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Research report

Short-term galvanic vestibular stimulation promotes functional recovery and neurogenesis in unilaterally labyrinthectomized rats

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ABSTRACT

Current experimental research on the therapeutic effects of galvanic vestibular stimulation (GVS) has mainly focused on neurodegenerative disorders. However, it primarily stimulates the vestibular nuclei and could be potentially effective in modulating imbalance between them in the case of unilateral labyrinthectomy (UL).

Fifty male Wistar rats (180–220 g) were used in 5 groups of 10: intact, sham, right-UL (RUL; without intervention), and two other right-UL groups with GVS intervention [one group treated with low rate GVS (GVS.LF; 6–7 Hz), and the other treated with high rate GVS (GVS.HF; 17–18 Hz)]. The UL models were prepared by intratympanic injection of sodium arsanilate. GVS protocols were implemented 30 min/day and continued for 14 days via ring-shaped copper electrodes inserted subcutaneously over each mastoid. Functional recovery was assessed by several postural tests including support surface area, landing and air-righting reflexes, and rotarod procedure. Immunohistochemical investigations were performed on ipsi- and contra-lesional medial vestibular nuclei (MVN) using bromodeoxyuridine (BrdU) and Ki67, as markers of cell proliferation.

Behavioral evaluations showed significant functional recovery of GVS-treated groups compared to RUL group. The percent of marked cells with BrdU and Ki67 were significantly higher in the ipsilesional MVN of both GVS-treated groups compared with other groups.

Our findings confirmed the effectiveness of GVS-intervention in accelerating static and dynamic vestibular compensation. This could be explained by the cell proliferation in ipsilesional MVN cells and rapid rebalancing of the VNs and the modulation of their motor outputs. Therefore, GVS could be promising for rehabilitating patients with unilateral vestibular weakness.

1. Introduction

Passing a brief electrical current between two electrodes on both mastoids or in both ears using bilateral bipolar galvanic vestibular stimulation (GVS) resulted in a simultaneous increase and decrease in the vestibular afferents' firing rate on the cathode-side and anode-side, respectively (Goldberg et al., 1982; Courjon et al., 1987).

GVS acted upon the spike trigger zone, which is situated between the vestibular sensory epithelium and the afferent terminals (Goldberg et al., 1984; Eatock et al., 2008). Therefore, post-synaptic mechanisms should be considered in explanations of GVS-evoked phenomena (Goldberg et al., 1984; Curthoys, 2010).

GVS activates all primary vestibular afferents (Goldberg et al., 1984; Curthoys and Macdougall, 2012), but the irregularly discharging (phasic) vestibular afferents have a higher galvanic sensitivity and a lower stimulation threshold compared to the regular ones (Goldberg et al., 1982; Goldberg et al., 1984; Minor and Goldberg, 1991; Kim and Curthoys, 2004). However, non-vestibular inputs are not significantly affected by GVS (Wardman and Fitzpatrick, 2002). Furthermore, GVS-induced modulation projects to the vestibular nuclei (VNs), secondary projection neurons from the VNs (Highstein et al., 1987), and the multisensory vestibular centers, including the temporo-insular and temporo-parietal

Abbreviations: GEHM, galvanic-evoked head movement; GVS, galvanic vestibular stimulation; GVS.HF, right labyrinthectomized rat stimulated by high-rate GVS; GVS.LF, right labyrinthectomized rat stimulated by low-rate GVS; RUL, right UL; UL, unilaterally labyrinthectomized; VC, vestibular compensation; VN, vestibular nucleus; VNs, vestibular nuclei

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cortical areas (Dieterich and Brandt, 2008). Therefore, GVS could be considered a powerful and relatively pure vestibular stimulator impacting output motor actions of the vestibular system (the vestibulo-ocular and vestibulo-spinal reflexes) (Fitzpatrick and Day, 2004) and therapeutically impacting the widespread central vestibular network in various neurological and cognitive disorders (Rorsman et al., 1999; Wilkinson et al., 2005; Yamamoto et al., 2005; Pan et al., 2008; Wilkinson et al., 2010).

Unilateral labyrinthectomy (UL) resulted in several static and dynamic balance symptoms in animals. Static symptoms are generated at rest, while dynamic symptoms are generated during motion (Beraneck and Idoux, 2012). Previous studies indicated that intratympanic injection of sodium arsanilate is very effective in inducing unilateral or bilateral chemical labyrinthectomy in rats (Horn et al., 1981; Hunt et al., 1987; Besnard et al., 2012; Vignaux et al., 2012). The researchers also noted that the damage was more extensive in the region of type I hair cells (Vignaux et al., 2012), which mainly connected to irregular discharge afferents known to be the main neural facilitators of GVS (Goldberg et al., 1982; Goldberg et al., 1984). Moreover, static and dynamic symptoms induced by unilaterally injected sodium arsanilate do not recover until about 36 and 42 days after injection, respectively (Liberge et al., 2010). Therefore, sodium arsanilate injections provide a long-term UL model for evaluating the potential therapeutic effects of GVS on UL.

The central nervous system (CNS), through a plasticity process known as vestibular compensation (VC), attempts to resolve UL-induced symptoms (Dutia, 2010). Of the structures involved in VC, including the VN, spinal cord, cerebellum, and cortical areas (Dutia, 2010; Lambert and Straka, 2012; Smith and Curthoys, 1989; Curthoys and Halmagyi, 1995), the VNs play the most important role in VC (Lambert and Straka, 2012) via the vestibular commissural inhibitory system that reciprocally connected bilateral VNs (Dutia, 2010). Thus, through a controlled excitatory-inhibitory stimulation like as GVS (Wardman and Fitzpatrick, 2002), it may be possible to constructively modulate the vestibular commissural inhibitory system to facilitate the recovery of static and dynamic vestibular symptoms.

The natural VC phenomenon could be effective in relieving static symptoms of UL (Smith and Curthoys, 1989; Curthoys and Halmagyi, 1995). However, evidence suggests that the modulation of phasic input gain to the contralesional VN is vital in compensating for dynamic symptoms following UL (Sadeghi et al., 2007; Cullen et al., 2009) because of the fact that after UL, the neuronal behavior of the contralesional and ipsilesional VN become more phasic and tonic, respectively (Sadeghi et al., 2007; Cullen et al., 2009). Accordingly, it is interesting whether GVS, as a potential modulator of phasic inputs into VN, could be really constructive in rebalancing the phasic-tonic imbalance between VNs and consequently in accelerating static and dynamic post-UL VC. In addition, it is unclear whether any sign of plasticity could be traced in VNs, as a main area for VC, following GVS intervention.

To evaluate these assumptions, we explored the effectiveness of a short-term GVS intervention on functional behavioral recovery (using static and dynamic postural tests) and neurogenesis (using BrdU and Ki67 markers) in rats with chemical UL induced by intratympanic injections of sodium arsanilate.

2. Results

2.1. Behavioral observations

Behavioral observations of the UL rats clearly showed an apparent head-tilt (in the planes of roll and yaw), circling and falling toward the labyrinthectomized side. When the UL rats were lifted by the tail, they spin around the long axis of their body for about 20–30 s. The limbs on the lesioned side were in flexion and adduction, while limbs on the intact side were extended and abducted. Although the circling and falling toward the lesioned side were predominantly observed during the first 3 days after TT injection, other signs were detectable until the final evaluations. None of these signs (i.e. classical postural and locomotor deficits (Liberge et al., 2010)) were seen in the intact or sham groups.

During the procedure of GVS threshold detection in the intervention groups, we observed that the GEHM is almost always a leftward (i.e., contralesional) head rotation in the roll plane. Interestingly, the spatial plane and direction of the induced-GEHM was the same for both right-anodal and left-anodal stimulation.

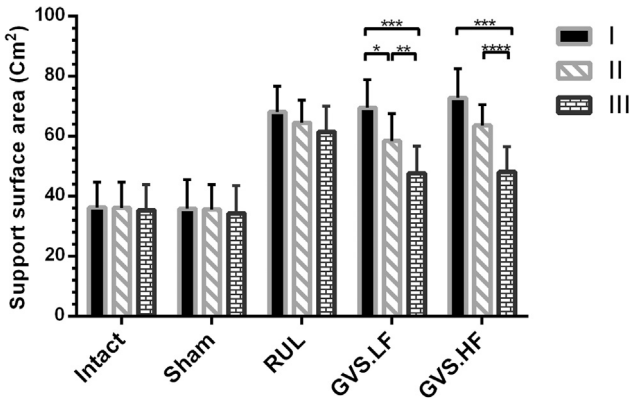

Fig. 1. Evaluation of the support surface area (SSA). The measurement (cm^2; mean ± SD; n = 10) was performed on three occasions (I, II, and III). The histograms reveal significant SSA-decrease in both intervention groups from I-to-III experiments (*$p < 0.0141$, **$p = 0.0183$, ***$p < 0.0001$, ****$p = 0.0003$). There was not any significant difference between them in either of three evaluations ($p > 0.9999$). A slight SSA-decrease (i.e. I–III difference) is observable for intact (9708), sham ($p > 0.9283$) and RUL ($p = 0.2128$) groups which is not statistically significant. I: before GVS intervention; II: one week after GVS intervention; and III: two weeks after GVS intervention.

2.2. Outcomes of the support surface measurement

Evaluation of the support surface area (SSA; in terms of cm^2) was performed with 4 groups at three time points (I, II, and III). As shown in Fig. 1, the mean SSA was about 35 cm^2 in intact and sham groups at all evaluations (note: SD of the SSA mean was nearly similar in all groups). Therefore, there was no significant difference ($p > 0.9999$) between their SSA at evaluations I, II, or III. The mean SSA was about 68, 64, and 61 cm^2 at three time points respectively in RUL group. Moreover, there was no significant difference ($p > 0.9999$) between SSA of intact and sham groups at each evaluation. A slight decrease in SSA occurred between evaluations I–III in the intact ($p = 0.9708$), sham ($p = 0.9283$) and RUL groups ($p = 0.2128$), which was not statistically significant. Furthermore, it was about 70, 60, and 47 cm^2 at three time points respectively in both intervention groups. Thus, SSA significantly decreased in both intervention groups (GVS.LF, GVS.HF) from time points I–III ($p < 0.0001$). At time points I and II, there was no significant difference ($p > 0.9999$) between the RUL-GVS.LF and RUL-GVS.HF groups. However, a significant difference was seen in the RUL-GVS.LF ($p = 0.0055$) and RUL-GVS.HF ($p = 0.0080$) groups in evaluation III. Sham-RUL differences were statistically significant for each evaluation ($p < 0.0001$). Sham-GVS.LF and sham-GVS.HF differences were statistically significant ($p < 0.0001$) in evaluations I and II. The sham-GVS.LF and sham-GVS.HF differences were also significant ($p = 0.0089$, $p = 0.0062$) for evaluation III. The almost similar results were obtained for comparison of intact group with intervention groups.

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