# EXPERIMENTAL INVESTIGATION

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# Inhibition of Dendritic L-Type Calcium Current by Memantine in Frog Tectum

**Armantas Baginskas[1], Antanas Kuras[2], Artūras Grigaliūnas[1]**

*[1]Department of Physics, Mathematics and Biophysics, Medical Academy, Lithuanian University of Health Sciences, [2]Laboratory of Neurophysiology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Lithuania*

***Key Words:*** *frog tectum; dendritic L-type calcium current; memantine; Alzheimer's disease.*

***Summary.*** *The aim of the study was to explore the effects of memantine on responses elicited in the frog tectum by the bursts of spikes of moderate strength of a single retina ganglion cell and to gain an insight about the effect of memantine on the L-type $Ca^{2+}$ current.*

*Material and Methods. The experiments were performed in vivo on adult frogs (Rana temporaria). An individual retina ganglion cell (or its retinotectal fiber) was stimulated by current pulses delivered through a multichannel stimulating electrode positioned on the retina. Responses to the discharge of a single retinal ganglion cell were recorded in the tectum by an extracellular carbon-fiber microelectrode positioned in the terminal arborization of the retinotectal fiber in the tectum layer F. The solution of memantine (1-amino-3,5-dimethyladamantane) hydrochloride (30 or 45 μM) was applied onto the surface of the tectum by perfusion at a rate of 0.4 mL/min.*

*Results. Memantine (30–45 μM) largely inhibited the L-type $Ca^{2+}$ channel-mediated slow negative wave and late discharges seen in the tectum responses without any effect on fast synaptic retinotectal transmission.*

*Conclusions. Our results suggest that the neuroprotective effect of memantine could arise not only through the inhibition of the NMDA receptor current but also through the suppression of the L-type $Ca^{2+}$ current.*

## Introduction

Memantine (1-amino-3,5-dimethyladamantane) shows clinical efficacy in patients with Alzheimer's disease (1, 2). Most studies describe its neuroprotective effects through the inhibition of extrasynaptic NMDA receptors (3, 4). However, memantine seems to generate multiple other effects. It blocks $\alpha 7$ nicotinic acetylcholine (5) and 5-$HT_3$ (6) receptors, stimulates cholinergic receptors (7), increases the production of kynurenic acid in the brain (8), inhibits ATP-dependent $K^+$ conductance (9), depresses glutamate release (10), decreases the basal level of intracellular calcium (11), reduces the secretion of Alzheimer's amyloid precursor protein and amyloid-$\beta$ peptide (12), and relieves microglia-associated inflammation and stimulates neurotrophic factor release from astroglia (13).

We have previously demonstrated that a discharge of action potentials of a single frog retina ganglion cell can evoke a long-lasting suprathreshold excitation of recurrent neurons of the tectum column (14) due to the activation of a slow dendritic L-type $Ca^{2+}$ current seen in the recordings of tectal responses as a slow negative wave (sNW) (15). The excitation of the recurrent neurons is evidenced in the recordings as recurrent synaptic potentials (rSPs) superimposed on the sNW. In the present study, we aimed at exploring the action of memantine on the sNW of frog tectal responses and thus at gaining an insight about the effect of memantine on the L-type $Ca^{2+}$ current.

Correspondence to A. Baginskas, Department of Physics, Mathematics and Biophysics, Medical Academy, Lithuanian University of Health Sciences, Eivenių 4, 50161 Kaunas, Lithuania
E-mail: baginskas@med.kmu.lt

## Material and Methods

The experimental procedure is described in detail in our earlier papers (16, 17). Here we shortly emphasize the main points.

The experiments were performed in vivo on 7 adult frogs (*Rana temporaria*). All the experiments in this study were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication No. 86-23 revised in 1985) and the European Communities Council Directive (November 24, 1986; 86/609/EEC) and were approved by the Animal Care and Use Committee of the State Food and Veterinary Service of Lithuania (No. 0167). During surgical manipulations, frogs were anesthetized with a high concentration of $CO_2$. The dorsal tectum was exposed in the manner described by Maturana et al. (18). The retina, contralateral to the opened tectum, was prepared in the way de-

scribed by George and Marks (19). The eyeball cavity was filled, and the exposed dorsal tectum was perfused with Ringer's solution (in mM: 116, NaCl; 2.5, KCl; 1.8, $CaCl_2$; 1.0, $MgCl_2$; 1.2, $NaHCO_3$; and 0.17, $NaH_2PO_4 \times 2H_2O$; pH, 7.3–7.4). Frogs were immobilized using an intramuscular injection of d-tubocurarine (0.15-0.3 mg), intubated and ventilated by a mechanical ventilator with a tidal volume of 2-3 mL at a frequency of 6-10 breaths per minute. The subsequent injections of d-tubocurarine (0.05-0.1 mg) were applied every hour to keep the frog immobilized during the experiment. Frogs were slightly anesthetized by placing them into the solution of MS-222 at a concentration of 50 mg/L (20). All the recordings were carried out in the dark at ambient temperatures of 17°C-22°C. At the end of the experiments, the animals were sacrificed with an overdose of an anesthetic.

An 8-channel electrode was used for the stimulation of the retina. The electrode consisted of 8 tungsten wires (channels) 40 $\mu$m in diameter bunched at 50–150 $\mu$m distances between the centers of different wires. The stimulating electrode was placed on the nasoventral quadrant of the naked retina. The single current pulses of magnitude of 11.8-22.5 $\mu$A and duration of 50 $\mu$s, or a train of 2–4 of such pulses were applied to the retina through a pair of stimulating electrode channels using an isolator (World Precision Instruments). The excitation of a single ganglion cell or its axon was achieved by slightly moving the stimulating electrode, switching between 8 channels of the electrode, and changing the strength of the current pulse.

The solution of memantine (1-amino-3,5-dimethyladamantane) hydrochloride (30 or 45 $\mu$M) was applied to the surface of the tectum by perfusion at a rate of 0.4 mL/min. It took approximately 3 minutes to replace the solution superfusing the tectum. The solution of the drug was prepared just before the use. Memantine hydrochloride was purchased from Sigma-Aldrich Co.

Responses evoked in the tectum layer F were recorded using a carbon-fiber microelectrode with a 50–70-$\mu$m tip. Responses consisted of individual retinotectal presynaptic action potentials (APs) followed by individual fast synaptic potentials (fSPs), the sNW with a duration of hundreds of ms, and rSPs superimposed on this wave (Fig. 1A). The amplitudes of the AP, fSP, and sNW, and the duration of the sNW were measured. The amplitude of the sNW ($A_{sNW}$) was evaluated as an average voltage in the time interval of the response from 60 to 120 ms. The duration of the sNW ($T_{sNW}$) was measured as the time interval from the peak of the last fSP in the burst to the end of the wave that was determined as the time moment when the level of the wave approached the baseline within a noise level of ~10 $\mu$V.

Data are expressed as mean±SE. The paired *t* test with the confidence level of 0.95 was used for the evaluation of the statistical significance of the results.

## Results

Recordings from 7 individual retinotectal axon terminals located in the dorsal tectum zones (areas 1, 3, 4, or 6) of 7 frogs were obtained. The stimulation of a single retina ganglion cell or its axon was considered reliable because an increase in the stimulation strength by up to 6 $\mu$A had no effect on the response, and a decrease by 0.2–0.8 $\mu$A completely abolished it. It was also confirmed that recordings were taken precisely at the tectum layer F: the measured depth of the tip of the recording microelectrode from the tectum surface was 260–280 $\mu$m, and the latency of the individual action potentials in respect of the stimulus artifact was 7.47±0.19 ms (range, 6.7–8.1 ms).

An individual retina ganglion cell was stimulated by a train of 2–4 current pulses with the interpulse intervals of 10 ms to evoke tectal responses with the sNW present (Fig. 1A). Then, 30 $\mu$M of memantine was added to the perfusion solution, and a change in the amplitude and duration of the sNW and fSPs was monitored during the perfusion when repeating the same stimulus (Figs. 1B and C). In 3 experiments, the concentration of memantine was increased afterward to 45 $\mu$M (Figs. 1D and E). Figs. 1 and 2A show that the amplitude of the fSP, $A_{SP}$, did not change significantly during the perfusion with the memantine solution at a concentration of 30 $\mu$M (134±16 $\mu$V in control versus 140±17 $\mu$V with memantine; n=7; $P$=0.1). The amplitude and duration of the sNW, $A_{sNW}$ and $T_{sNW}$, decreased from 93±21 $\mu$V and 157±20 ms in control to 36±9 $\mu$V (n=7, $P$=0.006) and 84±17 ms (n=7, $P$=0.0002) when the memantine solution (30 $\mu$M) was applied (Figs. 1B, 2B, and 2C). An increase in the concentration of memantine to 45 $\mu$M virtually abolished the sNW ($A_{sNW}$=0.8±5 $\mu$V, n=3, $P$=0.04; $T_{sNW}$=31±12 ms, n=3, $P$<0.05) (Figs. 1E, 2B, and 2C). In all the experiments, the effect of memantine became obvious within the first 15 minutes of perfusion with the memantine solution. It took 12 to 50 minutes (31±6 minutes on the average) for the effect to develop fully. The dependence of amplitude of the fSP, and amplitude and duration of the sNW on the time of perfusion with the memantine solution is shown in Fig. 3, demonstrating that the amplitude of the retinotectal synaptic transmission remained unchanged and the amplitude and duration of the sNW decreased during the perfusion.

The surface of the frog brain was washed with Ringer's solution for 1 hour after the perfusion with the memantine solution, but no change in the tectal

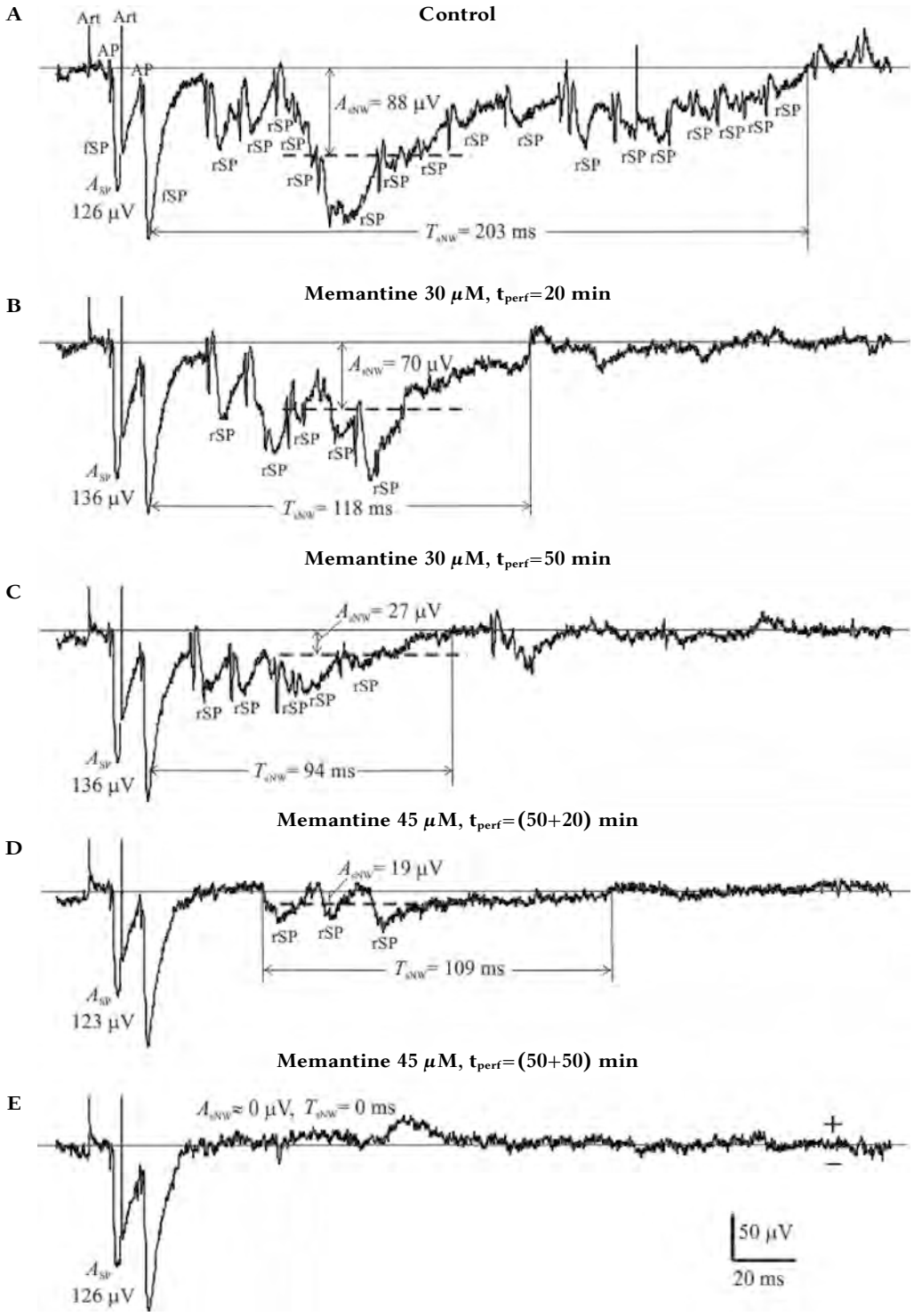


*Fig. 1.* Effects of memantine on tectal responses

**A**, tectal response to the stimulation of an individual retina ganglion cell by a pair of current pulses with an interpulse interval of 10 ms under control conditions. The response consists of individual retinotectal action potentials (AP), fast retinotectal synaptic potentials (fSP), slow negative wave (sNW), and recurrent synaptic potentials (rSP) superimposed on this wave. $A_{SP}$, amplitude of the first fSP; $A_{sNW}$, amplitude of the sNW; $T_{sNW}$, duration of the sNW; Art, stimulus artifact; $t_{perf}$, duration of perfusion with the memantine solution. **B**, tectal response after 20-minute perfusion with 30-μM memantine ($t_{perf}$=20 min). **C**, tectal response after 50-minute perfusion with 30-μM memantine ($t_{perf}$=50 min). **D**, tectal response after 20-minute perfusion with the memantine solution at a greater concentration (45 μM) ($t_{perf}$=50+20 min). Onset of the sNW can be delayed when the dendritic L-type $Ca^{2+}$ currents decreased sufficiently. **E**, tectal response after 50-minute perfusion with the memantine solution at a greater concentration (45 μM) ($t_{perf}$=50+50 min). The application of memantine (30 μM) resulted in a decrease in the amplitude and duration of the sNW more than 3 and 2 times, respectively. An elevation in the concentration to 45 μM abolished the sNW completely. A number of the rSPs decreased following a decrease in the sNW.

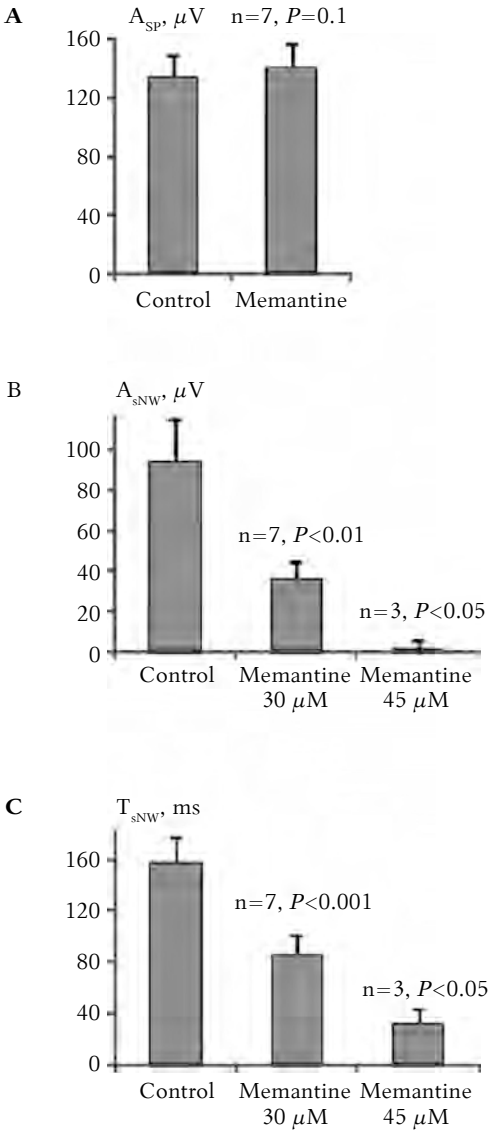


*Fig. 2.* Effects of memantine on the amplitude of the first fast synaptic potential ($A_{SP}$), amplitude of the slow negative wave ($A_{sNW}$), and duration of the slow negative wave ($T_{sNW}$)

**A**, mean amplitude of the fSP in control and after the application of memantine (134±16 μV versus 140±17 μV, n=7, $P$=0.1). **B**, mean amplitude of the sNW in control and after the application of 30-μM and 45-μM memantine (93±21 μV versus 36±9 μV [n=7, $P$<0.01] and 0.8±5 μV [n=3, $P$<0.05], respectively). **C**, mean duration of the sNW in control and after the application of of 30-μM and 45-μM memantine (157±20 ms versus 84±17 ms (n=7, $P$<0.001) and 31±12 ms [n=3, $P$<0.05], respectively).

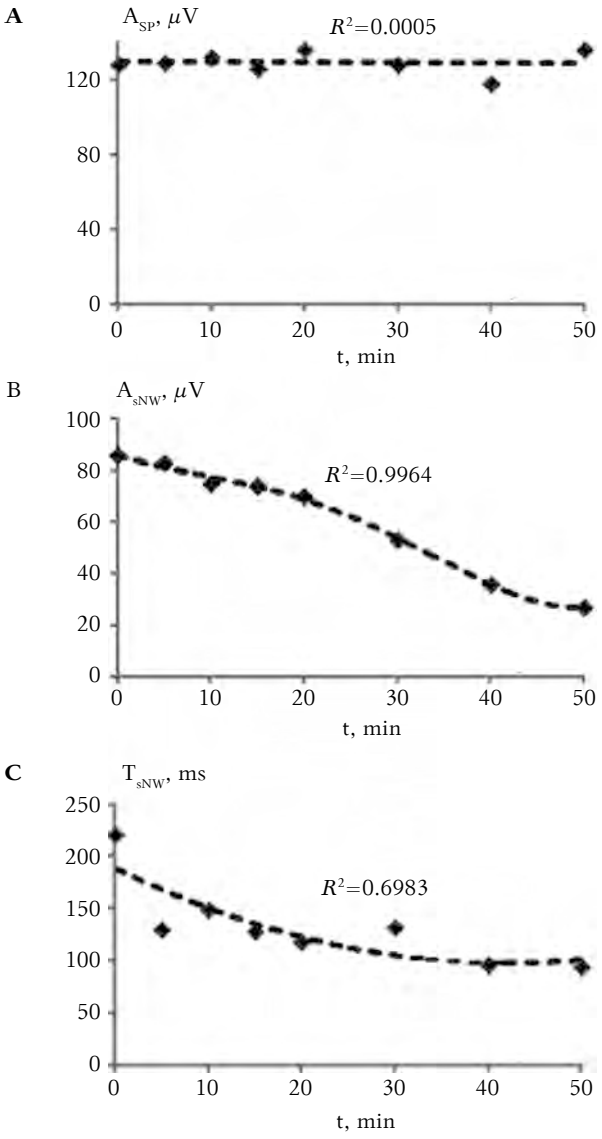


*Fig. 3.* Dependence of amplitude of the synaptic potential of retinotectal transmission, $A_{SP}$ (**A**), and the amplitude and duration of the slow negative wave, $A_{sNW}$ (**B**) and $T_{sNW}$ (**C**), respectively, on the time of perfusion with the memantine solution at a concentration of 30 μM

Dashed lines show trends of the data points with $R^2$ indicated next to them.

responses was seen. Therefore, the effect of memantine failed to be wash out.

## Discussion

The obtained results clearly show that the sNW of frog tectal responses was decreased or even abolished by memantine. This indicates that memantine inhibits calcium influx through dendritic L-type $Ca^{2+}$ channels, since the sNW is generated by the activation of the L-type calcium current in the dendrites of recurrent pear-shaped neurons of the frog tectum column as it was demonstrated in our previous study (15). It is evident from the latter and other our studies (16, 17) that the sNW remains stable within at least 2 hours of stimulation and recordings under control conditions. Thus, the time interval of

12 to 50 minutes (31±6 minutes on the average) of perfusion with the memantine solution, required for the effect to develop, was smaller than the time interval during which the stable control responses are usually recorded. Moreover, this matched the period needed for the development of the effect of nifedipine, a specific L-type $Ca^{2+}$ channel blocker (15, 16). However, contrarily to the effect of nifedipine, we did not manage to wash out the effect of memantine. This agrees with the results of other studies. For example, Frankiewicz et al. (21) noted that the recovery of the response was seen 4 hours after the washout of memantine. If it took so long in the in vitro experiments on thin (~400 $\mu$m) hippocampal slices, it is hardly possible to expect washout of the effect of memantine in the in vivo experiments on the whole frog brain when only part of the upper brain surface was perfused with Ringer's solution. The failure to wash out the effect of memantine points out another important aspect of the action of memantine, namely, its potent noncompetitive nature of binding to receptors or other effectors.

It is important to note that the strength of the retinotectal synaptic transmission (amplitude of synaptic potential, $A_{SP}$) was not significantly affected by memantine (Fig. 2A). A small insignificant increase in this amplitude could be attributed to the enhanced tonic potentiation (22) of the retinotectal transmission due to an increased ambient concentration of acetylcholine induced by a relatively intense stimulation of the tectum column.

The concentrations of memantine (30 and 45 $\mu$M) used in our experiments were similar to the concentrations used by other researchers to investigate the effect of memantine on the NMDA receptor current in cell cultures (23) and brain slices (21).

The data of our experiments do not answer the question whether memantine directly blocks L-type $Ca^{2+}$ channels or whether its inhibitory effect on the L-type calcium current is indirect, through some signal transduction pathways targeting the gating and/or conductance of L-type $Ca^{2+}$ channels. A diverse action of memantine discussed in the introduction favors the latter point of view. For example, it has been established that the activity of L-type $Ca^{2+}$ channels is positively modified by neuromodulators such as acetylcholine, serotonin, noradrenaline, and glutamate metabotropic receptors, and negatively modified by GABA(B) receptors (24). However, we have not found in the literature any evidence on the activation of GABA(B) receptors by memantine. Considering approximately equal times required for the development of the effect of memantine and nifedipine, an L-type $Ca^{2+}$ channel blocker (15, 16), the direct action of memantine on L-type $Ca^{2+}$ channels may be inferred. Certainly, in vitro experiments with the intracellular recordings of dendritic L-type $Ca^{2+}$ currents need to be done to address this question.

Experimental data show that L-type $Ca^{2+}$ channels are involved in the pathogenesis of Alzheimer's disease: amyloid-$\beta$ peptides, a causative factor for Alzheimer's disease, potentiate the influx of calcium into neurons via L-type $Ca^{2+}$ channels (25). The blockers of those channels inhibit the accumulation and aggregation of amyloid-$\beta$ peptides (26) and prevent neurons from amyloid-$\beta$ peptide-induced apoptosis (27). Researchers suggest that the specific L-type $Ca^{2+}$ channel blockers could be used for the treatment of Alzheimer's disease (27, 28). Our findings provide evidence to support this view. It has been established that L-type $Ca^{2+}$ channels are expressed in human neurons. Studies indicate that the activity of L-type $Ca^{2+}$ channels is altered by a number of neuromodulators in the brain of reptiles and mammals (24, 25, 29, 30). Therefore, we expect that the effect of memantine we have described in frogs may be seen in human neurons, too. In such a case, our results may be useful for the development of new approaches to the treatment of Alzheimer's disease.

## Conclusions

Memantine decreased the sNW elicited in frog tectal responses by a burst of action potentials of a single retina ganglion cell. As the sNW is generated by the dendritic L-type $Ca^{2+}$ current in pear-shaped neurons of the tectum column, this shows that memantine suppresses the L-type $Ca^{2+}$ current. Thus, the neuroprotective effect of memantine could be mediated not only through the inhibition of the NMDA receptor current but also through the suppression of the L-type $Ca^{2+}$ current.

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## Statement of Conflict of Interest

The authors state no conflict of interest.

## References

1. Smith SA. Treatment of Alzheimer's disease in the long-term-care setting. Am J Health Syst Pharm 2009;66:899-907.
2. Massoud F, Léger GC. Pharmacological treatment of Alzheimer disease. Can J Psychiatry 2011;56:579-88.
3. Xia P, Chen HS, Zhang D, Lipton SA. Memantine preferentially blocks extrasynaptic over synaptic NMDA receptor currents in hippocampal autapses. J Neurosci 2010;30:11246-50.
4. Bordji K, Becerril-Ortega J, Buisson A. Synapses, NMDA

receptor activity and neuronal Aβ production in Alzheimer's disease. Rev Neurosci 2011;22:285-94.
5. Aracava Y, Pereira EF, Maelicke A, Albuquerque EX. Memantine blocks alpha7* nicotinic acetylcholine receptors more potently than n-methyl-D-aspartate receptors in rat hippocampal neurons. J Pharmacol Exp Ther 2005;312: 1195-205.
6. Reiser G, Binmöller FJ, Koch R. Memantine (1-amino-3,5-dimethyladamantane) blocks the serotonin-induced depolarization response in a neuronal cell line. Brain Res 1988; 443:338-44.
7. Drever BD, Anderson WG, Johnson H, O'Callaghan M, Seo S, Choi DY, et al. Memantine acts as a cholinergic stimulant in the mouse hippocampus. J Alzheimers Dis 2007;12:319-33.
8. Kloc R, Luchowska E, Wielosz M, Owe-Larsson B, Urbanska EM. Memantine increases brain production of kynurenic acid via protein kinase A-dependent mechanism. Neurosci Lett 2008;435:169-73.
9. Giustizieri M, Cucchiaroni ML, Guatteo E, Bernardi G, Mercuri NB, Berretta N. Memantine inhibits ATP-dependent K+ conductances in dopamine neurons of the rat substantia nigra pars compacta. J Pharmacol Exp Ther 2007; 322:721-9.
10. Lu CW, Lin TY, Wang SJ. Memantine depresses glutamate release through inhibition of voltage-dependent Ca2+ entry and protein kinase C in rat cerebral cortex nerve terminals: an NMDA receptor-independent mechanism. Neurochem Int 2010;57:168-76.
11. Blanchard AP, Guillemette G, Boulay G. Memantine potentiates agonist-induced Ca2+ responses in HEK 293 cells. Cell Physiol Biochem 2008;22:205-14.
12. Ray B, Banerjee PK, Greig NH, Lahiri DK. Memantine treatment decreases levels of secreted Alzheimer's amyloid precursor protein (APP) and amyloid beta (A beta) peptide in the human neuroblastoma cells. Neurosci Lett 2010; 470:1-5.
13. Wu HM, Tzeng NS, Qian L, Wei SJ, Hu X, Chen SH, et al. Novel neuroprotective mechanisms of memantine: increase in neurotrophic factor release from astroglia and anti-inflammation by preventing microglial activation. Neuropsychopharmacology 2009;34:2344-57.
14. Kuras A, Baginskas A, Batuleviciene V. Suprathreshold excitation of frog tectal neurons by short spike trains of single retinal ganglion cell. Exp Brain Res 2004;15:509-18.
15. Baginskas A, Kuras A. Single retina ganglion cell evokes the activation of L-type Ca2+-mediated slow inward current in frog tectal pear-shaped neurons. Neurosci Res 2008; 60:412-21.
16. Baginskas A, Kuras A. L-Type Ca2+ current in frog tectal recurrent neurons determines the NMDA receptor activation on efferent neuron. Exp Brain Res 2009;193:509-17.
17. Baginskas A, Kuras A. Muscarinic inhibition of recurrent glutamatergic excitation in frog tectum column prevents NMDA receptor activation on efferent neuron. Exp Brain Res 2011;208:323-34.
18. Maturana HR, Lettvin JY, McCulloch WS, Pitts WH. Anatomy and physiology of vision in the frog (Rana pipiens). J Gen Physiol 1960;43:129-75.
19. George SA, Marks WB. Optic nerve terminal arborizations in the frog: shape and orientation inferred from electrophysiological measurements. Exp Neurol 1974;42:467-82.
20. Cakir Y, Strauch SM. Tricaine (MS-222) is a safe anesthetic compound compared to benzocaine and pentobarbital to induce anesthesia in leopard frogs (Rana pipiens). Pharmacol Rep 2005;57:467-74.
21. Frankiewicz T, Potier B, Bashir ZI, Collingridge GL, Parsons CG. Effects of memantine and MK-801 on NMDA-induced currents in cultured neurones and on synaptic transmission and LTP in area CA1 of rat hippocampal slices. Br J Pharmacol 1996;117:689-97.
22. Kuras A, Gutmaniene N. N-cholinergic facilitation of glutamate release from an individual retinotectal fiber in frog. Vis Neurosci 2001;18:549-58.
23. Kaufman AM, Milnerwood AJ, Sepers MD, Coquinco A, She K, Wang L, et al. Opposing roles of synaptic and extrasynaptic NMDA receptor signaling in cocultured striatal and cortical neurons. J Neurosci 2012;32:3992-4003.
24. Alaburda A, Perrier JF, Hounsgaard J. Mechanisms causing plateau potentials in spinal motoneurones. Adv Exp Med Biol 2002;508:219-26.
25. Kim S, Rhim H. Effects of amyloid-β peptides on voltage-gated L-type Ca(V)1.2 and Ca(V)1.3 Ca(2+) channels. Mol Cells 2011;32:289-94.
26. Paris D, Bachmeier C, Patel N, Quadros A, Volmar CH, Laporte V, et al. Selective antihypertensive dihydropyridines lower Aβ accumulation by targeting both the production and the clearance of Aβ across the blood-brain barrier. Mol Med 2011;17:149-62.
27. Yagami T, Kohma H, Yamamoto Y. L-type voltage-dependent calcium channels as therapeutic targets for neurodegenerative diseases. Curr Med Chem 2012;19:4816-27.
28. Anekonda TS, Quinn JF. Calcium channel blocking as a therapeutic strategy for Alzheimer's disease: the case for isradipine. Biochim Biophys Acta 2011;1812:1584-90.
29. Abbinanti MD, Zhong G, Harris-Warrick RM. Postnatal emergence of serotonin-induced plateau potentials in commissural interneurons of the mouse spinal cord. J Neurophysiol 2012;108(8):2191-202.
30. Bell DC, Butcher AJ, Berrow NS, Page KM, Brust PF, Nesterova A, et al. Biophysical properties, pharmacology, and modulation of human, neuronal L-type (alpha(1D), Ca(V)1.3) voltage-dependent calcium currents. J Neurophysiol 2001;85:816-27.

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