Original Article

Does Chronic Administration of Sodium Valproate to Juvenile Rats Induce Movement Disorder and Cognitive Dysfunction during Adulthood?

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ABSTRACT

Background: Children with seizure disorder are often treated with sodium valproate (SV) on long-term basis. SV acts mainly through gamma amino butyric acid pathways, reducing the excitatory neurotransmission and modifying the monoamine concentration. Altered monoamine concentration by SV is expected to cause movement disorder and cognitive dysfunction, considered reversible after the withdrawal of treatment, but some claim it to be irreversible. It is not clear whether such adverse effects continue during adulthood. The aim of this study was to investigate whether chronic administration of SV in juvenile rats causes movement disorder and cognitive dysfunction during their early adulthood.

Methods: Sixteen-day-old male *Wistar* rats from the central animal house, KMC, Mangalore, India in 2015, received either 200 or 400 mg/kg dose of SV for 45 consecutive days and another group served as control. Thirty days after discontinuation of the drug, at postnatal day 90, the rats were tested for movement disorder and cognitive function.

Results: Chronic SV treatment in juvenile rats resulted in slow movement, tremors during adulthood but did not affect muscle tone, locomotor and exploratory activities. It also caused cognitive dysfunction in adult rats.

Conclusion: Despite the reported safety of chronic SV therapy, its adverse effects such as Parkinsonism symptoms or cognitive dysfunctions should be of concern in all young patients treated with SV for many years. Persistence of cognitive impairment, tremors and generalized slow movement during adulthood after cessation of treatment that was observed in this study, warrants a close monitoring system in children who receive long-term sodium valproate.

Keywords: Chorea, Cognitive Manifestation, Movement Disorders, Parkinsonian Disorders, Rats, Valproic Acid.

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INTRODUCTION

Despite more than 40 yr of clinical use, sodium valproate (SV)-induced movement disorder in children is still of concern. SV is the sodium salt of valproic acid (VPA) and is an anticonvulsant mainly used in the treatment of epilepsy as well as panic attack, anxiety disorder, anorexia nervosa, migraine, bipolar disorder, acute mania and acute stress reaction [1]. It is considered the first-choice drug for many forms of symptomatic and idiopathic generalized epilepsies [2, 3]. The mechanism of action of SV is based on selective potentiation of postsynaptic inhibition mediated by gamma-aminobutyric acid (GABA) or on direct increase of GABA in the brain [4]. Valproate also acts by reducing excitatory neurotransmission and modifying monoamine concentration [5].

Though SV is a broad-spectrum antiepileptic drug, its neurotoxic effects in young children is of concern. These effects could range from tremors to involuntary movements [6]. The SV induced extrapyramidal symptoms include Parkinson's like symptoms and less commonly choreiform movements. [7]. The risk of parkinsonism is 10-fold higher in those taking valproate as compared with other antiepileptic drugs [8]. In a case series reported by Lancman (1994), patients exhibited choreic movements involving the mouth, tongue, head, trunk and limbs [7]. Patients can also develop bradykinesia, rigidity, hyperreflexia, dysphagia, anarthria and aphonia, which constitute Parkinsonism plus Syndrome [9]. The movement disorder after chronic SV treatment is attributed to its ability to reduce D_2 signalling in the brain [10].

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Most of the reported data claim that these neurotoxic effects are reversible. Parkinson like symptoms are mostly hypertonic and hypokinetic, while chronic symptoms are hypotonic and hyperkinetic. It would be interesting to evaluate the side effects of SV by analysing movement and muscle tone after chronic treatment in juvenile rats. Hence, in the present study we focused on SV induced movement disorder and muscle tone using standard behavioural psychopharmacological studies.

The objectives of this study were to evaluate whether chronic treatment of SV in juvenile rats cause movement disorder. Further, we tested chronic SV treatment effects on muscle tone, muscle strength and cognition during early adulthood.

MATERIALS AND METHODS

Animals

In-house bred male albino *Wistar* rats from central animal house, KMC Mangalore, India in 2015 were used in the present study. Rats were fed with water and food pellets *ad libitum*. The rats were maintained under controlled conditions of light-dark cycles (12:12), temperature (22±3°C), humidity (approximately 50±10%) and pathogen free environment. Polypropylene cages with paddy husk as bedding material was used for housing the rats.

The approval of Animal Ethics Committee of our institution was obtained before commencing the experiment.

Animal Groups

The experiment consists of the following animal groups (n=6).

Group-1: Control rats
Group-2: Rats received 200mg SV for 45 days
Group-3: Rats received 400mg SV for 45 days

SV Administration

The SV was obtained from Sun Pharmaceuticals, India and was dissolved in water and administered via intraperitoneal route. SV administration began from postnatal day 16, and continued until the postnatal day 60 because at this age the maturation level of brain corresponds to that of adolescent humans [9].

Study Parameters

The open field test, movement analysis test and catalepsy test were performed one month after the termination of SV administration, at postnatal day (PND) 90 while T-maze test was performed at PND 100.

Measurement of Body Weight

The body weight was noted at different intervals throughout the experiment, as SV is known to cause abnormal weight gain [11].

Open Field Test

Open-field test is one of the most widely used methods to assess the motor, exploratory activities and emotional reactivity of rodents [12].

Apparatus: A rectangular box (100×100×40 cm) with the floor consisting of 25 equal squares (5×5 cm) of fine unit wire mesh was used. Illumination was provided with 100 watts bulb, fixed 60 cm above the centre of the field.

Procedure: The rats were placed in one corner of the chamber. The number of peripheral and central crossings in five-minute durations was recorded. Rearing (elevated hind limb & pelvis with elevation of fore limb) and grooming (use of head, tongue and forelimb for the process of cleaning various part of the body) activities were recorded.

A. Movement Analysis

Rats were placed individually in a Plexiglas cages for movement assessment. The severity of motor abnormalities was evaluated using a quantitative neurological scale [13]:

Quantitative Neurological Scale:

- Normal behaviour – score 1
- General slowness of displacement resulting from mild hind limb impairment – score 2
- Incoordination and marked gait abnormalities – score 3
- Hindlimb paralysis – score 4
- Incapacity to move, resulting from forelimb and hindlimb impairment – score 5

B. Oro-Facial Movements

Rats were placed individually in a Plexiglas cage for assessment of vacuous chewing movements [14]. The floor and the back of the cage consisted of mirrors to permit observation of vacuous chewing movements when the rats were faced away from the observer. These movements were measured continuously for five-minute periods.

Inclined Plane Test/Catalepsy Test

Catalepsy.is a nervous condition characterized by muscular rigidity and fixity of posture regardless of external stimuli, as well as decreased sensitivity to pain. Catalepsy was assessed in terms of the time the rats maintained an imposed position with both

front limbs extended and resting on a 4-cm high wooden bar. The endpoint of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner. A cut-off time of 180 seconds was applied during the recording of observations. The rats were returned to their individual home cages between determinations. All observations were made between 10.00 and 16.00 h in a soundproof room at 23-25 °C.

Assessment method: If the treatment groups of rats maintained the imposed posture for longer durations as compared to control or untreated rats, it would indicate muscular rigidity. However, if the treatment groups of rats maintain the imposed posture for lesser durations as compared to control or untreated rats, it would indicate decreased muscle tone. This assessment method was modified for rats from earlier studies with mice [15, 16].

All the tests were video recorded and were conducted by a trained researcher blinded to the treatment schedule.

Spatial Learning Test (T-Maze Test)

To assess the spatial learning ability, rats were subjected to spontaneous alternation and rewarded alternation tests. The T-Maze consisted of a start box, 15×12 cm in size, a stem area (35×12 cm), a choice area (15×12 cm) and two arms (35×12 cm each), at the end of which were the goal areas (15×12 cm each), containing food pellets. The sidewalls were 40 cm in height. A sliding door separated the stem, from the start box. The T-maze was kept in a sound attenuated room [17].

I) Spontaneous Alternation Test: The rats were starved for two days prior to the test in order to motivate them for food reward. Subsequently food was restricted so that the body weight was maintained at 85% of pre-test weight. Rats were placed in the T-maze for 30 minutes daily, for 2 days, to orient them to the T-maze environment. During these sessions, 15 pellets of food (10 mg each) were kept in each goal area. On the following 4 days, six trials were performed daily. In each trial, the rats were placed in the start box and the door was opened, thus allowing it to enter into the stem and arms of the T-maze. After the rats ate the pellet in the goal area, they were placed back in the start box. In each trial, the arm chosen by the rat and the number of alternations made, were noted. The inter-trial intervals were one minutes. The rat was considered to have entered into a particular arm when it entered that arm with all its four limbs. Percentage bias was calculated for each rat using the following formula:

$$\%\,\text{bias} = \frac{\text{Total number of choices more frequentely chosen side} \times 100}{\text{Total number of trials}}$$

With higher numbers of alternations, a lesser % bias was considered as an index of improved learning ability.

II) Reward alternation test: This test is performed after completion of spontaneous alternation test. The test consisted of 6 trials/d, for four consecutive days. Each trial included two runs, viz. forced run and choice run. In the forced run, the rats were forced to one of the arms by blocking the other arm and allowing them to consume the pellets there. In the choice run, the forced arm is kept empty and pellets are placed in the opposite arm. Both the arms were free for the rats to roam. Now, the rat had to enter the arm, opposite to the forced one, as "correct response". The forced arm was predetermined and it was the same for all rats in each day, but it changed every other day. The experiment was repeated on 4 successive days. The "Percentage of correct responses" was calculated for each rat by using the following formula:

$$\%\,\text{of correct response} = \frac{\text{Total number of choices of correct response} \times 100}{\text{Total number of trials}}$$

Increase in mean % correct response was considered as improved learning and memory.

Statistical Analysis

The data was expressed as mean ± SE. The significance of differences among the groups were assessed using one-way analysis of Variance (ANOVA) test followed by Bonferroni's multiple comparison post hoc test. *P* values <0.05 were considered as significant. Graph Pad version 3 was used for statistical analysis.

RESULTS

Comparison of Mean Percentage of Body Weight Gain during Different Period of Postnatal Development among Various Animal Groups (Fig.1)

Day-16: At day 16 rat pups were randomly allocated into three groups (n=6), but their initial body weight were not the same. Rats designated to receive SV-400 had a significantly higher body weight compared to controls ($P<0.05$) and SV-200 groups ($P<0.01$). Hence, at later stages of postnatal development body weights of different rats groups were not compared as their base line weights were

not the same. However, percentage of body weight gain was calculated at PND-21, PND-60 and PND-90.

Comparison between PND-16 and PND-21: The percentage of body weight gain was maximum in controls (68.66%), followed by SV-200 (47.75%) and SV-400 (21.74%).

Comparison between PND-21 and PND-35: The percentage of body weight gain was maximum in SV-200 (120.37%), followed by SV-400 (76.42%) and controls (30.15%).

Comparison between PND-35 and PND-49: During this period the percentage of body weight gain was maximum in SV-400 (120.16%), followed by controls (111%) and SV-200 (74.36%).

Comparison between PND-49 and PND-60: The percentage of body weight gain was maximum in the control group (92.98%), followed by SV-200 (41.68%) and SV-400 (10.25%).

Comparison between PND-60 and PND-90: The percentage of body weight gain was maximum in SV-200 (92.18%), followed by SV-400 (82.72%) and controls (68.18%).

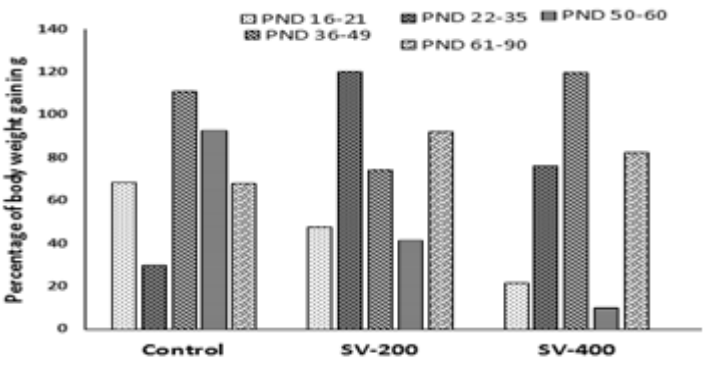

Figure 1. Comparison of percentage of body weight gain during postnatal development in rats (n=6).

Results of the Open Field Activities

The number of peripheral square crossings did not differ between controls and SV treated rats, which demonstrated that SV-200 or SV-400 did not affect locomotor or exploratory activities in rats. SV at both doses increased the number of central square crossings, which indicated that SV did not produce any anxiety-induced behaviour. Time spent in central square area was also not significantly different (P>0.05) between controls and SV treated rats. This also indicated that SV did not cause any anxiety like behaviour (Fig. 2& 3).

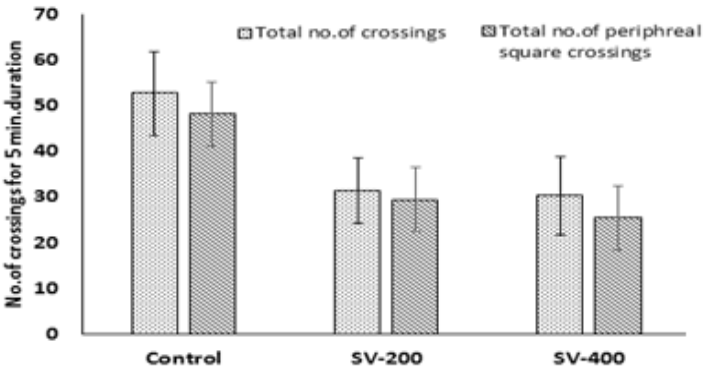

Figure 2. Results of the open field activity of rats for 5 min. durations (n=6). Values are expressed as means and error bars indicate ±SE. For total No. of line crossings, P=0.2027. For total No. of peripheral line crossings, P=0.1541.

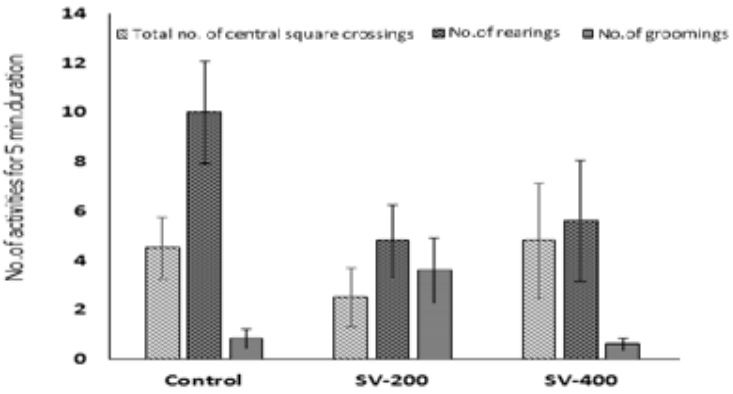

Figure 3. Results of the open field activity by rats for 5 min. durations (n=6). Values are expressed as means and error bars indicate ±SE. For total No. of central square crossings, P=0.5377. For total No. of grooming activity, P=0.0328 and for rearing activities, P=0.1786.

Results of Movement Analysis

The severity of motor abnormality was evaluated using a quantitative neurological scale [13]. No severe movement abnormality was observed in sodium valproate treated rats. The mean score for control rats were 1 and they did not show any abnormal movements. The mean score for SV-200 was 1.6±0.24 and for SV-400 were 2. This indicated that sodium valproate at both the doses resulted in general slowness of displacement, resulting in hindlimb impairment. There was no incoordination or gait abnormalities in sodium valproate treated rats.

There were no abnormal chewing (oro-facial) movements in either of the sodium valproate treated groups. Tremors were observed in both SV-200 and SV-400 group, but it was more pronounced in SV-400 group.

Results of Catalepsy/Inclined Plane Test

This test demonstrates muscular rigidity or hypotonia in animals. The mean duration of time by which rats were imposed on inclined plane did not differ between controls and SV-200 or SV-400 groups. This indicatee that sodium valproate neither caused muscular rigidity nor hypotonia (Table.1).

Table 1. Catalepsy/Inclined Plane Test**.** The values are expressed as mean of duration of time (in seconds) by which the rats were imposed to be in inclined position ±SE. (P=0.3496).

Groups	Time spent (seconds)
Normal Control	149.33±13.77
Sodium valproate 200 mg/kg	105.8±31.86
Sodium valproate 400 mg/kg	143.4±16.65

Results of the T-Maze Test

There was no significant (P>0.05) difference in the mean number of alterations between control rats and sodium valproate treated ones (either 200mg/kg dose or 400 mg/kg dose). However, a marginally significant (P<0.05) increase in mean number of alteration was observed in rats treated with 400mg/kg dose compared to 200 mg/kg dose treated group (Fig.4).

The mean number of correct response showed a reduction (P<0.01) in SV-200mg/kg dose treated rats but not in SV-400 mg/kg dose treated rats (P=0.0002) when compared to the control group (Fig.4).

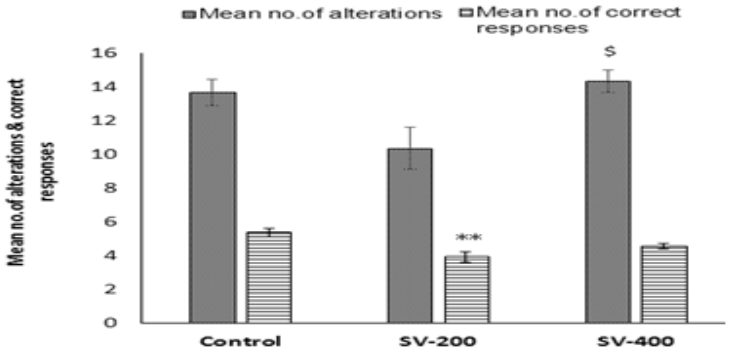

Figure 4. Mean number of alteration and mean number of correct response by rats in T-maze test. Values are expressed as mean ± SE. Comparison between controls vs. SV-200, ** = P<0.015 and comparison between SV-200 vs. SV-400, P=0.05, for mean No. of alteration, P=0.019, and for mean No. of correct response, P= 0.002, (n=6).

The mean percentage of bias data, showed that rats treated with SV-200mg/kg demonstrated less percentage bias (P<0.05) as compared to controls. However, SV-400mg/kg ones did not show such reduction in the percentage of bias (Fig.5).

The mean percentage of correct response was significantly (P<0.01) reduced in rats treated with SV-200 mg/kg dose compared to control rats. However, SV-400 mg/kg did not show any reduction (Fig.5).

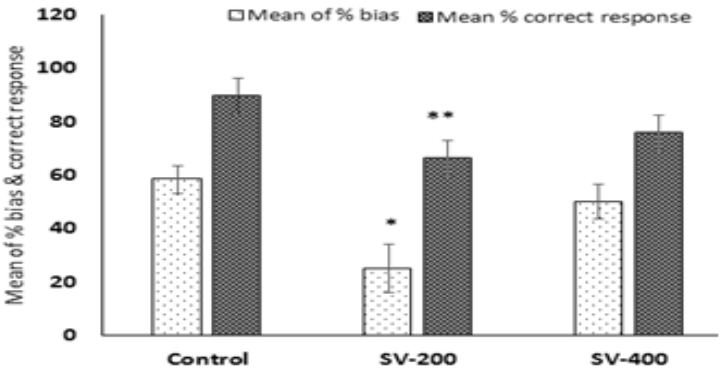

Figure 5. Mean of percentage bias and mean percentage of correct response by rats in T-maze test. Values are expressed as mean ± SE. Comparison between controls vs. SV-200, *=P<0.05, ** = P<0.015. For mean of % bias P= 0.0128 and for percentage of correct response P= 0.0031 (n=6).

DISCUSSION

The comparison of percentage of body weight gain after 90 days showed that SV at 200mg/kg dose resulted in maximum gain (92.18%), followed by SV at 400 mg/kg dose (82.72%) and controls (68.18%). SV is a frequently used antiepileptic drug used for different types of epilepsy. It has several side effects, including increase in body weight, metabolic and endocrine disorders and metabolic syndrome. A recent cross-sectional, study by Carmona et al. [18] in children receiving only SV for epileptic disorder, demonstrated that 17% of them developed obesity, 25% had metabolic syndrome and 6% of the children were significantly overweight [18]. The authors suggested that children treated with SV who became obese, might develop metabolic syndrome, hence careful monitoring is required. There are many such reports with respect to valproate-monotherapy-induced fat deposition and abnormal weight gain [19]. Possible reasons for the weight gain could be an increase in the consumption of calorie-rich foods and beverages as a result of a greater appetite and thirst, defective sympathetic nervous system activity, impaired beta-oxidation of fatty acids caused by

carnitine deficiency, ineffective leptin activity in spite of high leptin levels (leptin-resistance) or increased secretion of β cells causing hyperinsulinemia [20, 21].

SV treatment during the juvenile period did not affect locomotor and exploratory activities in adult rats. They also did not develop any anxiety like behaviour after SV treatment during adulthood. Our findings were consistent with a few earlier studies. Acute sodium valproate administration (320 mg/kg, intraperitoneally) decreased the frequency and the time spent in grooming, even though it did not modify locomotion, rearing or defecation, 15 and 60 min after acute treatment [22]. Prolonged (30 days) valproate treatment for 30 consecutive days with increasing doses from 40 to 320 mg/kg, did not modify rat behaviour in the open-field test, from the first to the fourteenth day of the test [22]. Acute (one injection), subacute (three injections) and chronic treatment with sodium valproate did not cause stimulation of motor activity in the open-field test, but resulted in reduced immobility time [23].

Concerning the adverse effects of SV on extrapyramidal system, both Parkinsonism syndromes and choreiform movements have been reported in adults. A study reported 10 cases of extrapyramidal disorders in elderly after various durations of treatment (from 6 months to 10 years) [24]. Apart from extrapyramidal disorders, cognitive dysfunction similar to dementia was also observed in six patients. However, the signs and symptoms improved some weeks or months after discontinuation of SV treatment. Armon et al. described various abnormal signs and symptoms related to cognitive and motor function impairment in patients with chronic SV treatment [25]. There are many such reports with respect to SV induced Parkinsonism related symptoms in adults, most often reversible [26 – 30]. Whether treatment with SV juvenile period causes Parkinsonism and cognitive dysfunction in adulthood has not been adequately studied. In the present paper, we did not observe any severe movement abnormality in SV treated rats. Although, general slowness of movements was evident in rats treated with both doses of SV, there were no incoordination or gait abnormalities. We did not observe any abnormal chewing (oro-facial) movements in either of the SV treated groups. Most of the reports regarding valproate induced Parkinsonism symptoms were related to adults. Our concern was the effects of long-term usage of SV in children and its consequences during adulthood. Except for tremors and poor movement coordination, we did not observe any severe form of extrapyramidal system dysfunction one month after termination of treatment in the present study.

The most common adverse effect of SV on the central nervous system is tremor [6]. This is also the most common side effect of valproate compared to other antiepileptic drugs [31]. In the present study tremors were observed in both SV-200 and SV-400 groups, but it was more pronounced in SV-400 ones. Valproate-induced tremor is said to appear within a month to 14 months after the start of therapy [28]. It is typically an intention tremor (while executing a movement or a posture), but can be present at rest as well. It has also been suggested that valproate can either induce tremor or unmask an already existing, clinically silent essential tremor. The severity of SV-induced tremor can range from minimal to severe and is usually dose dependent. In our study, tremors were evident in both doses of SV treatment; and we could not distinguish the severity between the two tested doses.

The movement disorder associated with SV treatment is attributed to changes in the basal ganglia motor circuit secondary to dopaminergic receptor blockade. Although the mechanism of SV-induced parkinsonism is not known, several biological theories have been proposed to explain this increased risk, including an effect on complex I activity in the electron transport chain of mitochondria [25] and on GABAergic pathways in the basal ganglia [32]. Apart from that, both oxidative stress and mitochondrial dysfunction have are also been attributed [27]. Functional neuroimaging in a few cases has shown that the striatonigral function appeared to be minimally affected. Easterford et al., [33] detected Parkinsonism in three out of 50 patients receiving chronic valproate therapy. In all three cases, beta-CITSPECT scans were normal, indicating that dopaminergic neuronal loss was not the causal mechanism for the disorder. Interestingly SV is known to exert neuroprotection by reversing the behavioural and neurochemical alterations observed in rats treated with 6-OHDA, a Parkinson model [34].

Another extrapyramidal dysfunction, the choreiform movements secondary to long-term valproate administration is less frequently reported [7]. Lancman et al., presented three patients who developed choreic movements after long-term treatment with SV [7]. These patients had severe brain damage and one had a unilateral vascular

lesion in the caudate nucleus. The choreiform movements involved the mouth, tongue, head, trunk and limbs bilaterally in two cases and contralaterally in the patient with the caudate lesion. Between the two major manifestations of extrapyramidal dysfunctions, Parkinsonism symptoms are associated with increased muscle tone (hypertonic), reduced movement (hypokinetic) while choreiform movements are associated with decreased muscle tone (hypotonic), and increased movement (hyperkinetic). In our study, we observed generalized poor movement in SV treated rats. Further to test the muscle tone and muscular rigidity, we performed catalepsy test. In this test, SV caused neither muscular rigidity nor hypotonia. Hence, the present study demonstrated that chronic treatment of SV in juvenile rats caused Parkinsonism like symptoms (tremors and slow movements) during adulthood even after one month of terminating administration.

Another adverse effect associated with long term SV treatment is cognitive impairment [24]. The Spontaneous Alternation Test suggested that treatment with SV in juvenile period did not affect learning abilities in adult rats. While, 400 mg/kg dose of SV showed marginal increases in mean number of alternations compared to 200 mg/kg dose, this could not be considered an evidence for enhanced learning ability, since the value did not differ from control counterparts. The percentage of bias was reduced in adult rats who received 200mg/kg of SV during juvenile period compared to controls. This might be considered as an index of improved learning ability. However, the mean number of correct responses decreased in rats treated with 200 mg/kg dose of SV, not in the 400mg/kg group. The mean percentage of correct response also reduced significantly in rats treated with 200 mg/kg SV but not in 400 mg/kg treated ones. Except for the reduction in percentage of bias by rats treated with of 200 mg/kg SV, no other parameter clearly demonstrated any improvement in learning ability. However, rats that received 200 mg/kg SV demonstrated a significant reduction in mean number of alternations, mean number of correct response and mean percentage of correct response. Hence, it is evident that chronic SV treatment (PND-15 to 60) (200 mg/kg dose) in juvenile rats affects their learning and memory abilities in adulthood (at PND 90). Interestingly the higher dose did not show such effect. In contrast to our findings, another experiment performed in 2002 reported that SV treatment from PND-4 to PND-10 impaired learning and memory abilities in Morris water maze when tested at PND-23 to PND-30. However, these changes were reversed when retested at PND-35 [35]. Our study demonstrated persistence of cognitive decline even in adulthood.

Despite the reported safety of chronic SV therapy, its adverse effects such as Parkinsonism symptoms or cognitive dysfunctions should be of concern in all young patients who are treated with SV for many years.

CONCLUSION

Treatment with SV during Juvenile period could cause generalized slow movement, tremors and cognitive dysfunction during adulthood. Even after discontinuation of the drug, persistence of cognitive impairment, tremors and generalized slow movement observed in this study warrants a close monitoring system in children who receive long-term sodium valproate treatment. Further studies in understanding the mechanisms associated with these adverse outcomes and to minimize such effects should be considered.

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REFERENCES

1. Fagundes, SBR. Valproic Acid: Review. Rev Neurocienc 2008; 16(2): 130-6.
2. Perruca E. Pharmacological and therapeutic properties of valproate: A summary after 35 years of clinical experience. CNS Drugs 2002; 16: 695-714.
3. King M. The new patient with a first seizure. Aust fam physician 2003;32(4):221-2.
4. Fariello RG, Varasi M, Smith MC. Valproic acid: Mechanism of action. Antiepileptic Drugs 1995; 4: 581-8.
5. Johanessen CU, Johanessen SI. Valproate: Past, Present, and Future. CNS Drug Rev 2006; 9(2): 199-216.
6. Hyuman NM, Dennis PD, Sinclair KGA. Tremor due to sodium valproate. Neurology 1979; 29: 1177-80.
7. Lancman ME, Asconape JJ, Penry JK. Choreiform movements associated with the use of valproate. Arch Neurol 1994; 51: 702-4.
8. Zadikoff C, Munhoz RP, Asante AN, Politzer N, Wennberg R, Carlen P, et al. Movement disorders in patients taking anticonvulsants. J Neurol Neurosurg Psychiatry 2007;78(2):147-51.
9. Khwaja GA, Ranjan R, Gupta M, Chowdhry D, Hirva M. Valproate-induced reversible

'Parkinsonism Plus' syndrome. JIACM 2010;11:235-8.
10. Ramadan E, Basselin M, Taha AY, Cheon Y, Chang L, Chen M, Rapoport SI. Chronic valproate treatment blocks D2-like receptor-mediated brain signaling via arachidonic acid in rats. Neuropharmacology 2011; 61(8):1256-64.
11. Kanemura H, Sano F, Maeda Y, Sugita K, Aihara M. Valproate sodium enhances body weight gain in patients with childhood epilepsy: A pathogenic mechanisms and open-label clinical trial of behavior therapy. Seizure 2012; 21(7):496-500.
12. Madhyastha S, Somayaji SN, Rao MS, Nalini K, Bairy KL. Hippocampal brain amines in methotrexate-induced learning and memory deficit. Can J Physiol Pharmacol 2002; 80: 1076-84.
13. Ludolph AC, Seelig M, Ludolph A, Novitt P, Allen CN, Spencer PS, et al. 3-Nitropropionic acid decreases cellular energy levels and causes neuronal degeneration in cortical explants. Neurodegeneration 1992; 1: 155-61.
14. Naidu PS, Singh A, Kulkarni SK. Quercetin, a bioflavonoid, attenuates haloperidol-induced orofacial dyskinesia. Neuropharmacology 2003; 44(8): 1100-6.
15. Gopala Krishna HN, Pemminati S, Dorababu P, Pai MR, Colaco N, Vineetha V. The effect of acute and chronic administration of the aqueous extract of Triphala on haloperidol induced catalepsy in mice. J Clin Diagn Res 2010; 3: 2134-8.
16. Ferre S, Guix T, Prat G, Jane F, Casas M. Is experimental catalepsy properly measured?. Pharmacol Biochem Behav 1990; 35(4): 753-7.
17. Rai KS, Murthy KD, Karantha KS, Rao MS. Clitoriaternatea (Linn) root extract treatment during growth spurt period enhances learning and memory in rats. Indian J Physiol Pharmacol 2001; 45(3): 305-13.
18. Carmona-Vazquez CR, Ruiz-Garcia M, Pena-Landin DM, Diaz-Garcia L, Greenawalt SR. The prevalence of obesity and metabolic syndrome in paediatric patients with epilepsy treated in monotherapy with valproic acid. Rev Neurol 2015; 61(5): 193-201.
19. Petty SJ, Kantor S, Lawrence KM, Berkovic SF, Collins M,Hill KD, et al.Weight and fat distribution in patients taking valproate: a valproate-discordant gender-matched twin and sibling pair study. Epilepsia 2014; 55(10): 1551-7.
20. Biton V. Effect of antiepileptic drugs on bodyweight: overview and clinical implications for the treatment of epilepsy. CNS Drugs 2003; 17: 781-91.
21. Luef GJ, Lechleitner M, Bauer G, Trinka E, Hengster P. Valproic acid modulates islet cell insulin secretion: a possible mechanism of weight gain in epilepsy patients. Epilepsy Res 2003; 55: 53-8.
22. Barros HM, Tannhauser SL, TannhauserMA,Tannhauser M. Effect of sodium valproate on the open-field behavior of rats. Braz J Med Biol Res 1992; 25(3): 281-7.
23. Teruel AF, Boix F, Escorihuela RM, Yanez P, Tobena A. Sodium valproate reduces immobility in the behavioral 'despair' test in rats. Eur J Pharmacol 1988; 152(1-2): 1-7.
24. Masmoudi K, Gras-Champel V, Masson H, Andrejak M. Parkinsonism and/or cognitive impairment with valproic acid therapy: a report of ten cases. Pharmacopsychiatry 2006; 39(1): 9-12.
25. Armon C, Shin C, Miller P, Carwile S, Brown E, Edinger JD, et al. Reversible Parkinsonism and cognitive impairment with chronic valproate use. Neurology 1996; 47: 626-35.
26. Schreur L, Middeljans-Tijssen C, Hengstman G, Olde RM. Cognitive impairment and parkinsonism due to use of sodium valproate. Tijdschr Gerontol Geriatr 2009;40(1):29-33.
27. Jamora D, Lim SH, Pan A, Tan L, Tan EK. Valproate-induced Parkinsonism in epilepsy patients. Mov Disord 2007: 22: 130-3.
28. Onofrj M, Thomas A, Paci C. Reversible Parkinsonism induced by prolonged treatment with valproate. J Neurol 1998; 245 (12): 794-6.
29. Sasso E, Delsoldato S, Negrotti A, Mancia D. Reversible valproate-induced extrapyramidal disorders. Epilepsia 1994; 35: 391-3.
30. Alvarez-Gomez MJ, Vaamonde J, Narbona J, Barao M, Barona P, Branna T, et al. Parkinsonism syndrome in childhood after sodium valproate administration. Neuropharmacol 1993; 16: 451-5.
31. Karas BJ, Wilder BJ, Hammond EJ, Bauman AW. Valproate tremors. Neurology 1982; 32: 428-32.
32. Pineda-Trujillo N, Carvajal-Carmona LG, Buritica O, Moreno S, Uribe C, Pineda D, et al. A novel Cys212Tyr founder mutation in parkin and allelic heterogeneity of juvenile Parkinsonism in a population from North West Colombia. Neurosci Lett 2001; 298(2): 87-90.
33. Easterford K, Clough P, Kellett M, Fallon K, Duncan S. Reversible Parkinsonism with normal β-CIT–SPECT in patients exposed to sodium valproate. Neurology 2004; 62: 1435-7.
34. Ximenes JCM, Neves KRT, Leal LKA, do Carmo MRS, Brito GAdC, Naffah-Mazzacoratti MdG, et al. Valproic acid neuroprotection in the 6-OHDA model of Parkinson's disease is possibly related to its anti-inflammatory and HDAC inhibitory properties. J Neurodegener Dis 2015;2015:1-13.
35. Filgueiras CC, Pohl-Guimaraes F, Krahe TE, Medina AE. Sodium valproate exposure during the brain growth spurt transiently impairs spatial learning in prepubertal rats. Pharmacol Biochem Behav 2013; 103(3): 684-91.