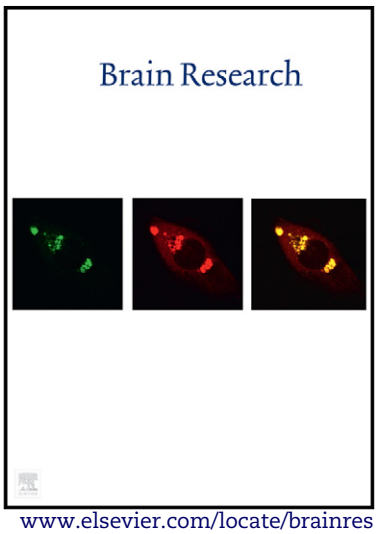

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PII: S0006-8993(15)00596-X
DOI: http://dx.doi.org/10.1016/j.brainres.2015.07.051
Reference: BRES44395

To appear in: *Brain Research*

Accepted date:
30 July 2015

Cite this article as: Shukhrat S. Uzakov, Andrey D. Ivanov, Sergey V. Salozhin, Vladimir A. Markevich, Natalia V. Gulyaeva, Lentiviral-mediated overexpression of nerve growth factor (NGF) prevents beta-amyloid [25–35]-induced long term potentiation (LTP) decline in the rat hippocampus, *Brain Research,* http://dx.doi.org/10.1016/j.brainres.2015.07.051

Research Report

Lentiviral-mediated overexpression of nerve growth factor (NGF) prevents beta-amyloid [25-35]-induced long term potentiation (LTP) decline in the rat hippocampus

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Running title: Protective impact of NGF against acute toxic action of Aβ.

Abstract

We have explored the potential neuroprotective effect of local lentiviraly-mediated overexpression of nerve growth factor (NGF) on *in vivo* long-term potentiation (LTP) in the rat hippocampus under pathological conditions. The suspension of lentiviral particles was prepared using a genetic construct containing the human NGF gene under the control of a neuron-specific CaMKII promoter. Two weeks after the viral injection NGF concentration in the hippocampus doubled. *In vivo* recordings of total electrical activity in the dentate gyrus were performed. While the increased expression of NGF did not affect the amplitude of evoked postsynaptic potentials recorded after a high-frequency stimulation of the perforant path, it prevented the LTP decline induced by the i.c.v. administration of 50 nM beta-amyloid (25-35) 1 hour prior to tetanization. Our results demonstrate that increased endogenous NGF concentration can rescue hippocampal neuronal function from beta-amyloid peptide induced impairment.

Keywords: hippocampus; plasticity; LTP; beta-amyloid; NGF; neurotrophins; lentivirus.

1. Introduction

Alzheimer's disease (AD) is a widespread incurable neurodegenerative disorder leading to memory loss, cognitive functions decline, and premature death. The pathogenesis of AD is believed to be associated with the abnormal metabolism of the amyloid precursor protein yielding to beta-amyloid (Aβ) peptide oligomers accumulation. Aβ is found in the senile plaques in AD brains, but soluble isomers of Aβ are also present in neurons (Castellani et al., 2008). The majority of researchers agree that Aβ is directly involved in neuronal degeneration and synaptic plasticity failure in AD (Cullen et al., 1996; Chen et al., 2000; Freir et al., 2001; Fukuta et al., 2001; Kim et al., 2003; Garcia-Rates et al., 2013). Most of the *in vivo* and *in vitro* models of AD are based on alteration of amyloid precursor protein metabolism and accumulation of Aβ (Larson et al., 1999; Moechars et al., 1999; Chapman et al., 2001; Rowan et al., 2003). Soluble Aβ oligomers were repeatedly shown to induce long-term potentiation (LTP) impairment in both the dentate gyrus and the CA1 region of the hippocampus (Wang et al., 2002; Kimura et al., 2012). The possible mechanisms of this effect may include abnormal [Ca^{2+}] elevation, AMPA receptors phosphorylation and trafficking corruption and GSK-3 kinase activation (Hsiesh et al., 2006; D'Amelio et al., 2011; Hu et al., 2014).

Potential therapeutic strategies for AD include delivery of an adequate and long lasting trophic support to the neurons in affected brain regions. Neurotrophic factors, in particular, neurotrophins belong to the group of secretory proteins essential for CNS development and functioning. Of the major mammalian neurotrophins nerve growth factor (NGF) is the most common and well studied. NGF controls the differentiation of vast majority of the CNS neurons during ontogenesis, while in the adult brain it ensures normal synaptic plasticity and survival of mature neurons. In rat brain NGF augmentation facilitates LTP induction, while NGF blockade inhibits LTP and spatial memory (Conner et al., 2009).

It has been shown that neurotrophins, especially NGF and BDNF, can have a neuroprotective effect in AD-like conditions (Wang et al., 2002; Skaper, 2008; Bruno et al., 2009). NGF injection prevents degeneration of cholinergic neurons after fornix lesion or administration of toxins (Williams et al., 1986; Koliatsos et al., 1990; Charles et al., 1996; Blesch et al., 2005; Skaper,

2008). Successful experiments on NGF-producing fibroblasts transplantation into the affected brain structures were reported (Rosenberg et al., 1988; Chen, Gage, 1995; Tuszynski et al., 1996). These data suggest that elevation of NGF in the hippocampus can prevent plasticity failure induced by Aβ. Here we show that lentiviral-mediated hNGF gene expression in the rat hippocampus significantly increases NGF concentration and exerts a neuroprotective effect preventing Aβ-induced failure of LTP induction and maintenance.

2. Results

2.1. Aβ-peptide reduces LTP in vivo

To study the dose-dependent negative effect of Aβ[25-35] administration on LTP dynamics we injected Aβ in the right lateral ventricle 1 hour prior to HFS. Three different concentrations of Aβ[25-35] were used: 25 nM, 50 nM, and 100 nM. None of them showed any effect on the baseline PS evoked by low frequency (0.033 Hz) stimulation.

Animals from the naive group demonstrated strong HFS-induced LTP with PS amplitude 354 ± 33% (n=9, mean ± SE, percent to baseline). Aβ[25-35] significantly impaired LTP even at the lowest dose of 25 nM: 218 ± 19% (n=8, $F_{1,15}$=15.00, P=0.0015 as compared to the naive control group). At higher doses (50 or 100 nM) Aβ[25-35] induced a further dose-dependent LTP decrease: 143 ± 15% (n=9, $F_{1,16}$=40.75, P=0.00001 as compared to the naive control group) and 121 ± 12% (n=8, $F_{1,15}$=48.81, P=0.00001 as compared to the naive control group) respectively (Fig.1).

Group	n	PS amplitude (% of baseline) after LTP induction, M±SEM	Inter-group significance (ANOVA) as compared to the naive control group
naïve (saline)	9	354 ± 33	-
Aβ [25-35] 25nM	8	218 ± 19	$F_{1,15}$=15.00, P=0.0015
Aβ [25-35] 50nM	9	143 ± 15	$F_{1,16}$=40.75, P=0.00001
Aβ [25-35] 100nM	8	121 ± 12	$F_{1,15}$=48.81, P=0.00001
Aβ [35-25] 100nM	7	270 ± 34	Non significant

Table 1. Dose-dependent Aβ-induced impairment of *in vivo* LTP in the dentate gyrus.

Administration of the reverse peptide Aβ[35–25] (100 nM) 1 hour prior to the HFS had no significant effect on the baseline PS as well as on the LTP (270 ± 34% n=7, non significant when compared to the saline control group). No statistically significant difference was observed between Aβ[25-35] 25 nM and Aβ[35–25] 100 nM treated groups, as well as between 50 and 100 nM Aβ[25-35] treated groups. However, a significant difference was demonstrated between the effects of Aβ[35–25] (100 nM) and Aβ[25-35] (50 or 100 nM) ($F_{1,14}$=14.39, P=0.002 and $F_{1,13}$=20.06, P=0.0006 respectively). Based on these results we have chosen the 50 nM concentration for the further experiments.

2.2. NGF overexpression protects in vivo LTP against Aβ-induced impairment

A lentiviral delivery of NGF gene to hippocampus was performed to investigate whether NGF interferes with Aβ-induced LTP impairment. pCaMKII or pCaMKII-NGF constructs containing enhanced green fluorescent protein (EGFP) alone or in combination with human NGF were used for preparation of high-titer lentiviral suspensions. Two weeks prior to the electrophysiological experiments the suspensions were injected unilaterally into the right dentate gyrus of experimental animals, while Aβ or saline was injected in the right ventricle one hour before HFS.

To evaluate the impact of NGF overexpression itself on hippocampal LTP in normal conditions we compared LTP dynamics in sham + saline and pCaMKII + saline control groups with the pCaMKII-NGF + saline experimental group (Fig. 2). Mean PS amplitudes were the following: 191 ± 12% n=6, 183 ± 10% n=7 and 257 ± 22% n=8. No statistically significant difference was found between both control groups' results, while the experimental group significantly differed from them ($F_{1,12}$=6.61, P=0.025 and $F_{1,13}$=9.45, P=0.009 respectively).

Group	n	PS amplitude (% of baseline) after LTP induction, M±SEM	Inter-group significance (ANOVA) as compared to the naive control group
Aβ [25-35] 50nM	9	143 ± 15	Non significant
pCaMKII + Aβ	12	123 ± 9	-
pCaMKII + saline	7	183 ± 10	$F_{1,17}$=29.24, P=0.00005
pCaMKII-hNGF + Aβ	11	217 ± 23	$F_{1,17}$=19.92, P=0.0002
pCaMKII-hNGF + saline	8	257 ± 22	$F_{1,17}$=47.33, P=0.00001
Sham + saline	6	191 ± 12	$F_{1,17}$=34.27, P=0.00002

Table 2. NGF overexpression impact on *in vivo* LTP in normal conditions and under Aβ-induced impairment.

As mentioned above, administration of 50 nM Aβ[25-35] resulted in significant impairments in LTP PS amplitude in naïve rats (143 ± 15% n=9). Similar effect was observed in the control pCaMKII + Aβ group where PS amplitude after 50 nM Aβ injection (123 ± 9% n=12) was close to that observed in the naïve + Aβ group animals (non significant).

However, animals of the experimental pCaMKII-NGF + Aβ group demonstrated much stronger LTP in the same conditions (217±23% n=11; $F_{1,21}$=19.92, P=0.0002 as compared to the pCaMKII + Aβ group). This result can be explained by either a protective effect of NGF overexpression or by NGF-mediated LTP magnitude enhancement occurring independently from Aβ impairment. The second possible explanation seem more realistic as the difference between the experimental pCaMKII-NGF + saline and pCaMKII-NGF + Aβ did not reach statistical significance, whereas the Aβ-induced impairment LTP in the control pCaMKII group was significant ($F_{1,17}$=29.24, P=0.00005). These data suggest that elevated concentrations of NGF in the transduced hippocampus induce higher activation of TrkA-dependent signaling interfering with the effect of Aβ.

2.3. NGF levels increase as a result of the leniviral transduction

To prove the transduction efficacy and estimate hippocampal levels of NGF we performed an ELISA assay. After the electrophysiological experiments hippocampi were removed, its left and right parts dissected and homogenized separately. The assay revealed a nearly double increase in NGF concentration in the hippocampi of the experimental pCaMKII-NGF group (367.9±24.2 pg/ml; $F_{3,12}$=9.84, P=0.001) as compared to its contralateral hippocampi (218.0±14,1 pg/ml), as well as compared to the control pCaMKII group (188.7±26.4 pg/ml and 165.8±43.4 pg/ml for lentivirally transduced and control hippocampi, respectively) (Fig. 4).We conclude that the lentiviral transduction resulted in a strong and sustained increase of NGF levels in the hippocampi of the experimental animals.

3. Discussion

A number of reports suggest that a long-term increase in neurotrophin concentration can rescue hippocampal neurons from AD-related pathology (see Lu et al., 2013 for review). While brain derived neurotrophic factor (BDNF) is believed to directly affect synaptic transmission, acting as a trigger of the transcription dependent, late phase of LTP (Lu et al., 2008), the putative role of NGF is related to neuronal survival and protection as well as normal plasticity maintenance (Conner et al., 2009). This study is the first to show the consequences of a permanent long-term NGF elevation in the *in vivo* model of Aβ toxicity.

We used Aβ [25-35] to induce amyloid toxicity. Several groups demonstrated that basal synaptic transmission was unaffected by Aβ during the 1 h pre-HFS registration period (Cheng et al., 2009; Zhang et al., 2009; Guo et al., 2010). Aβ can reduce baseline hippocampal transmission only 24 h after an *i.c.v.* injection (Cullen et al., 1996, 1997; Rowan et al., 2003). A strong dose-dependent negative effect of Aβ[25-35] on LTP generation and maintenance observed in our experiments confirms the data received previously by other groups (Gault, Hölscher. 2008; Jing et al.. 2009).

The effect of Aβ on LTP induction was evident already 15 min after the *i.c.v.* injection (Freir et al., 2001; Gengler et al., 2007). To investigate interactions of NGF-dependent signaling with Aβ-induced pathological mechanisms we used a lentiviral-mediated gene delivery expressing human NGF gene in hippocampus. A double increase in NGF concentration in the hippocampi was

achieved. This continuous NGF overexpression prevented Aβ-induced failure of the *in vivo* LTP induction. It should be noted that animals of both control and experimental groups demonstrated weaker LTP than the animals of the naïve group, and this could be due to a surgery-related damage. However, in the NGF-overexpressing group injected with Aβ, LTP was almost as high as in the experimental group without Aβ, while in the control group, administration of Aβ robustly impaired LTP. We must also note that in a recent paper by our group we did not observe any visible impact of NGF overexpression on cholinergic transmission in acute brain slices *in vitro* (Ivanov et al., 2015). This may lead to a conclusion, that in the present *in vivo* work the NGF protective effect is TrkA-dependent and occurs simultaneously with the hippocampal cholinergic system over-activation.

The neuroprotective function of NGF and its clinical relevance has been described in numerous papers (see Aloe et al., 2012 for review). NGF synthesis stimulation by propentofylline was shown to partially compensate Aβ-induced learning and memory deficits in rats (Yamada et al., 1998).

In Yamada's work NGF-dependent signaling was stimulated indirectly, that could have some extra unknown consequences and/or side effects. Taking into account the current progress of molecular and biochemical methods, we suppose that a direct increase of NGF concentration via viral transduction is now more perspective. Moreover, Yamada's group focused on long-term learning and memory disruption by Aβ administration. It is well-known, that Aβ can have both long-term and acute short-term pathological action in the CNS (Yamada, Nabeshima, 2000; Rowan et al., 2003). Long-term neurotoxicity is associated with Aβ aggregation into fibrils, which easily form amyloid plaques and provoke oxidative stress and apoptosis (Trubetskaya et al., 2003). Soluble Aβ in non-toxic by itself, but compromises cholinergic transmition by alternating cellular ionic metabolism and suppressing acetylcholine synthesis, uptake and release (Hoshi et al., 1997; Kar et al., 1998). Continuous i.c.v. infusion of Aβ used in Yamada's team experiments clearly leaded to chronic neurotoxicity and neurodegeneration. The observed protective effect of NGF synthesis stimulation by propentofylline can be thus associated with some sort of compensatory circuits alteration, as Aβ-induced ChAT activity reduction in the rat hippocampuses was not rescued.

In our work, we modeled an acute and rapid synaptic plasticity disruption by Aβ. Our results correspond well with Yamada's paper, but reveal another aspect of NGF neuroprotecive action. We can assume that NGF protective effect may rescue neuronal function both under acute impairment and during chronic pathogenesis. The rapid protective effect can be related to ionic metabolism maintenance and cholinergic transmition rescue. Long-term neuroportection may be explained by oxidative stress prevention or increased compensatory circuit alterations potential.

4. Experimental Procedure

4.1. Animals

Seventy nine adult male Wistar rats 350–450 BW supplied by the 'Stolbovaya' Breeding Center (Moscow, Russia) were used in this study. The animals were housed five per cage under a day-night cycle 12h:12h with free access to food and water. The experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by IHNA Bioethics Commission. All efforts were made to minimize animals' suffering and to reduce the number of animals used.

4.2. Plasmids synthesis and virus preparation

Lentiviral packaging plasmids pCSC, pVSV-G, pMDL, pRev and pLenti-CaMKIIa were a kind gift from Dr. Inder M.Verma (Salk Institute for Biological Studies, La Jolla, CA). Neuron-specific promoter CaMKIIa was cloned into the pCSC plasmid instead of CMV promoter by BamHI and ClaI restriction sites to generate control empty plasmid pCaMKII. Human NGF was amplified from IMAGE clone: 8991864 (OpenBiosystems) with primers with BamHI-sites that allowed subsequent cloning of the product under control of CaMKIIa promoter. Resultant clones were verified by restriction analysis and sequencing for proper orientation of insert and lack of mistakes in the coding sequence.

To check biological activity of cloned hNGF PC12 cells were transfected with pCaMKII or pCaMKII-NGF plasmid. Only transfection with pCaMKII-NGF induced growth of transfected cells' neurites.

The viral suspension was obtained by polyethylenimine co-transfection of ten 150 mm dishes of HEK293T cells with the following plasmids (per one dish): 12 μg of pCAMKII or pCaMKII-NGF, 7.8 μg of pMDL, 4.2 μg of pVSVG and 3 μg of pRev. Two supernatants collected 24 and 48 hours after a transfection were combined and filtered through a 0.45 μm filter, the viral suspension was concentrated by ultracentrifugation at 50000 x g for 2 hours, resuspended in 300 μl of OPTI-MEM, aliquoted and stored at - 80 ºC. Viral titer was determined by ELISA against p24 antigen.

4.3. Stereotaxic injections of lentiviral suspension

The rats were divided into 3 groups: naïve, control pCaMKII and experimental pCaMKII-NGF. Rats were anesthetized with 8% chloralhydrate solution intraperitoneally and fixed in a Kopf stereotaxis. A saggital cut of the skin was made from Bregma to Lambda and the upper surface of the skull was cleaned from periosteum and dried with 3% hydrogen peroxide. The coordinates of the injection sites were measured from Bregma according to Paxinos and Watson rat brain atlas (Paxinos, Watson, 2005). The holes in the skull were made manually using a hand-drill. 2 μl of concentrated titer-matched lentiviral suspension were injected by an automatic nanoinjector into the right dentate gyrus (AP –3.0 mm; L 2.0 mm; H 3.6 mm from dura) at a rate of 0,2 μl/min. The animals were taken into the experiment two weeks after the injection.

4.4. Aβ preparation and stereotaxic injection

The Aβ [25-35] fragment containing the "active" amino acid sequence responsible for Aβ neurotoxicity (Pike et al. 1995) was used. Aβ [25-35] and Aβ [35-25] as a control sequence (Bachem, Bubendorf, Switzerland) were dissolved in sterile bidistilled water.

The rats were divided in 5 groups: naïve (saline), Aβ [25-35] 25 nM, Aβ [25-35] 50 nM, Aβ [25-35] 100 nM and control Aβ [35-25] 100 nM. All injections were made according to the above protocol (paragraph 2.3). Five μl of Aβ or saline were injected in the right lateral cerebral ventricle (AP –0.8mm; L 1.5mm; H 3.8mm from dura) at a rate of 0.5 μl/min. The injections were performed 60 minutes prior to LTP induction.

4.5. Electrophysiology

Rats were anaesthetized with urethane (1.75 g/kg, i.p.). A monopolar recording electrode (80 μm insulated nichrome wire) was stereotaxically implanted into the hilus (AP –2.8; L 1.8; H 3.2-3.5 from dura) of the right dentate gyrus, a bipolar stimulation electrode was implanted into the right medial perforant path (AP –6.9; L 4.1; H 2.2-2.5 from dura). A 200 μm stainless steel ground electrode was implanted in the rat skull. Electrode plugs were fixed on the skull with dental cement and connected to a custom-made electrophysiological recording device consisting of an amplifier (bandpass 0.5-1000Hz), a multichannel stimulator, analog-to-digital and digital-to-analog converters and a PC.

Before each experiment, a test of the population-spike amplitude (PS) was performed and a calibration input/output curve was created. Biphasic constant current pulses (0.1 msec per half-wave, intensity between 0.13 and 0.2 mA) were applied to the perforant path in order to evoke dentate gyrus field potentials (5-20 mV) making up 40% of the maximum PS amplitude.

After recording a stable baseline for 1 h (stimulation frequency 1 test stimulus per 15 min), LTP was induced by high frequency stimulation (HFS, 8 bursts of 15 pulses each, 200 Hz, stimulus duration 0.1 msec, 10 sec interburst intervals) with the same stimulus intensity as used for the test stimuli (0.2–0.4 mA). Two minutes after tetanization and further every 15 minutes, trains of ten test stimuli with 10 seconds interpulse interval were applied. Field potentials were recorded during 4 hours after tetanization. The data was normalized to average baseline PS for each animal individually. Examples of individual fEPSPs before and after tetantisation are shown on fig. 3.

4.6. Transduction efficiency control

A biochemical control of viral transduction efficiency was performed after the electrophysiological experiments. To determine NGF concentrations in rat hippocampi a standard Enzyme-linked immunosorbent assay (ELISA) protocol was used.

The rats were decapitated immediately after the last PS recording, their brains were removed and frozen in liquid nitrogen. A Millipore CYT304 ChemiKine Nerve Growth Factor, Sandwich ELISA kit was used according to the manufacturer protocol. Briefly, hippocampi were homogenized in the

ice cold homogenization buffer 1:10. The supernatant (100 µl) was decanted and transferred into wells preconjugated with anti-NGF antibodies. The plate was incubated at +4oC overnight and then washed 5 times with the Millipore wash buffer. Primary mouse-anti-NGF antibodies were diluted 1:100 with the assay diluent immediately before use, and 100 µl of diluted antibodies were distributed in each well. The plate was incubated at +37oC for 3 hours on a shaker and then washed 5 times with the Millipore wash buffer. Secondary donkey-anti-mouse antibodies were diluted 1:1000 with the assay diluent immediately before use and 100 µl of diluted antibodies were distributed in each well. The plate was incubated at +37oC 3 hours on a shaker and then washed 5 times with the Millipore wash buffer. TBM/E solution (100 µl) was added into each well. After 10 min of incubation at room temperature, 100 µl of the stop solution was added into each well and the plate was immediately placed in Wallac Victor plate-reader. The plate was read using the Luminescence protocol (1sec, 450 nm), and the results were analyzed using a standard calibration curve of NGF concentration. All measurements were done in duplicates.

4.7. Statistical analysis

All data expressed as mean ± standard error. ANOVA was used for comparison between pre- and post- HFS population spikes.

Conclusion

NGF, naturally synthesized in the affected brain structures and acting through existing metabolic pathways, is regarded as a promising candidate for AD treatment. Our data demonstrating that hippocampal neurons can also be protected by *in vivo* derived NGF support this concept and can contribute to the rationale for the efficacy of genetic manipulation in modulation of exogenous neurotrophins in the brain.

Acknowledgements

The authors are grateful to Dr. Inder M.Verma (Salk Institute for Biological Studies, La Jolla, CA) for providing the lentiviral packaging plasmids and Dr. Alon Chen (Weizmann Institute, Rehovot, Israel) for sharing the lentiviral technology.

We greatly appreciate the help of Gulnur R. Tukhbatova (Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia) with plasmids synthesis and virus preparation.

The study was supported by RFBR (09-04-01-157-a and 12-04-32121 mol_a) and the Presidium of RAS ("Fundamental sciences for medicine" Program).

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Figure captions

Fig. 1. Dose-dependent impairment of in vivo LTP in the dentate gyrus induced by Aβ. 25 nM, 50 nM and 100 nM Aβ[25-35] or control 100 nM Aβ [35-25] was injected i.c.v. 1h prior HFS.

Fig. 2. NGF overexpression impact on in vivo LTP in normal conditions and under Aβ-induced impairment. Animals received either saline (control) or 50nM Aβ[25-35] i.c.v. injection 1h prior to LTP induction.

Fig. 3. Individual traces examples before (grey line) and after tetantisation (black line). Top row: naïve, Aβ [35-25] 100 nM, Aβ [25-35] 25 nM, Aβ [25-35] 50 nM, Aβ [25-35] 100 nM,. Bottom row: sham + saline, pCaMKII + saline, pCaMKII + Aβ, pCaMKII-NGF + saline, pCaMKII-NGF + Aβ.

Fig. 4. NGF concentration increase induced by leniviral transduction. In both experimental and control groups viral injections were performed in the right hippocampus, leaving the contralateral left hippocampus intact for control.

Group	n	PS amplitude (% of baseline) after LTP induction, M±SEM	Inter-group significance (ANOVA) as compared to the naive control group
naïve (saline)	9	354 ± 33	-
Aβ [25-35] 25nM	8	218 ± 19	$F_{1,15}$=15.00, P=0.0015
Aβ [25-35] 50nM	9	143 ± 15	$F_{1,16}$=40.75, P=0.00001
Aβ [25-35] 100nM	8	121 ± 12	$F_{1,15}$=48.81, P=0.00001
Aβ [35-25] 100nM	7	270 ± 34	Non significant

Table 1. Dose-dependent Aβ-induced impairment of in vivo LTP in the dentate gyrus.

Group	n	PS amplitude (% of baseline) after LTP induction, M±SEM	Inter-group significance (ANOVA) as compared to the naive control group

Aβ [25-35] 50nM	9	143 ± 15	Non significant
pCaMKII + Aβ	12	123 ± 9	-
pCaMKII + saline	7	183 ± 10	$F_{1,17}$=29.24, P=0.00005
pCaMKII-hNGF + Aβ	11	217 ± 23	$F_{1,17}$=19.92, P=0.0002
pCaMKII-hNGF + saline	8	257 ± 22	$F_{1,17}$=47.33, P=0.00001
Sham + saline	6	191 ± 12	$F_{1,17}$=34.27, P=0.00002

Table 2. NGF overexpression impact on in vivo LTP in normal conditions and under Aβ-induced impairment.

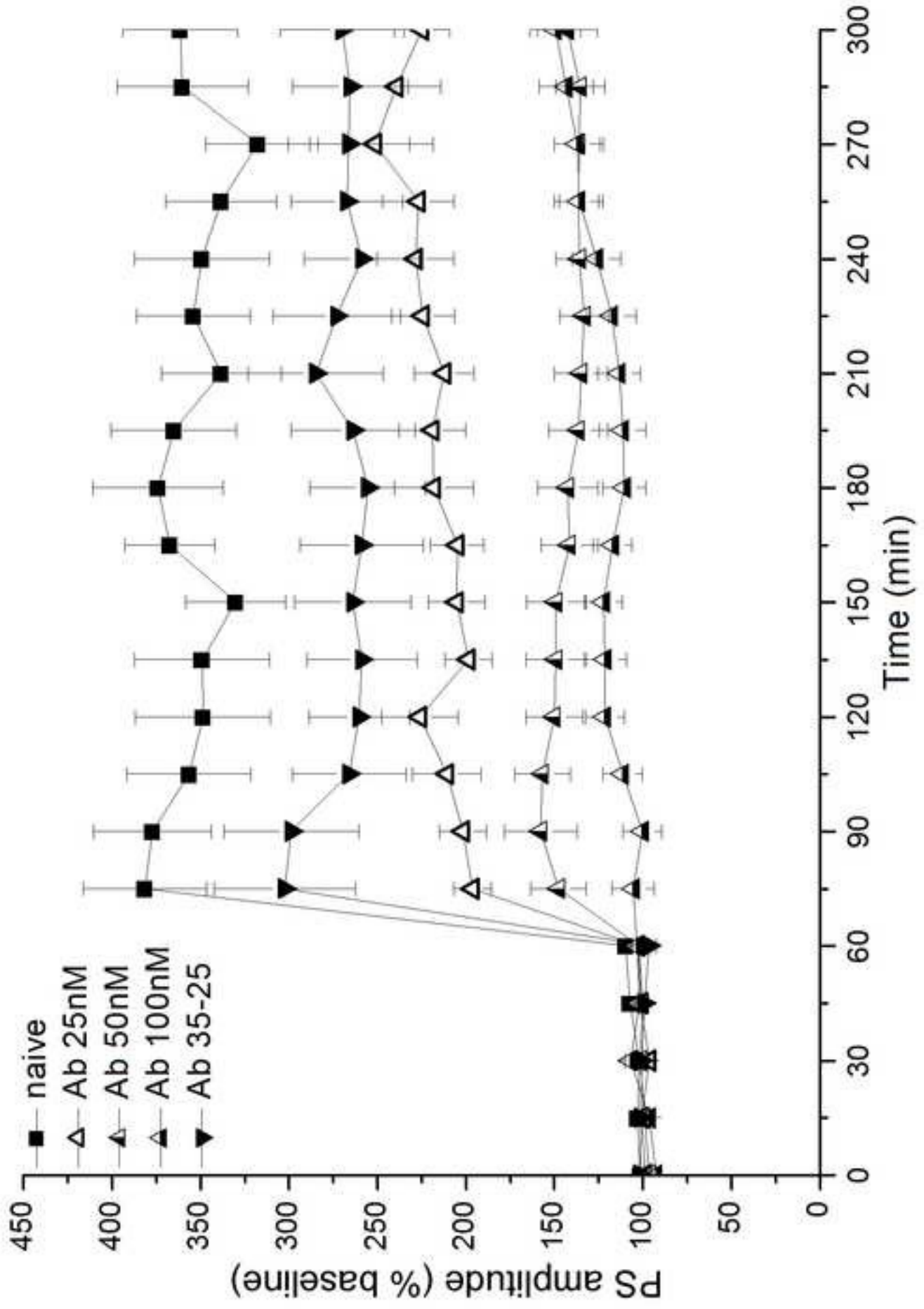

naive
Ab 25nM
Ab 50nM
Ab 100nM
Ab 35-25
PS amplitude (% baseline)
Time (min)
0
50
100
150
200
250
300
350
400
450
0
30
60
90
120
150
180
210
240
270
300

Figure 1

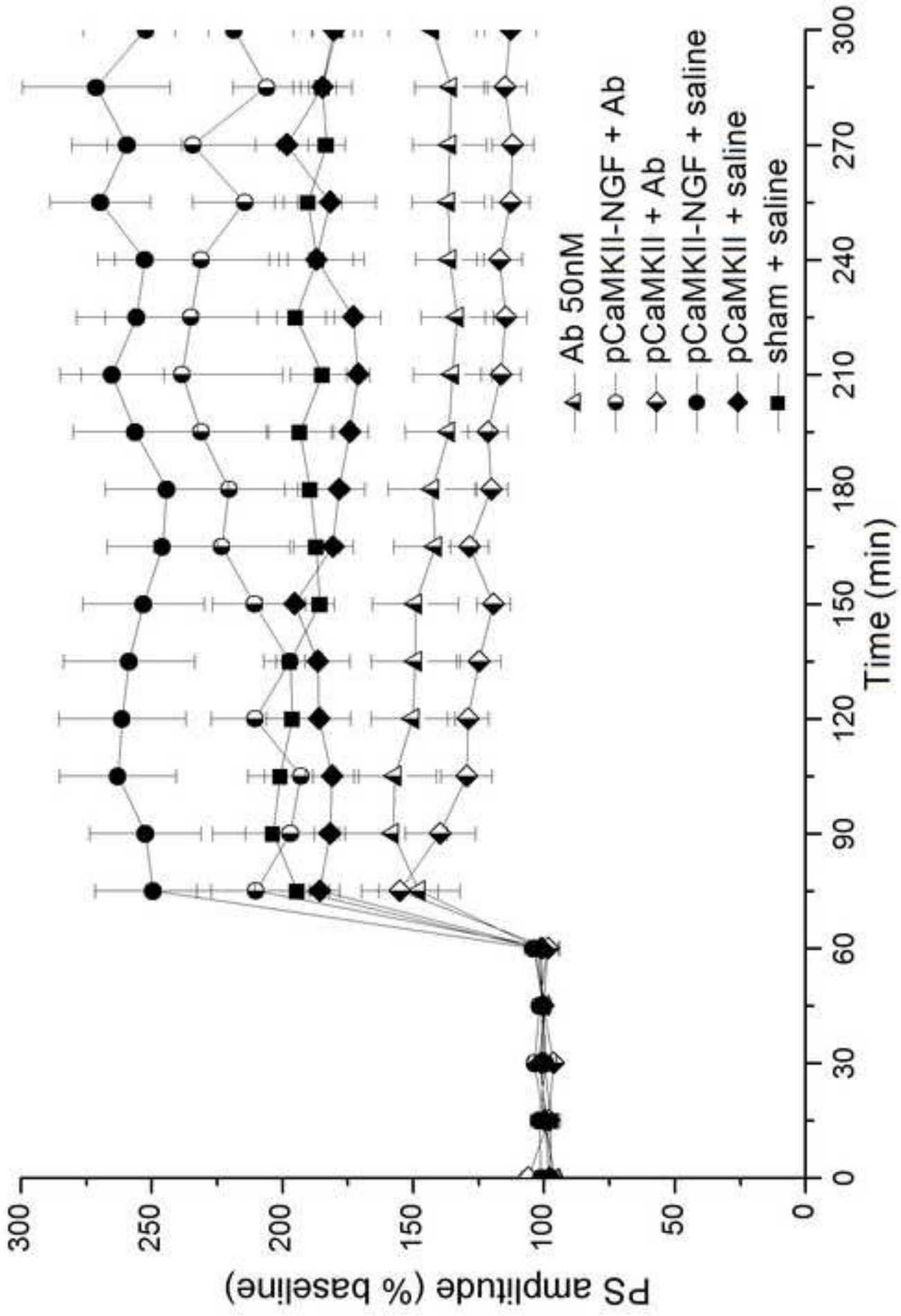
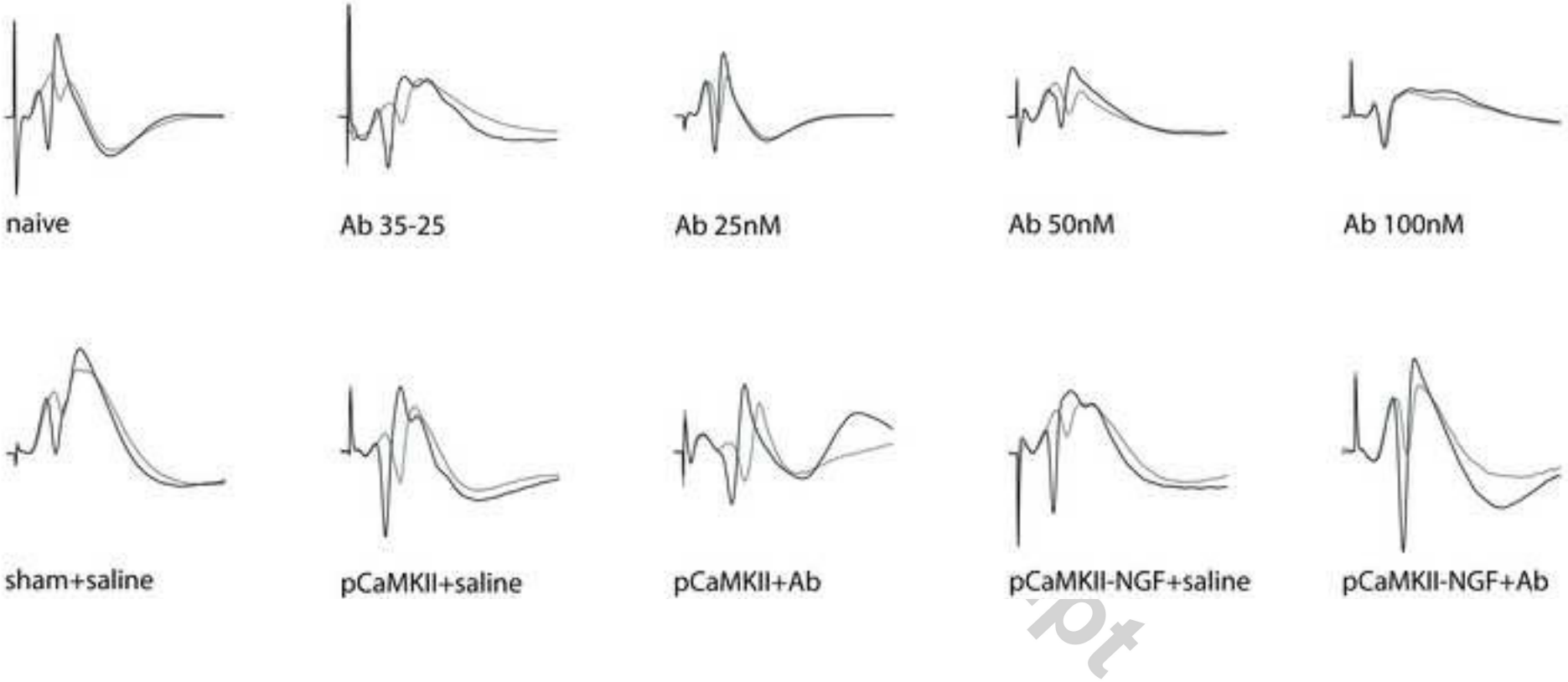

PS amplitude (% baseline)
300
250
200
150
100
50
0
0
30
60
90
120
150
180
210
240
270
300
Time (min)
Ab 50nM
pCaMKII-NGF + Ab
pCaMKII + Ab
pCaMKII-NGF + saline
pCaMKII + saline
sham + saline

Figure 2

Figure 3



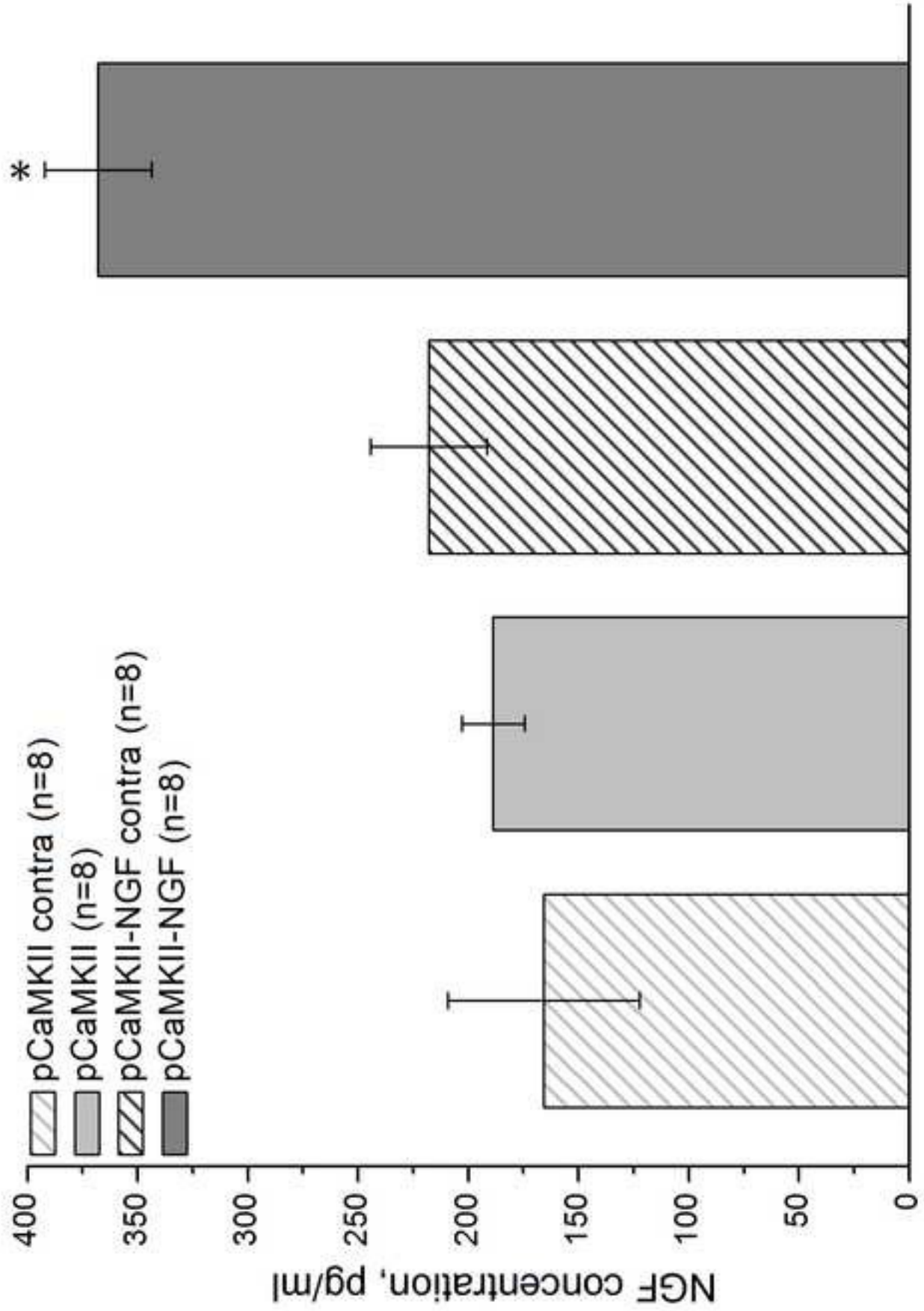

pCaMKII contra (n=8)
pCaMKII (n=8)
pCaMKII-NGF contra (n=8)
pCaMKII-NGF (n=8)
NGF concentration, pg/ml
400
350
300
250
200
150
100
50
0
*

Figure 4

Highlights

- NGF concentrations in rats' hippocampuses were doubled using lentiviral gene transfer.
- Beta-amyloid dose-dependent *in vivo* hippocampal LTP impairment was explored. 50 nM Aβ (25-35) concentration was chosen for modeling of pathological conditions.
- Increased endogenous NGF concentration can rescue hippocampal neuronal function from Aβ-induced impairment.