBM: Nucleus Basalis Magnocellularis; OD: Optical Density; WBV: Whole Body Vibration

Statistical analyses

Data were presented as mean \pm standard error of the mean (SEM). Statistical analyses were performed by Student's t-test. $P < 0.05$ was considered significant. Data were analyzed by using Microsoft Excel and SPSS23.

RESULTS AND DISCUSSION

No differences in body weight gain (due to growth during the five weeks intervention period) were found between the pseudo-WBV and WBV group (respectively 6.5% [from 24.1 to 26.88 g] and 6.0% [from 23.7 to 25.13 g]). ChAT OD and ChAT-immunostaining are depicted in Figure 1 for the NBM, cortex and amygdala. The ChAT staining pattern was similar as previously observed by us and others [13]. ChAT immunostaining in the NBM showed intense labeling in the cholinergic cell bodies and relatively less intensely stained processes (not shown). To discriminate between these structures the staining intensity of the cell bodies were measured separately from the processes. No differences were found in the staining intensity between pseudo-WBV (corrected OD: cell bodies 1.30 ± 0.04 SEM; processes 0.41 ± 0.06 SEM) and WBV groups (corrected OD: cell bodies 1.32 ± 0.04 SEM; processes 0.39 ± 0.05 SEM). In the two projection areas of interest of the NBM only ChAT-positive axons were observed. ChAT-immunostaining was significantly higher by 23% in the WBV group in layer 5 of the somatosensory cortex (WBV: corrected OD = 0.23 ± 0.01 SEM; pseudo-WBV: corrected OD = 0.19 ± 0.01 SEM; $p < 0.05$; Student's t-test). In the basolateral amygdala ChAT-immunoreactivity was also significantly increased by 21% in the WBV group (WBV: corrected OD = 0.33 ± 0.013 SEM; pseudo-WBV: corrected OD = 0.27 ± 0.012 SEM; $p < 0.01$; Student t-test).

The absence of a WBV-induced difference in the NBM 24 hours after the last WBV session is probably due to transport of the ChAT protein, produced in the cell bodies, towards the axons and their nerve terminals in the projection regions, where the enzyme is responsible for the production of acetylcholine. Although axonal transport of ChAT is relatively slow (for example, 1.25 mm/day in rat sciatic nerve axons [14], this rate of transport may explain why enhanced ChAT-immunoreactivity is not observed in the NBM 24 hours after the last WBV session. Most likely the enhanced ChAT-immunoreactivity in the NBM target regions reflects accumulation of ChAT in existing cholinergic fibers rather than a more widespread presence of cholinergic fibers via axonal sprouting. The increase in ChAT-immunostaining indicates a higher level of ChAT activity and acetylcholine release, and hence a higher activity of the cholinergic cells, as it has been shown that ChAT-immunoreactivity correlates positively with ChAT enzyme activity and hence cholinergic activity [12]. Nevertheless, as OD measurements of ChAT-immunoreactivity provides semi-quantitative values, future research using a quantitative assay of ChAT activity after WBV is needed to confirm the functional consequence of the observed increased ChAT OD in terms of enhanced cholinergic activity. If in humans WBV does enhance cholinergic activity as well, it could explain why cognitive performance in the Stroop test was improved by WBV in healthy

children [9] and young adults [7], but also in young adults with attention-deficit/hyperactivity disorder [7]. A link between cholinergic functioning and Stroop test performance has been shown [15] and ADHD-related deficits are reduced by cholinergic stimulation [16].

CONCLUSION

The results of this study reveal that the positive effects of WBV on attention may be (at least in part) mediated by an increased activity of the NBM cholinergic system. WBV could therefore be a suitable intervention strategy in conditions where a reduced cholinergic forebrain activity plays a role.

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