Enhancement of LTP in aged rats is dependent on endogenous BDNF

Maria José Diógenes, Ana Rita Costenla, Luísa Vaqueiro Lopes, André Jerónimo-Santos, Vasco C. Sousa, Bruno Miguel Fontinha, Joaquim Alexandre Ribeiro, Ana Maria Sebastião

1-Title Page

Title of the article: Enhancement of LTP in aged rats is dependent on endogenous BDNF

Abbreviated running title: Enhanced LTP in ageing is dependent on endogenous BDNF

Authors:

Correspondent author: Maria José Diógenes, PhD, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal, Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, diogenes@fm.ul.pt

Author: Ana Rita Costenla, PhD student, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, arcostenla@gmail.com

Author: Luísa V. Lopes, PhD, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, lvlopes@fm.ul.pt

Author: André Jerónimo-Santos, PhD student, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, andresantos@fm.ul.pt

Author: Vasco C. Sousa, PhD student, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, vascman@gmail.com

Author: Bruno Miguel Fontinha, PhD student, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, bruno.fontinha@gmail.com

Author: Joaquim Alexandre Ribeiro, MD, PhD, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, jaribeiro@fm.ul.pt

Author: Ana Maria Sebastião, PhD, Institute of Pharmacology and Neurosciences, Faculty of Medicine, and Unit of Neurosciences, Institute of Molecular Medicine, University of Lisbon, Portugal. Av. Professor Egas Moniz, 1649-028 Lisbon - Portugal , Tel. +351 217985183, Fax +351 217999454, anaseb@fm.ul.pt

 Corresponding author: Maria José Diógenes

2. Abstract and Keywords

Abstract:

Long-term potentiation (LTP), considered the neurophysiological basis for learning and memory, is facilitated by brain-derived neurotrophic factor (BDNF), an action more evident when LTP is evoked by weak θ-burst stimuli and dependent upon co-activation of adenosine A_{2A} receptors ($A_{2A}R$), which are more expressed in aged rats. Since θ-burst stimuli also favour LTP in aged animals, we hypothesised that enhanced LTP in ageing could be related to changes in neuromodulation by BDNF. The magnitude of CA1 LTP induced by a weak theta burst stimuli delivered to the Schaffer collaterals was significantly higher in hippocampal slices taken from 36-38 and from 70-80 week old rats, when compared to LTP magnitude in slices from 4 or 10-15 week old rats; this enhancement does not impact in cognitive improvement since aged rats revealed an impairment on hippocampal dependent learning and memory performance, as assessed by the Morris water maze tests. The scavenger for BDNF, TrkB-Fc, and the inhibitor of Trk phosphorylation, K252a, attenuated LTP in slices from 70-80 week old rats, but not from 10-15 week old rats. When exogenously added, BDNF significantly increased LTP in slices from 4 and 10-15 week old rats but did not further increased LTP in 36-38 or 70-80 week old rats. The effects of exogenous BDNF on LTP were prevented by the $A_{2A}R$ antagonist, SCH58261. These results indicate that the higher LTP magnitude observed upon ageing, which does not

translate into improved spatial memory performance, is a consequence of an increase in the tonic action of endogenous BDNF.

Key words:

LTP, ageing, BDNF, adenosine, A_{2A} receptors, hippocampus

 Corresponding author: Maria José Diógenes

3. Introduction

Long-term potentiation (LTP) is a form of synaptic plasticity widely accepted as one of the initial events required for memory encoding. Accordingly, LTP is impaired in animal models of ageing associated to neurodegenerative diseases such as Alzheimer's or Huntington's diseases (Auffret*, et al 2010* ; Rasmussen*, et al* 1996; Balkowiec and Katz, 2002; Rex*, et al* 2005; Simmons*, et al* 2009). However, when healthy ageing is concerned, LTP is found to be impaired (Barnes, 1979; Rex*, et al* 2005), not altered (Costenla*, et al* 1999; Rex*, et al* 2005; Kumar*, et al* 2007) or even increased (Costenla*, et al* 1999; Kumar and Foster, 2004; Huang and Kandel, 2006). The magnitude of the deficits may depend on the synaptic circuit that is being potentiated (Rex*, et al* 2005) or on the used stimulation pattern (Costenla*, et al* 1999). Thus, at a given pathway, as the CA3-CA1 synapses in the hippocampus, LTP induced by strong high-frequency stimulus is not affected by ageing (Costenla*, et al* 1999; Rex*, et al* 2005), whereas LTP induced by the weaker theta-burst (θ-burst) stimulus is enhanced by ageing (Costenla*, et al* 1999; Huang and Kandel, 2006).

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that acts as a regulator of synaptic plasticity in the adult brain. It was shown that hippocampal LTP is strongly impaired in BDNF (Korte*, et al* 1995; Figurov*, et al* 1996; Patterson*, et al* 1996) knockout mice, a deficit that could be rescued by increasing BDNF expression (Korte*, et al* 1996), or even by administration of exogenous BDNF (Patterson*, et al* 1996). In addition, in BDNF TrkB receptor knockout mice, LTP is also severally affected (Xu*, et al* 2000; Minichiello*,*

et al 2002), Moreover, up-regulation of BDNF rescues synaptic plasticity deficits in an animal model of Huntington's disease (Simmons*, et al* 2009). These positive actions of BDNF upon synaptic plasticity, being it endogenous (Balkowiec and Katz, 2002) or exogenously added (Fontinha*, et al* 2008), are particularly favored in weak θ-burst stimuli in the CA1-CA3 synapse. The influence of this fast acting neurotrophin on synapses requires co-activation of adenosine A_{2A} receptors ($A_{2A}R$) (Diogenes*, et al* 2004; Fontinha*, et al* 2008; Tebano*, et al* 2008) and this interaction is markedly influenced by ageing (Diogenes*, et al* 2007). Neuromodulation through $A_{2A}R$ is increased upon ageing (Rebola*, et al* 2003). Therefore, higher levels of $A_{2A}R$ seen in aged animals facilitate synaptic effects of BDNF (Diogenes*, et al* 2007), in spite of lower levels of BDNF receptors in the hippocampus upon ageing.

Given that 1) age-related increases in LTP induction (Kumar and Foster, 2004) are mostly evident when LTP is triggered by weak θ-bust stimulus (Costenla*, et al* 1999), 2) this weaker pattern of LTP induction favors the facilitatory action of BDNF upon LTP (Fontinha*, et al* 2008) and 3) the effects of BDNF are potentiated or dependent on $A_{2A}R$ activation (Sebastiao and Ribeiro, 2009) which are more relevant in aged subjects, we hypothesized that the enhanced LTP in aged rats could be due to an higher facilitatory influence of BDNF. To test this hypothesis we evaluated age-related changes in magnitude of LTP induced by weak θ-burst stimuli and tested the influence of endogenous and exogenously added BDNF upon LTP in hippocampal slices taken from infant (4 weeks) to aged (1.7 years) rats. In addition, we also evaluated how the effect of BDNF upon LTP in

the different age groups was affected by tonic $A_{2A}R$ activation, and whether the change in LTP upon ageing impacts upon alteration in learning and memory.

4. Materials and methods

Animals. Experiments were performed in rat (male, Wistar, Harlan Interfauna Iberica, SL, Barcelona) hippocampi from infant rats (4 weeks old) and from three adult rat groups: young adult (10-15 weeks), age where reproductive behaviour is fully established (Havenaar, 1993)**,** old adult (36-38 weeks), time where rats get the third part of life expectancy (Havenaar, 1993), and aged rats (70-80 weeks old). Animals were handled according to European Community guidelines and Portuguese law on Animal Care and anaesthetized with halothane before decapitation. Two hippocampi were isolated, one for electrophysiological recordings, and the other one to evaluate BDNF immunocontent.

Ex-vivo electrophysiological recordings. The hippocampus was dissected free within ice-cold Krebs' solution composed of (mM): NaCl 124; KCl 3; NaH_2PO_4 1.25; $NaHCO_3$ 26; $MgSO_4$ 1; $CaCl_2$ 2; and glucose 10, previously gassed with 95%O_2 and 5%CO_2, pH7.4. Slices (400µm thick) were cut perpendicularly to the long axis of hippocampus with a McIlwain tissue chopper and allowed to recover functionally and energetically for 1h in a resting chamber, filled with same solution, at room temperature. Slices were transferred to a recording chamber for submerged slices and continuously superfused at 3ml/min with gassed bathing solution at 32°C; drugs were added to this superfusion solution in an open circuit. When the effect of BDNF scavenger (Trk-Fc) was studied, slices were continuously superfused at same rate, temperature and oxygenation but

in a closed circuit. Appropriate controls have been performed to ensure that there were no time-dependent changes in fEPSPs under either the open or the closed circuit conditions. Recordings were obtained with an Axoclamp 2B amplifier and digitized (Axon Instruments, Foster City, CA). Individual responses were monitored, and averages of eight consecutive responses continuously stored on a personal computer with the LTP software (Anderson and Collingridge, 2001).

Basal synaptic transmission. Field excitatory post-synaptic potentials (fEPSPs) (Figure 1B) were recorded through an extracellular microelectrode (4M NaCl, 2–6MΩ resistance) placed in *stratum radiatum* of CA1 area (Figure 1A). Stimulation (rectangular 0.1ms pulses, once every 15s) was delivered through a concentric electrode placed on the Schaffer collateral-commissural fibers, in *stratum radiatum* near CA3–CA1 border (Figure 1A). The intensity of stimulus (80–200μA) was initially adjusted to obtain a large fEPSP slope with a minimum population spike contamination. Alteration on synaptic transmission induced by drugs was evaluated as the % change of average slope of fEPSP in relation to the average slope of the fEPSP measured during the 10min that preceded the addition of drugs as previously described (Diogenes, *et al* 2004).

Long-term potentiation (LTP) induction and quantification. fEPSPs (Figure 1B) were recorded through extracellular microelectrode (4M NaCl, 2–6MΩ resistance) placed

in *stratum radiatum* of CA1 area as represented in figure 1A. In LTP experiments, stimulation (rectangular 0.1ms pulses, once every 10s) was delivered alternatively to two independent pathways through bipolar concentric electrodes placed on Shaffer collateral/commissural fibers in *stratum radiatum* (figure 1A). LTP was induced by theta-burst protocol consisting of three trains of 100Hz, 3 stimuli, separated by 200ms as showed in figure 1C.

The intensity of the stimulus was maintained during the induction protocol. LTP was quantified as % change in the average slope of the fEPSP taken from 46-60min after LTP induction in relation to the average slope of the fEPSP measured during the 14min that preceded the induction of LTP. In each individual experiment the same LTP-inducing paradigm was delivered to each pathway. One hour after LTP induction in one of the pathways, BDNF (20ng/ml) was added to the superfusion solution and LTP was induced in the second pathway, no less than 30min after BDNF perfusion and only after stability of fEPSP slope values was observed for at least 10min. The effect of BDNF upon LTP was evaluated by comparing the magnitude of LTP in the first pathway in the absence of BDNF (control pathway), with the magnitude of LTP in the second pathway in the presence of BDNF (test pathway); each pathway was used as control or test on alternate days. To test the modification of the effect of BDNF upon LTP, the modulatory drugs were added to superfusing bath at least 30min before induction of LTP in the first pathway and remained in bath up to the end of the experiment. BDNF was added, as usual, 60min after induction of LTP in the first pathway. Thus, modulatory drugs were present during both LTP-

inducing periods, whereas BDNF was only present during the second induction of LTP. This protocol allows the comparison between the effect of BDNF upon LTP under different experimental conditions, keeping the magnitude of LTP under the same drug condition, without BDNF in the same slice as an internal control. When testing the effect of a drug upon LTP (rather than the modulation of the BDNF effect on LTP), the drug was added to bath 30min before induction of LTP in the second pathway and the magnitude of the resulting LTP was compared with that previously obtained (first pathway) in the absence of the drug (see Fontinha*, et al* 2008). In control experiments performed with hippocampal slices taken from any age group, we confirmed that when the θ-burst paradigm was sequencially applied to each of the two pathways in the absence of any drug, the synaptic potentiation obtained in the first pathway was similar to that achieved in the second pathway.

Input/output curve. Input/output curves in slices from different age groups were performed to ensure that modifications in LTP magnitude were not due to changes in basal synaptic transmission. After obtaining a stable baseline for at least 15min, the stimulus delivered to the slice was decreased until disappearance of the fEPSPs. The stimuli delivered to the slice were successively increased by steps of 20μA. For each stimulation condition, data from three consecutive averaged fEPSP (each averaged fEPSP is the computerized mean of 8 individual fEPSP) were stored. The range of all inputs delivered to the slice was typically from 60μA to a supra maximum stimulation amplitude of 300μA.

The input/output curve was plotted as the relationship of fEPSP slope versus stimulus intensity (i.e. fiber volley amplitude), which provides a measure of synaptic efficiency.

Western Blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to evaluate levels of BDNF immunoreactivity. Hippocampi were disrupted in sucrose-Tris solution with a Teflon pestle. Proteins on hippocampal homogenate were quantified using Bio-Rad assay according to Bradford (Bradford, 1976). A total of 70µg of protein, for each sample, were separated on 12% polyacrylamide gels, and then transferred onto nitrocellulose membranes. After blocking with 5% milk solution, the membranes were incubated (overnight at 4°C) with primary rabbit polyclonal antibodies recognizing BDNF or α-tubulin (Abcam) and then incubated with anti-rabbit secondary antibody conjugated with horseradish peroxidase for 1h at room temperature. Proteins of interest were detected using ECL-Plus (Amersham Biosciences). Intensities of bands were quantified using ImageJ program. The BDNF intensity values were divided by correspondent α-tubulin intensities (loading control).

Morris water maze. Young adult or aged rats (8 animals in each age group) were first habituated to handling during 5 days before testing began. The protocol used was the classical Morris water maze test, that is sensitive to hippocampal-dependent spatial learning and memory (Morris*, et al* 1982). The maze consisted of a large circular tank (1.8m in diameter, 0.6m in height) of water (temperature, 25±1ºC) made opaque with the addition of

non-toxic water-based black paint. An escape platform (10cm in diameter) was submerged 1cm below the water. Several visual cues were placed on the walls of the testing room, to be used by the animals as spatial references. An automated tracking system (Smart 2.5, PanLab, Barcelona) monitored all performances in the following parameters: swim path length; escape latency; average speed; and percentage of time spent in each quadrant. All subjects were first given a pre-training habituation session of 2 trials in which they were placed in the tank and allowed 60s to swim to the platform located at the centre of the tank. Rats were then given spatial (acquisition) training consisting of four trials/day for 5 days, in which the platform was placed at a fixed position in centre of one of 4 quadrants of the tank (platform Q, left, right and across). The starting position, at which subjects were placed in the tank facing the wall, was randomly chosen across trials between 4 possible locations. Inter-trial interval was 15min, during which animals were towel-dried and placed in a heated incubator (25ºC) to prevent hypothermia. The maximum trial duration was 60s, after which animals were manually guided to the platform if they failed to locate it. Once animals reached the platform, they were allowed to remain there for 20s. A probe test was given after the last trial on day 5 of the acquisition. In this test, the platform was removed from the tank and animals were allowed to swim freely for 60s, during which the percentage of time spent on each quadrant was recorded. The results are expressed as mean±SEM from *n* experiments.

Drugs. BDNF was supplied in a 1.0mg/mL stock solution in 150mM NaCl, 10mM sodium phosphate buffer, and 0.004%Tween-20, provided by Regeneron Pharmaceuticals (Tarrytown, NY). 2-[*p*-(2-carboxyethyl)phenethylamino]-5-*N*-ethylcarboxamido adenosine (CGS21680) was purchased from Sigma (St. Louis, MO). K252-a and {7-(2-phenylethyl)-5-amino-2-(2-furyl)-pyrazolo-[4,3-e]-1,2,4-triazolo[1,5-c]pyrimidine} (SCH58261) were from Tocris Cookson (Ballwin, MO, USA). The recombinant human TrkB/Fc chimera, Trk-Fc, was purchased from R&D systems (Minneapolis, USA). CGS21680 and SCH58261 were made up in a 5mM stock solution in DMSO. K252-a was made in a 2.5mM stock solution in DMSO. TrkB-Fc was made up in a 50µg/mL stock solution in PBS with 0.1% BSA. The concentration of DMSO added to slices (0.001%v/v) was bellow the concentration that influences glutamatergic synaptic transmission (0.02%v/v, Tsvyetlynska, et al, 2005). Aliquots of stock solutions were stored at -20ºC until use.

Data analysis. Values are expressed as mean±SEM from *n* slices. The significance of differences between the mean values obtained in two different conditions in same slice was evaluated by the Student´s *t*-test. For multiple comparisons, statistical significance was assessed by the one-way ANOVA with Bonferroni´s correction. Values of $P<0.05$ were considered to represent statistically significant differences.

For results obtained in morris water maze a 2-way ANOVA repeated measures was used to compare acquisition data from the two age groups. A student's *t*-test was used to compare time spent in the platform quadrant between groups.

5. Results

LTP induced by θ-burst stimulus, but not synaptic efficiency, is increased upon ageing

The mild θ-burst stimulation used in the present work induced a small, but statistically significant ($P<0.05$ as compared with baseline), LTP in slices taken from 4 week old rats (10 ± 3.6% increase in fEPSP slope 46-60 min after θ-burst, n=8, figure 2A) and 10-15 week old rats (14 ± 1.8% increase in fEPSP slope, n=7, figure 2B). In contrast, in hippocampal slices taken from older rats the same θ-burst elicited a robust LTP, so that 46-60 min after θ-burst stimulation the fEPSP slope was increased by 35 ± 4.6 % (36-38 week old rats, n=7, figure 2C) or 43 ± 4.9%, (70-80 week old rats, n=7, figure 2D). As summarized in Figure 2E, LTP magnitude attained in slices from the two oldest groups was not significantly different, but it was significantly different ($P<0.01$) from that attained in slices from younger animals (4 weeks or 10-15 weeks). The fEPSP recorded from slices from old animals tended have more population spikes than in fEPSP recorded in younger animals, and this was particularly evident after LTP induction (Figure 2A-D). Indeed, a higher LTP in these slices increases the probability to detect population spike contamination. A lower GABAergic inibition in aged rat hippocampus (Billard, *et al* 1995) might also contribute to higher population spikes.

To evaluate if the age-related differences in LTP magnitude could be due to changes in baseline synaptic efficiency, input/output curves in slices taken from 10-15 and 70-80 week old rats were performed. Increasing stimulus intensities were delivered to the slices, as

described in methods. For each intensity applied, the slope of the fEPSP (taken as a measure of the strength of the postsynaptic response) was plotted against the fiber volley amplitude (which reflects the level of presynaptic activation). No differences were found in input/output curves obtained in slices taken from both age groups (n=3, P>0.05, figure 2F) indicating that the age-related changes in LTP cannot be accounted by differences in synaptic efficiency.

Aged rats show deficits on hippocampal dependent learning and memory, when compared with young adult rats

To evaluate whether the increased LTP observed in aged rats was accompanied by enhanced skills at hippocampal-dependent learning and memory tasks, we compared the performance of young adult (10-15 week old) and aged (70-80 week old) rats at the Morris water maze. As shown in figure 3A, animals of both age groups improved their daily performance during acquisition phase of the Morris water maze task, but aged rats showed a much slower learning profile. Analysis of variance (ANOVA) revealed significant differences in latency throughout the acquisition days ($F(4.58)=29.9$, P<0.01, n=8, figure 3A) and an overall effect of age ($F(1.58)=12.74$, P<0.01; n=8). In probe test, both young and aged rats spent a significantly higher percentage of time in the training quadrant. However, young rats spent significantly more time (56.5±3.7% n=8) in the training quadrant than aged rats (43.4±3.7%, P<0.01, n=8, figure 3B), which indicates that aged rats have a deficient spatial memory, compared to young rats. The repeated measures analysis

of variance has revealed a consistent pattern across all the four quadrants regardless of the age factor (P>0.05).

In aged rats tonic influence of endogenous BDNF upon LTP is enhanced

Weak θ-burst stimulation, favors the release of BDNF (Balkowiec and Katz, 2002; Gartner and Staiger, 2002), as well as the facilitatory action of this neurotrophin upon LTP (Fontinha*, et al* 2008). To investigate whether the age-related increase in LTP magnitude could be related to a higher influence of endogenous BDNF, we evaluated the influence of BDNF's scavenger, TrkB-Fc (2 μg/ml), upon LTP in hippocampal slices from 10-15 and 70-80 week old rats. TrkB-Fc (2 μg/ml) was added to hippocampal slices 30 min before θ-burst stimulation and remained in the bath up to the end of experiment in a close circuit as described in methods. Under these conditions and in the absence of the scavenger LTP magnitude in slices taken from young adult rats was 19 ± 14% (Figure 4B,E, n=3, $P<0.05$ as compared with baseline) and in slices from 70–80 weeks old rats it was 59 ± 14% (Figure 4D,E, n=3, $P<0.05$ as compared with baseline), therefore within the same values as that found in the first group of experiments (Figure 2). The scavenger was devoid of effect on hippocampal synaptic transmission in slices taken from either group of animals (figure 4A,C, $n=3$). Furthermore, TrkB-Fc (2 μg/ml) did not change LTP magnitude (Figure 4B,E, $n=3$, $P>0.05$ as compared with LTP magnitude in the absence of the scavenger) in slices taken from 10-15 week old rats. However, as shown in figure 4D,E, TrkB-Fc (2 μg/ml) significantly decreased ($P<0.05$) the magnitude of LTP in slices taken from aged rats. Note,

however, that under those conditions a small but significant LTP (22 ±5.5%, n=3, $P<0.05$ as compared with baseline) still occurred in slices from aged rats, the magnitude being similar to that observed in slices from young adults (Figure 4E).

To further evaluate the contribution of endogenous BDNF to θ-burst-induced LTP in the two age groups (10-15 and 70-80 week old rats), we tested the effect of an inhibitor of Trk phosphorylation, K252a (Knusel and Hefti, 1992). K252a was used at a concentration (200 nM) known to prevent BDNF actions on hippocampal synaptic transmission (e.g. Kang and Schuman, 1995; Diogenes*, et al* 2004) and plasticity (Fontinha*, et al* 2008), without directly affecting LTP in slices from infant rats (Fontinha*, et al* 2008). The inhibitor of Trk phosphorylation was added to superfusing bath at least 30 min before induction of LTP in the second pathway, remaining in the bath up to the end of experiment, and was devoid of effect upon synaptic transmission in slices from either young adult (Figure 5A) or aged (Figure 5C) rats. Also, and in accordance with previous observations in infant rats (Fontinha*, et al* 2008), K252a (200nM) did not significantly ($P>0.05$) affect LTP magnitude in slices taken from 10-15 week old rats (Figure 5B,E). In contrast, in slices taken from older (70-80 weeks) rats, K252a (200 nM) significantly decreased LTP (figure 5D,E $n=3$, $P<0.05$), in agreement with data obtained with BDNF scavenger (Figure 4), which reinforces the conclusion that endogenous BDNF is involved in the enhancement of LTP upon ageing. It is worthwhile to note that in the absence of K252a, LTP magnitude in these experiments was statistically significant ($P<0.05$) either in slices from young adult (20 ± 7.1%, $n=3$) or aged (48 ± 8.5%, $n=3$) rats, and that similarly to what occurred in the

presence of TrkB-Fc, in the presence of K252a a small but significant LTP (21 ± 3.0%, *n*=3, P<0.05 as compared with baseline) was still detected in slices from aged rats (Figure 5D,E).

The age-related modifications of endogenous BDNF levels might be confined to synaptic areas since no significant differences (*P*>0.05, *n*=5) between BDNF immunoreactivity on hippocampal homogenates from the four age groups were detected (Figure 6).

Changes in effect of exogenous BDNF upon LTP are age-related dependent.

We next evaluated whether any effect of exogenously added BDNF upon LTP was also influenced by age. BDNF (20 ng/mL) was added to slices 60 min after LTP induction in first pathway and 30 min before LTP induction in second pathway and remained in the bath up to the end of experiments. As illustrated in figure 7A, in hippocampal slices from young animals (4 weeks old) BDNF caused a marked facilitation of LTP, in accordance with previous reports (Fontinha*, et al* 2008). In hippocampal slices taken from 10-15 week old rats, the θ-burst stimulus applied in the presence of BDNF also induced a robust LTP (33 ± 3.2 % increase of fEPSP slope, *n*=5, figure 7B), which was significantly higher (*P*<0.05, figure 7E) than that obtained in the other pathway in the absence of BDNF (13 ± 1.8 % increase in fEPSP slope).

In contrast, when applied to hippocampal slices taken from 36-38 week old rats, BDNF did not cause a further increase in LTP (figure 7C,E). Indeed, θ-burst stimulation

induced a 42 ± 7.0 % increase in fEPSP slope (n=4) in the presence of BDNF and 37 ± 7.4% increasing in fEPSP slope, in the same slices without applying BDNF (P>0.05). Similar results were obtained with the oldest group (70–80 week old) of rats, where the presence of BDNF also did not induce any further significant increase in LTP magnitude (37 ± 1.4 % or 35 ± 3.5 % increase in fEPSP slope 60 min after the θ-burst in the presence or absence of BDNF, respectively, n=4, P>0.05, figure 7D,E).

It is worthwhile to note that magnitude of LTP in slices taken from 36-38 or 70-80 week old rats under control conditions (no BDNF added) is similar to that observed in slices taken from 10-15 week old rats but in the presence of BDNF.

The effect of BDNF upon LTP, whenever observed (4 weeks and 10-15 weeks old rats), was prevented by blockade of $A_{2A}R$ with the selective $A_{2A}R$ antagonist, SCH 58261 (100mM) (Figure 8), which is in conformity with previously reported influence of $A_{2A}R$ upon the effect of BDNF on synaptic transmission (Diogenes, *et al* 2004; PMID: 17421024 Diogenes, *et a* 2007) and LTP (Fontinha*, et al* 2008).

Influence of adenosine A_{2A} receptor blockade upon θ-burst-induced LTP is aged-related dependent

Since the effect of BDNF upon LTP requires co-activation of adenosine $A_{2A}R$ (fig. 8 and (Fontinha*, et al* 2008), blockade of $A_{2A}R$ should markedly reduce the magnitude of LTP in

old animals if, as data with the BDNF scavenger and Trk phosphorylation inhibitor (fig. 4 and 5) suggested, the boosted LTP at these ages results from an enhanced action of endogenous BDNF upon LTP. To directly test that hypothesis, we compared the effect of the $A_{2A}R$ antagonist upon LTP in hippocampal slices taken from the different age groups. SCH 58261 was used at a concentration (100 nM) selective for rat $A_{2A}R$ (Zocchi*, et al* 1996), and was added to perfusion solution 60 min after induction of LTP in the first pathway and at least 30 min before LTP induction in the second pathway. As sumarized in figure. 9, the $A_{2A}R$ antagonist significantly ($P<0.05$) reduced LTP magnitude in slices from 36-38 week old and 70-80 week old rats, but was virtually devoid of effect on LTP in slices from younger animals (4 weaks and 10-15 weaks). Interestingly, in the presence of SCH 58261 (100nM), LTP magnitude in aged rats (≥36 weeks old) was similar to that observed in younger animals either in the presence or absence of SCH 58261 (figure 9).

Corresponding author: Maria José Diógenes

6. Discussion

The main finding of the present work is that the higher LTP observed after theta-burst stimulation upon ageing is a consequence of an increase in facilitation of LTP by endogenous BDNF. However, the increased LTP does not translate into improved spatial memory performance of the aged animals.

It is classically established that LTP in the hippocampus is a prototypical experimental model that translates into forms of learning and memory associated with that brain area (Lynch, 2004) including spatial memory as assessed by the Morris Water maze (Morris, *et al* 1982). However, LTP magnitude and memory should not be linearly related, as higher LTP in old animals does not necessarily translate into improved memory upon ageing. As pointed out by Huang and Kandel (1996), who observed an age-related increase of LTP induced by a brief 1Hz paired pulse stimulation (PP-1Hz), there might be forms of LTP that are negatively coupled with age related memory loss and that may, therefore, have a negative role in memory storage. On the other hand, changes in LTP can vary in different areas of the dendritic tree since age-related decreases in basal but not apical dendrites of CA1 pyramidal neurons have been shown (Lynch, *et al* 2006). It is therefore possible that the now reported age-dependent increase in weak theta burst induced LTP of apical dentrites of CA1 neurons reflects, at least in part, LTP failure in some other anatomical areas contributing to the learning and memory paradigms under assay. Clearly, memory

impairment in aging is not solely related to a loss of synaptic plasticity (see also Lynch, *et al* 2006).

It is known that in old rats there is no loss of hippocampal CA1 pyramidal cells (Rapp and Gallagher, 1996; Rasmussen, *et al* 1996), that most biophysical properties of old pyramidal cells do not differ from those in young cells, and that there is no change in spontaneous firing rates of single cells in the hippocampus of freely behaving old rats comparing with young ones (see Barnes, 2003). With ageing there is a decline in expression of mRNA for different NMDA receptors subunits (Magnusson, 2000; Bai, *et al* 2004) as well as a decrease in several NMDA subunits (Sonntag, *et al* 2000; Clayton, *et al* 2002), which might contribute to a decrease in LTP magnitude upon ageing. However, LTP involves many steps besides NMDA receptors activation. We focused on the possibility that the neuromodulatory influence of BDNF on LTP could be affected by ageing because the age-related increases in susceptibility to LTP induction (Kumar and Foster, 2004) are more evident when LTP is triggered by weak θ-burst stimuli (Costenla, *et al* 1999), which favours the release of endogenous BDNF (Gartner and Staiger, 2002; Aicardi, *et al* 2004) as well as facilitatory actions of exogenous BDNF on LTP (Kramar, et al 2004; Fontinha, *et al* 2008). Our results clearly show that the BDNF scavenger, Trk-Fc and the inhibitor of Trk phosphorylation, K252a caused a marked decrease in LTP in slices from old but not from young rats, compatible with previous observations that early LTP in young animals is not dependent on endogenous BDNF signaling (Fontinha*, et al* 2008) and suggestive that upon ageing there is an enhanced component of LTP that is dependent upon endogenous

BDNF. Interestingly, in the presence of these inhibitors of endogenous BDNF action, the magnitude of LTP in young or aged animals becomes similar. Furthermore, upon addition of exogenous BDNF, LTP magnitude in slices from young animals is markedly increased becoming comparable to that observed in slices from aged animals.

Taken together these results strongly suggest that the age-related increases in susceptibility to LTP induction by weak θ-burst stimuli involves an exacerbation of the facilitatory action of endogenous BDNF.

BDNF release can occur through two different pathways: 1) a constitutive pathway, where this molecule is spontaneously secreted or 2) a regulated pathway, where it is secreted in response to depolarization-induced transient changes in the Ca2+ concentration at release sites (see Lessmann, *et al* 2003). Therefore, secretion of neurotrophins is regulated by neuronal activity being dependent on the stimulus-frequency (Lim, *et al* 2003) and facilitated by θ-burst stimulation (Gartner and Staiger, 2002; Aicardi, *et al* 2004). This leads to fast and highly localized changes in synaptic BDNF levels that might not reflect changes in total tissue levels of the neurotrophin. Indeed, we could not detect appreciable age-related changes in the levels of BDNF in hippocampal homogenates. So, the higher influence of endogenous BDNF upon LTP in aged animals might be due to an increased activity of the regulated pathway leading to an enhanced release of BDNF at synapses, accompanied by an enhanced release of adenosine, which facilitates the action of BDNF in spite of the age-dependent decline in TrkB receptors (Diogenes, *et al* 2007). Upon ageing, there is a deficit in the regulation of calcium concentration (Landfield and Lynch, 1977)

leading to increased levels of intracellular calcium in neurons (Hajieva, *et al* 2009). Whether this is a cause for an increase in BDNF release through the regulated pathway requires future investigation.

Whether the enhanced action of endogenous BDNF is a compensatory phenomenon physiologically aimed to increase LTP and ameliorate learning and memory deficits or, in alternative, whether the enhanced action of BDNF is itself the source of an age-related increased but dysfunctional LTP remains to be established. Interestingly, in the dentate gyrus, BDNF-induced signaling and plasticity is impaired in aged rats (Gooney, *et al* 2004) suggesting that deficits in spatial learning could be due to deficits in BDNF/TrkB signalling in other hippocampal areas or even in other brain regions. As discussed above, memory impairment in aging is not solely related to a loss of synaptic plasticity, and surely, spatial learning does not rely exclusively on LTP in the CA1 hippocampal area.

Synaptic plasticity is under tight control by adenosine inhibitory A_1 receptors (A_1R) and facilitatory $A_{2A}R$ (see e.g. Costenla, *et al* 2010). In has been repeatedly observed that synaptic influences of BDNF are dependent on $A_{2A}R$ receptors activation (Diógenes, *et al* 2004; Diógenes, *et al* 2007; Fontinha, *et al* 2008; Tebano, *et al* 2008; Assaife-Lopes, *et al* 2010), and we could confirm these observations in the present work, expanding them to LTP in aged animals. Therefore, the marked LTP inhibition by a selective $A_{2A}R$ antagonist in aged but not young animals may be due, at least in part, to blockade of the component of LTP that is dependent upon endogenous BDNF. The density of $A_{2A}R$ (Cunha, *et al* 1995; Lopes, *et al* 1999; Diogenes, *et al* 2007) as well as their coupling to G-proteins (Lopes, *et*

al 1999; Rebola, *et al* 2003; Rodrigues, *et al* 2008) is increased in the hippocampus of aged animals. Age-induced impact on density and function of $A_{2A}R$ may underlie the preventive effects of caffeine in age-related memory impairments in rodents (Prediger, *et al* 2005; Costa, *et al* 2008), since it is mimicked by $A_{2A}R$ antagonists but not by A_1R antagonists (Costa, *et al* 2008). Here we report that an $A_{2A}R$ antagonist has a marked inhibitory effect on LTP in aged rats where endogenous BDNF actions seems to be also more evident and where impaired hippocampal dependent memory was detected as assessed by the Morris water maze. One may therefore speculate that the positive actions of $A_{2A}R$ antagonists, including caffeine, upon memory in aged subjects can be, at least in part be related to an inhibition of dysfunctional higher tonic actions of BDNF upon plasticity.

The animals used in the present study were undergoing a non-pathological ageing process, rather than being a model of neurodegenerative disease, such as Alzheimer´s disease where total BDNF levels in the cortex and hippocampus are known to be decreased (Connor*, et al* 1997; Ferrer*, et al* 1999). Interestingly, in animal models of Alzheimer´s disease, LTP is markedly decreased (Chapman*, et al* 1999; Oddo*, et al* 2003; Gureviciene*, et al* 2004; Trinchese*, et al* 2004), reinforcing the idea of a key role for a fine tune regulation of synaptic BDNF levels for proper LTP and, eventually, for memory function. In what concerns influence of BDNF upon LTP, it should be stressed that not only the amount but also the timing and pattern of BDNF release are crucial for shaping the actions of BDNF at synapses (Ji, *et al* 2010).

In conclusion, we show for the first time that, in a situation of non-pathological ageing, the actions of BDNF are increased upon synaptic plasticity in aged animals. Since no changes in total hippocampal levels of this neurotrophin where detected, this enhanced effects are probably due to an increased tonic action of BDNF upon high frequency neuronal firing.

Corresponding author: Maria José Diógenes

7. Disclosure/Conflicts of interest

All authors state the absence of actual or potential conflicts of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence (bias) their work. The authors' institution has no contracts relating to this research through which it or any other organization may stand to gain financially now or in the future. None of the authors or their institutions has any other agreements that could be seen as involving a financial interest in this work.

8. Acknowledgments

The work was supported by Fundação para a Ciência e Tecnologia, Fundação Calouste Gulbenkian and EU (COST B-30 concerted action). André Jerónimo-Santos and Vasco Sousa were supported by Fundação para a Ciência e Tecnologia (AJS: SFRH / BD/ 62828 / 2009; VCS: SFRH/BD/21359/2005, PhD fellowships). We thank Regeneron for the gift of brain-derived neurotrophic factor and W. W. Andersen (University of Bristol, Bristol, UK) for the gift of the data analysis (LTP) program. We also acknowledge Alexandre de Mendonça and Antonina Pereira for scientific discussions. The animal housing facilities of the Institute of Physiology of the Faculty of Medicine of the University of Lisbon are also acknowledged.

 Corresponding author: Maria José Diógenes

9. References

Aicardi, G., Argilli, E., Cappello, S., Santi, S., Riccio, M., Thoenen, H. and Canossa, M. (2004) Induction of long-term potentiation and depression is reflected by corresponding changes in secretion of endogenous brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A,* **101,** 15788-15792.

Anderson, W. W. and Collingridge, G. L. (2001) The LTP Program: a data acquisition program for on-line analysis of long-term potentiation and other synaptic events. *J Neurosci Methods,* **108,** 71-83.

Assaife-Lopes, N., Sousa, V. C., Pereira, D. B., Ribeiro, J. A., Chao, M. V. and Sebastiao, A. M. (2010) Activation of adenosine A2A receptors induces TrkB translocation and increases BDNF-mediated phospho-TrkB localization in lipid rafts: implications for neuromodulation. *J Neurosci,* **30,** 8468-8480.

Auffret, A., Gautheron, V., Mattson, M. P., Mariani, J. and Rovira, C. Progressive age-related impairment of the late long-term potentiation in Alzheimer's disease presenilin-1 mutant knock-in mice. *J Alzheimers Dis,* **19,** 1021-1033.

Bai, L., Hof, P. R., Standaert, D. G., Xing, Y., Nelson, S. E., Young, A. B. and Magnusson, K. R. (2004) Changes in the expression of the NR2B subunit during aging in macaque monkeys. *Neurobiol Aging,* **25,** 201-208.

Balkowiec, A. and Katz, D. M. (2002) Cellular mechanisms regulating activity-dependent release of native brain-derived neurotrophic factor from hippocampal neurons. *J Neurosci,* **22,** 10399-10407.

Barnes, C. A. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol,* **93,** 74-104.

Barnes, C. A. (2003) Long-term potentiation and the ageing brain. *Philos Trans R Soc Lond B Biol Sci,* **358,** 765-772.

Billard, J. M., Lamour, Y. and Dutar, P. (1995) Decreased monosynaptic GABAB-mediated inhibitory postsynaptic potentials in hippocampal CA1 pyramidal cells in the aged rat: pharmacological characterization and possible mechanisms. *J Neurophysiol,* **74,** 539-546.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem,* **72,** 248-254.

Chapman, P. F., White, G. L., Jones, M. W. et al. (1999) Impaired synaptic plasticity and learning in aged amyloid precursor protein transgenic mice. *Nat Neurosci,* **2,** 271-276.

Chen, L.Y., Rex, C.S., Pham, D.T., Lynch, G. and Gall, C.M. (2010) BDNF signaling during learning is regionally differentiated within hippocampus. *J Neurosci* **30**,15097-15101.

Clayton, D. A., Mesches, M. H., Alvarez, E., Bickford, P. C. and Browning, M. D. (2002) A hippocampal NR2B deficit can mimic age-related changes in long-term potentiation and spatial learning in the Fischer 344 rat. *J Neurosci,* **22,** 3628-3637.

Connor, B., Young, D., Yan, Q., Faull, R. L., Synek, B. and Dragunow, M. (1997) Brain-derived neurotrophic factor is reduced in Alzheimer's disease. *Brain Res Mol Brain Res,* **49,** 71-81.

Costa, M. S., Botton, P. H., Mioranzza, S., Souza, D. O. and Porciuncula, L. O. (2008) Caffeine prevents age-associated recognition memory decline and changes brain-derived neurotrophic factor and tirosine kinase receptor (TrkB) content in mice. *Neuroscience,* **153,** 1071-1078.

Costenla, A. R., de Mendonca, A. and Ribeiro, J. A. (1999) Adenosine modulates synaptic plasticity in hippocampal slices from aged rats. *Brain Res,* **851,** 228-234.

Costenla, A.R., Cunha, R.A. and de Mendonça, A. (2010) Caffeine, adenosine receptors and synaptic plasticity. *J Alzheimer Dis*, **20**(Suppl.1), S25-S34.

Cunha, R.A., Constantino, M.C., Sebastião, A.M. and Ribeiro, J.A. (1995) Modification of A1 and A2a adenosine receptor binding in aged striatum, hippocampus and cortex of the rat. *Neuroreport,* **6**, 1583-1588.

Diógenes, M. J., Assaife-Lopes, N., Pinto-Duarte, A., Ribeiro, J. A. and Sebastiao, A. M. (2007) Influence of age on BDNF modulation of hippocampal synaptic transmission: interplay with adenosine A2A receptors. *Hippocampus,* **17,** 577-585.

Diógenes, M. J., Fernandes, C. C., Sebastiao, A. M. and Ribeiro, J. A. (2004) Activation of adenosine A2A receptor facilitates brain-derived neurotrophic factor modulation of synaptic transmission in hippocampal slices. *J Neurosci,* **24,** 2905-2913.

Ferrer, I., Marin, C., Rey, M. J., Ribalta, T., Goutan, E., Blanco, R., Tolosa, E. and Marti, E. (1999) BDNF and full-length and truncated TrkB expression in Alzheimer disease. Implications in therapeutic strategies. *J Neuropathol Exp Neurol,* **58,** 729-739.

Figurov, A., Pozzo-Miller, L. D., Olafsson, P., Wang, T. and Lu, B. (1996) Regulation of synaptic responses to high-frequency stimulation and LTP by neurotrophins in the hippocampus. *Nature,* **381,** 706-709.

Fontinha, B. M., Diógenes, M. J., Ribeiro, J. A. and Sebastiao, A. M. (2008) Enhancement of long-term potentiation by brain-derived neurotrophic factor requires adenosine A2A receptor activation by endogenous adenosine. *Neuropharmacology,* **54,** 924-933.

Gartner, A. and Staiger, V. (2002) Neurotrophin secretion from hippocampal neurons evoked by long-term-potentiation-inducing electrical stimulation patterns. *Proc Natl Acad Sci U S A,* **99,** 6386-6391.

Gooney, M., Messaoudi, E., Maher, F.O.,Bramham, C.R. and Lynch, M.A. (2004) BDNF-induced LTP in dentate gyrus is impaired with age: analysis of changes in cell signaling events. *Neurobiol Aging*, **25**, 1323-1331

Gureviciene, I., Ikonen, S., Gurevicius, K., Sarkaki, A., van Groen, T., Pussinen, R., Ylinen, A. and Tanila, H. (2004) Normal induction but accelerated decay of LTP in APP + PS1 transgenic mice. *Neurobiol Dis,* **15,** 188-195.

Hajieva, P., Kuhlmann, C., Luhmann, H. J. and Behl, C. (2009) Impaired calcium homeostasis in aged hippocampal neurons. *Neurosci Lett,* **451,** 119-123.

Havenaar, R., Meijer, J.C., Morton, D.B., Ritskes-Hoitinga, Zwart, P. (1993) *Principles of Laboratory Animal Science. A Contribution to the humane Use and Care of Animals and to the quality of Experimental Results*: Biology and Husbandry of Laboratory Animals. Elsevier, New York.

Huang, Y. Y. and Kandel, E. R. (2006) Age-related enhancement of a protein synthesis-dependent late phase of LTP induced by low frequency paired-pulse stimulation in hippocampus. *Learn Mem,* **13,** 298-306.

Ji, Y., Lu, Y., Yang, F., Shen, W., Tang, T. T., Feng, L., Duan, S. and Lu, B. Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. *Nat Neurosci,* **13,** 302-309.

Kang, H. and Schuman, E. M. (1995) Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science,* **267,** 1658-1662.

Knusel, B. and Hefti, F. (1992) K-252 compounds: modulators of neurotrophin signal transduction. *J Neurochem,* **59,** 1987-1996.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H. and Bonhoeffer, T. (1995) Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A,* **92,** 8856-8860.

Korte, M., Griesbeck, O., Gravel, C., Carroll, P., Staiger, V., Thoenen, H. and Bonhoeffer, T. (1996) Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proc Natl Acad Sci U S A,* **93,** 12547-12552.

Kramar, E. A., Lin, B., Lin, C. Y., Arai, A. C., Gall, C. M. and Lynch, G. (2004) A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J Neurosci,* **24,** 5151-5161.

Kumar, A. and Foster, T. C. (2004) Enhanced long-term potentiation during aging is masked by processes involving intracellular calcium stores. *J Neurophysiol,* **91,** 2437-2444.

Kumar, A., Thinschmidt, J. S., Foster, T. C. and King, M. A. (2007) Aging effects on the limits and stability of long-term synaptic potentiation and depression in rat hippocampal area CA1. *J Neurophysiol,* **98,** 594-601.

Landfield, P. W. and Lynch, G. (1977) Impaired monosynaptic potentiation in in vitro hippocampal slices from aged, memory-deficient rats. *J Gerontol,* **32,** 523-533.

Lessmann, V., Gottmann, K. and Malcangio, M. (2003) Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol,* **69,** 341-374.

Lim, K. C., Lim, S. T. and Federoff, H. J. (2003) Neurotrophin secretory pathways and synaptic plasticity. *Neurobiol Aging,* **24,** 1135-1145.

Lopes, L. V., Cunha, R. A. and Ribeiro, J. A. (1999) Increase in the number, G protein coupling, and efficiency of facilitatory adenosine A2A receptors in the limbic cortex, but not striatum, of aged rats. *J Neurochem,* **73,** 1733-1738.

Lynch, M. A. (2004) Long-term potentiation and memory. *Physiol Rev,* **84,** 87-136.

Lynch, G., Rex, C.S. and Gall, C.M. (2006) Synaptic plasticity in early aging. Ageing Res Rev, 5, 255-280.

Magnusson, K. R. (2000) Declines in mRNA expression of different subunits may account for differential effects of aging on agonist and antagonist binding to the NMDA receptor. *J Neurosci,* **20,** 1666-1674.

Minichiello, L., Calella, A. M., Medina, D. L., Bonhoeffer, T., Klein, R. and Korte, M. (2002) Mechanism of TrkB-mediated hippocampal long-term potentiation. *Neuron,* **36,** 121-137.

Morris, R. G., Garrud, P., Rawlins, J. N. and O'Keefe, J. (1982) Place navigation impaired in rats with hippocampal lesions. *Nature,* **297,** 681-683.

Oddo, S., Caccamo, A., Shepherd, J. D. et al. (2003) Triple-transgenic model of Alzheimer's disease with plaques and tangles: intracellular Abeta and synaptic dysfunction. *Neuron,* **39,** 409-421.

Patterson, S. L., Abel, T., Deuel, T. A., Martin, K. C., Rose, J. C. and Kandel, E. R. (1996) Recombinant BDNF rescues deficits in basal synaptic transmission and hippocampal LTP in BDNF knockout mice. *Neuron,* **16,** 1137-1145.

Prediger, R. D., Batista, L. C. and Takahashi, R. N. (2005) Caffeine reverses age-related deficits in olfactory discrimination and social recognition memory in rats. Involvement of adenosine A1 and A2A receptors. *Neurobiol Aging,* **26,** 957-964.

Rapp, P. R. and Gallagher, M. (1996) Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci U S A,* **93,** 9926-9930.

Rasmussen, T., Schliemann, T., Sorensen, J. C., Zimmer, J. and West, M. J. (1996) Memory impaired aged rats: no loss of principal hippocampal and subicular neurons. *Neurobiol Aging,* **17,** 143-147.

Rebola, N., Sebastiao, A. M., de Mendonca, A., Oliveira, C. R., Ribeiro, J. A. and Cunha, R. A. (2003) Enhanced adenosine A2A receptor facilitation of synaptic transmission in the hippocampus of aged rats. *J Neurophysiol,* **90,** 1295-1303.

Rex, C. S., Kramar, E. A., Colgin, L. L., Lin, B., Gall, C. M. and Lynch, G. (2005) Long-term potentiation is impaired in middle-aged rats: regional specificity and reversal by adenosine receptor antagonists. *J Neurosci,* **25,** 5956-5966.

Rodrigues, R. J., Canas, P. M., Lopes, L. V., Oliveira, C. R. and Cunha, R. A. (2008) Modification of adenosine modulation of acetylcholine release in the hippocampus of aged rats. *Neurobiol Aging,* **29,** 1597-1601.

Sebastiao, A. M. and Ribeiro, J. A. (2009) Triggering neurotrophic factor actions through adenosine A2A receptor activation: implications for neuroprotection. *Br J Pharmacol,* **158,** 15-22.

Simmons, D. A., Rex, C. S., Palmer, L., Pandyarajan, V., Fedulov, V., Gall, C. M. and Lynch, G. (2009) Up-regulating BDNF with an ampakine rescues synaptic plasticity

and memory in Huntington's disease knockin mice. *Proc Natl Acad Sci U S A,* **106,** 4906-4911.

Sonntag, W. E., Bennett, S. A., Khan, A. S., Thornton, P. L., Xu, X., Ingram, R. L. and Brunso-Bechtold, J. K. (2000) Age and insulin-like growth factor-1 modulate N-methyl-D-aspartate receptor subtype expression in rats. *Brain Res Bull,* **51,** 331-338.

Tebano, M. T., Martire, A., Potenza, R. L. et al. (2008) Adenosine A(2A) receptors are required for normal BDNF levels and BDNF-induced potentiation of synaptictransmission in the mouse hippocampus. *J Neurochem,* **104,** 279-286.

Trinchese, F., Liu, S., Battaglia, F., Walter, S., Mathews, P. M. and Arancio, O. (2004) Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice. *Ann Neurol,* **55,** 801-814.

Xu, B., Gottschalk, W., Chow, A. et al. (2000) The role of brain-derived neurotrophic factor receptors in the mature hippocampus: modulation of long-term potentiation through a presynaptic mechanism involving TrkB. *J Neurosci,* **20,** 6888-6897.

Zocchi, C., Ongini, E., Conti, A., Monopoli, A., Negretti, A., Baraldi, P. G. and Dionisotti, S. (1996) The non-xanthine heterocyclic compound SCH 58261 is a new potent and selective A2a adenosine receptor antagonist. *J Pharmacol Exp Ther,* **276,** 398-404.

 Corresponding author: Maria José Diógenes

10- Titles and legends of the figures

FIGURE 1- Extracellular recordings in hippocampal slices. Schematic representation of a hippocampal transverse slice preparation showing the recording configuration used to obtain extracellular responses in the CA1 dendritic layer (stratum radiatum) evoked by stimulation of two (**A**) separate sets of the Schaffer pathway (S1 and S2). (**B**) Example of representative trace obtained after stimulation are composed of the stimulus artifact (**1**), followed by the presynaptic volley (**2**) and the fEPSP (**3**). A schematic representation of the stimulation paradigms used in plasticity experiments is represented in **C**.

FIGURE 2 - Theta-burst induced LTP but not synaptic efficiency is increased upon ageing. Left panels in **A-D** show averaged time courses changes of fEPSP slope induced by a θ-burst stimulation in the absence of any drug. The ordinates represent normalized fEPSP slopes where 0% corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: -0.61 ± 0.06 mV/ms, *n*=8 (**A**), -0.58 ± 0.06 mV/ms, *n*=7 (**B**), -0.69 ± 0.07 mV/ms, *n*=7 (**C**) and -0.69 ± 0.07 mV/ms, *n*=7 (**D**) and the abscissa represents the time of recording. Representative traces from representative experiments are shown in the right panels in **A-D**; each trace is the average of eight consecutive responses obtained before (**1**) and 46-60 minutes after (**2**) LTP induction, and is composed of the stimulus artifact, followed by the presynaptic volley and the fEPSP. Panel **E** depicts the magnitude of LTP (change in fEPSP slope at 46-60 minutes) induced by θ-burst stimulation in relation to pre-

θ-burst values (0%) in the absence of any drugs in hippocampal slices taken from the different rat ages as indicated below each column. *P<0.01 and **P<0.001 (one-way ANOVA with the Bonferroni´s correction). All values are mean ± SEM. In **F** arerepresented input-output curves obtained in hippocampal slices of young adult rats (10-15 weeks old; (○)) and aged rats (70-80 weeks old; (●)). Input/output curves are displayed as the relationship between fEPSP slope (ordenates) and stimulus intensity (measured as the amplitude of the presynaptic volley, in the abscissa) in the two age groups. After obtaining a stable baseline for at least 15 min, the input delivered to the slice was decreased until the slope of the fEPSP was zero. Afterwards, the current delivered to the slice was increased by steps of 20 μA at the time, with three data points collected at each stimulation amplitude (each data point being the average of 8 individual fEPSP). The range of all the input delivered to the slice was typically from 60 μA to a supra maximum intensity amplitude of 300 μA. For each age group the data are mean±SEM (both of fiber volley and fEPSP slope in each data point) of n=3 slices taken from different animals.

FIGURE 3. Comparison of the performance by young and aged rats in the spatial learning protocol of the Morris Water maze. A) Learning curve of the mean escape latencies (± SEM) over the five days of acquisition training for young (○) and aged (●) animals (n=8). * P<0.01 between the groups, 2-way ANOVA repeated measures test; **B**) Percentage of time spent in each quadrant in hidden platform test (probe test), which was performed after the acquisition period. *p>0.05, Student t-test.

FIGURE 4- The BDNF scavenger, TrkB-Fc, decreases the LTP magnitude in slices taken from 70-80 week-old rats but not in slices taken from 10-15 week old rats. Panels **A** and **C** illustrate virtual absence of changes in fEPSP slope when TrkB-Fc (2μg/ml) was applied to hippocampal slices taken from 10-15 (**A**) or from 70-80 (**C**) week old rats. **B** and **D** show averaged time courses changes of fEPSP slope induced by the θ-burst stimulation in the absence (○) or in the presence (●) of TrkB-Fc (2μg/ml) in slices taken from 10-15 (**B**) or from 70-80 (**D**) week old rats. Ordinates represent normalized fEPSP slopes where 0% corresponds to the averaged fEPSP slopes recorded for 10 minutes immediately before TrkB-Fc, and were: -0.43±0.01 mV/ms, (*n*=3,**A**) and -0.53±0.10 mV/ms, (*n*=3,**C**) or corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: -0.49±0.05 mV/ms, *n*=4 (B,●) -0,50±0.06 mV/ms, *n*=4 (**B**,○) -0.55±0.01 mV/ms, *n*=3 (**D**,●), -0.59±0.02 mV/ms, *n*=3 (**D**,○). The abscissas represent the time of every recordings. Representative traces from a representative experiment are shown below the panel **D**; each trace is the average of eight consecutive responses obtained before (1 and 3) and 46-60 minutes after (2 and 4) LTP induction, and is composed of the stimulus artifact, followed by the presynaptic volley and the fEPSP. Panel **E** depicts the magnitude of LTP (change in fEPSP slope at 46-60 minutes) induced by θ-burst stimulation in relation to pre-θ-burst values (0%) in the absence of any drugs (control) and in the presence of TrkB-Fc (2μg/ml) in slices from 10-15 week and 70-80 week old rats. **P*<0.05 (paired Student´s *t* test). All values are mean ± SEM

Corresponding author: Maria José Diógenes

FIGURE 5- The inhibitor of Trk phosphorilation, K252A, decreases the LTP magnitude in slices taken from 70-80 weekold rats but not in slices taken from 10-15 week old rats. A and **C** show averaged time courses changes of fEPSP slope induced by K252a (200 nM) when applied to hippocampal slices taken from 10-15 (**A**) or from 70-80 (**C**) week old rats. **B** and **D** show averaged time courses changes of fEPSP slope induced by the θ-burst stimulation in the absence (○) or in the presence (●) of K252a (200 nM) in slices taken from 10-15 week old (**B**) or 70-80 (**D**) week old rats. Ordinates represent normalized fEPSP slopes where 0% represents the averaged fEPSP slopes recorded for 10 minutes immediately before K252a administration; and were, -0.72±0.11 mV/ms, *n*=4 (**A**) and -0.72±0.07 mV/ms, *n*=4 (**C**) or represent normalized fEPSP slopes where 0% corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: -0.74±0.01 mV/ms, *n*=4 (**B**,●), -0.62±0.02 mV/ms, *n*=4 (**B**,○), and -0.55±0.01 mV/ms, *n*=3 (**D**,●), -0.69±0.02 mV/ms, *n*=3 (**D**,○). The abscissa represent the time of every recordings. Panel **E** depicts the magnitude of LTP (change in fEPSP slope at 46-60 minutes) induced by θ-burst stimulation in relation to pre-θ-burst values (0%) in the absence of any drugs (control) and in the presence of K252a (200nM) in slices from 10-15 and 70-80 week old rats. All values are mean ± SEM. **P*<0.05 (paired Student´s *t* test).

Corresponding author: Maria José Diógenes

FIGURE 6- Expression levels of endogenous BDNF in the different age groups. In (**A**) are shown the Western blots of BDNF (14 KDa) and β-tubulin (55 Kda, loading control), in homogenates of rat hippocampus taken from 4, 10-15, 36-38 and 70-80 week old rats, as indicated above each lane. In (**B**) are shown the averaged of BDNF levels (n=4-5). All values are mean ± SEM. The levels of BDNF in infant (4 week old) rats were taken as 1. The hippocampus homogenates were taken from the same animals that were used in electrophysiology experiments.

FIGURE 7- Age-dependent effects of BDNF (20 ng/mL) on θ-Burst induced LTP. Panels **A-D** show averaged time courses changes of fEPSP slope induced by the θ-burst stimulation in the absence (○) or in the presence (●) of BDNF (20 ng/mL) in hippocampal slices taken from 4 (**A**), 10-15 (**B**), 36-38 (**C**) and 36-38 (**D**) week old rats. BDNF (20 ng/mL) was applied 60 minutes after the induction of LTP in the first pathway (○) and at least 30 minutes before induction of LTP in the second pathway (●). The ordinates represents normalized fEPSP slopes where 0% corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: -0.61 ± 0.06 mV/ms, *n*=4 (**A**,○), -0.52 ± 0.06 mV/ms, *n*= 4 (**A**,●), -0.58 ± 0.06 mV/ms, *n*=5 (**B**,○), -0.57 ± 0.06 mV/ms, *n*= 5 (**B**,●), -0.69 ± 0.07 mV/ms, *n*=4 (**C**,○), -0.68±0.05 mV/ms, *n*=4 (**C**,●), -0.69 ± 0.07 mV/ms, *n*= 4 (**D**,○) and -0.68 ± 0.07 mV/ms, *n*= 4 (**D**,●) and the abscissa represents the time of every recordings.

All values are mean ± SEM. Panel **D** depicts the magnitude of LTP (change in fEPSP slope at 46-60 minutes) induced by θ-burst stimulation in relation to pre-θ-burst values (0%) in the absence of any drugs (open bars), or in the presence of BDNF (20 ng/mL, filled bars) alone. Note that BDNF significantly increases LTP magnitude in hippocampal slices taken from 4 (*n*=4) and 10-15 (*n*=5) week old rats but not in slices taken from 36-38 (*n*=4) and 70-80 (*n*=4) week old rats. $^{**}P<0.005$ and $^{*}P<0.05$ (paired Student´s *t* test) as compared with absence of BDNF in the same experiments (adjacent open column to the left).

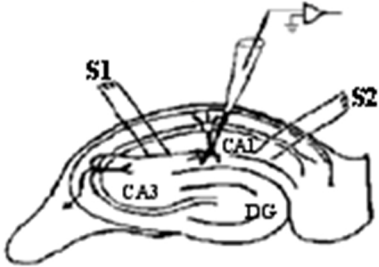
FIGURE 8. The antagonist of adenosine A_{2A} receptors, SCH 58261, prevents the effect of BDNF upon theta burst induced LTP. Panels **A** and **C** show averaged time courses changes of fEPSP slope induced by a θ-burst stimulation in the absence (○) or in the presence (●) of BDNF (20 ng/mL) in experiments where $A_{2A}R$ had been blocked by the selective antagonist, SCH 58261 (100 nM). The antagonist was applied 30 minutes before the LTP induction in the first pathway (○) and remained in the bath up to the end of the experiment being therefore present during LTP induction in the first and second pathways. BDNF (20 ng/ml) was added (●) 60 minutes after the induction of LTP in the first pathway (○) and remained up to the end of the experiment. The ordinates represents normalized fEPSP slopes where 0% corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: - 0.52 ± 0.03 mV/ms, *n*=3 (**B**,○), -0.55 ± 0.08 mV/ms, *n*=3 (**B**,●), -0.54

± 0.03 mV/ms, *n*=4 (**B**,○) and -0.63 ± 0.07 mV/ms, *n*=4 (**B**,●) and the abscissa represents the time of recordings. Panels **B** and **D** depict the magnitude of LTP (change in fEPSP slope at 46-60 minutes) induced by θ-burst stimulation in relation to pre-θ-burst values (0%) in the absence of any drugs (control), in the presence of BDNF (20 ng/mL) alone, in the presence of SCH 58261 (100 nM) alone or BDNF (20 ng/mL) together with SCH 58261 (100 nM) as indicated below each column. All values are mean ± SEM. **P*<0.05 (one-way ANOVA with the Bonferroni´s correction).

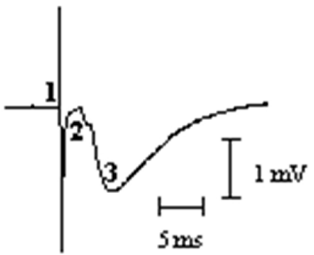
FIGURE 9- Age-dependent effects of SCH 58261 (100 nM) effect on θ-Burst induced LTP. **A**, **B**, **C** and **D** show averaged time courses changes of fEPSP slope induced by the θ-burst stimulation in the absence (○) or in the presence (●) of SCH 58261 (100 nM) in hippocampal slices taken from 4 (**A**), 10-15 (**B**), 36-38 (**C**) and 70-80 (**D**) week old rats. SCH 58261 (100nM) was applied (●) 60 minutes after the induction of LTP in the first pathway. The ordinates represents normalized fEPSP slopes where 0% corresponds to the averaged slopes recorded for 14 minutes before θ-burst stimulation: - 0.56 ± 0.03 mV/ms, *n*=4 (**A**,○), -0.57 ± 0.03 mV/ms, *n*= 4 (**A**,●), -0.56 ± 0.01 mV/ms, *n*=3 (**B**,○), 0.52 ± 0.06 mV/ms, *n*=3 (**B**,●), -0.59 ± 0.06 mV/ms, *n*=3 (**C**,○), -0.65 ± 0.09 mV/ms, *n*=3 (C, ●), -0.60 ± 0.12 mV/ms, *n*=3 (**D**,○) and -0.59 ± 0.05 mV/ms, *n*= 3 (**D**,●) and the abscissa represents the time of recordings. Panel D depicts the magnitude of LTP (change in fEPSP slope at 46-

60 minutes) induced by θ-burst stimulation in relation to pre-θ-burst values (0%) in the absence of any drugs (control) or in the presence of SCH 58261 (100 nM). Note that SCH 58261 significantly decreased LTP magnitude in hippocampal slices taken from 36-38 (*n*=3) and 70-80 (*n*=3) week old rats but not in slices taken from 4 (*n*=4) and 10-15 (*n*=3) week old rats. All values are mean ± SEM. **P*<0.05 (paired Student´s *t* test) as compared with absence of SCH 58261 in the same experiments (adjacent open column to the left).

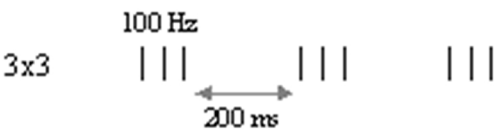
A- Recording configuration

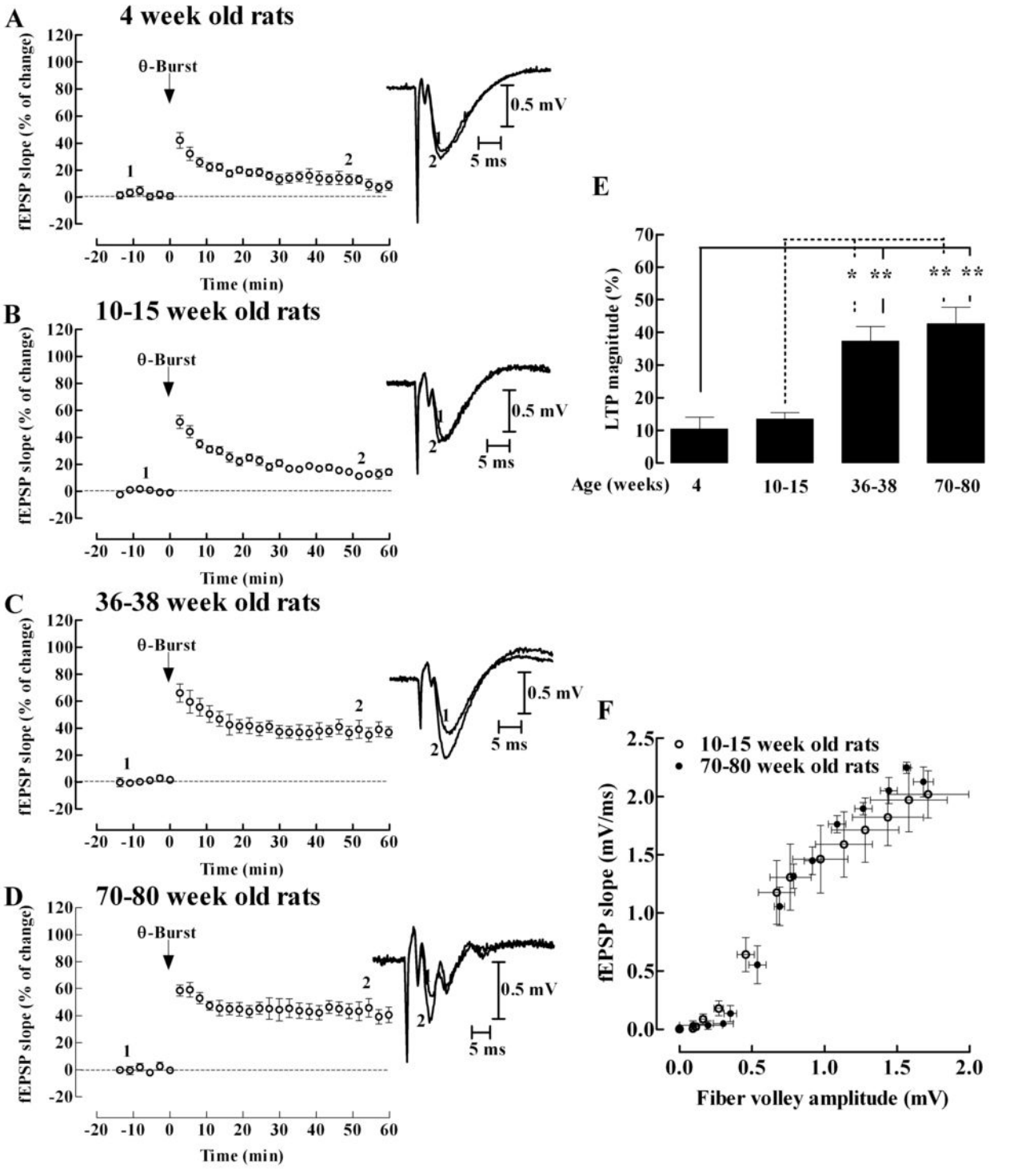


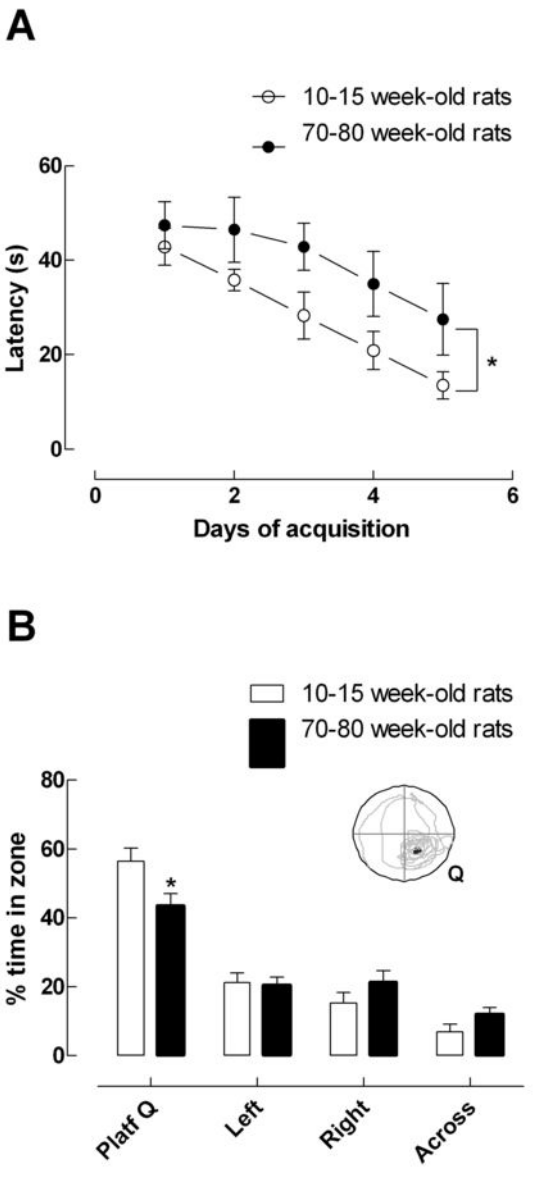
B- Signal recorded

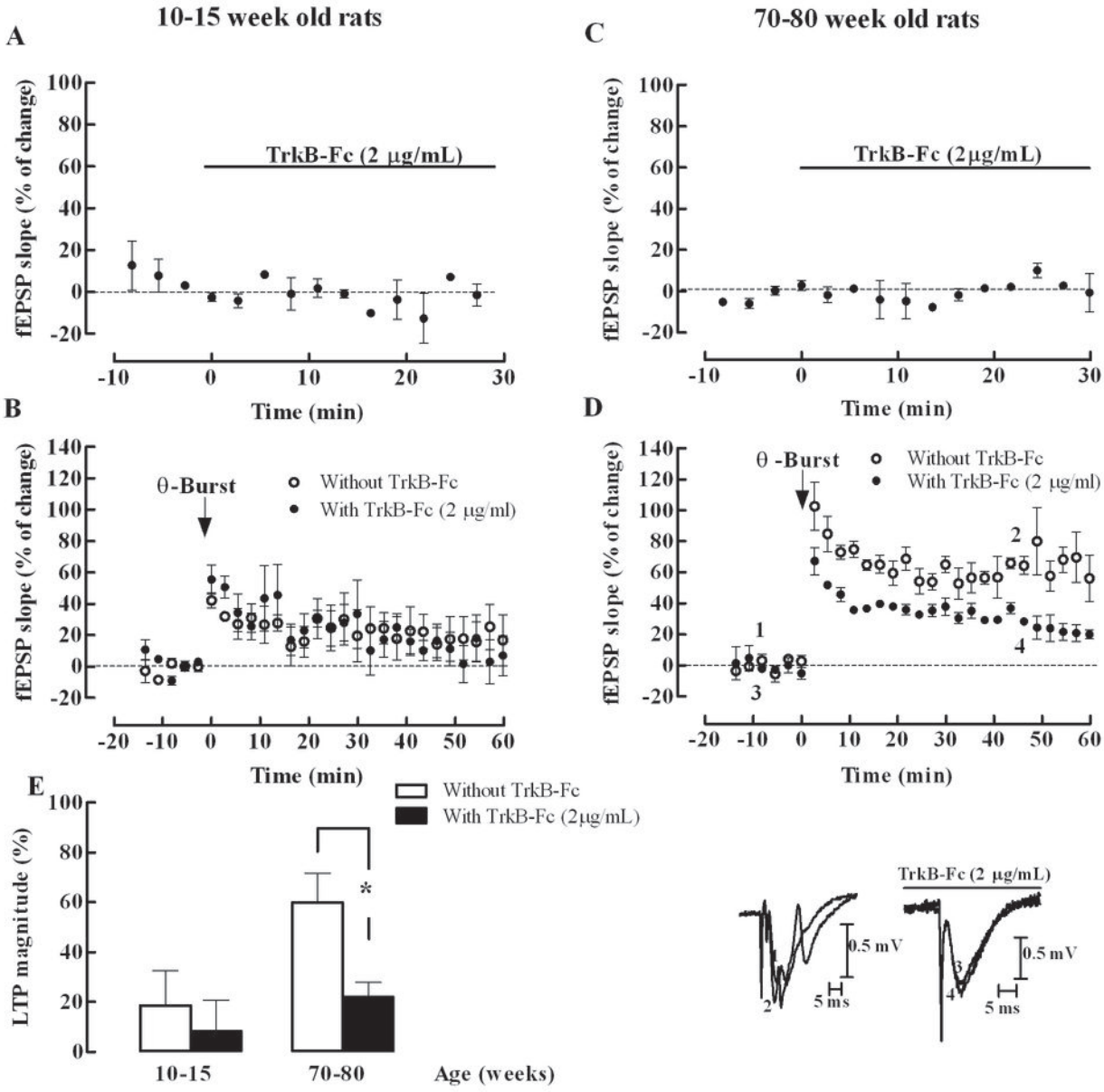


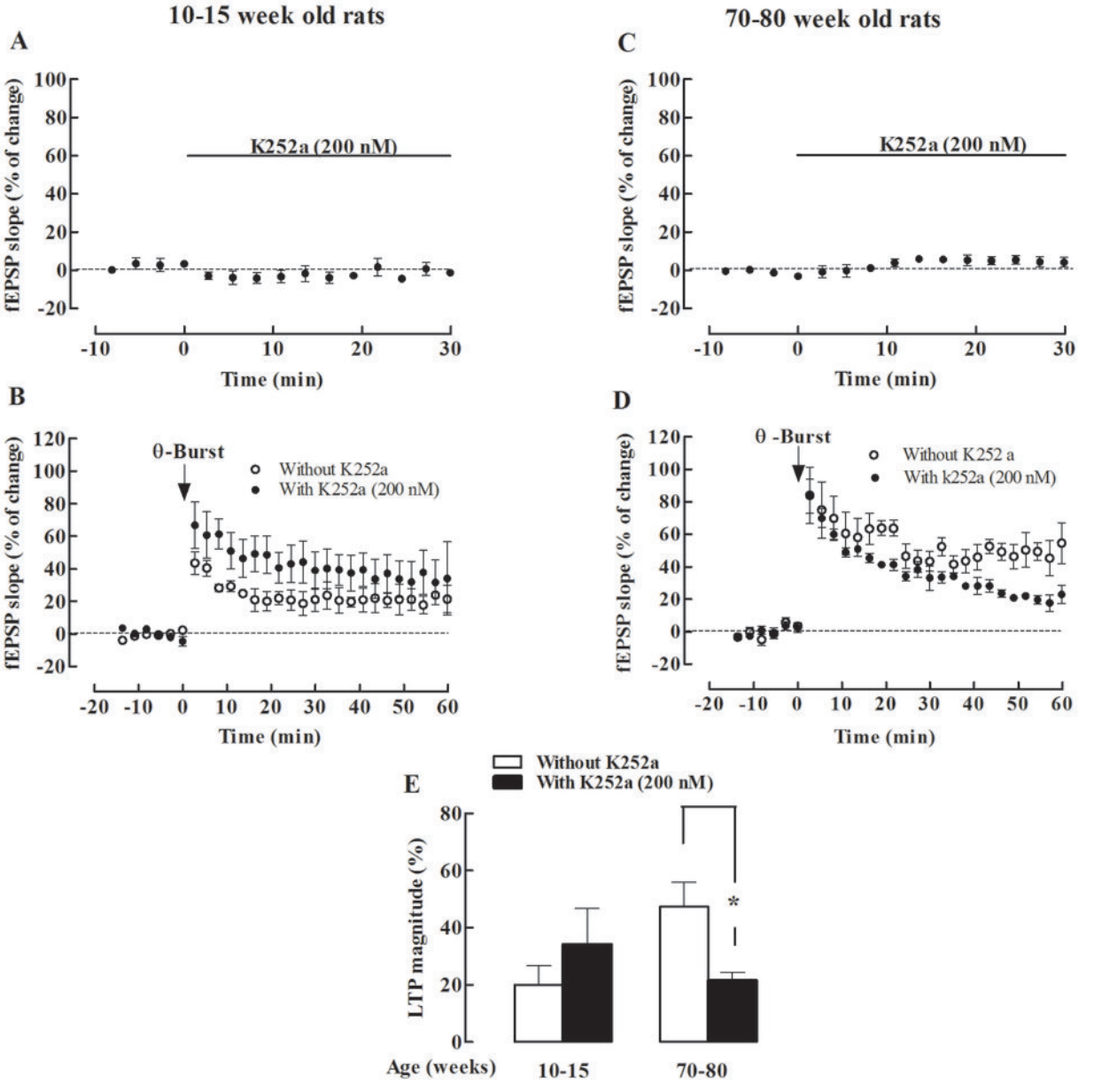
C- θ-Burst stimulation paradigm

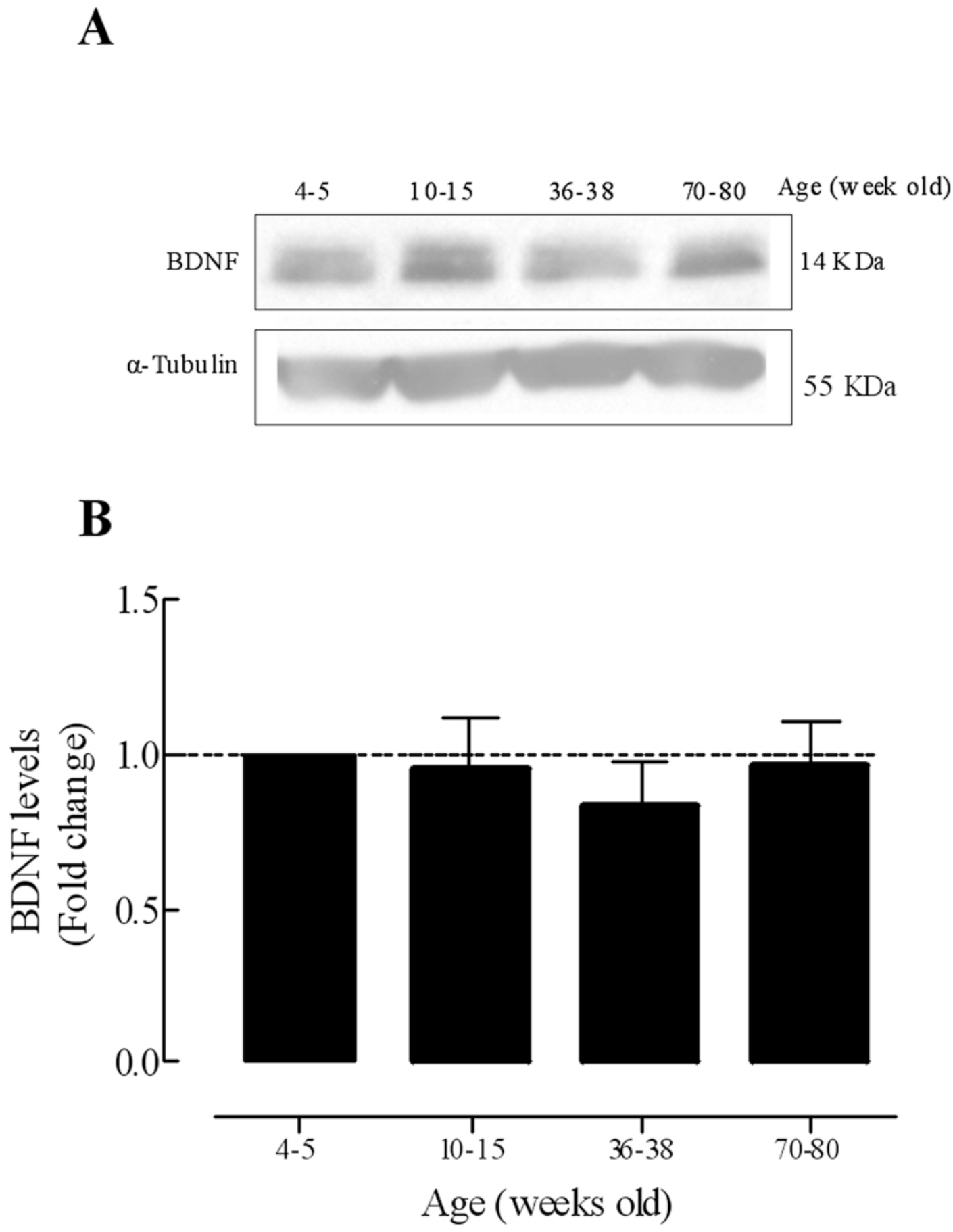


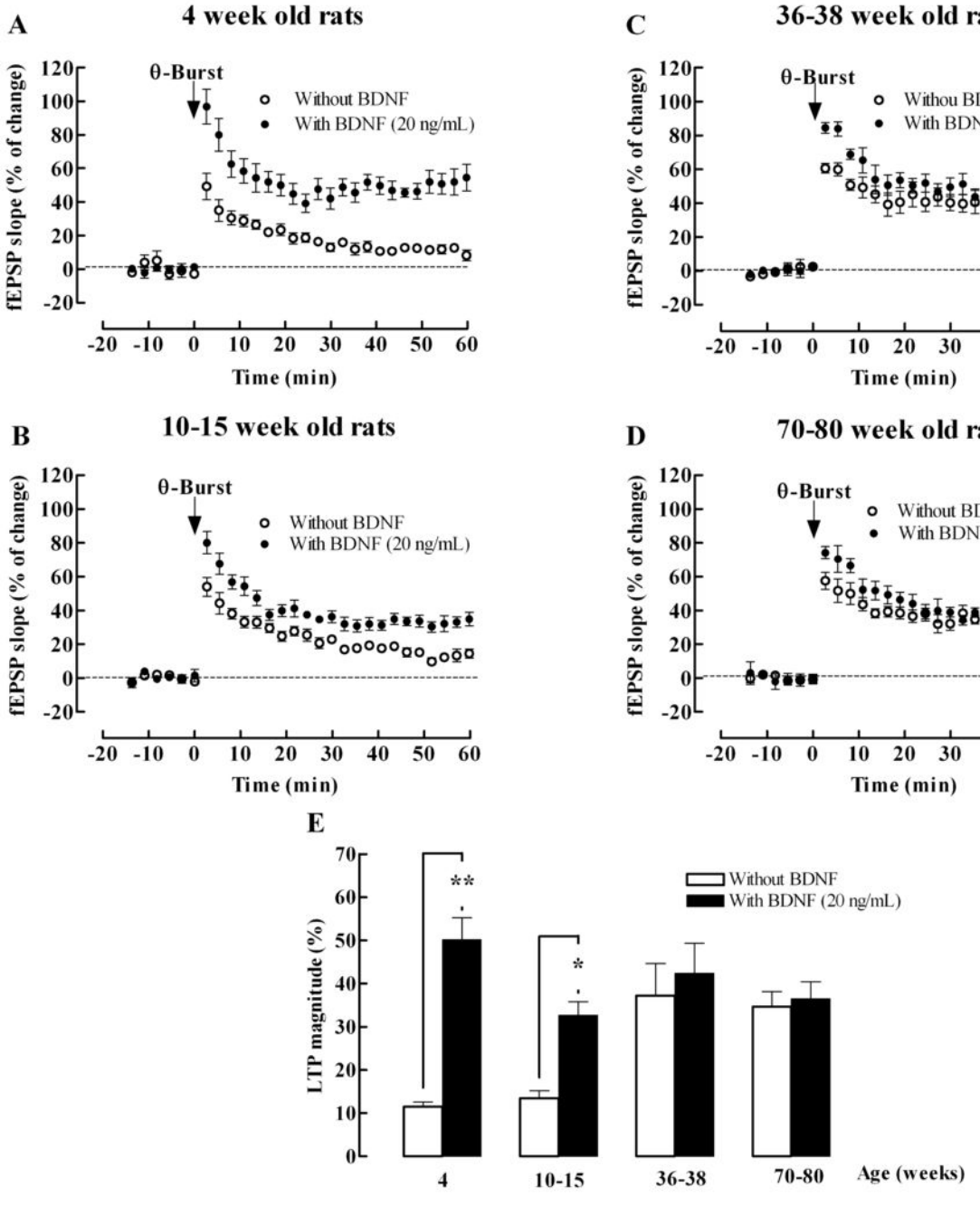

A
4 week old rats
θ-Burst
fEPSP slope (% of change)
Time (min)
0.5 mV
5 ms
B
10-15 week old rats
C
36-38 week old rats
D
70-80 week old rats
E
LTP magnitude (%)
Age (weeks)
4
10-15
36-38
70-80
F
10-15 week old rats
70-80 week old rats
fEPSP slope (mV/ms)
Fiber volley amplitude (mV)

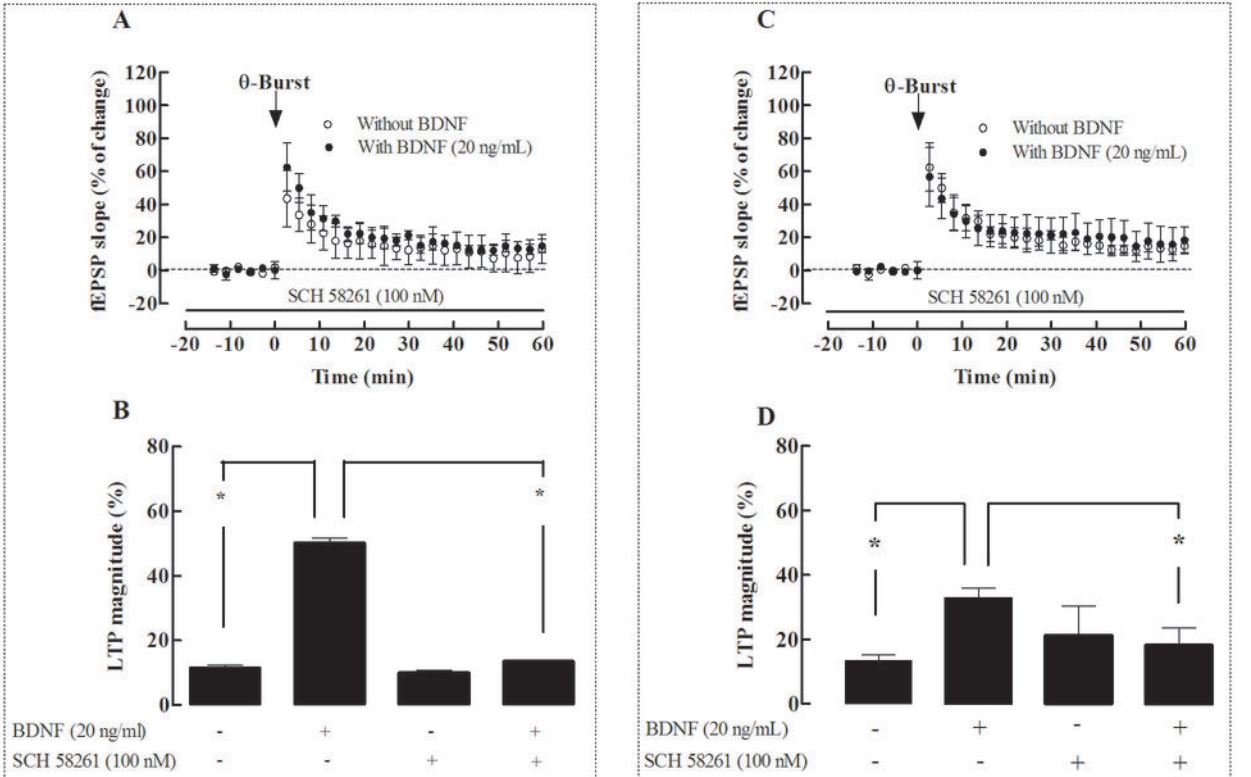

A
10-15 week-old rats
70-80 week-old rats
Latency (s)
60
40
20
0
*
0
2
4
6
Days of acquisition
B
10-15 week-old rats
70-80 week-old rats
% time in zone
80
60
40
20
0
*
Q
Platf Q
Left
Right
Across

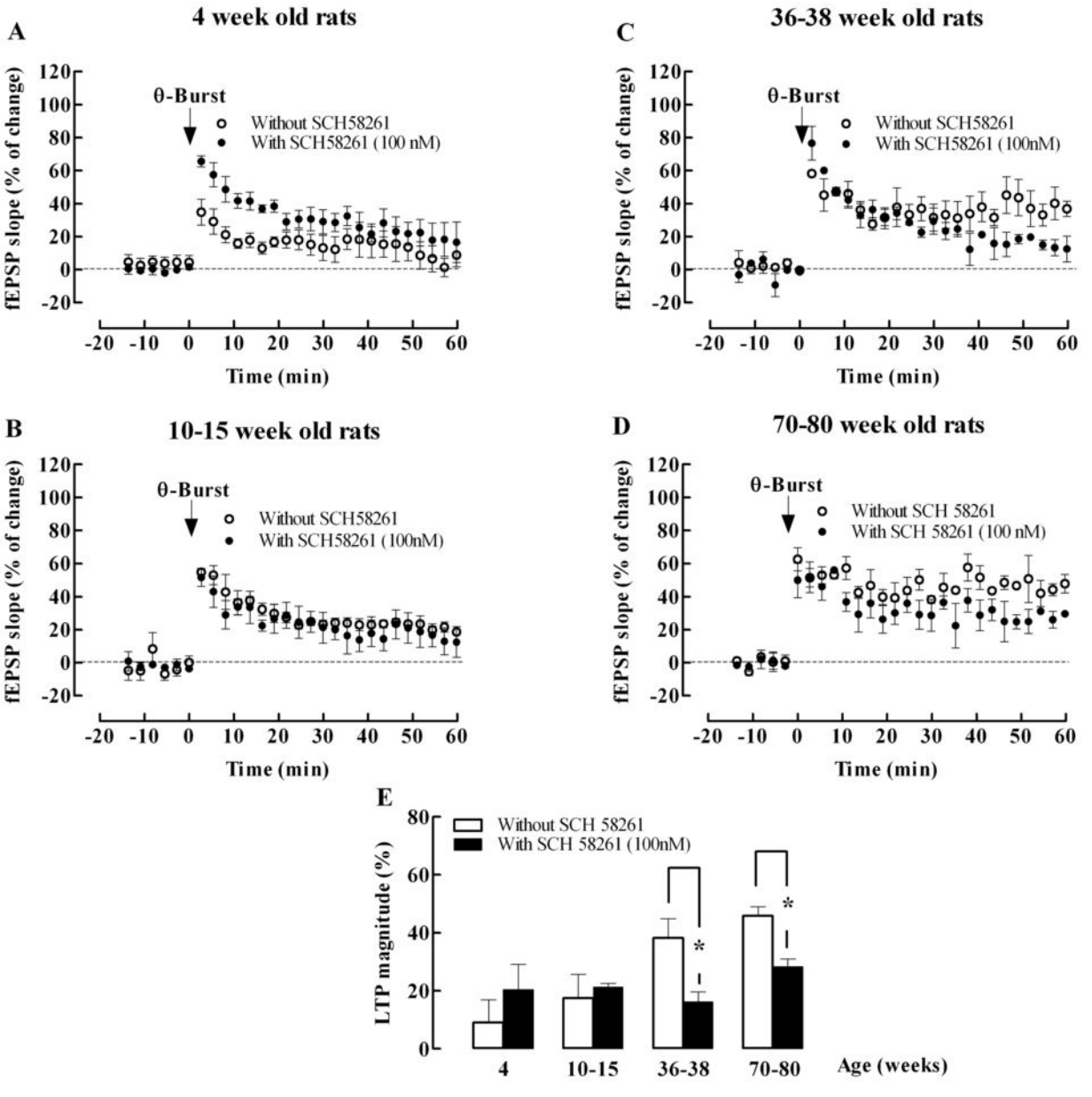

A
10-15 week old rats
TrkB-Fc (2 μg/mL)
fEPSP slope (% of change)
Time (min)
C
70-80 week old rats
TrkB-Fc (2μg/mL)
fEPSP slope (% of change)
Time (min)
B
θ -Burst
Without TrkB-Fc
With TrkB-Fc (2 μg/ml)
fEPSP slope (% of change)
Time (min)
D
θ -Burst
Without TrkB-Fc
With TrkB-Fc (2 μg/ml)
1
2
3
4
fEPSP slope (% of change)
Time (min)
E
Without TrkB-Fc
With TrkB-Fc (2μg/mL)
LTP magnitude (%)
*
10-15
70-80
Age (weeks)
TrkB-Fc (2 μg/mL)
0.5 mV
5 ms
0.5 mV
5 ms
2
3
4


10-15 week old rats
70-80 week old rats
A
C
B
D
E
fEPSP slope (% of change)
K252a (200 nM)
Time (min)
θ-Burst
Without K252a
With K252a (200 nM)
Without K252 a
With k252a (200 nM)
LTP magnitude (%)
Age (weeks)
10-15
70-80
*


A
4-5
10-15
36-38
70-80
Age (week old)
BDNF
14 KDa
α-Tubulin
55 KDa
B
1.5
1.0
0.5
0.0
BDNF levels
(Fold change)
4-5
10-15
36-38
70-80
Age (weeks old)


A
4 week old rats
θ-Burst
Without BDNF
With BDNF (20 ng/mL)
fEPSP slope (% of change)
Time (min)
B
10-15 week old rats
θ-Burst
Without BDNF
With BDNF (20 ng/mL)
fEPSP slope (% of change)
Time (min)
C
36-38 week old rats
θ-Burst
Withou BDNF
With BDNF (20 ng/mL)
fEPSP slope (% of change)
Time (min)
D
70-80 week old rats
θ-Burst
Without BDNF
With BDNF (20 ng/mL)
fEPSP slope (% of change)
Time (min)
E
Without BDNF
With BDNF (20 ng/mL)
LTP magnitude (%)
**
*
4
10-15
36-38
70-80
Age (weeks)


4 week old rats
10-15 week old rats
A
C
θ-Burst
Without BDNF
With BDNF (20 ng/mL)
fEPSP slope (% of change)
SCH 58261 (100 nM)
Time (min)
B
D
LTP magnitude (%)
BDNF (20 ng/ml)
BDNF (20 ng/mL)
SCH 58261 (100 nM)


A
4 week old rats
θ-Burst
Without SCH58261
With SCH58261 (100 nM)
fEPSP slope (% of change)
Time (min)
B
10-15 week old rats
θ-Burst
Without SCH58261
With SCH58261 (100nM)
fEPSP slope (% of change)
Time (min)
C
36-38 week old rats
θ-Burst
Without SCH58261
With SCH58261 (100nM)
fEPSP slope (% of change)
Time (min)
D
70-80 week old rats
θ-Burst
Without SCH 58261
With SCH 58261 (100 nM)
fEPSP slope (% of change)
Time (min)
E
Without SCH 58261
With SCH 58261 (100nM)
LTP magnitude (%)
4
10-15
36-38
70-80
Age (weeks)