Oxytocin facilitates the induction of long-term potentiation in the accessory olfactory bulb

Long-Yun Fang[a,b], Rong-Dan Quan[a,c], Hideto Kaba[a,d,*]

[a]*Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan*

Departments of [b]*Orthopaedic Surgery and* [c]*Obstetrics and Gynecology, College of Medical, Yanbian University, 119 Juzijie, Yanjishi, Jilin province, China*

[d]*Division of Adaptation Development, Department of Developmental Physiology, National Institute for Physiological Sciences, Okazaki 444-8585, Japan*

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Corresponding author:

Hideto Kaba

Department of Physiology, Kochi Medical School, Nankoku, Kochi 783-8505, Japan

Tel: +81 88-880-2305

Fax: +81 88-880-2307

E-mail address: kabah@kochi-u.ac.jp

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Abstract

When female mice are mated, they form a memory to the pheromonal signal of their male partner. Several lines of evidence indicate that the neural changes underlying this memory occur in the accessory olfactory bulb (AOB) at the first stage of the vomeronasal system. The formation of this memory depends on the mating-induced release of noradrenaline in the AOB. In addition to noradrenaline, the neuropeptide oxytocin (OT) is also released within the central nervous system during mating. Because OT has been implicated in social memory and its receptors are expressed in the AOB, we hypothesized that OT might promote the strength of synaptic transmission from mitral to granule cells in the AOB. To test this hypothesis, we analyzed the lateral olfactory tract-evoked field potential that represents the granule cell response to mitral cell activation and its plasticity in parasagittal slices of the AOB. Of the 10-, 20-, 50-, and 100-Hz stimulations tested, the 100-Hz stimulation was optimal for inducing long-term potentiation (LTP). OT paired with 100-Hz stimulation that only produced short-term potentiation enhanced LTP induction in a dose-dependent manner. OT-paired LTP was blocked by both the selective OT antagonist desGly-NH_2,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]-ornithine vasotocin and the *N*-methyl-D-aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonovaleric acid. These results indicate that OT can function as a gate to modulate the establishment of NMDA receptor-dependent LTP at the mitral-to-granule cell synapse in the AOB.

There are a number of ways to examine how an organism's interaction with its environment modifies its nervous systems. One of these has been the study of learning and memory mechanisms, an endeavor that has contributed significantly to the comprehensive understanding of the neural basis of behavior. In this context, one of the best-characterized forms of mammalian learning is the pheromonal recognition memory in mice. This memory is formed by newly mated female mice to the male pheromones that are present in the period immediately after mating. These pheromones are present in the urine of male mice and normally those of the mating male. When a female mouse impregnated by a male of one strain is exposed to pheromones from a male of another strain within 3 days after mating, the pregnancy is blocked and the female returns to estrus, a phenomenon first reported by Bruce [9]. Pheromones of a mating male have the capacity to block pregnancy by a male of a different strain while not blocking pregnancy that he himself has induced. Of particular interest to us are the mechanisms by which the female recognizes the mating male's pheromones and gates their effects so as to maintain the pregnancy [1,2,4,5,7,8,16,20,24].

Pheromonal memory is acquired with one-trial learning, depends upon the occurrence of mating, and lasts for several weeks [26,31]. The neural changes underlying the memory occur in the accessory olfactory bulb (AOB), the first relay in the vomeronasal system [3,6,25,34,35]. Microcircuits in the AOB include the prominent dendrodendritic reciprocal synapse between mitral cells, a single class of projection neurons, and granule cell interneurons. Glutamate released from mitral cell dendrites activates the dendrites of granule cells, which in turn mediate GABAergic dendrodendritic inhibition back onto mitral cell dendrites [17,42]. This feedback inhibition at the reciprocal synapses regulates mitral cell activity [17,42]. The formation

of the pheromonal memory requires the association between the pheromonal and mating signals in the AOB. The mating signal is conveyed, at least in part, by noradrenergic projections to the AOB. Artificial vaginocervical stimulation [37] or mating [6] promotes the release of noradrenaline in the AOB. The blockade of α-adrenoceptors in the AOB immediately after mating [23] prevents the formation of pheromonal memory, as does removal of noradrenergic innervation of the AOB prior to mating [37]. Furthermore, memory formation is associated with neurochemical and morphological changes at the mitral-granule cell reciprocal synapses [6,34,35].

Like noradrenaline, oxytocin (OT), is released within the central nervous system in response to vaginocervical stimulation [29,30,38]. Centrally released OT, a major source of which is the paraventricular nucleus (PVN) of the hypothalamus, has been implicated in a number of mnemonic and social processes including reproductive and parental behaviors, the formation of social bonds, the management of stressful experiences, and trust in social interaction [10,13,14,18,27,28,32,33,41,43,44]. Recently, mapping OT receptor gene expression in OT receptor knockout-lacZ reporter mice revealed the expression of OT receptors in the AOB [15]. This raises the possibility that OT is involved in the synaptic plasticity associated with pheromonal recognition memory. The purpose of this study was to attempt to answer, using electrophysiological methods, the following questions. (1) Does plasticity, in the form of long-term potentiation (LTP), occur at the mitral-to-granule cell synapse in parasagittal slices of the AOB? (2) If so, what stimulation frequency is optimal for inducing LTP? (3) Does OT modulate LTP induction?

Balb/c mice (postnatal days 21-42) were anesthetized with diethyl ether and decapitated. The AOB, together with the main olfactory bulb, was rapidly dissected out

from the skull and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1.0 $MgSO_4$, 2.5 $CaCl_2$, 26 $NaHCO_3$, and 11 D-glucose (pH 7.3-7.4). Parasagittal slices (350 μm) were prepared, incubated in oxygenated ACSF at 32 ± 1°C for at least 1 h and then transferred into a recording chamber for field potential recordings at room temperature (22-26°C). Oxygenated ACSF was continuously perfused through the chamber at 1.0-1.5 ml/min. All procedures were approved by the Kochi Medical School Animal Care and Use Committee.

A stainless steel concentric bipolar stimulating microelectrode (USK-10: inner diameter 30 μm, outer diameter 100 μm with two 50 μm contacts separated by 50 μm insulation; Unique Medical, Tokyo, Japan) was positioned on the lateral olfactory tract (LOT) to stimulate the axons of mitral cells antidromically. A glass microelectrode (~2 MΩ) was placed in the anterior or posterior subdivisions of the external plexiform layer (EPL) to record field excitatory postsynaptic potentials (fEPSPs). Baseline fEPSPs were elicited every 30 s by applying a stimulus intensity (0.03-0.08 mA, 50 μs duration) sufficient to elicit an fEPSP slope that was 50-60% of the maximum.

Evoked field potentials were recorded, digitized at 10 kHz, and analyzed using the PowerLab/4sp system (ADInstruments, Castle Hill NSW, Australia). One-minute records (two traces) were averaged. The maximal initial slope of the fEPSP was measured to monitor the strength of synaptic transmission. Unless otherwise noted, the magnitude of potentiation was calculated by comparing the average of fEPSP slope measurements between 150 and 160 min after the completion of tetanic stimulation with the average for the 10 min preceding tetanic stimulation or drug application. Potentiation was elicited with the same intensity and pulse duration as the test stimuli.

Data are expressed as mean ± S.E.M. A statistical comparison of the averages was performed by ANOVAs followed by a post-hoc analysis using the Tukey-Kramer test. Significance was assessed at $p < 0.05$.

The drugs used in this study included 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), DL-2-amino-5-phosphonovaleric acid (AP5), and OT, all from Sigma (St. Louis, MO, USA), tetrodotoxin (TTX) from Latoxan (Valence, France), and the selective OT antagonist desGly-NH_2,d$(CH_2)_5$[Tyr(Me)^2,Thr^4]-ornithine vasotocin (desOVT) kindly provided by Dr M. Manning (Medical University of Ohio, Toledo, OH, USA).

The mitral cell output neurons of the AOB project mainly to the amygdala via the LOT. First, we analyzed field potentials elicited by electrical stimulation of the LOT in parasagittal slices of the mouse AOB. LOT stimulation, while recording from the EPL containing the dendrodendritic reciprocal synapses, evoked two sequential deflections similar to those reported in the rat [17] (Fig. 1). Immediately after the stimulus artifact, there is a brief negativity, which represents antidromic activation of the mitral cells. This is followed by a slower negative deflection, which represents an EPSP evoked in the granule cells, as the corresponding potential has been shown to closely correlate with the intracellularly recorded EPSP [17]. As expected, the synaptic response was little affected by the *N*-methyl-D-aspartate (NMDA) receptor antagonist AP5, but completely blocked by the non-NMDA receptor antagonist CNQX. The initial response, however, was resistant to both AP5 and CNQX, but eliminated by the sodium channel blocker TTX (Fig. 1B).

Second, we tested the effectiveness of four different stimulation frequencies on LTP induction by applying 10-, 20-, 50-, and 100-Hz stimulations, each consisting of

20-pulse trains (except for the 100-Hz stimulation, which consisted of 100-Hz trains) spaced 3-min apart (Fig. 2). Neither 200 nor 400 pulses at 10 Hz was effective at inducing LTP. In contrast, 200 pulses at 20, 50 or 100 Hz induced only short-term potentiation of the synaptic responses that decayed back to their control values, whereas 400 pulses at each of these three stimulation frequencies induced LTP that remained potentiated for at least 3 h. The magnitudes of LTP induced by the 20-, 50-, and 100-Hz stimulations were 149 ± 4% (n = 8), 153 ± 11% (n = 5) and 165 ± 9% (n = 10), respectively (Fig. 2).

Since OT has been implicated in social memory, we reasoned that OT might promote the induction of LTP as the leading biological model of memory formation. Finally, we tested this possibility by pairing OT with 100-Hz stimulation, which was optimal for LTP induction (Fig. 3). Indeed, robust LTP was induced when sub-threshold stimulation (200 pulses at 100 Hz) that only produced short-term potentiation was paired with OT. OT itself had no effect on synaptic transmission in the absence of tetanic stimulation. OT-paired LTP was induced in a dose-dependent manner and blocked by the selective OT antagonist desOVT. OT-paired LTP was also blocked by the NMDA receptor antagonist AP5, indicating that this LTP is dependent on the NMDA receptor.

The present study demonstrates that OT facilitates the induction of NMDA receptor-dependent LTP at the mitral-to-granule cell synapse via the activation of the OT receptor in the AOB. This raises several important questions. First, is OT actually involved in the pheromonal memory in the context of pregnancy block? The formation of this memory requires prolonged pheromone exposure during a sensitive period of about 3-4 hours immediately after mating. Whether or not a female infused with the OT

antagonist desOVT into the AOB immediately after mating fails to form pheromonal memory can be tested by re-exposing the female to the impregnating male for 48 h beginning the day following mating: if the pregnancy is blocked by this re-exposure, it can be said that desOVT has interfered with memory formation. Our previous study showed that the activation of the metabotrophic glutamate receptor mGluR2 in the AOB leads to the formation of pheromonal memory, independently of noradrenaline [21]. Given that OT is implicated in pheromonal memory, our findings support a model in which pheromonal memory is mediated by a redundant mechanism involving noradrenaline- and OT-coupled, NMDA receptor-dependent and mGluR2-dependent pathways. Redundant systems would improve signal-to-noise, leading to a robust memory that is resistant to interference.

Second, what is the route by which OT originating in the PVN exerts its effect on AOB neurons? One such route might consist of direct projections of OT fibers from the PVN to the AOB. Scattered OT fibers have been observed in the main olfactory bulb [39]. However, convincing evidence for the presence of OT fibers in the AOB has not appeared. A second route might be the cerebrospinal fluid (CSF). Morphological studies have revealed OT fiber endings in the immediate vicinity of the cerebroventricular walls close to the PVN, and some of these endings seem to penetrate into the ventricle [39]. Electrical stimulation of the rat PVN elicits a release of OT in the CSF [19]. Our previous studies suggested that activation of the PVN releases OT into the CSF and that the CSF OT reaches the main olfactory bulb, where the OT induces a rapid onset of maternal behavior in rats [45,46]. A third route might be the systemic circulation. It is generally recognized that OT does not cross the blood-brain barrier. However, complicating factors are the presence of an OT-like peptide and the appearance of

conversion products of OT that possibly exert their effects on AOB neurons [14]. Moreover, OT fragments such as OT-(1-6) or OT-(7-9) could cross the blood-brain barrier more easily [12].

Third, does OT act on the presynaptic or postsynaptic site of the mitral-to-granule cell synapse? The OT receptor-LacZ reporter mouse exhibited OT receptor-expressing neurons in regions that have not been described previously [15]. These new sites include the AOB. However, there is no convincing evidence regarding the cellular localization of OT receptors in the AOB. Our previous study showed that OT enhances presynaptic and postsynaptic glutamatergic transmission between rat main olfactory bulb neurons in culture [36]. Therefore, the possibility that OT may act on the presynaptic and postsynaptic sites of the mitral-to-granule cell synapse in the AOB must be considered. Additionally, the OT receptor expression reported was from animals 12 h following parturition, which may have induced OT receptor expression. The present results imply that OT receptor expression is not restricted to this post parturition period.

Finally, why is the optimal stimulation frequency for LTP induction different between parasagittal and coronal slices? The results presented here show that the effective induction of LTP in parasagittal slices is achieved at 100 Hz and not at 10 Hz. This contrasts with the finding that LTP in coronal slices is induced at 10 Hz but not at 100 Hz [22]. As the anterior and posterior AOB may well be functionally separate [40], this could be a factor influencing the frequency dependence of LTP induction. However, we failed to show a relationship between them. Another factor might be a strict balance between excitatory and inhibitory inputs that is essential for the normal functioning of the central nervous system. However, transient changes in this balance are necessary to allow long-term synaptic modifications to occur. Such beneficial alterations in synaptic

function usually result from specific patterns of repetitive afferent input, which transiently up- and down-regulate excitatory and inhibitory synaptic transmission in a frequency-dependent manner [11]. Although speculative, slice orientation might influence the strict balance between excitatory and inhibitory inputs to the granule cells in the AOB, leading to a change in the optimal stimulation frequency for LTP induction.

Acknowledgements

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Figure legends

Fig. 1. Schematic diagram of major synaptic interconnections in the AOB and pharmacological properties of LOT-evoked field potentials recorded in the EPL of a parasagittal AOB slice.

(A) Positions of the recording (Rec) and stimulating (Stim) electrodes in the AOB are depicted. GC, granule cell; GL, glomerular layer; Glu, glutamate; GRL, granule cell layer; IPL/LOT, internal plexiform layer/lateral olfactory tract; MC, mitral cell; VNL, vomeronasal nerve layer. (B) Stimulation of the LOT evokes negative field potentials, N_1 and N_2. Only TTX (1 μM) blocks N_1, whereas N_2 is blocked by CNQX (30 μM) but not by AP5 (50 μM). Calibration: 10 ms, 1 mV.

Fig. 2. Comparison of potentiation induced by different stimulation frequencies.

(A) 10 (○, n = 11) and 20 (●, n = 6) trains of 10-Hz, 20-pulse stimulation. (B) 10 (○, n = 8) and 20 (●, n = 8) trains of 20-Hz, 20-pulse stimulation. (C) 10 (○, n = 8) and 20 (●, n = 5) trains of 50-Hz, 20-pulse stimulation. (D) Two (○, n = 9) and four (●, n = 10) trains of 100-Hz, 100-pulse stimulation. Note that 100-Hz stimulation produces stable and maximal LTP. In this and the following figures, arrows represent the times at which tetanic stimulation started.

Fig. 3. OT enhances the induction of NMDA receptor-dependent LTP via the oxytocin receptor.

(A) OT administered in the absence of tetanic stimulation elicits no change in synaptic transmission (96 ± 3%, n = 11). OT paired with two trains of 100-Hz stimulation that

only produced short-term potentiation results in the generation of robust LTP in a dose-dependent manner (156 ± 8% for 2 μM OT, n = 12; 119 ± 1% for 0.2 μM OT, n = 10). Both the selective OT antagonist desOVT (2 μM, 100 ± 3%, n = 9) and the NMDA receptor antagonist AP5 (50 μM, 104 ± 4%, n = 8) block OT-paired LTP. The inset shows superimposed traces taken at the indicated times in a 100-Hz + 2 μM OT experiment. The horizontal bar indicates drug application. (B) Summary bar graph depicting levels of potentiation seen in 150-160 min post-tetanic stimulation in the presence of OT and selective antagonists. $^{**}P < 0.01$ when compared with control (2 trains of 100-Hz, 100-pulse stimulation, data from figure 2D) and all other experiments, one-way ANOVA with Tukey-Kramer post-hoc test. There is no significant difference between 0.2 μM OT and control.

Figure1

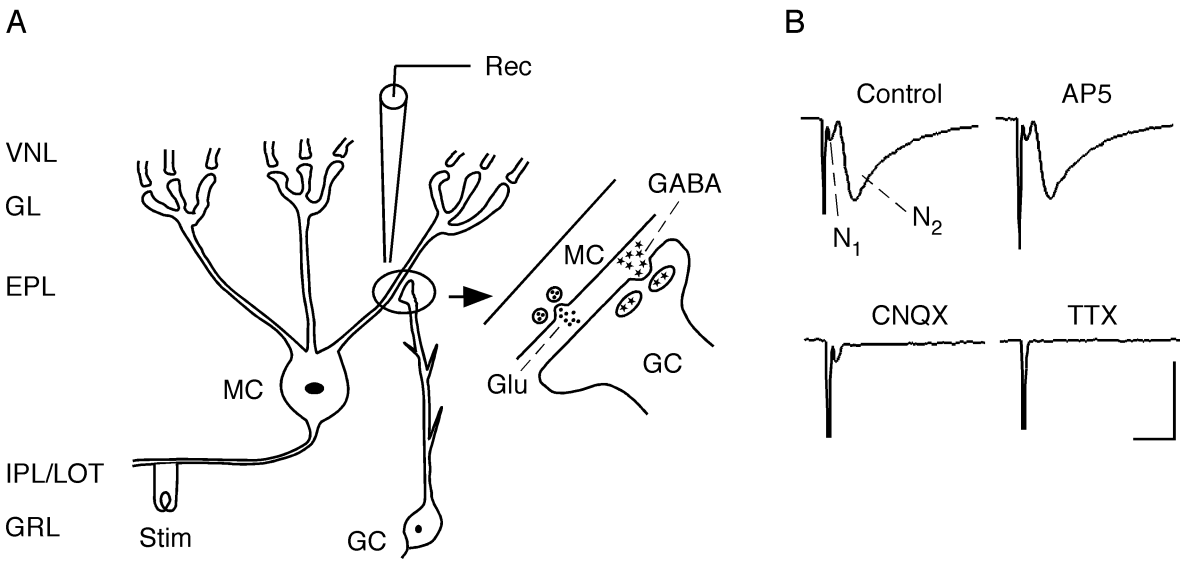
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Figure 2

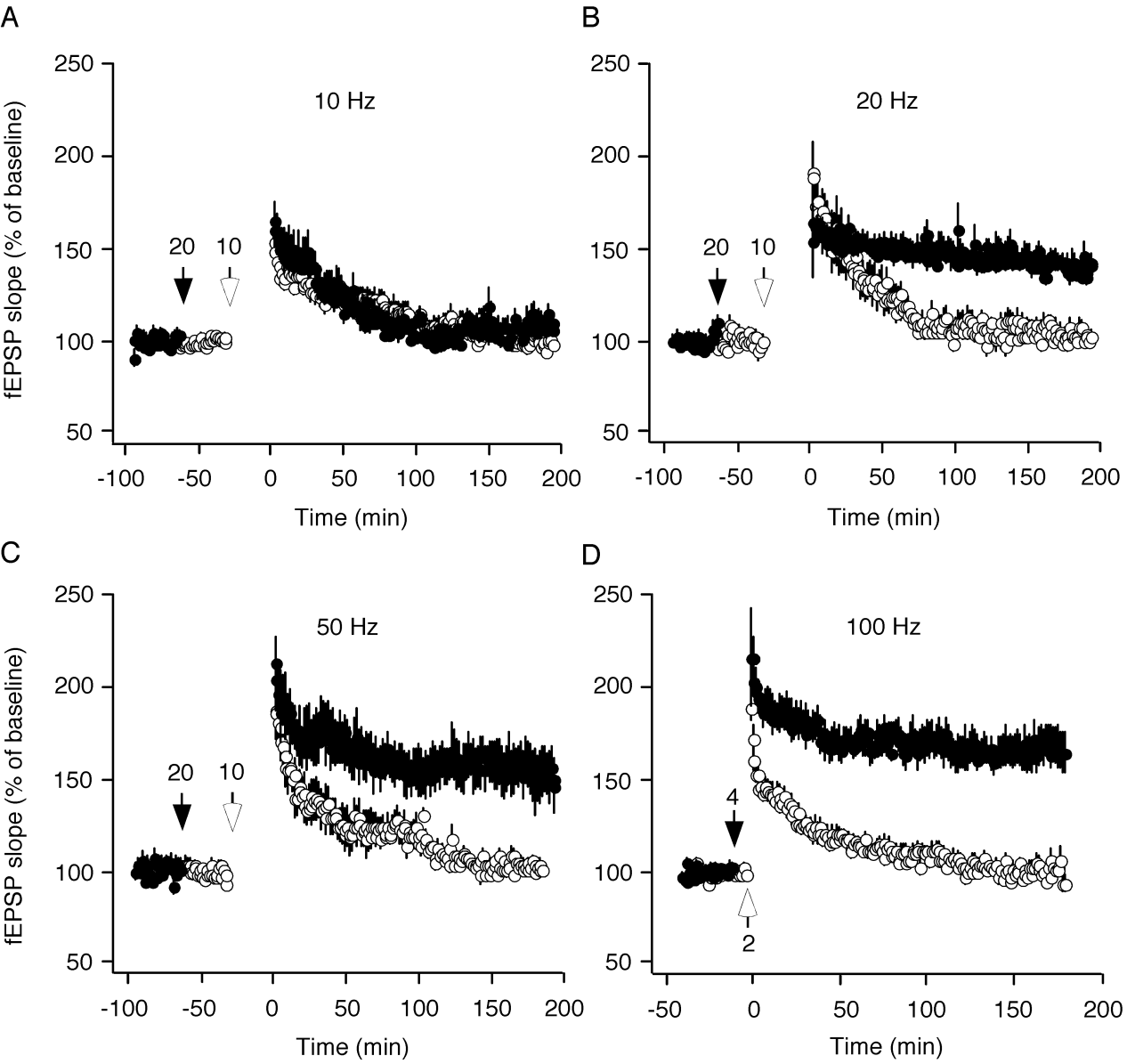
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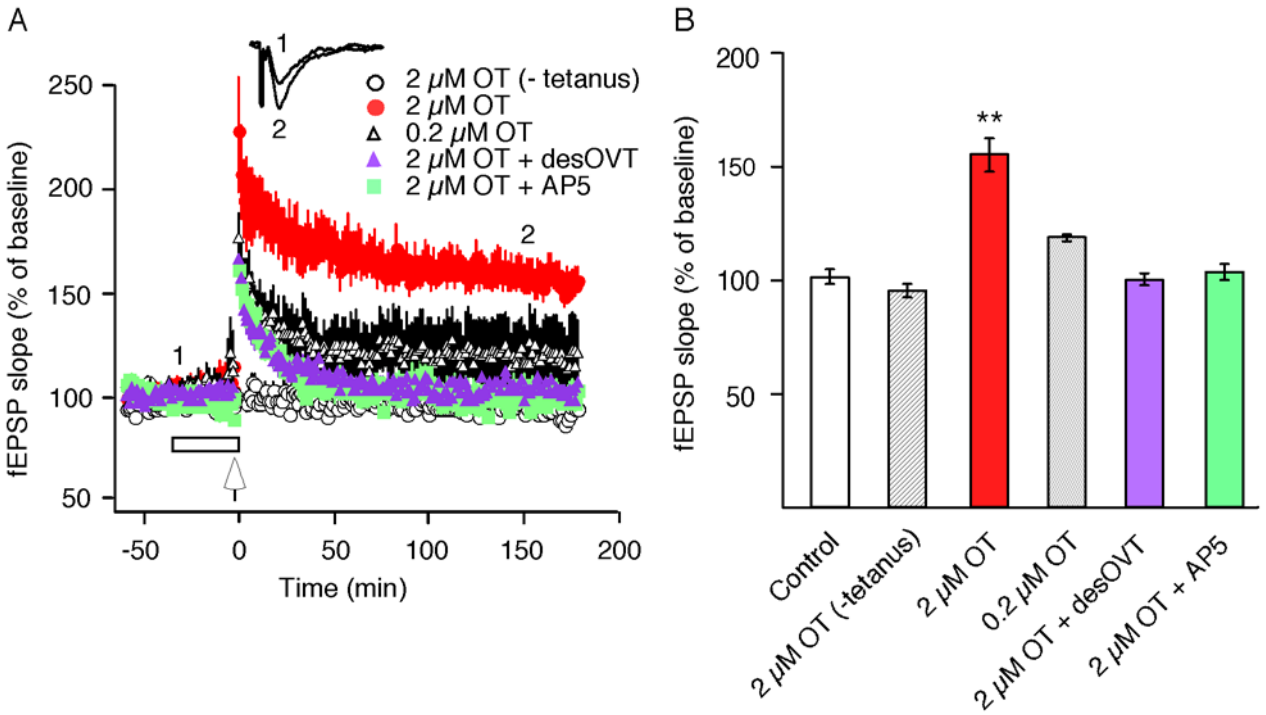
Figure 3


A
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2 μM OT (- tetanus)
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2 μM OT + desOVT
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-50
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50
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150
200
Time (min)

B
fEPSP slope (% of baseline)
200
150
100
50
**
Control
2 μM OT (-tetanus)
2 μM OT
0.2 μM OT
2 μM OT + desOVT
2 μM OT + AP5