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Research Article

EFFECT OF VESTIBULAR STIMULATION ON BEHAVIORAL CHANGES IN PARKINSON DISEASE-INDUCED MICE

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ABSTRACT

Objective: This prospective study was conducted to provide an authoritative database for beneficial effects of vestibular stimulation, a simple non-invasive method to alleviate the behavioral changes in Parkinson's disease (PD).

Methods: Vestibule is stimulated by caloric vestibular stimulation (CVS). Pesticide Rotenone is used to induce PD. Open field test and elevated plus maze were used to access learning, memory, and behavior.

Results: Behavioral scores were taken before and after stimulation of the vestibular system. The scores were significantly different between rotenone-induced PD, control, and hot water vestibular stimulation groups ($p<0.05$).

Conclusion: This study categorically confirms that CVS with hot water causes behavioral changes in PD. This study certainly merits further studies with higher sample sizes to confirm the effect of CVS for enhancement of learning, memory, and behavior in patients with Parkinsonism.

Keywords: Vestibular stimulation, Parkinson's disease, Behavioral changes, Elevated plus maze, Open field test.

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INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disease characterized by rigidity, tremor, postural imbalance, gradualness of kineticism ("bradykinesia"), and other non-motor symptoms such as pain, fatigue, restless legs, and cognitive impairment [1]. The incidence rate of Parkinson's disease is roughly 13.4/100,000, and it increases rapidly beyond the age of 60 [2,3].

Postural instability is one of the main symptoms of the disease, which is due to the dysfunction of the vestibular system. Evidence for the influence of the vestibular system in PD is still emerging. The fact that vestibular dysfunction may occur in PD has a perplexingand long history. Many controversial findings have been reported by the previous studies regarding vestibular involvement in PD [4].

Studies have discovered that, in PD, vestibular information is transmitted to the striatum of the basal ganglia, in which the dopaminergic input is lost. However, corroboration of such a pathway has been slow to emerge, with cold caloric stimulation, electrophysiological, and neuro-tracer studies. The vestibular apparatus consisting of three semi-circular canals connected at their base to the utricle, saccule, and endolymphatic sac [5,6]. Extensive connections are present between the vestibular system and anterior cingulated cortex, hippocampus, locus coeruleus, cerebellum, insular cortex, raphe nucleus, amygdala, thalamus, prefrontal cortex, parietal lobe, occipital cortex, putamen, and other areas of encephalon which has a significant role in cognition [7,8]. Effects of vestibular stimulation may vary from regulating blood pressure, heart rate, enhancing spatial and verbal recollection, motor coordination, endocrine secretion, and postural instability [9]. Vestibular stimulation additionally helps in mitigating stress, apprehensiveness, and depression and vertigo. Vestibular stimulation modulates cognitive and sensory functions in normal healthy individuals and encephalon-damaged patients [10]. Vestibular system modulates cognition through hippocampus, through hypothalamic-pituitary-adrenal axis, and through limbic system and neo-cortex. Vestibular stimulation activates areas of the encephalon which are involved in learning and recollection [11].

Caloric vestibular stimulation (CVS) is a non-invasive and safe method of stimulating encephalon areas cognate to balance and coordination function. CVS is done by the irrigation of the external auditory canal with dihydrogen monoxide, which may induce a temperature change that leads to convection currents in the semi-circular canals [12]. This study was conducted to provide an authoritative database for benign effects of vestibular stimulation to alleviate motor dysfunction and behavioral disorders in PD. There is evidence that vestibular symptoms are present in PD, also from the neuropathology of PD in the central vestibular system [10].

The effect of vestibular stimulation on rigor in PD creates more interest in research in this field. Studies have suggested that early adjunctive treatment such as vestibular stimulation may be of great significance in PD, which may delay the desideratum for treatment with drugs such as L-DOPA and ropinirole. Higher doses of drugs can be "saved" for later use in the course of treating PD.

Aim and objectives

The objective of this study is to assess the interaction between vestibular function and PD.

METHODS

Ethical approval

The study was approved by the Institutional Animal Ethical Committee of Saveetha Medical College and Hospital (Approval number - SU/CLAR/RD/017/2016). The study was conducted in accordance with Committee for the purpose of control and supervision on experimentation on animals. All measures were taken to minimize the number of animals used and their suffering.

Animals

This interventional study was done in18 healthy and adult male Swiss albino mice with body weight between (25 and 40 g). Under standard laboratory conditions, the mice were housed with water and food provided ad libitum.

Mice were randomly grouped into four groups with six animals in each group.

Group I (Control group)
It administered with olive oil as vehicle.

Group II (PD group)
PD group injected with rotenone intraperitoneally (IP)

Group III (PD+CVS)
Rotenone (IP) injection and CVS with hot water for 30 days.

Method of administration of rotenone

Rotenone solution was initially made as a 50% stock in 100% dimethyl sulfoxide (DMSO) and then diluted in olive oil and medium-chain triglyceride to obtain a final concentration of 2.5 mg/kg rotenone in 98% olive oil and 2% DMSO. The solution is vortexed to generate a stable emulsion of DMSO, olive oil, and rotenone. Fresh solution was produced 2–3 times per week and stored in an amber septa vial that was kept out of direct sunlight and flipped many times before injection to prevent settling [13].

CVS

Using hot water, irrigate the mice's middle ear cavity (40°C). In a 5 mL syringe, 0.5 mL of water was drawn with the needle removed. The mice's ears were irrigated with water drop by drop using the syringe. The earlobes of mice were gently shook. The technique was then repeated on the other ear [14].

Materials

Behavioral parameters were carried on day 0.15 and 30 at Department of Physiology, Saveetha Medical College. All the test protocols were carried out between 9:00 am and 12:00 pm. Animals were habituated with the tests as in the training protocol for all the test parameters.

Elevated plus maze
The equipment included four arms, each measuring 6 cm wide and 18 cm long. It is 60 cm above the floor and built of wood covered with impermeable Formica. The closed arms are the two arms that are encircled by 6 cm high walls. The open arms are the two opposite arms without the surrounding walls. All four arms are connected by a central platform. For 5 min, each mouse was held in the center of the maze, facing a closed arm, and their movement and behavior were observed. Take note of the length of time spent in each closed and open arm, as well as the number of entries [15].

Open field test
The open field device is made of white plexiglass that is 36 × 36 cm and has 36 cm walls. The floor was split into 16 9 × 9 cm squares by lines establishing a center red square (9 cm × 9 cm). The open field maze was cleaned between each mouse with 70% ethyl alcohol. Mice were placed in one of the four corners of the open field or in the center and given 5 min to roam around the device. The mice were exposed to the apparatus for 5 min on 2 consecutive days to measure the habituation process to the arena. Line crossing, entering the center square, remaining in the center square for an extended period of time, rearing, stretch attempt postures, urinating, and excrement were all recorded [16].

Statistical analysis

The collected data were analyzed with IBM.SPSS statistics software 23.0 version. Descriptive statistics were done. The values were expressed as mean ± SD. To find out, the significant difference between the various group's multivariate analysis by Kruskal–Walli's test was done followed by the Mann–Whitney U test. For the repeated measures (0^{th} day, 15^{th} day, and 30^{th} day), the Friedman test was used. The probability value at $p<0.05$ was considered statistically significant.

RESULTS

In open field test, line crossing, rearing, and center square entries were found to be reduced in the PD group when compared to the control group animals. Vestibular stimulation for 15 days ($p<0.01$) and 30 days ($p<0.01$) has consistently increased when compared to Group II (PD). There was no significant difference found in the center square duration in all three groups (control, PD, and CVS + PD group) (Table 1).

In elevated plus maze, the number of entries into closed arm and the number of entries into the open arm, duration of stay in the closed arm and duration of stay in the open arm were reduced significantly ($p<0.01$) in PD group when compared with control group animals on 15^{th} and 30^{th} day. Number of entries into the open arm, time spent in the open arm, and time spent in the closed arm were increased significantly in CVS PD group ($p<0.01$) on 15^{th} and 30^{th} day, when compared with PD group. There was no significant difference found in animal's entries into the closed arm spending behavior in the closed arm on 15^{th} day and 30^{th} day (Table 2).

DISCUSSION

The present study was done to learn the efficacy of CVS on the behavior of mouse induced with rotenone. Normally, the CVS leads to activation of the afferents from vestibuli and these changes in the vestibular input exerts vigorous influence on posture and balance of the individual.

Structures of extrapyramidal system, namely, limbic system, basal ganglia, pedunculopontine nucleus, and spinal cord are activated by CVS through the eighth nerve (vestibular division). Due to this, an incrementation in the axial motor function occurs which may lead to the postural instability of the individual [12]. Tremor, dizziness, tinnitus, and gait unbalance are the common symptoms in Parkinsonian patients.

Pedunculopontine nucleus-thalamus controls the *postural sensory integration* in PD and not the cholinergic innervations from the cortex. Disturbances in these connections and differents from thalamus can cause postural instability in these patients [17]. In Parkinsonism, reduction in excitability of vestibular nuclei was observed, which can be modulated by inducing DOPA. The previous studies have shown that PD patients with lateral trunk flexion have defective processing of vestibular information. This partially leads to abnormality in patients' posture. The previous studies have stated that vestibular stimulation can be the one of the treatment options for PD. Visual and spatial abilities are affected due to and frontoparietal and frontal-basal ganglionic system dysfunction [18].

India has a low prevalence of PD when compared to Western countries and occupied the 16^{th} rank in the year 2002–2011 mainly in the in ecumenical context. Studies have stated that the truncation in the coalesced effect of the muscle vigor, the decreased visual sense and disturbed proprioception resulted in narrow base support which, in turn, leading to the imbalance in PD [19]. Despondence, anhedonia, cognitive disorders, apprehensiveness, psychosis, suicidal tendencies, apathy, incremented verbalization impairment, impulse control disorders, and restless leg syndrome are seen commonly in these patients. There are evidences for dysfunction in cardiac autonomic division in patients with parkinsonism.

A significant role is played by the vestibular system in different range of functions from reflexes to the highest perception and consciousness. Even used to treat the developmentally delayed children [20]. The previous studies have stated that application of controlled vestibular stimulation is not a mere intervention for the impaired cognition but

Table 1: Data were expressed as mean±SD. It shows the comparison of behavioral analysis (line crossing, center square entries, center square duration and rearing [open field test]), and their significance between the groups on 0th, 15th, and 30th day

Parameters	0th day			15th day			30th day		
	Control group	PD group	CVS+PD group	Control group	PD group	CVS PD group	Control group	PDgroup	CVS+PD group
Open field test									
Line crossing (in numbers)	13.5±3.32	10.17±3.43	10±3.12	14.25±0.96	10.17±1.47**	12.38±3.16	17.25±7.14	12.67±1.63	16.13±2.7$^{\#}$
Centre square entries (in numbers)	2.25±0.96	1±0.89	1.25±1.49	3.75±1.26$	1.17±0.75**	1.38±1.30	5.25±2.22$^{\$}$	1.83±0.75**	2.75±0.89$^{\#}$
Centre square duration (in sec)	13.5±5.26$^{\$}$	9.33±3.78	4.38±4.98	14.50±3.79	9.50±6.66	10.25±4.40	13.75±3.77	8.67±3.44	11±2
Rearing (in numbers)	3±0.82	0.83±0.75**	2.63±0.92$^{\#\#}$	3.50±1.91	0.83±0.98*	2.75±1.67$^{\#}$	4.25±1.5	1.17±0.98**	2.50±1.6

Data were expressed as mean±SD. Significance between the control group and PD only group (*p<0.05, **p<0.01, ***p<0.001), significance between the control and CVS PD groups ($^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001), and significance between the PD only and CVS PD groups ($^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001) are also indicated in the table, CVS: Caloric vestibular stimulation, PD: Parkinson's Disease

Table 2: Data were expressed as mean±SD. It shows the comparison of behavioural analysis (no. of entries into open arm, no. of entries into closed arm, time spent on the open arm, and time spent on the closed arm [elevated plus maze]), and their significance between the groups on 0th, 15th, and 30th day

Parameters	0th day			15th day			30th day		
	Control group	PD group	CVS PD group	Control group	PD only group	CVS PD group	Control group	PD only group	CVS PD group
Elevated plus maze									
No.of entries into open arm (numbers)	13.25±4.86$^{\$\$}$	6.17±2.48**	6.75±4.10	14.25±1.71$^{\$}$	6.17±2.32**	9.25±3.65	13±3.92	7±1.26**	10.38±1.77
No.of entries into closed arm (numbers)	13.5±7.14	5.17±1.33	8.25±4.68	12±5.35	5.17±1.47*	7.75±6.39	11.5±2.52$^{\$}$	6.33±1.75**	6.88±3.18
Time spent on the open arm (sec)	124±24.39$^{\$\$}$	41.83±31.44**	63.63±44.23	116±4.97	42±25.37**	89±37.60$^{\#}$	131±21.2$^{\$\$}$	53±17.93**	92±15.08$^{\#\#\#}$
Time spent on the closed arm (sec)	176±24.39$^{\$\$}$	258.17±31.44**	236.38±44.23	184±4.97	258±25.37**	211±37.6$^{\#}$	169±21.2$^{\$\$}$	247±17.93**	208±15.08$^{\#\#\#}$

Data were expressed as mean±SD. Significance between the control group and PD only group (*p<0.05, **p<0.01, ***p<0.001), significance between the control and CVS PD groups ($^{\$}$p<0.05, $^{\$\$}$p<0.01, $^{\$\$\$}$p<0.001), and significance between the PD only and CVS PD groups ($^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001) are indicated in the table, CVS: Caloric vestibular stimulation, PD: Parkinson's Disease

also it can be used to alleviate stress, cancer pain, insomnia, ameliorates immunity, and treats endocrine dysfunction.

In Wistar albino rats, the stress-induced changes in immunological parameters can be experimentally proven with cold water stimulation [21]. The previous studies have proven the beneficial effect of hot and cold water stimulation on lipid profile in these animals. In Wistar albino rats, the hot water CVS has improved the cognitive domain in scopolamine induced partial amnesia. CVS has been used to treat a variety of sensory and cognitive functions in normal subjects and also in encephalon damaged patients [9].

Vestibular nuclei give projections the dorsal motor vagal nucleus, nucleus tract of Solitarus, nucleus raphe magnus, nucleus equivocal/para-equivocal and also to ventrolateral medullary reticular formation, and lateral medullary tegmentum. Because of the projections from the vestibular nuclei to these sites, cold vestibular stimulation of the vestibular nucleus, in turn, causes stimulation in motor activity and arousal of the animal.

Vestibular stimulation enhances *Long-term Potentiation* in hippocampus especially by activating increase in synaptic transmission of Ach. Studies shows that there will be decrement in the serotonin and encephalon derived neurotrophic factors, which vigorously affects the regulation of synaptic plasticity and neurogenesis. Many experiments have proven that vestibular stimulation is benign in incrementing serotonin level in encephalon [22].

CVS causes release of acetylcholine (ACh) from rat hippocampus. Effects of ACh on CNS cause changes in neuronal excitation, presynaptic release of neurotransmitters, neuronal development, and coordinates the firing of different groups of neurons. This, in turn, activates cortical neurons, which helps in increasing attention and memory of the animal. Earlier studies stated that an exercises with a systematic program can amend the UPDRS scores, daily activities, and additionally gait of PD patients. PD shoe which is a wireless vibratory feedback system and partial weight fortified treadmill gait training such as physical therapies were efficacious in treating arduous manifestations of Parkinsonism such as freezing and gait disturbances [23].

CONCLUSION

This study categorically confirms that the CVS with hot water enhances learning, memory, and behavior in PD-induced mice when assessed

using open field test and elevated plus maze test. Hence, this study certainly merits future studies with translational research among human subjects to confirm the effect of CVS for enhancement of learning, memory, and behavior in individuals with PD.

CONFLICT OF INTERESTS

Nil.

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Self.

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