

ORIGINAL ARTICLE

# Evaluation of Chronic and Acute Effects of Gabapentin on Passive Avoidance Learning Process in Mice

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## KEYWORDS

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**ABSTRACT:** Gabapentin (GBP) is one of the new antiepileptic drugs (AEDs) applied extensively in neurology and psychiatry. The advantage of new AEDs includes newer mechanism of action, broad spectrum of anti-seizure effects, lesser drug interactions and fewer side effects. GBP is a cyclized analogue of GABA but it does not interact with GABA receptors, nor does it inhibit GABA uptake or prevent the degeneration of GABA. Restricted studies have been performed on acute and chronic effects of GBP on passive avoidance (PA) learning and little is known about its chronic phase. Therefore, the aim of the present study was to evaluate acute and chronic effects of GBP on passive avoidance learning in 100 mice (w=30 gr). Ten mg/kg GBP were injected interaperitoneally for assessment of memory in three steps (acquisition, consolidation and retrieval). Shuttle box trial was used for PA task assessment. Retrieval memory was tested 24h after injection, and the results indicated increased Step Through Latency (STL), showing the enhancement of memory. Moreover, in acute phase of PA, GBP enhanced acquisition and retrieval of memory. In chronic phase of PA, GBP showed no effect on memory. The present study suggests that GBP exerted no destructive effects on cognition; however, it improved emotional cognitive performance in mice in PA tasks.

## INTRODUCTION

Seizure is one of the most common neurological disorders and its control and treatment is important in terms

of medical and social complications. Although a variety of factors contribute to cognitive deficits in patients with epilepsy, the adverse effects of antiepileptic drugs (AEDs) on cognitive function are important. The older AEDs are known to produce untoward cognitive effects which are clinically significant in some patients [1].

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Older AED namely carbamazepine (CBZ) have been shown to influence acquisition of recent memory, thus one would suspect that they might also affect practice effects [2, 3]. Several newer AEDs are well-tolerated in patients and demonstrate few adverse cognitive effects, compared with placebo and older AED [4]. Therefore, a selection of appropriate antiepileptic drug seems logical. In clinical trials, GBP has been generally well-tolerated without significant complication [5, 6].  $\gamma$ -aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system. The termination of GABA transmission is through the action of a family of membrane proteins, called GABA transporters (GAT1–4). GABA system is involved in the modulation of memory [7]; however, GBP does not act on the same brain receptors of GABA. Gabapentin from newer antiepileptic drug was initially synthesized chemical structure of the neurotransmitter gamma-aminobutyric acid (GABA), widely used to treat epilepsy, neuropathic pain, migraine, tremor, phobia, mania and refractory partial seizures [8, 9 and 10].

Cognitive effects of GBP and other AEDs have been compared in a number of clinical studies. GBP as add-on treatment has been shown to have favorable effects on cognition in clinical studies in humans. In a double-blind, add-on, crossover study of patients with refractory partial seizures, GBP had no negative side effects on cognition, except for an increase in drowsiness at 2400 mg/day, but in smaller doses (600, 1200mg/day) had no adverse effects [11]. In a small randomized double blind placebo controlled crossover study wherein healthy volunteers were treated by single low dosages (50-400 mg) of GBP, EEG findings showed a subtle positive psychotropic effects (e.g., improved concentration and attention) [12]. Elsewhere, a double-blind randomized crossover study comparing GBP with carbamazepine (CBZ) in healthy adults with 5 weeks treatment showed a statistically better performance for GBP, compared to CBZ [13]. In contrast, in a randomized double-blind parallel

study in healthy volunteers, administration of CBZ and GBP during 12 weeks was compared using quantitated EEG and a cognitive battery and no significant differences were found between CBZ and GBP treatments [14]. In a double-blind randomized placebo-controlled parallel study in healthy volunteers, the effects of topiramate on cognitive abilities were compared with GBP and the results showed that GBP was significantly effective than topiramate [15]. Gabapentin acutely harms neuronal pathway via adjustment of mechanism of neuronal cognitive pathway in different steps of learning tasks [16].

Several studies using GBP as add-on therapy in patients with epilepsy reported subjective improvements in well-being [17]. There are few systematic data on the effects of GBP on specific cognitive domains. Despite the fact that primary results are promising with GBP for its effect on memory storage (acquisition, consolidation and retrieval) the effects of GBP still remains to be explored emotional learning and memory functions in mice.

The aim of this study was to investigate the acute and chronic effects of GBP on cognitive performance in passive avoidance (PA) tasks in naive mice.

## MATERIALS AND METHODS

One hundred male mice weighing 25-30 g were housed five per home cages in an animal colony facility. The animals were maintained in constant room temperature ( $22\pm 2$  °C) under a 12-h light/dark cycle (light onset at 07:00 h) all animals were naive to tests. Each mouse was tested individually. Experiments were conducted between 10:00 and 14:00 h. The animals were randomly assigned to different case and control groups.

GBP was purchased from Sigma Chemical Company and dissolved in saline GBP freshly prepared and administered intraperitoneally (10 mg/kg). Control groups received the same volume of saline.

Animals were trained in a one-trial, step-through, PA apparatus for evaluating memory based on fear conditioning and instrumental learning [18]. Decrease in retention latency indicates impairment in memory in the PA task and increase in retention latency indicates improvement in memory. Shuttle box is a common apparatus for the evaluation of memory used by multiple investigations [19]. The apparatus contains a box with an illuminated part (L 19×10×h 16 cm) and a dark part (L 21×10×h 16 cm), both equipped with a grid floor composed of steel bars spaced 0.5 cm apart. The inhibitory avoidance task consisted of two trials. On the first day of training, mice were placed individually into the light compartment and allowed to explore the boxes. The inter compartment door was opened after a 60-s acclimation period. During the training process the animals received electrical shock (0.5 MA) for 3 s. In the acquisition trial, each mouse was placed in the illuminated compartment lighted by a bright bulb. The animals received drugs 30 min prior to acquisition training. The retention trial started 24 h subsequent to the end of the acquisition trial. In consolidation trial immediately after training the animal received drug and thereafter retention trial 24 h started after the end of trial. In retrieval trial 24 h after training and 30 min before retention the animal received the drug. Each mouse was placed in the illuminated compartment as in the training trial. The door was opened after a 30-s acclimation period. The STL in the retention trial (with a maximum 360 s cutoff time) was used as the index of retention of the learned experience. Shock was not applied at the retention trial. For evaluation of chronic phase of PA learning, the animals were treated by GBP 10 mg/kg/day for 10 day for each trial same as above.

One-way analysis of variance (ANOVA) and post-hoc Tukey test were used for analyses of PA tests data. Data are expressed as the mean values  $\pm$ SEM and  $P < 0.05$  was considered as statistically significant.

### **Test 1**

Sixty male mice were randomly divided into 6 groups as follows:

- 1- Control group with saline injection performed immediately after acquisition (n=10)
- 2- Treatment group with GBP 10 mg/kg immediately after acquisition (n=10)
- 3- Control group with saline injection performed 30 min before acquisition (n=10).
- 4- Treatment group with GBP 10 mg/kg 30 min before acquisition (n=10)
- 5- Control group with saline injection performed 30 min before retrieval (n=10)
- 6- Treatment group with GBP 10 mg/kg 30 min before retrieval (n=10)

### **Test 2**

Forty male mice were randomly divided into 4 groups as follows:

- 1- Control group acquisition test was performed after 10 daily dose saline injection (n=10)
- 2- Treatment group acquisition test was performed after 10 daily dose GBP 10 mg/kg/d injection (n=10)
- 3- Control group acquisition test was performed before 10 daily dose saline injection (n=10)
- 4- Treatment group acquisition test was performed before 10 daily dose GBP10 mg/kg/d injection (n=10)

## **RESULTS**

GBP (10 mg/kg i.p.) increased STL in acquisition and retrieval of acute PA learning model compared to control group (ANOVA posthoc Tukey n=10  $P=0.023$  & n=10  $P=0.001$ ).

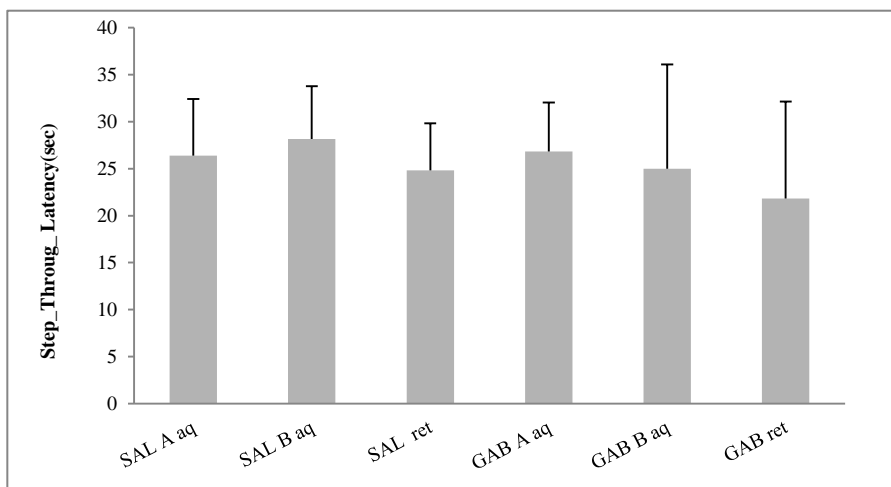
GBP (10 mg/kg i.p.) administration showed no effect on consolidation step of acute PA learning model compared to control group. (ANOVA posthoc Tukey n=10 &  $P=0.99$ ).

Figure 1a shows the effect of GBP (10 mg/kg, i.p.) on the first day latency (STL) of mice in PA task. GBP

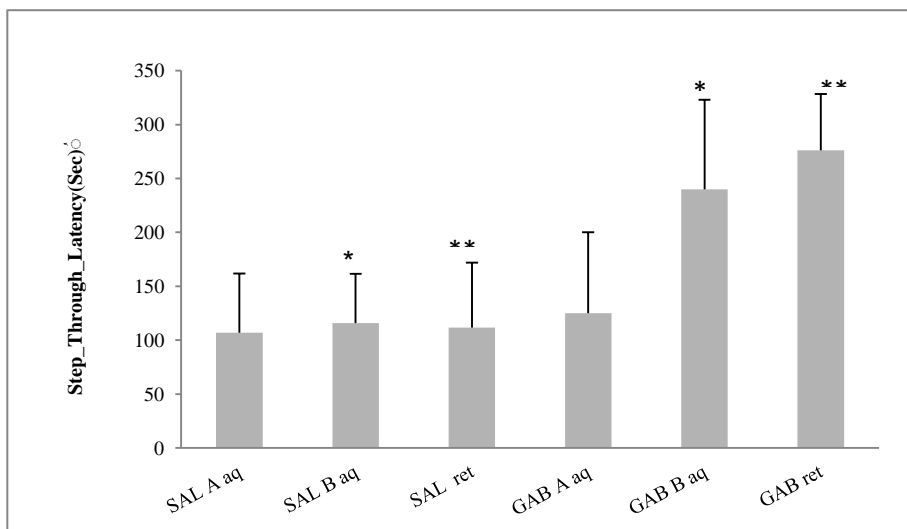
showed no effect on first day latency in training session of PA task (n = 10, P = 0.45).

Figure 1b shows the effect of GBP (10 mg/kg, i.p.) on the retention latency during retention test of PA task.

GBP (10 mg/kg) significantly increased the retention latency (sec) on 2nd day, compared to controls. (n=10 F (2, 27) = 29, 716, P< 0.0001 ANOVA followed by post-hoc Tukey test).



**Figure 1a.** Effect of GBP (10mg/kg i.p.) in three step of memory compared with control group in acute phase of passive avoidance learning (PA) showed in STL. Figure 1a shows the effect of GBP (10 mg/kg, i.p.) on the first day latency (STL) of mice.



**Figure 1b.** The effect of GBP (10 mg/kg i.p.) on the retention latency during retention of PA task\*significantly decreased STL in acquisition compared with control\*\* significantly decreased STL in retrieval compared with control

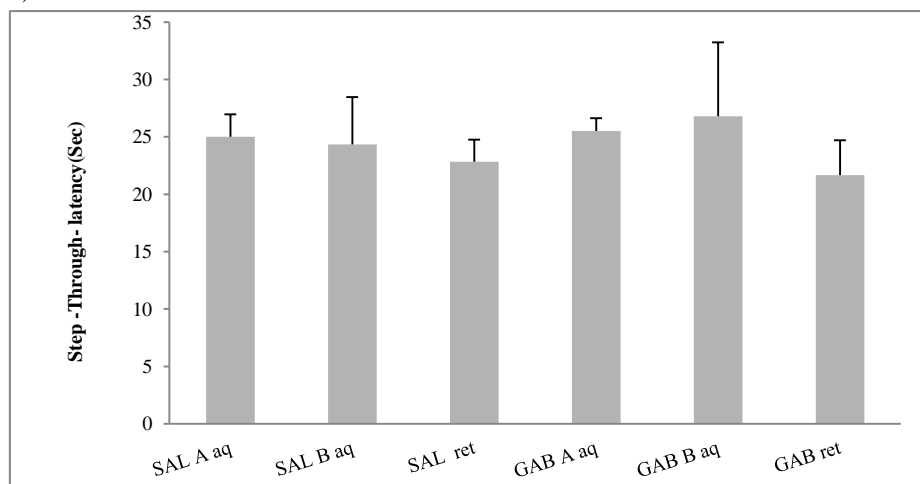
GBP (10 mg/kg i.p.) administration exerted no effect on acquisition step of chronic passive avoidance learning model compared to control group (ANOVA post hoc Tukey  $n=10$   $P=0.99$ ).

GBP (10 mg/kg i.p.) administration had no effect on consolidation step of chronic PA learning model, compared to the control group (ANOVA post hoc Tukey  $n=10$   $P=0.97$ ).

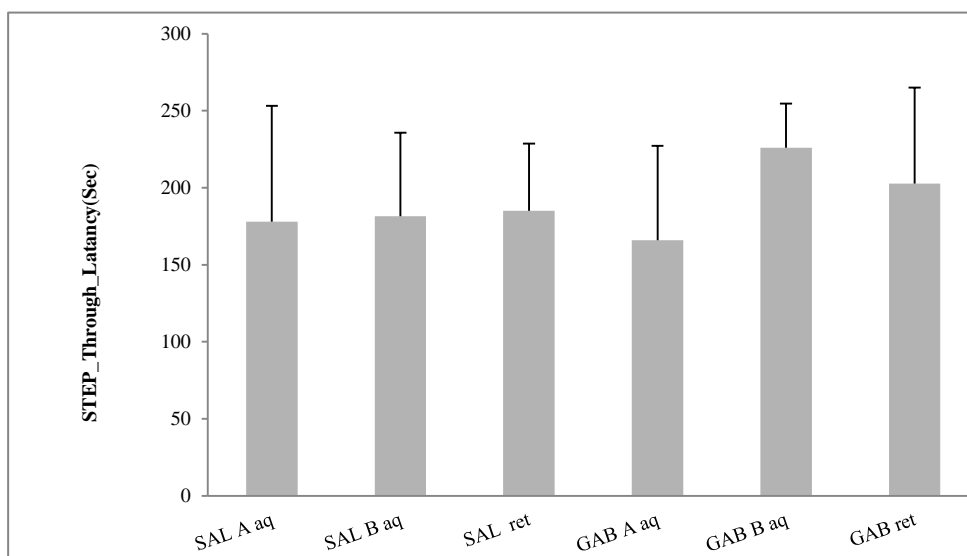
GBP (10 mg/kg i.p.) administration did not affect retrieval step of chronic passive avoidance learning model, compared to the control group (ANOVA post hoc Tukey  $n=10$   $P=0.91$ ).

Figure 2a shows the effect of GBP (10 mg/kg, IP) on the first day latency (STL) of mice in PA task. GBP showed no effect on the first day latency on training session of PA task ( $n = 10$ ,  $P = 0.45$ ).

Figure 2b shows the effect of GBP (10 mg/kg, i.p.) on the retention latency during retention test of PA task after 10 mg/kg daily for 10 day injection. GBP (10 mg/kg) no significantly increase the retention latency (sec) on 2nd day, compared to controls ( $n=10$ ,  $F(2, 27) = 0.81$   $P = 0.99$ , ANOVA followed by post-hoc Tukey test).



**Figure 2a.** Effect of GBP (10mg/kg i.p.) in three step of memory compared with control group in chronic phase of passive avoidance learning (PA) showed in STL. Figure 2a shows the effect of GBP (10 mg/kg, i.p.) on the first day latency (STL) of mice in PA task.



**Figure 2b.** The effects of GBP (10 mg/kg/d, i.p.) on retention latency during retention test of PA task after 10 mg/kg daily for 10 day injection

## DISCUSSION

The present study assessed GBP effects on acute and chronic PA learning in non-epileptic healthy mice. Our results showed that acute and intraperitoneal administrations of GBP (10 mg/kg) positively affect and facilitate acquisition and retrieval emotional learning-memory function in PA test in naive mice. In chronic phase, intraperitoneal administration of GBP (10 mg/kg) exerted no destructive effects on cognition. PA learning was studied in a step-through type task utilizing the natural preference of mice in a dark environment. Hippocampal and amygdale play basic roles in instrumental learning dependent test such as PA tasks [20]. In this test, the animals learn to avoid an inescapable electrical shock, and longer retention latencies indicate a better learned experience. In this study, GBP (10 mg/kg) enhanced learning and memory performance in PA test in mice. Our results in acute phase of effects of GBP on memory were in accordance with those of Celiyuret et al. [21]. The authors demonstrated that memory was enhanced in acquisition phase while our results showed that acquisition and retrieval phases were enhanced [21]. Our results can also be compared with those of Acosta et al. [22] in which GBP with unknown mechanism facilitated acquisition of learning but exerted no effects on retrieval step. In a study, impairment of retention performance in PA task in mice induced by repeated administration of GBP doses affected memory retrieval; however, no influence was shown on memory consolidation [23]. This impairment may be attributable to a reduction in central cholinergic activity [23]. Our results showed that GBP in chronic model PA task had no destructive effects on cognition. In another study, GBP in chronic model of PA task reiterated memory [24]. Our results were consistent with those of Blake et al. [25], which showed maintenance of stable GBP plasma levels protecting against seizures without causing memory impairment. Elsewhere, GBP enhanced retention performance in

CF-1 mice using an inhibitory avoidance task and such an effect was prevented by atropine [24]. It was also shown that neither methylatropine nor mecamlamine, or hexamethonium prevented the effects of post training GBP on retention performance. This effect was not influenced by neostigmine either; suggesting that central muscarinic cholinergic mechanism is possibly involved in memory consolidation [24]. GBP is an analogue of GABA but it does not interact with GABA receptors, nor does it inhibit GABA uptake or prevent the degradation of GABA [26]. However, in vivo GBP increases the GABA accumulation in rat brains [27]. The exact mechanism of GBP action is still unknown, but its therapeutic action on neuropathic pain is thought to involve voltage-gated N-type calcium ion channels [13]. GBP treatment interferes metabolism or concentration of some neurotransmitters such as glutamate, glycine or GABA in brain tissues [28].

Cognitive function is influenced by multiple factors such as underlying pathology of seizure, type and effects of AED. In the treatment of epilepsy, the physician's goal is the control of seizures without side effects or with minimal side effects of drugs. The efficacy of AED should be considered for selection of drugs for patients. There are concerns regarding cognitive and behavioral toxicity of AED because most patients remain on therapy for years to decades.

## CONCLUSION

GBP acutely enhances acquisition and retrieval PA learning in naive mice and had no destructive effects on its consolidation step. However, in chronic model of PA task, it exerted neither destructive nor constructive effects on all three steps of PA learning. Further studies should be performed to investigate the effects of GBP treatment on memory. Comparison of GBP effects on memory with those of at least one conventional

antiepileptic drug in the same task both in naive animals and in epileptic models would be of value.

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