



Vestibular stimulation induced alteration in glutamate levels improves memory and anxiety scores in scopolamine induced dementia rats

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ABSTRACT

Glutamate is an excitatory neurotransmitter which is essential for cognition but, at the same time, a neurotoxin if accumulated beyond a certain level. The derangement of glutamate level in brain is closely associated with Alzheimer's disease. Vestibular stimulation is known to stabilize various neuro-chemical transmission in central nervous system, especially effective in enhancing acetyl choline level and reduction of acetyl cholinesterase level. So the current study has undertaken to evaluate glutamate level in dementia and vestibular stimulation groups and its role in improving memory and anxiety scores. In the present study, 32 Wistar rats were used. Scopolamine was used to induce dementia and caloric stimulation (bilateral, unilateral right and unilateral left) was used to stimulate vestibular system. Behavioral parameters like water maze used to assess memory and elevated plus maze was used to assess anxiety in our study. Glutamate was quantified by spectrofluorimetry and histopathology of hippocampus and cortex were assessed. Caloric vestibular stimulation effectively reduced glutamate level near to normal values and this result reflected in increased memory scores in water maze and increased exploratory activities in elevated plus maze. Especially unilateral left vestibular stimulation has found beneficial in reducing glutamate level. All these positive outcomes have proven that vestibular stimulation can increase memory and anxiety scores in dementia and thereby can be used as an alternative therapy in this field. Further animal studies and human studies are needed to dig deep into the molecular mechanisms behind its actions.



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INTRODUCTION

It is definite that a cluster of neuronal networks with vast variety of neurotransmitter system involved in learning and memory, especially cholinergic neurotransmitter. The severe cholinergic damage was always pointed towards dementias, particularly in age-related cognitive decline and Alzheimer's disease. The symptomatic improvement by cholinesterase inhibitors in Alzheimer's patients validated this cholinergic hypothesis even more (Summers *et al.*, 1986). So any decrease or blockade of different receptors in cholinergic

system is always associated with impaired learning and memory. But recently, it has been found that glutamatergic system plays a key role in the pathophysiology of dementia. Glutamate is an excitatory neurotransmitter which is essential for cognition but at the same time a neurotoxin if accumulated beyond a certain level. Glutamate at high concentrations in the synaptic cleft is toxic and may result in neuronal death, a phenomenon generally termed excitotoxicity. Research had proven that in uptake and recycling system of glutamate is severely impaired by toxic $A\beta$ may allow more glutamate availability in Alzheimer's disease and CSF glutamate levels were found to be significantly elevated in patients with AD in comparison to patients with mild cognitive impairment (Parpura-Gill *et al.*, 1997; Fernández-Tomé *et al.*, 2004; Kaiser *et al.*, 2010).

Vestibular stimulation is known to stabilize various neuro-chemical transmission in central nervous system, especially effective in enhancing acetyl choline level and reduction of acetyl cholinesterase level (Horii *et al.*, 1994; Devi and Mukkadan, 2016). But there is not much literature which shows the advantage of vestibular stimulation in enhancing the glutamate level in brain and mechanism through which it can be beneficial in improving cognition. Detailed studies should be done in this area in order to prove effectiveness of vestibular stimulation in increasing glutamate level and its mechanism.

MATERIALS AND METHODS

Experimental Animals

A total of 36 male Wister rats (three months old) with body weight ranging between 150-200 g were used in the present study. All animals were housed for one week in the laboratory animal room prior to study. The selected animals were provided with standard environmental conditions. All the readings were taken during the same time of the day, that is, between 9 am and 5 pm.

30 days Study

G1: control group

G2: Scopolamine induced dementia

[3mg/kg scopolamine for 3 days and no intervention]

G3: Scopolamine induced dementia+ Donepezil

[3mg/kg scopolamine for 3 days and 5mg/kg donepezil for 14days]

G4: Scopolamine induced dementia +bilateral hot water vestibular stimulation (30days)

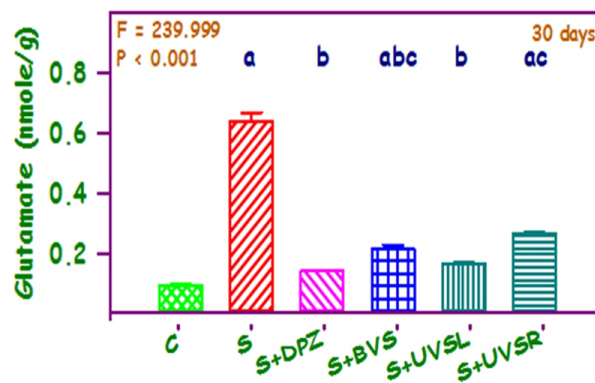


Figure 1: Glutamate levels in control, dementia and vestibular stimulation groups

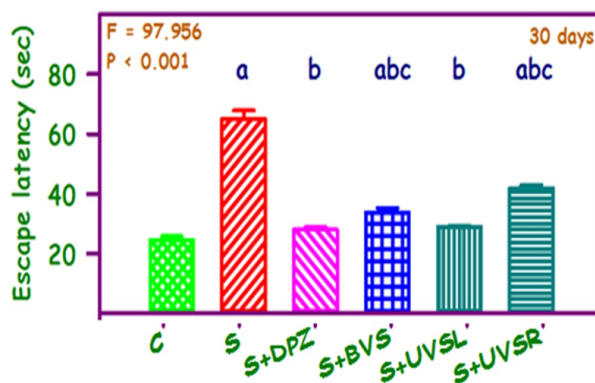


Figure 2: Escape latency in control, dementia and vestibular stimulation groups

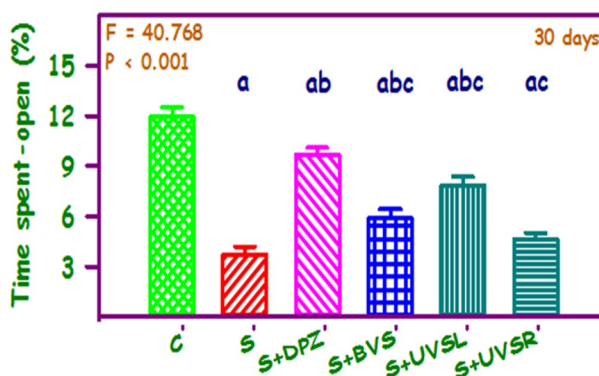


Figure 3: Elevated plus maze-Percentage time spent in open arm in control, dementia and vestibular stimulation groups

[3mg/kg scopolamine for 3 days and 30days of bilateral caloric vestibular stimulation]

G5: (n=6) Scopolamine induced dementia +Unilateral Left ear hot water vestibular stimulation (30days)

[3mg/kg scopolamine for 3 days and 30days of unilateral left ear caloric vestibular stimulation]

G6: (n=6) Scopolamine induced dementia +unilateral right ear hot water vestibular stimulation

(30days)

[3mg/kg scopolamine for 3 days and 30days of unilateral right ear caloric vestibular stimulation]

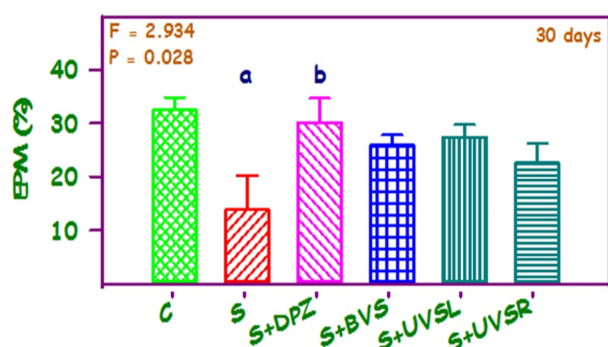


Figure 4: Elevated plus maze-Percentage entries in open arm in open arm in control, dementia and vestibular stimulation groups

Caloric Vestibular Stimulation

Caloric vestibular stimulation was administered by irrigating the middle ear cavity with hot water with a temperature of 45°C with the help of syringe for 30 days.

Glutamate

The level of Glutamate was estimated by spectrofluometry (Butcher and Lowry, 1976).

Procedure

To 0.2 ml aqueous phase, 0.25 ml of Ophthaldehyde (OPT) reagent was added. The fluorophore was developed by heating to 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, readings were taken at 515 nm for Glutamate in the spectrofluorimeter.

Morris Water Maze Test

Water maze was developed by Richard Morris. The maze was designed to assess spatial or place learning. MWM testing was conducted in an open circular pool of 214 cm in diameter, about 91cm and 10cm² platform and filled approximately half-way with water. Distal visual clues were placed (like X, O etc.). The three pre-training trials was conducted in order to orient the animals about these properties of the task. They were placed into a pool of tepid water and swim around for a minute and removed after. After pre-training, maze was filled up in such a way that the platform gets immersed one inch below the water. 125 milliliters of non-toxic white tempera paint was used to make the water opaque. Each animal underwent three consecutive 3 trials/day for 4days. Animal was allowed to swim for 120seconds. If animal was not able to find the hidden platform, it was recorded as 120 seconds and

the animal was placed on the platform for 15 seconds. Once the rodent reached the platform, the time was recorded. Trials were done before the intervention and a retention test was carried out after 30 days (Morris, 1984).

Elevated Plus Maze

In order to measure the anxiety level all intervention groups, % time spent in open arm and % entries in the open arm in EPM had been chosen, as this tool is widely accepted for anxiety measure for various pharmacological and non-pharmacological agents. EPM had been cleaned before the test and dried. Rats were taken out of the cage and placed at the junction of the open and closed arms. Rats were allowed to move around for 5 minutes. The observer noted the number of entries into the open and closed arms and the time spent on the open arm and closed arm. Maze is cleaned with hypochlorous acid between the runs in order to remove odor thereby to prevent a bias based on olfactory cues (Walf and Frye, 2007). Basic values were recorded before the intervention and 30days and 60 days' values were noted post-intervention.

Histopathology

Brains were dissected and fixed in 10% formalin for histopathological processing. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 microns' thickness were obtained, mounted on glass slides and stained with the routine haematoxylin and eosin technique.

RESULTS AND DISCUSSION

Glutamate

Glutamate activity significantly ($p < 0.001$) increased in scopolamine group by nearly 6 folds compared to the control group. Donepezil, bilateral and unilateral left vestibular stimulation significantly ($p < 0.001$) reduced the glutamate activity by 78%, 66% and 73% respectively (Figure 1). In both the Donepezil and unilateral left vestibular stimulation group the glutamate activity like that observed in the control group. While the efficacy in the bilateral vestibular stimulation group was marginally inferior ($p < 0.001$) to Donepezil group. There was no significant difference between the glutamate activity observed in the unilateral left vestibular stimulation and Donepezil group ($p = 0.146$). In unilateral right vestibular stimulation group glutamate activity was significantly ($p < 0.001$) reduced by 58% compared to the scopolamine group. Although the efficacy in the unilateral right vestibular stimulation group was inferior compared to Donepezil and bilateral and

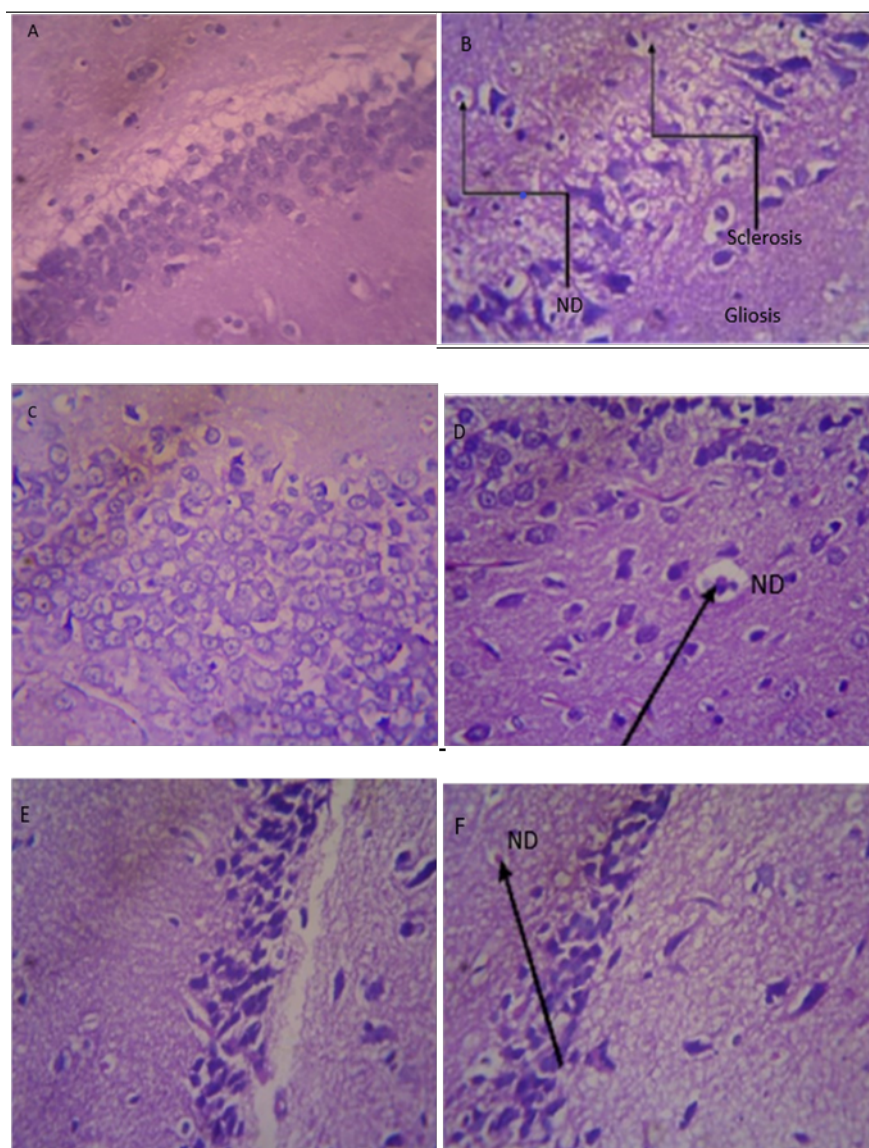


Figure 5: Effect of 30 days of vestibular stimulation on histopathological changes in hippocampus. A. Control B. Scopolamine induced neurodegeneration C. Donepezil treated group moderate gliosis D, E & F : S+BVS, S+UL, S+UR respectively-mild neuronal degeneration

unilateral-left vestibular stimulation groups. The order of glutamate activity observed in the various groups is as follows: C > DPZ > UL > BVS > UR.

Morris Water Maze Test-Escape latency

Measure of escape latency is an indicator of spatial memory. Scopolamine significantly ($p < 0.001$) increased the escape latency by 2.5 folds compared to the values in the control group. Donepezil significantly ($p < 0.001$) restored the escape latency time by 56% when compared with scopolamine group. Bilateral and left vestibular stimulation also significantly ($p < 0.001$) restored the escape latency time by 49% and 56% respectively. Unilateral left vestibular stimulation significantly reduced escape latency similar to that observed in the standard treatment group (Donepezil). Unilateral right vestibular stim-

ulation group significantly ($p < 0.001$) decreased the latency time by 36% when compared with scopolamine only group. All the interventions tested were efficacious in improving the spatial memory and the order of scored observed were as follows: C > DPZ = UL > BVS > UR as given in (Figure 2).

The percentage time spent in open arm is a measure of anxiety levels in elevated plus maze. Scopolamine administration significantly ($p < 0.001$) reduced the time spent in open arm by 68% compared to the control group. Donepezil therapy significantly ($p < 0.001$) increased the percentage time spent in open arm by 61%. Bilateral vestibular stimulation enhanced percentage time spent in open arm but was significantly ($p < 0.001$) inferior to the standard treatment group (Donepezil). Unilateral

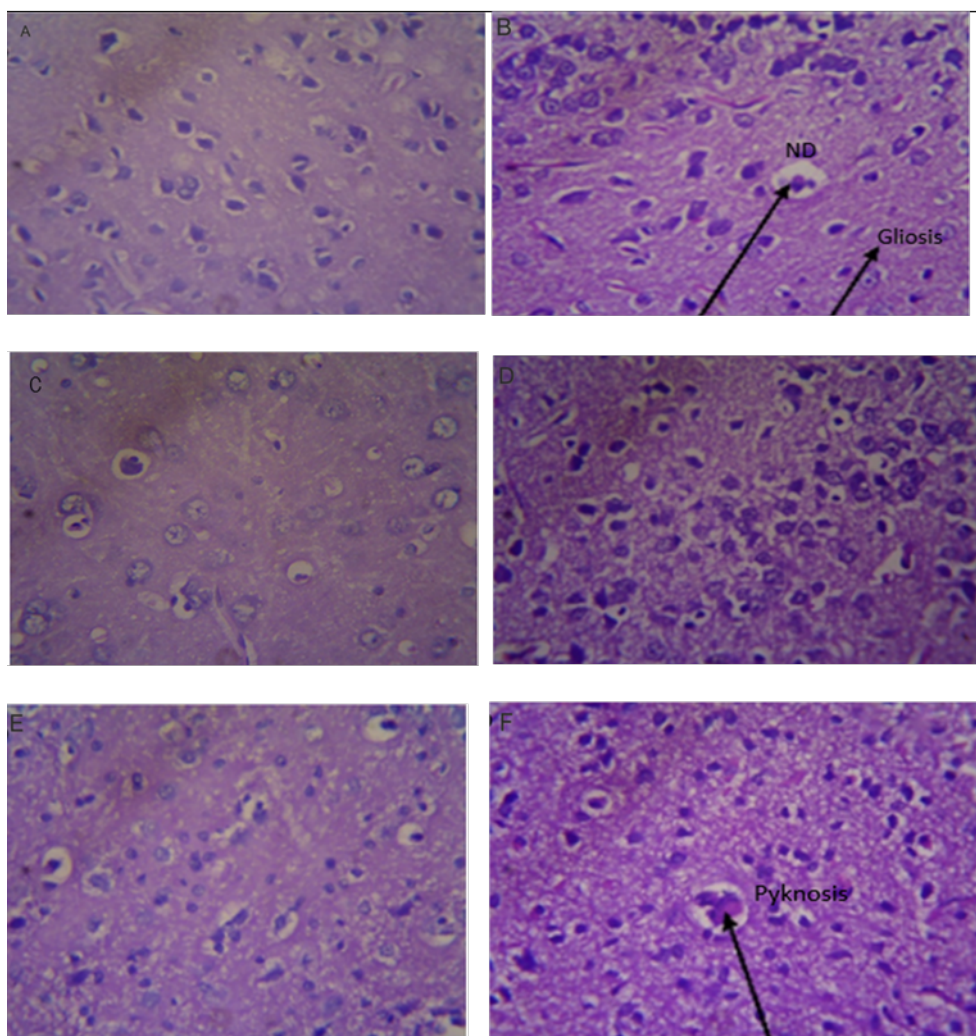


Figure 6: Effect of 30 days of vestibular stimulation on histopathological changes in cerebral cortex. A. Control B. Scopolamine induced neurodegeneration , severe gliosis with sclerotic changes C. S+DPZ- mild gliosis D, E & F : S+BVS, S+UL, S+UR respectively- Mild neuronal degeneration with moderate gliosis and mild sclerotic changes

left vestibular stimulation significantly ($p < 0.001$) enhanced the percentage time spend in open arm by 52%, which was inferior to the standard treatment group (Donepezil) but superior to bilateral vestibular stimulation group. In contrast, unilateral right vestibular stimulation was observed to have limited beneficial effects in improving anxiety levels. Donepezil and unilateral and bilateral vestibular stimulation therapies was efficient in reducing anxiety levels in scopolamine induced dementia and almost restored the scores to near normal values with a p value of ($p = 0.0293$) and ($p < 0.001$). In the present 30 days' study, the order of therapeutic efficacy was $DPZ = UL > BVS > UR$ as observed in (Figure 3).

The percentage entry into the open arm is another measure of anxiety level. Scopolamine significantly ($p = 0.021$) reduced the number of entries into the

open arm by 57% when compared with control group (Figure 4). Donepezil, bilateral and unilateral left and right vestibular stimulation enhanced the percentage entries into the open arm by 117%, 86%, 97% and 62% respectively, when compared to scopolamine group. All intervention groups showed no significant difference with control and donepezil group. Hence the data suggests that 30 days of vestibular stimulation by enhancing entries into open arm was effective in reducing anxiety levels in scopolamine induced dementia.

Histopathology-Normal histological features were observed in the hippocampus and cerebral cortex of the control group. The histological features in the hippocampus and cerebral cortex were significantly altered in the scopolamine group. Scopolamine administration induced moderate neurodegeneration, severe gliosis and sclerosis in the hippocam-

pus. The hippocampal pyramidal cells also showed features of shrinkage and eosinophilic infiltration in its cytoplasm. Scopolamine induced neurodegeneration and gliosis was also observed in the cortex. In all the intervention groups, mild gliosis and mild neurodegeneration in hippocampus and cortex were observed. But Compared with the scopolamine group, all the treatment groups showed considerable improvement towards normal histological features (Figures 5 and 6).

This study measured the neurotransmitter glutamate involved in cognitive process to understand the mechanisms of benefits observed following caloric vestibular stimulation (CVS) on memory and anxiety in scopolamine-induced dementia in rats. Glutamate, Water maze for memory and Elevated Plus maze (Percentage time spent in open arm and Percentage entry into the open arm) and histopathology of hippocampus and cortex were assessed and compared in six groups. Glutamate levels were corrected to normal level after unilateral and bilateral vestibular stimulation. The beneficial effects of CVS on memory and anxiety can be attributed to changes in neurotransmitters, more so due to changes in glutamate levels as normal level of glutamate is essential for learning and memory. Vestibular stimulation revived hippocampus and cortex back to normal, which is the reflection of restoration of neurotransmitter levels.

Glutamate is a synaptic transmitter (Hayazhi, 1954; Olney, 1969) and is the major mediator of excitatory signals in the mammalian central nervous system. Glutamate is essential for normal brain function including brain development, synaptic plasticity, cellular survival, cognition, memory and learning. Glutamate is known to have toxic effects at both high and low levels. Hence it is crucial that glutamate level is maintained at the right concentrations at the right locations for the right amount of time (Danbolt, 2001). More recently glutamate is gaining increasing attention for its role in the pathogenesis of dementia and this has led to the use of glutamate antagonists for the treatment of dementia (Kelly et al., 2006). High levels of glutamate lead excitotoxicity (neuronal injury) and apoptosis (cell death) are considered as the key events in the pathogenesis of dementia. Consistent with this, in the present study significant increase in glutamate levels and associated decline in cognition and spatial memory was observed in the scopolamine-administered rats. CVS for 30 significantly reduced the glutamate levels and improved memory scores and anxiety level. There are multiple mechanisms involved in how glutamate leads to excitotoxicity and apoptosis. The major mechanism is abnor-

mally high glutamate level stimulates N-Methyl-D-aspartate (NMDA) receptors excessively, leading to overload of calcium in the cell and impairs neuronal homeostasis. This continuous interruption of calcium homeostasis leads to neuronal damage and apoptosis (Rothman et al., 1987; Procter, 2000). It has been also found that the presence of A β exacerbating the glutamate toxicity in Alzheimer's disease (Mattson et al., 1992; Velliquette, 2005). In addition, glutamate and excessive activation of the NMDA receptor are found to enhance the production of A β and tau protein in Alzheimer's disease and also increases tau immunoreactivity (Sindou et al., 1994; Couratier et al., 1996). However, it seems that this vicious cycle continuous in dementia where each pathologic condition tends to exacerbate the other (Greenamyre et al., 1988). Likely, interventions such as CVS or any other forms of vestibular stimulation can potentially interfere with several molecular pathways linked to glutamate signaling. Hence the beneficial effects of CVS observed in this study are mediated by not only the reduction in glutamate level but may also involve other associated collateral pathways (Sindou et al., 1994; Couratier et al., 1996).

The discussion about glutamate is never complete without mentioning about gamma-aminobutyric acid (GABA). Glutamate and GABA are excitatory and inhibitory neurotransmitters with homeostatic relationship, which balances the level of brain activity. Hence GABA is equally important neurotransmitter, which inhibits brain activity and enabling relaxation. Although, in the present study, we didn't evaluate the GABA levels, it is very likely that CVS may have an influence in regulation of GABA. This interesting hypothesis will be worth exploring in future studies. A large number of post-mortem studies of AD patients have shown moderate to significant reductions in GABA levels in various cortical areas of the brain (Perry et al., 1987; Mohanakrishnan et al., 1995; Yew et al., 1999). Hence potentiating GABAergic inhibition can potentially counteract elevated glutamate excitation and decrease excitotoxicity in cortical circuits (Gu et al., 2003). It is hence likely that CVS by reducing the glutamate levels may indirectly potentiate GABAergic signaling. GABA not only counterbalances excitatory effect of glutamate but also a supplementary role of GABA is in modulating Ach, Serotonin and dopamine levels (Decker and McLaugh, 1991; Zorumski and Isenberg, 1991).

As CVS increased the Ach levels and reduced in the glutamate levels in this study, the possibility of an additional influence on GABA is likely. More so because in addition to improvement in learning & memory function, in this study we also observed

reduction in anxiety scores, which is potentially mediated by GABA signaling (Lydiard, 2003; Gauthier and Nuss, 2015). This unique ability makes GABA a potential target to be modulated by CVS for controlling behavioral and psychological symptoms of dementia.

All these positive outcomes have proven that vestibular stimulation can increase memory and anxiety scores in dementia and thereby can be used as an alternative therapy in this field. The hemispheric dominance also plays a part in the effectiveness of vestibular stimulation. Throughout the study, unilateral left ear vestibular stimulation has shown better results, which is due to left hemispheric dominance in rats. The mechanism is still unknown and translational studies are essential to further establish the benefits observed in this study.

CONCLUSION

Many beneficial effects of vestibular stimulation are unexplained and have not been explored. Further animal studies and human studies are needed to dig deep into the molecular mechanisms behind its actions. As vestibular stimulation would be a simple and cost-effective intervention in reviving the adverse effects of dementia to a certain extent, this possibility should be further investigated.

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