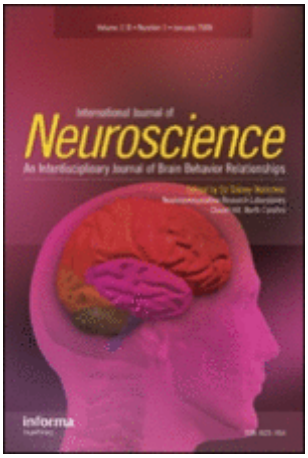

Spatial memory deficits initiated by agroclavine injection or olfactory bulbectomy in rats are characterized by different levels of long-term potentiation expression in the hippocampus

Journal:	*The International Journal of Neuroscience*
Manuscript ID	GNES-2018-OR-0677.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Vorobyov, Vasily; Institute of Cell Biophysics Medvinskaya, Natalia; Institute of Cell Biophysics Deev, Alexander; Institute of Theoretical and Experimental Biophysics Sengpiel, Frank; School of Biosciences and Neuroscience & Mental Health Research Institute Bobkova, Natalia; Institute of Cell Biophysics Lunin, Sergey; Institute of Cell Biophysics
Keywords:	learning, Morris test, neuronal plasticity

Spatial memory deficits initiated by agroclavine injection or olfactory bulbectomy in rats are characterized by different levels of long-term potentiation expression in the hippocampus

Vasily Vorobyov[a], Natalia Medvinskaya[a], Alexander Deev[b], Frank Sengpiel[c], Natalia Bobkova[a], Sergey Lunin[a]

[a]*Institute of Cell Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*

[b]*Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, 142290 Pushchino, Moscow Region, Russia*

[c]*School of Biosciences and Neuroscience & Mental Health Research Institute, Cardiff University, Museum Avenue, Cardiff CF10 3AX, UK*

Contact information:

Dr. V. Vorobyov, PhD

Institute of Cell Biophysics

142290 Pushchino, Russia

Tel: 007-4967-739-442

Fax: 007-4967-330-509

E-mail: vorobyovv2@gmail.com

Spatial memory deficits initiated by olfactory bulbectomy or pretreatment with an ergot alkaloid, agroclavine, in rats are characterized by different expression of long-term potentiation in the hippocampus

ABSTRACT. To clarify whether long-term potentiation (LTP) is the mechanism underpinning mnemonic processes we studied LTP in hippocampal slices from rats whose spatial memory deficit was produced by either olfactory bulbectomy (OBX) or pretreatment with an ergot alkaloid, agroclavine. OBX is accompanied by cholinergic system inhibition whereas agroclavine predominantly activates dopaminergic mediation. The both have been shown to be involved in learning/memory and LTP mechanisms. In OBX- *vs.* sham-operated rat, we have revealed significant reduction of LTP in hippocampal CA1 region. In contrast, no LTP differences in agroclavine- *vs.* vehicle-treated rats were observed. These results demonstrate that LTP expression in the hippocampus is dependent on the origin of spatial memory impairment. Furthermore, they suggest that pharmacological and neurodegenerative models of AD might be useful approach for discovery of both AD mechanisms and mixed pathology dementias.

Keywords: learning; Morris test; LTP; neuronal plasticity

Introduction

The phenomenon of "long-term potentiation" (LTP) is supposed to be linked with the mechanisms of learning/memory formation and its impairment [1]. However, in patients suffered from Alzheimer's disease (AD) and in animal models of AD, the memory deficit has been shown to correlate with suppression of cortical LTP [2] while age-related memory loss is accompanied by enhanced hippocampal LTP [3]. Furthermore, different learning paradigms have different effects on LTP [4] while drugs, affecting spatial memory, have no influence on LTP, and *vice versa* [5].

To clarify whether LTP in the hippocampus is the mechanism underpinning learning and/or memory processes we studied LTP in rats whose spatial memory deficits were produced by either olfactory bulbectomy (OBX), a model of AD [6], or pretreatment with an ergot alkaloid, agroclavine [7]. The olfactory bulbs damage initiates a retrograde degeneration of cholinergic neurons, thereby leading to memory impairment and suppression of hippocampal LTP [8]. On the other hand, the hippocampus is well known to receive dopaminergic (DA) input from midbrain DA neurons [9] and exposure to novelty, stimulating DA release [10], facilitates LTP induction [11]. Furthermore, DA modulates synaptic plasticity in the prefrontal cortex [12] and DA mediation in the hippocampus is involved in memory performance [13]. Surprisingly, agroclavine, a DA receptor agonist [7], has been revealed to produce spatial memory loss in mice [14].

Thus, our present study aims to elucidate the question whether there is a difference between hippocampal LTP in brain slices from OBX- *vs.* agroclavine-treated rats characterized by comparable extents of spatial memory impairment.

Methods

Subjects

Twenty five male Wistar rats bred in a colony (donated by Charles Rivers Laboratories, Wilmington, MA, USA) under control barrier conditions were used in this study. The animals were given food and water *ad libitum* and reared in a standard 12-h/12-h day/night cycle. All manipulations were carried out in accordance with the principles enunciated in National Institutes of Health Publications No. 80-23, revised 1978, for animal experiments and approved by the Institutional Ethical Review Committee.

Surgical procedure

At age of two months, the rats were anaesthetised with intraperitoneal (i.p.) injection of Nembutal at dose of 60 mg/kg and two holes (2 mm in diameter) were drilled symmetrically over the olfactory bulbs (8 mm anterior to the bregma and 2 mm laterally from the midline). The bulbs were aspirated carefully under visual control through a blunt needle attached to a water pump ("OBX" group of 6 rats). In nineteen sham-operated rats, small drops of brain liquor were aspirated through the holes.

Spatial discrimination testing

At the age of fourteen months, learning/memory abilities of rats were tested with the Morris water paradigm (for details, see [15]). On the next day after training session, sham-operated rats were randomly divided on three groups. One of them was used as a control for OBX-rats ("ShX" group, N=7). In another group ("ShAg", N=5), the animals were treated with agroclavine (50 µg/kg, i.p.), whereas all others ("ShVh" group, N=7) and from ShX and OBX groups were injected with the vehicle (0.3 ml, i.p.). We used agroclavine synthesized microbiologically by strain Claviceps sp. BKM F-2609 [16]. The injections were performed one hour before testing procedure. Total time spent in each quadrant and the number of inward crossings of its borders was estimated by use a digital camera (Logitech QuickCam 3000, 800x600 pixels, 15 fps) and custom-developed video tracking software.

Electrophysiology

Immediately after memory testing, the rat was deeply anaesthetized with ether and decapitated. Its brain was cooled at 4°C in artificial cerebrospinal fluid (ACSF). The whole brain was prepared into coronal slices of 400 µm, further placed into a holding chamber with ACSF bubbled continuously with carbogen.

After at least 1.5 hour of equilibration in the holding chamber, the slice was transferred into a submerged recording chamber and perfused at a rate of 0.8 ml/min with warmed ACSF (30±1°C). The glass micropipettes (1 MΩ) filled with 2 M NaCl, were positioned in the *stratum radiatum* of the CA1 region of the hippocampus for extracellular recording of field excitatory postsynaptic potentials (fEPSP), using a custom-made amplifier. In the CA1 region, the Schaffer collateral pathway was stimulated through a unipolar tungsten electrode (100-200 KΩ) positioned in the *stratum radiatum*, 2 mm apart from the recording electrode. Amplitude of stimulating 0.1-ms pulses was varied (0.06–0.4 mA) to elicit fEPSPs of ~1 mV. Testing stimulation

(0.05 Hz) was followed by a series (0.5 Hz) of eight 200-Hz trains with eight impulses in each train to produce LTP. The signals were digitized at 10 kHz using a CED1401 A/D converter (Cambridge Electronic Design Ltd, Cambridge, UK) and analyzed with custom-developed software. The baseline activity was recorded for at least 20 min before LTP induction; four 0.2-Hz biphasic, constant current pulses (0.1-ms per polarity) were used for baseline recording and testing every 0.5 min after tetanus up to 90 min. The fEPSP was defined as the maximal slope of the rising phase, and further, in percentage of the mean values *vs.* those in baseline recordings. Stimulus intensity of the test pulse was adjusted so that it produced a fEPSP of 45-50% of the maximal fEPSP amplitude.

Statistics

The data were evaluated by non- parametric Mann-Whitney U-test and one-/two-way ANOVA for repeated measures, where appropriate. The group data were expressed as the means ± SEM; differences were considered significant at $p < 0.05$. The statistical analyses were performed using STATISTICA, version 10 (StatSoft, Inc., Tulsa, OK, USA).

Results

In rats from different groups, locomotor capabilities, evaluated by the latency in the reaching the *visible* platform, were in the range of 10.3±1.7 - 12.2±2.3 sec for all groups. In the daily training trials with the *hidden* platform (Fig. 1A), decreasing the latencies over time in the reaching of the platform, were observed in all groups (1-way ANOVA: $F_{5,36,30,36,24} > 46$, $p < 0.001$, for all). Despite significant delay in correct responses of OBX-rats *vs.* sham-operated animals (ShX, ShVh, and ShAg) on Day 3 and Day 4, by the end of training (Day 6), they were able to find the platform with relatively short delay, comparable with rats from other groups. Memory testing on Day 7 (without the platform in the pool) revealed that the rats from ShX and ShVh control groups spent more time in the target quadrant (Fig. 1B, 1-way ANOVA: $F_{3,24,24} > 46$, $p < 0.001$, for both) and visited it more frequently (Fig. 1C, $F_{3,24,24} > 10.1$, $p < 0.001$, for both) than the other quadrants. However, this was not the case for the animals from OBX and ShAg groups (1-way ANOVA: $F_{3,20,16} < 2.8$, $p > 0.05$, for both) Thus, both OBX and agroclavine produced evident spatial memory impairment in rats.

Time course and magnitude of LTP in CA1 region were different in brain slices from OBX- *vs.* ShX-rats (Fig. 2A, 2-way ANOVA: $F_{1,495} = 716$, $p < 0.001$) while no evident differences in LTP between ShAg- and ShVh-rats were revealed (Fig. 2B, 2-way ANOVA: $F_{1,450} = 1.2$, $p = 0.3$). The LTP profiles were very similar in animals from both control groups (ShX *vs.* ShVh, 2-way ANOVA: $F_{1,540} = 2.8$, $p = 0.1$).

Discussion

In this study, we revealed an association between spatial memory impairment in OBX-rats and LTP suppression in their hippocampus. In contrast, no LTP modification was observed in agroclavine pretreated rats with similar memory deficit.

On OBX-rats, the rising level of Aβ in the cortex-hippocampus samples has been shown to correlate with the extent of spatial memory impairment in these animals [15]. This is in line with data suggesting an important role of Aβ in memory loss in AD patients and in LTP suppression [17]. The LTP difference in OBX- *vs.* agroclavine-pretreated rats might be linked with different baseline conditions during learning and testing sessions. Indeed, the mechanisms associated with learning and LTP in OBX-rats were chronically affected by Aβ and/or by neurotransmitter signaling abnormalities produced by elevated Aβ [17]. Different effects of agroclavine on memory and LTP might be explained by its dopamine-mimetic and alkaloid activities [7] involved in dopamine-cholinergic systems interaction [18]. However, we have revealed that agroclavine, injected before testing, initiates spatial memory loss in naïve rats. These suggest that agroclavine affects predominantly the retention stage of memory processing and, given LTP insensitivity to agroclavine, that LTP is not crucial for this stage.

In OBX-rats, with memory loss and chronically increased Aβ in the brain [15], LTP impairment has been shown in present study. This is in line with data obtained in transgenic mice model of AD [19]. Cholinergic system is well known to be involved in both the LTP induction [20] and interaction between the hippocampus and the olfactory bulbs [21]. Given these, we suggest that OBX initiates a retrograde degeneration of cholinergic neurons and, in turn, the malfunctioning of both intrinsic circuitry in the hippocampus and its interrelation with other brain areas, required for functioning of learning/memory mechanisms. One of the possible sources potentiating these pathological changes after OBX seems to be linked with the enhanced Aβ level. This, in turn, might be associated with partial calcium/calmodulin-dependent protein kinases

inactivation that has been supposed to mediate LTP impairment and the learning disability in OBX-mice [22].

Thus, OBX-animals, with chronically depleted cholinergic mediation, i.e., with inactivated calcium-dependent intracellular processing [23], suppose to be lacking in support for LTP, whereas agroclavine, with its dopamine-mimetic activity, is able to activate this processing and, in turn, to stabilise LTP profile. Given these and both the delay in learning of OBX-rats (see Fig. 1A, Days 2-4) and the lack of a link between impaired memory retention and unaltered LTP in agroclavine-injected rats (cf. Fig. 1B,C and Fig. 2B), we propose that OBX-rats are suffered from disturbances predominantly in the consolidation phase of memory processing. The LTP suppression revealed in OBX-rats on the next day after memory testing (see Fig. 2A) seems to characterize those alterations in the synaptic plasticity which are closely associated with the progression of the neurodegenerative processes (in the cholinergic system, in particular) initiated by the bulbectomy.

Our results highlight the necessity of taking into consideration a specificity of mechanisms involved in memory processing in different models of AD. Namely, a comparative analysis of findings obtained in pharmacological and neurodegenerative models of AD might be useful approach for both discovery of multiple mechanisms of the disease [24] and development of effective therapy for AD-associated and mixed pathology dementias [25].

Acknowledgment

This work was supported by the Russian Fund of Basic Research under Grant number 16-04-00942.

Conflict of interest

The authors declare that they have no conflict of interest

References

[1] Nicoll RA. A Brief History of Long-Term Potentiation. Neuron. 2017;93(2):281-290.

[2] Battaglia F, Wang HY, Ghilardi MF, Gashi E, Quartarone A, Friedman E, Nixon RA. Cortical plasticity in Alzheimer's disease in humans and rodents. Biol Psychiatry. 2007;62:1405-1412.

[3] Pinho J, Vale R, Batalha VL, Costenla AR, Dias R, Rombo D, Sebastião AM, de Mendonça A, Diógenes MJ. Enhanced LTP in aged rats: Detrimental or compensatory? Neuropharmacology. 2017;114:12-19.

[4] Korz V, Frey JU. Emotional and cognitive reinforcement of rat hippocampal long-term potentiation by different learning paradigms. Neuron Glia Biology. 2004;1:253-261.

[5] Kikusui T, Aoyagi A, Kaneko T. Spatial working memory is independent of hippocampal CA1 long-term potentiation in rats. Behav Neurosci. 2000;114:700-706.

[6] Yamamoto T, Jin J, Watanabe S. Characteristics of memory dysfunction in olfactory bulbectomized rats and the effects of cholinergic drugs. Behav Brain Res. 1997;83:57-62.

[7] Berde B, Schild HO, editors. Handbook of Experimental Pharmacology. Berlin: Springer, 1978. (Ergot Alkaloids and Related Compounds; 49).

[8] Doralp S, Leung LS. Cholinergic modulation of hippocampal CA1 basal-dendritic long-term potentiation. Neurobiol Learn Mem. 2008;90:382-388.

[9] Huang Q, Zhou D, Chase K, Gusella JF, Aronin N, DiFiglia M. Immunohistochemical localization of the D1 dopamine receptor in rat brain reveals its axonal transport, pre- and postsynaptic localization, and prevalence in the basal ganglia, limbic system, and thalamic reticular nucleus. Proc Natl Acad Sci USA. 1992;89:11988-11992.

[10] Ljungberg T, Apicella P, Schultz W. Responses of monkey dopamine neurons during learning of behavioral reactions. J Neurophysiol. 1992;67:145-163.

[11] Li S, Cullen WK, Anwyl R, Rowan MJ. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. Nat Neurosci. 2003;6:526–531.

[12] Otani S, Bai J, Blot K. Dopaminergic modulation of synaptic plasticity in rat prefrontal neurons. Neurosci Bull. 2015;31:183-190.

[13] Hotte M, Naudon L, Jay TM. Modulation of recognition and temporal order memory retrieval by dopamine D1 receptor in rats. Neurobiol Learn Mem. 2005;84:85-92.

[14] Bobkova NV, Medvinskaya NI, Nesterova IV, Arinbasarov MU. Lesioning of spatial memory in mice treated with agroclavin. Neurosci Behav Physiol. 2003;33:301-306.

[15] Bobkova N, Vorobyov V, Medvinskaya N, Aleksandrova I, Nesterova I. Interhemispheric EEG differences in olfactory bulbectomized rats with different cognitive abilities and brain beta-amyloid levels. Brain Res. 2008;1232:185-194.

[16] Vepritskaya IG, Boichenko LV, Arinbasarov MU, Zelenkova NF, Bobkova NV. Selection of Ergot Alkaloid Producers by Induced Mutagenesis. Appl Biochem Microbiol. 2002;38:28-31.

[17] Ondrejcak T, Klyubin I, Hu NW, Barry AE, Cullen WK, Rowan MJ. Alzheimer's Disease Amyloid beta-Protein and Synaptic Function. Neuromolecular Med. 2010;12:13-26.

[18] McGurk SR, Levin ED, Butcher LL. Cholinergic-dopaminergic interactions in radial-arm maze performance. Behav Neural Biol. 1988;49:234-239.

[19] Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J. Impaired learning and LTP in mice expressing the carboxyterminus of the Alzheimer amyloid precursor protein. Nature. 1997;387:500-505.

[20] Yamazaki Y, Jia Y, Hamaue N, Sumikawa K. Nicotine-induced switch in the nicotinic cholinergic mechanisms of facilitation of long-term potentiation induction. Eur J Neurosci. 2005;22:845-860.

[21] Carlsen J, De Olmos J, Heimer L. Tracing of the two-neuron pathways in the olfactory system by the aid of transneuronal degeneration. Projections to the amygdaloid body and hippocampal formation. J Comp Neurol. 1982;208:196-208.

[22] Moriguchi S, Han F, Nakagawasai O, Tadano T, Fukunaga K. Decreased calcium/calmodulin-dependent protein kinase II and protein kinase C activities mediate impairment of hippocampal long-term potentiation in the olfactory bulbectomized mice. J Neurochem. 2006;97:22-29.

[23] Welsby PJ, Rowan MJ, Anwyl R. Intracellular mechanisms underlying the nicotinic enhancement of LTP in the rat dentate gyrus. Eur J Neurosci. 2009;29:65-75.

[24] Chen YG. Research Progress in the Pathogenesis of Alzheimer's Disease. Chin Med J (Engl.). 2018;131:1618-1624.

[25] Luo J, Lee SH, VandeVrede L, Qin Z, Ben Aissa M, Larson J, Teich AF, Arancio O, D'Souza Y, Elharram A, Koster K, Tai LM, LaDu MJ, Bennett BM, Thatcher GR. A multifunctional therapeutic approach to disease modification in multiple familial mouse models and a novel sporadic model of Alzheimer's disease. Mol Neurodegener. 2016;11: 35, doi: 10.1186/s13024-016-0103-6.

Captions

Figure 1. Trial-by-trial evolution of latencies to reach the hidden platform during learning in Morris water maze (**A**) for olfactory bulbectomized rats (OBX) and sham-operated rats (ShX, ShVh, and ShAg) and during memory testing on Day 7 (**B** and **C**). Ordinate in **A** is latency of correct attempts to find the hidden platform, in seconds; abscissa shows the session days. Diamond denotes significant difference ($p < 0.001$; U-test) between OBX and ShX groups. Before testing session, the animals from OBX, ShX and ShVh groups were treated (one hour apart) with the vehicle (0.3 ml, i.p.), whereas those from ShAg group were injected with agroclavine (50 µg/kg, i.p.). Ordinate in **B** is duration, in seconds, spent in each water maze quadrant (grey bars, I - "Indifferent", black bars, T - "Target"); ordinate in **C** is percentage of inward crossings into a quadrant *vs.* crossings of all quadrants. Abscissa in **B** and **C** represents different quadrants and groups of rats. Diamonds denote significant differences I *vs.* T: one - $p < 0.05$, two - $p < 0.01$, three - $p < 0.001$; U-test). All data are represented as means (± SEM).

Figure 2. Different time courses of LTP induced by tetanic stimulation of the *stratum radiatum* of the hippocampal CA1 region in brain slices from the bulbectomized (**A**) and agroclavine-treated (**B**) rats. Ordinate is fEPSP slope expressed as the percentage of the baseline values registered for 20 min immediately before the tetanic stimulation (marked as "0" on abscissa). All data are represented as means (± SEM). Other details see in the legend to figure 1.

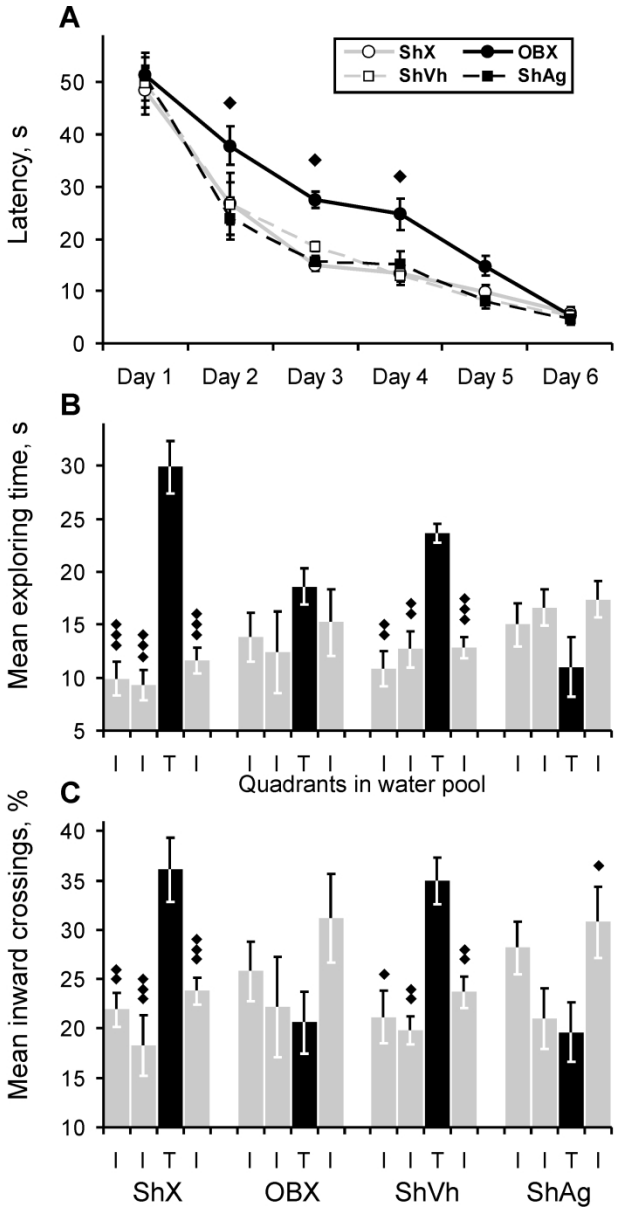

Figure 1. Trial-by-trial evolution of latencies to reach the hidden platform during learning in Morris water maze (A) for olfactory bulbectomized rats (OBX) and sham-operated rats (ShX, ShVh, and ShAg) and during memory testing on Day 7 (B and C). Ordinate in A is latency of correct attempts to find the hidden platform, in seconds; abscissa shows the session days. Diamond denotes significant difference ($p < 0.001$; U-test) between OBX and ShX groups. Before testing session, the animals from OBX, ShX and ShVh groups were treated (one hour apart) with the vehicle (0.3 ml, i.p.), whereas those from ShAg group were injected with agroclavine (50 μg/kg, i.p.). Ordinate in B is duration, in seconds, spent in each water maze quadrant (grey bars, I - "Indifferent", black bars, T - "Target"); ordinate in C is percentage of inward crossings into a quadrant vs. crossings of all quadrants. Abscissa in B and C represents different quadrants and groups of rats. Diamonds denote significant differences I vs. T: one - $p < 0.05$, two - $p < 0.01$, three - $p < 0.001$; U-test). All data are represented as means (± SEM).

99x177mm (600 x 600 DPI)

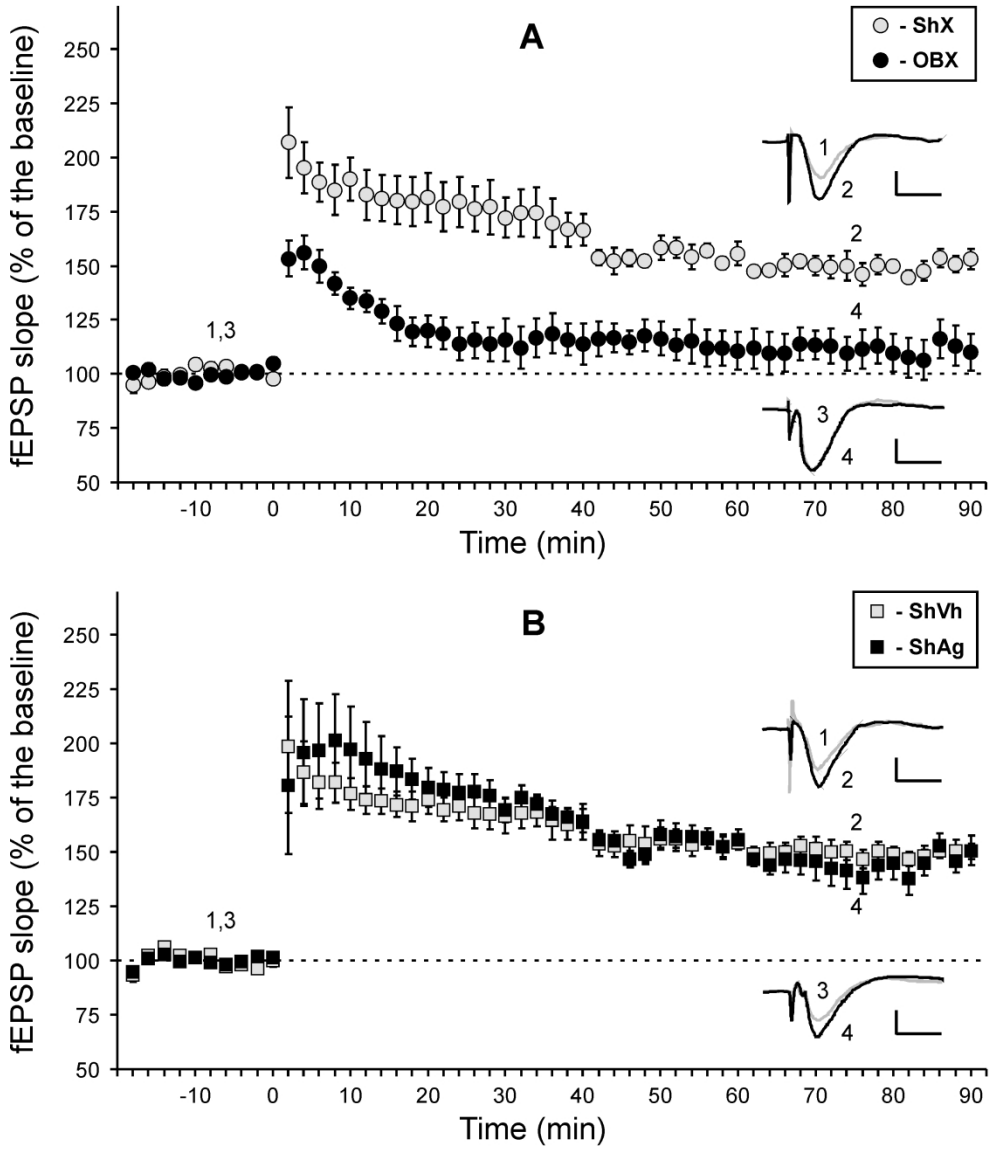

Figure 2. Different time courses of LTP induced by tetanic stimulation of the stratum radiatum of the hippocampal CA1 region in brain slices from the bulbectomized (A) and agroclavine-treated (B) rats. Ordinate is fEPSP slope expressed as the percentage of the baseline values registered for 20 min immediately before the tetanic stimulation (marked as "0" on abscissa). All data are represented as means (± SEM). Other details see in the legend to figure 1.

126x150mm (600 x 600 DPI)