

Research Article

Effect of GABA_B Receptor Antagonist (CGP35348) on Learning and Memory in Albino Mice

Quratulane Gillani,¹ Shahid Iqbal,¹ Fatima Arfa,¹ Saba Khakwani,¹ Atif Akbar,² Asmat Ullah,³ Muhammad Ali,⁴ and Furhan Iqbal¹

¹ Zoology Division, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan

² Department of Statistics, Bahauddin Zakariya University, Multan 60800, Pakistan

³ Department of Zoology, Quaid-I-Azam Campus, Punjab University, Lahore 54000, Pakistan

⁴ Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University, Multan 60800, Pakistan

Correspondence should be addressed to Furhan Iqbal; furhan.iqbal@bzu.edu.pk

Received 26 August 2013; Accepted 27 October 2013; Published 19 January 2014

Academic Editors: J. Kanwal, Ü. Tan, and Y. Yoshiyama

Copyright © 2014 Quratulane Gillani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The present study was designed to demonstrate the potential effect of CGP 35348 (GABA_B receptor antagonist) on the learning, memory formation, and neuromuscular coordination in albino mouse. Mice were intraperitoneally injected with 1mg CGP 35348/mL of distilled water/Kg body weight, while the control animals were injected with equal volume of saline solution. A battery of neurological tests was applied following the intraperitoneal injections. Results of rota rod indicated that CGP 35348 had no effect on neuromuscular coordination in both male ($P = 0.528$) and female ($P = 0.125$) albino mice. CGP 35348 treated females demonstrated poor exploratory behavior during open field for several parameters (time mobile ($P = 0.04$), time immobile ($P = 0.04$), rotations ($P = 0.04$), and anticlockwise rotations ($P = 0.038$)). The results for Morris water maze (MWM) retention phase indicated that CGP 35348 treated male mice took shorter latency to reach the hidden platform ($P = 0.04$) than control indicating improved memory. This observation was complemented by the swim strategies used by mice during training days in MWM as CGP 35348 treated males used more direct and focal approach to reach the platform as the training proceeded.

1. Introduction

Glutamate and gamma-aminobutyric acid (GABA) are among the most abundant neurotransmitters in our central nervous system. Glutamate play role in excitatory responses while the GABA act as inhibitory stimuli [1]. Approximately 30% of neurons in the brain produce GABA and almost every neuron can respond to GABA [2].

Two pharmacologically and molecularly distinct GABA receptors have been identified, GABA_A and GABA_B. GABA_B receptors are heterodimeric G protein-coupled sites, located both pre- and postsynaptically [3, 4]. GABA_B receptors are widely used in the treatment of neurologic and psychiatric disorders including absence seizures and gamma-hydroxybutyrate toxicity and more recently used for the treatment of autoimmune limbic encephalitis [5]. Activation of GABA_B receptors produces anesthetic effects in animals with

neuropathy and chronic inflammation [6]. GABA_B antagonists have antidepressant activity [1], cognition improvement [7–9], through inhibition of memory suppressor mechanisms [10, 11], and beneficial effects in rat models of absence epilepsy [12]. 3-Aminopropyl diethoxymethylphosphinic acid (CGP35348) is GABA mimetic containing a phosphinic acid moiety which is a centrally active blocker of GABA_B receptors. CGP35348 was the first GABA_B antagonist discovered which is able to cross blood brain barrier and is the most active GABA_B receptor antagonist *in vivo* [13]. The interaction of CGP35348 with other receptors appears to be negligible making it a selective GABA_B receptor antagonist [14]. Present study was designed to suppress the GABA_B receptor mediated activity by using CGP 35348 (GABA_B receptor antagonist) and to observe its potential effect on the learning, memory formation, and neuromuscular coordination in albino mouse.

2. Material and Method

2.1. Subjects. Eight-week old albino mice ($N = 40$, male and female 20 each) were used during these experiments. Mice were maintained in cages filled with wood chips at the core animal facility of Bahauddin Zakariya University, Multan. Mice were fed on standard rodent diet and water ad libitum and housed in individual cages. Room temperature was maintained at $22 \pm 1^\circ\text{C}$.

2.2. Chemical. On the 20th day of life, mice were separated from their parents and fed on normal mouse diet until 9th week of life when they received intraperitoneal injections of GABA_B receptor antagonist (CGP35348, 3-Aminopropyl diethoxymethyl phosphinic acid) or saline solution for 5 days at the rate of 1 mg/1 mL solvent/Kg body weight/day 30 minutes prior to behavioural testing.

2.3. Rota Rod. The rota rod apparatus test balance and coordination and comprised of a rotating drum which rotated at the speed of 40 rpm. The time at which each animal fell from the drum was recorded. Each animal received three pretraining trials. Subsequently, each mouse completed three more consecutive trials and the longest time on the drum was used for analysis [15].

2.4. Open Field (OF). Mice were observed using a video monitoring system consisting of a video camcorder coupled to computational tracking system (Any-Maze, USA). Standard parameters for locomotor activity (i.e., total distance covered, average speed, amount of large movement, amount of local movement, resting time, and frequency of spontaneous change in direction) and exploratory behaviour (i.e., rearing, crossing the center, and time spent in the margin) recorded [16].

2.5. Morris Water Maze (MWM). MWM consists of a circular pool (122 cm diameter, 76 cm deep) in which mice were trained to escape from water by swimming to a hidden platform (1.5 cm beneath water surface) whose location could be identified using distal extra-maze cues attached to the room walls. Visual cues had different colors and dimensions and kept constant during the whole experiment.

The pool was divided into four quadrants (compass locations, NE, NW, SW, and SE) by a computerised tracking/image analyser system coupled to computational tracking system. The platform was placed in the middle of the NE quadrant and remained at the same position during the whole experiment.

The spatial acquisition phase consisted of 16 training trials and 4 training trials per day for 4 days with an intertrial interval of 15 min. Mice were released randomly with their heads facing the pool wall from the four compass locations, and allowed to swim and search for the platform for 120 s. When mice did not locate the platform within 120 s, animals were manually placed on the platform and allowed to remain on it for another 30 s.

On day 5, after the acquisition phase, subject received a probe trial, in which the platform was removed. Mice were

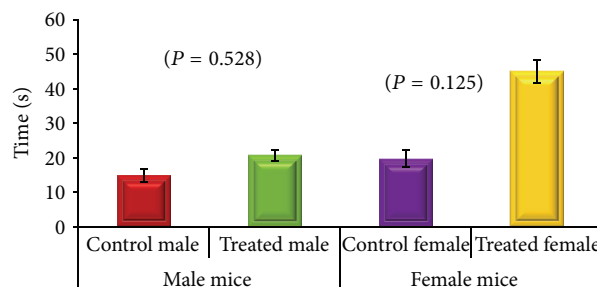


FIGURE 1: Comparison of rota rod test results control male/female with their respective GABA_B receptor antagonist (CGP 35348) treated male and female albino mice. Data is expressed as mean \pm standard deviation. P values indicate the results of two-sample t -test.

released from the south start point and were allowed to swim freely for 60 s. Parameters like time and path length to reach the platform area, number of times crossing the platform area, swimming strategies to reach platform area and swimming speed were recorded [17].

2.6. Statistical Analysis. All data are expressed as mean \pm standard deviation. For all the studied parameters of open field, rota rod MWM training, and probe trial parameters, two-sample t -test was applied to compare the results between treated male and females and their respected controls.

3. Results

3.1. Rota Rod. Results indicated that the CGP 35348 injection did not affect the neuromuscular coordination in male albino mice ($P = 0.528$), but female mice treated with GABA_B receptor antagonist performed better on rotating rod than saline treated female albino mice, and the difference in performance did not reach the statistical significance ($P = 0.125$) (Figure 1).

3.2. Open Field. There were interesting gender specific results regarding the open field test as all the studied parameters remained insignificantly different when compared between CGP 35348 and saline treated male mice (data not shown here). On the other hand, CGP 35348 treated females had demonstrated poor exploratory behavior as compared to saline treated females for several parameters (time mobile ($P = 0.04$), time immobile ($P = 0.04$), rotations ($P = 0.04$), anticlockwise rotations ($P = 0.038$)) (Table 1).

3.3. Morris Water Maze (MWM). Acquisition phase of MWM revealed gender specific results. For male albino mouse, total distance travelled ($P = 0.03$) and total time mobile ($P = 0.02$) were significantly different between CGP 35348 and saline treated albino mice (Figures 2 and 3) with saline treated male mice remained active for longer time and covered more distance in MWM than the GABA_B receptor antagonist treated male albino mice. For female mice, mean speed was the only parameter which significantly varied ($P = 0.003$) between saline and CGP 35348 treated females during training day 2 with control females swimming with more

TABLE 1: Comparison of the studied open field parameters between saline and CGP 35348 treated female albino mice. *P* values indicate the results of two-sample *t*-test.

Parameters	Control female (<i>N</i> = 5)	CGP 35348 treated female (<i>N</i> = 5)	<i>P</i> value
Distance (m)	21.4 ± 5.3	11.9 ± 8.2	0.072
Mean speed (m/sec)	0.04 ± 0.01	0.02 ± 0.01	0.071
Time mobile (sec)	492 ± 26.2	296.8 ± 148.4	0.044*
Time immobile (sec)	108 ± 26.2	303.3 ± 148.4	0.044*
Mobile episodes	29.4 ± 8.0	29.6 ± 14.1	0.979
Immobile episodes	29.4 ± 7.8	29.2 ± 14.3	0.979
Max speed (m/sec)	0.3 ± 0.2	0.2 ± 0.1	0.158
Rotations	25.6 ± 6.8	13.6 ± 8.3	0.041*
Clockwise rotations	9.6 ± 6.9	6.4 ± 3.4	0.397
Anticlockwise rotation	16.0 ± 1.1	7.2 ± 5.3	0.038*

P > 0.05 = nonsignificant; *P* ≤ 0.05 = least significant*.

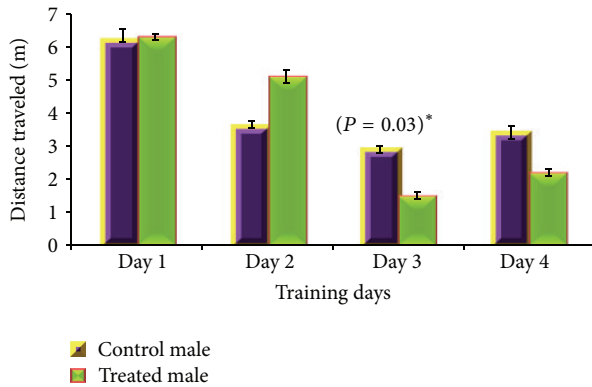


FIGURE 2: Comparison of total distance (m) travelled by GABA_B antagonist and saline treated male albino mouse during acquisition phase of Morris water maze test. Data is expressed as mean ± standard deviation. *P* value indicates the result of 2-sample *t*-test for the specific training day.

speed than treated ones (Figure 4), while all other studied parameters, for both male and female, remained insignificantly different between control and treated animals.

During probe trial, CGP 35348 treated male mice performed significantly better. They took shorter latency (*P* = 0.04) and remained less mobile (*P* = 0.04) than saline treated male as they found the hidden platform earlier than control animals (Figures 5 and 6), while none of the parameters studied in female mice reached statistical significance (data not shown here).

Results of swimming strategies during training days of MWM indicated that both CGP 35348 treated female and male albino mice had demonstrated improved memory formation as the direct and focal approach to the hidden platform increased, while random swimming and wall hugging decreased over the training period. Effect was more pronounced in treated male as the chaining and wall hugging strategies eliminated as the training proceeded indicating improved memory formation complementing the results of probe trial (Figures 7 and 8).

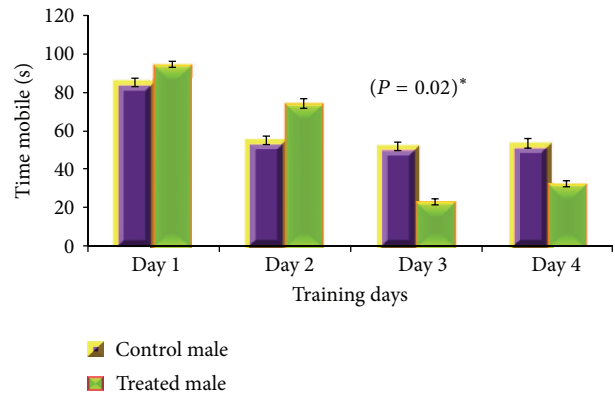


FIGURE 3: Comparison of time mobile (sec) by GABA_B antagonist and saline treated male albino mouse during acquisition phase of Morris water maze test. Data is expressed as mean ± standard deviation. *P* value indicates the result of 2-sample *t*-test for the specific training day.

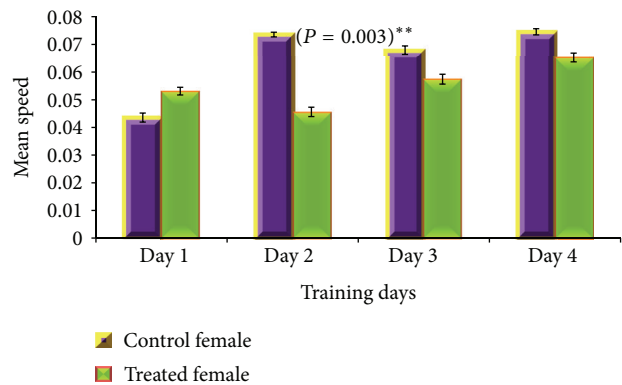


FIGURE 4: Comparison of mean speed by GABA_B antagonist and saline treated female albino mouse during acquisition phase of Morris Water Maze test. Data is expressed as mean ± standard deviation. *P* value indicates the result of 2-sample *t*-test for the specific training day.

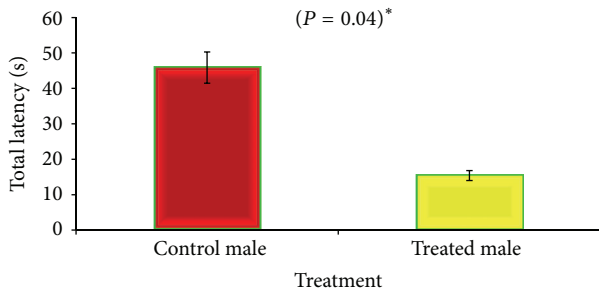


FIGURE 5: Comparison of total latency between GABA_B antagonist and saline treated male albino mouse during probe trial of Morris Water Maze test. Data is expressed as mean ± standard deviation. *P* value indicates the result of 2-sample *t*-test.

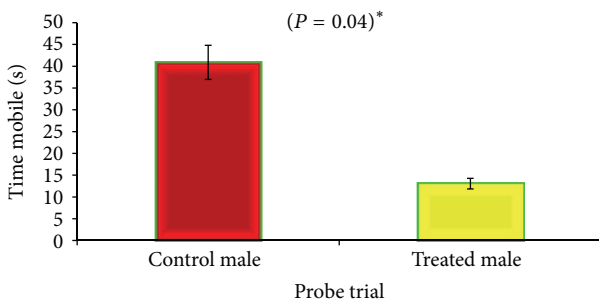


FIGURE 6: Comparison of time mobile between GABA_B antagonist and saline treated male albino mouse during probe trial of Morris Water Maze test. *P* value indicates the result of 2-sample *t*-test.

4. Discussion

In the central nervous system, GABA_B receptor regulates cyclic AMP (cAMP) levels through adenylyl cyclase activity. Activation of the cAMP regulatory pathway is vital for long-term memory formation across a variety of species [18, 19]. Recent studies indicated that metabotropic GABA_B receptors in the hippocampus are directly coupled to CREB-2 transcription factors which appear to serve as memory suppressors [20–22]. Removal of the repressive action of CREB-2 is thought to be mediated by protein kinase A (PKA, an intracellular second-messenger involved in long-term memory and short-term memory formation) and another protein, MAPK (catalytic subunit of PKA called mitogen-activated protein kinase) [10]. Present study was designed to suppress the GABA_B receptor mediated activity by using CGP 35348 (GABA_B receptor antagonist) and to observe its potential effect on the learning, memory formation, and neuromuscular coordination in albino mouse.

The rota rod test is widely used to determine the motor coordination in rodents [23]. It provides diverse measurable, continuous variables (time length) used for statistical purposes to appraise the effects of different conditions, procedures, and drug's effects [24]. Our results indicated no significant effect GABA_B receptor antagonist (CGP35348) in both male and female mice when compared with untreated

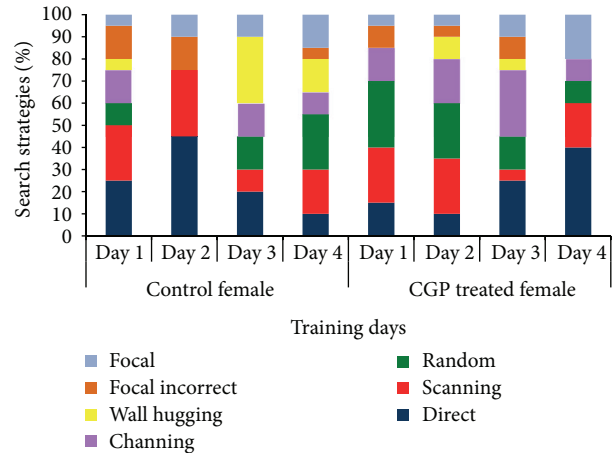


FIGURE 7: Swimming strategies of control and CGP 35348 treated female albino mouse during acquisition phase of Morris Water Maze test.

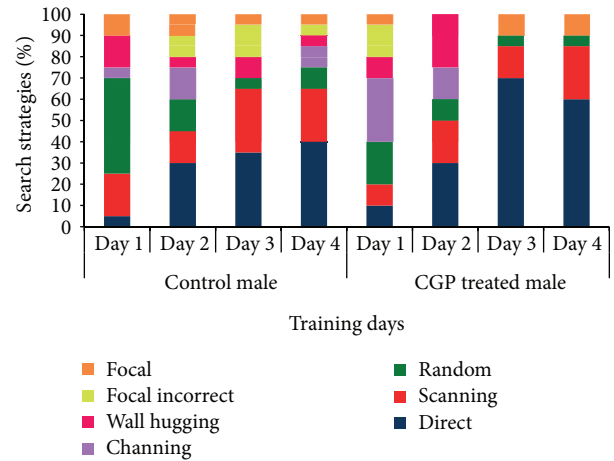


FIGURE 8: Swimming strategies of control and CGP 35348 treated male albino mouse during acquisition phase of Morris Water Maze test.

controls indicating that it has no influence on neuro-muscular coordination. This observation is in agreement with [14] which has reported that GABA_B receptor antagonist CGP 35348 is unable to evoke measurable effects on motor performance during the rota rod experiments.

While analyzing the exploratory behavior in mice through open field test, various parameters in the open field test were considered. Our results indicated gender specific effect of CGP 35348 injections as treated females had demonstrated poor exploratory behavior as compared to saline injected females for several parameters (time mobile (*P* = 0.04), time immobile (*P* = 0.04), rotations (*P* = 0.04), and anticlockwise rotations (*P* = 0.038)) (Table 1), while there was no effect of CGP 35348 supplementation in male albino mice (data not shown here). These results are in agreement with the findings of [25, 26] which had reported that GABA_B receptor antagonist had very low or no effect on the fundamental mouse behaviors.

In order to test hippocampal-dependent learning, including acquisition of spatial memory and learning for albino mouse, the Morris water maze (MWM) was applied. The CGP 35348 treatment led to the improvement of learning during the acquisition phase and significantly improved memory formation during probe trail in male albino mouse as they found the platform earlier and had shorter latency as compared to the saline treated male mice (Figures 5 and 6). These results are in agreement with those of [9] who had reported that GABA_B receptor antagonist CGP 35348 is capable of improving learning and memory in test of cognitive functions and formation of LTP in mice.

Various doses of CGP 35348 were applied [27] by pressure ejection to one of two recording sites in area CA1 of hippocampal slices in order to observe its effect on long term potentiation (LTP) and reported that memory was enhanced at intermediate dosages but not at very low and high concentrations. Similar observations were reported in rats as they had observed that very low and high doses did not help in memory retention [27, 28]. The dose applied in present study (1mg/mL of solvent/Kg body weight) is probably too low as only selective parameters of various studies neurological tests were affected by the application of CGP 35348.

5. Conclusion

We concluded that CGP 35348 has a potential to improve the various aspects of behavior in a gender specific manner in albino mice. It has improved the memory formation of male mice during retention phase of MWM, but has negatively affected the exploratory behavior of female albino mice, while the rota rod test remained unaffected in both genders. Repetition of these tests following the application of higher doses of CGP 35348 would reveal more interesting results.

Conflict of Interests

The authors declare that they do not have conflict of interests of any sort.

Authors' Contribution

Quratul Aain Gillani and Shahid Iqbal contributed equally to this paper.

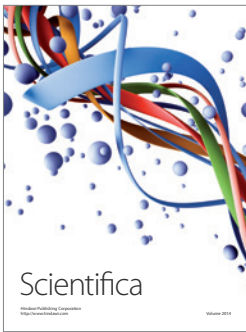
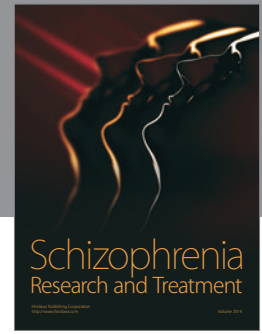
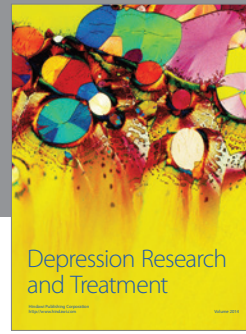
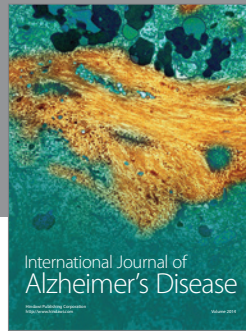
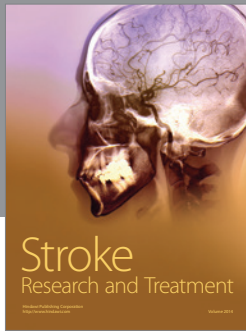
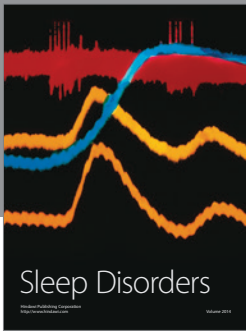
Acknowledgment

The Authors are thankful to Higher Education Commission of Pakistan for funding this project through indigenous Ph.D. scholarship scheme.

References

- [1] J. F. K. Cryan and K. Kaupmann, "A role for GABA_B receptors in anxiety and depression," *Trends in Pharmacological Sciences*, vol. 26, no. 1, pp. 36–43, 2005.
- [2] R. S. Kleppne and J. A. Tobin, *Encyclopedia of the Human Brain*, vol. 2, Elsevier Science, Philadelphia, Pa, USA, 2002.
- [3] S. J. Enna, "GABA_B receptor signaling pathways," in *Pharmacology of GABA and Glycine Neurotransmission*, H. Mohler, Ed., vol. 150, pp. 329–342, Springer, Heidelberg, Germany, 2001.
- [4] S. J. Enna, "A GABA_B mystery: the search for pharmacologically distinct GABA_B receptors," *Molecular Interventions*, vol. 1, no. 4, pp. 208–218, 2001.
- [5] E. Eduardo and E. E. Benarroch, "GABA_B receptors: structure, functions, and clinical implications," *Neurology*, vol. 78, no. 8, pp. 578–584, 2012.
- [6] J. P. L. Pin and L. Prezeau, "Allosteric modulators of GABA_B receptors, mechanism of action and therapeutic perspective," *Current Neuropharmacology*, vol. 5, no. 3, pp. 195–201, 2007.
- [7] C. Mondadori, G. Preiswerk, and J. Jaekel, "Treatment with a GABA_B receptor blocker improves the cognitive performance of mice, rats and rhesus monkeys," *Pharmacology Communications*, vol. 2, pp. 93–97, 1992.
- [8] N. J. DeSousa, R. J. Beninger, K. Jhamandas, and R. J. Boegman, "Stimulation of GABA_B receptors in the basal forebrain selectively impairs working memory of rats in the double Y-maze," *Brain Research*, vol. 641, no. 1, pp. 29–38, 1994.
- [9] W. Froestl, M. Gallagher, H. Jenkins et al., "SGS742: the first GABA_B receptor antagonist in clinical trials," *Biochemical Pharmacology*, vol. 68, no. 8, pp. 1479–1487, 2004.
- [10] E. Kandel, J. H. Schwartz, and T. M. Jessell, *Principles of Neural Science*, McGraw-Hill, New York, NY, USA, 2000.
- [11] K. A. Helm, R. P. Haberman, S. L. Dean et al., "GABA_B receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus," *Neuropharmacology*, vol. 48, no. 7, pp. 956–964, 2005.
- [12] J.-P. A. Manning, D. A. Richards, and N. G. Bowery, "Pharmacology of absence epilepsy," *Trends in Pharmacological Sciences*, vol. 24, no. 10, pp. 542–549, 2003.
- [13] N. Khadim, S. Iqbal, Q. U. A. Gillani, S. Safdar, and F. Iqbal, "Supplementation of GABA_B receptor antagonist (CGP 55845), following hypoxia ischemia encephalopathy, moderately effects the hematological and serum biochemical profile in albino mice," *Pakistan Journal of Zoology*, vol. 45, no. 4, pp. 1164–1167, 2013.
- [14] H.-R. Olpe, G. Karlsson, M. F. Pozza et al., "CGP 35348: a centrally active blocker of GABA_B receptors," *European Journal of Pharmacology*, vol. 187, no. 1, pp. 27–38, 1990.
- [15] B. Sunyer, S. Patil, C. Frischer et al., "Strain-dependent effects of SGS742 in the mouse," *Behavioural Brain Research*, vol. 181, no. 1, pp. 64–75, 2007.
- [16] R. Weitzdoerfer, H. Hoeger, E. Engidawork et al., "Neuronal nitric oxide synthase knock-out mice show impaired cognitive performance," *Nitric Oxide*, vol. 10, no. 3, pp. 130–140, 2004.
- [17] J. Kipnis, H. Cohen, M. Cardon, Y. Ziv, and M. Schwartz, "T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 21, pp. 8180–8185, 2004.
- [18] T. Abel and E. Kandel, "Positive and negative regulatory mechanisms that mediate long-term memory storage," *Brain Research Reviews*, vol. 26, no. 2-3, pp. 360–378, 1998.
- [19] A. J. Silva, J. H. Kogan, P. W. Frankland, and S. Kida, "CREB and memory," *Annual Review of Neuroscience*, vol. 21, pp. 127–148, 1998.

- [20] R. B. Nehring, H. P. M. Horikawa, O. El Far et al., "The metabotropic GABA_B receptor directly interacts with the activating transcription factor 4," *The Journal of Biological Chemistry*, vol. 275, no. 45, pp. 35185–35191, 2000.
- [21] E. Vernon, G. Meyer, L. Pickard et al., "GABA_B receptors couple directly to the transcription factor ATF4," *Molecular and Cellular Neuroscience*, vol. 17, no. 4, pp. 637–645, 2001.
- [22] J. H. White, R. A. J. McIlhinney, A. Wise et al., "The GABA_B receptor interacts directly with the related transcription factors CREB2 and ATFx," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 25, pp. 13967–13972, 2000.
- [23] H. Shiotsuki, K. Yoshimi, Y. Shimo et al., "A rotarod test for evaluation of motor skill learning," *Journal of Neuroscience Methods*, vol. 189, no. 2, pp. 180–185, 2010.
- [24] J. C. Crabbe, N. R. Rustay, and D. Wahlsten, "Assessment of genetic susceptibility to ethanol intoxication in mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 5, pp. 2917–2922, 2003.
- [25] A. Dalvi and R. J. Rodgers, "GABAergic influences on plus-maze behaviour in mice," *Psychopharmacology*, vol. 128, no. 4, pp. 380–397, 1996.
- [26] A. Partyka, A. Kłodzińska, B. Szewczyk et al., "Effects of GABA_B receptor ligands in rodent tests of anxiety-like behavior," *Pharmacological Reports*, vol. 59, no. 6, pp. 757–762, 2007.
- [27] M. R. Zarrindast, A. Bakhsha, P. Rostami, and B. Shafaghi, "Effects of intrahippocampal injection of GABAergic drugs on memory retention of passive avoidance learning in rats," *Journal of Psychopharmacology*, vol. 16, no. 4, pp. 313–319, 2002.
- [28] N. Pitsikas, A. E. Rigamonti, S. G. Cella, and E. E. Muller, "The GABA_B receptor and recognition memory: possible modulation of its behavioral effects by the nitreergic system," *Neuroscience*, vol. 118, no. 4, pp. 1121–1127, 2003.



Hindawi

Submit your manuscripts at
<http://www.hindawi.com>

