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Research report

TrkB in the hippocampus and nucleus accumbens differentially modulates depression-like behavior in mice

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ABSTRACT

Brain-derived neurotrophic factor (BDNF) exerts antidepressant-like effects in the hippocampus and pro-depressant effects in the nucleus accumbens (NAc). It is thought that downstream signaling of the BDNF receptor TrkB mediates the effects of BDNF in these brain structures. Here, we evaluate how TrkB regulates affective behavior in the hippocampus and NAc. We overexpressed TrkB by electroporating a non-viral plasmid in the NAc or hippocampus in mice. Depression- and anxiety-like behaviors were evaluated in the sucrose test (anhedonia), the forced swim test (despair) and the elevated zero maze (anxiety). Targeted brain tissue was biochemically analyzed to identify molecular mechanisms responsible for the observed behavior. Overexpressing TrkB in the NAc increased the number of young neuronal cells and decreased despair and basal corticosterone levels. TrkB overexpression in the hippocampus increased astrocyte production and activation of the transcription factor CREB, yet without altering affective behavior. Our data suggest antidepressant effects of BDNF-TrkB in the NAc, which could not be explained by activation of the transcription factors CREB or β-catenin. The effects TrkB has on depression-related behavior in different brain regions appear to critically depend on the targeted cell type.

1. Introduction

Major depression (MD) is a severe mental disorder expected to be the second leading cause of disability worldwide by 2020 [1]. According to the neurotrophin hypothesis of depression, stress-induced decreases in neurotrophic support may contribute to the pathophysiology of depression by impairing hippocampal plasticity [2,3]. Brain-derived neurotrophic factor (BDNF) is a well-studied neurotrophin directly implicated in the pathophysiology of MD and in the mechanism of action of antidepressants. Intra-hippocampal injection or viral overexpression of BDNF produce antidepressant effects in animal models for depression [4–7], while reducing BDNF by means of RNA interference in the DG or by a conditional BDNF deletion in the forebrain attenuates antidepressant efficacy [8,9]. A conditional knock-out of BDNF in the forebrain induces depression-like behavior in female mice [8], and local silencing of BDNF in the dorsal DG in male rats increases anhedonia and despair [10]. These studies provide evidence that BDNF is necessary and sufficient for anti-depressant efficacy, while BDNF reductions may be a contributing factor in MD.

Tropomyosin-related kinase B (TrkB), a member of the Trk family of receptor tyrosine kinases, is a high-affinity receptor for BDNF. Activation of TrkB is linked to multiple signaling cascades (PLCγ, Erk and Akt signaling) that increase neuronal synaptogenesis and neurogenesis, thus increasing overall plasticity in the brain [11]. These cascades are initiated by autophosphorylation of the TrkB receptor on different cytoplasmic Tyrosine residues, which recruit specific

Abbreviations: BDNF, brain-derived neurotrophic factor; DCX, doublecortin; DG, dentate gyrus; EZM, elevated zero maze; FST, forced swim test; MD, major depression; NAc, nucleus accumbens; OF, open field; SIT, sucrose intake test; TBS, tris-buffered saline; TrkB, tropomyosin-related kinase B; VTA, ventral tegmental area.

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binding proteins. Phosphorylation of Tyr785 triggers activation of PLCγ, which on its turn hydrolyses phosphatidyl inositides. This causes an intracellular release of Ca^{2+} and activation of CaM and CaMKK which results in phosphorylation and activation of the transcription factor CREB. Phosphorylation of Tyr490 in the cytoplasmic domain of TrkB recruits the Shc-Grb2-SOS binding complex which on its turn activates Ras or PI-3K. Activated Ras triggers activation of the Erk/MAPK cascade which eventually results in CREB phosphorylation. Phosphorylation of PI-3K activates PI-3K/Akt signaling which phosphorylates and inhibits GSK3β. Inhibition of GSK3β leads to an accumulation of the transcription factor β-catenin and activation of Wnt-regulated gene expression. Similar outcomes to BDNF have been recorded when studying TrkB in relation to depression. Transgenic mice overexpressing full-length TrkB in the hippocampus and cortex show increased latency to immobility in the forced swim test (FST) and reduced anxiety-like behavior [12–14]. In contrast, transgenic mice overexpressing dysfunctional TrkB in these brain regions are resistant to the effects of antidepressants [15].

During chronic exposure to stress, the expression of glucocorticoid receptors is downregulated, especially at the level of the hypothalamus and the pituitary leading to disinhibition and hyperactivity of the HPA-axis. Long-lasting increased glucocorticoid levels, possibly by negatively affecting BDNF-TrkB signaling, may eventually damage the hippocampus as evidenced by decreased hippocampal volume, cell proliferation, atrophy, cell loss, and reduced neuronal turnover in the DG [16–20]. The resulting damage in the hippocampus, an important brain area involved in learning, memory and mood, may therefore cause depressive-like states. This interaction between glucocorticoids and BDNF signaling works in both directions. Acute or chronic central administration of BDNF in the lateral ventricles in rats increases hypothalamic mRNA expression of corticotrophin-releasing hormone, and activates the HPA-axis as evidenced by increased plasma corticosterone levels [21–23].

In contrast to the hippocampus, viral overexpression or infusion of BDNF into the ventral tegmental area (VTA)-nucleus accumbens (NAc) system induces depression-like phenotypes and attenuates antidepressant efficacy in rats [24,25]. Also, viral-mediated deletion of BDNF in the VTA reduces social avoidance induced by social defeat stress in mice and elicits antidepressant effects in the FST and sucrose preference test in rats [24,26]. Viral overexpression of a dominant-negative form of TrkB in the NAc produces an antidepressant phenotype in rats [25]. Additionally, rats with viral-mediated silencing of CREB in the NAc display less depression-like behavior [27]. This suggests a pro-depressant role for BDNF-TrkB-CREB signaling in the VTA-NAc, in striking contrast to the antidepressant properties in the hippocampus. Interestingly, it has not been investigated whether overexpression of functional TrkB in the NAc negatively influences affect.

1.1. Aims of the study

In order to elucidate the exact role of TrkB in the hippocampus and the NAc in relation to depression in mice, we locally transfected a non-viral plasmid encoding TrkB in the dorsal dentate gyrus (DG) of the hippocampus or NAc-shell using micro-electroporation. We hypothesized that overexpression of TrkB would have opposite effects on measures of anxiety- and depression-like behaviors, with a behavioral phenotype depending on the manipulated brain region.

2. Methods and materials

2.1. Animals

Six weeks old, male C57BL/6 mice were purchased from Charles River (L' Arbresle, France). All animals were housed under an inverted 12 h light/dark cycle (lights on at 7:00 PM) with an average temperature of 22 °C and relative humidity of 42%. All experimental procedures were approved by the local ethical committee for animal experiments, according to governmental guidelines (EAA: DGVGZ/VVP-83267).

2.2. Plasmid DNA

pEF/BOS-TrkB.TK(+) encoding rat full length TrkB with a 5′ FLAG-tag was kindly provided by Dr. Alex Krüttgen from RWTH Aachen (Aachen, Germany). pVAX-LacZ plasmid encoding β-galactosidase was from Invitrogen (San Diego, CA). Plasmid DNA was prepared using the Qiaprep Spin Maxiprep kit (Qiagen, Venlo, the Netherlands) according to the manufacturer's instructions. Plasmid DNA was dissolved in saline (0.9% NaCl) at 2.5 μg/μl and verified by restriction analysis and western blotting.

2.3. In vivo *micro-electroporation*

Micro-electroporation does not induce inflammation or damage and offers a safe and efficient alternative to viral overexpression [28,29]. Bilateral *in vivo* micro-electroporation was performed as described previously [28,30]. Briefly, anesthetized animals were injected with 0.5 μl plasmid DNA or saline in the NAc-shell (AP + 1.28 mm, ML ± 1.0 mm, DV −4.75 mm) or in the DG (AP −2.06 mm, ML ± 1.5 mm, DV −2.0 mm) at 0.05 μl/min. After DNA injection, electroporation was performed with a pair of needle-like electrodes (Technomed, Beek, the Netherlands). Coordinates for the electrodes in the DG were: AP −1.34 mm, ML ± 1.0 mm, DV −2.0 mm (anode); AP −3.07 mm, ML ± 2.20 mm, DV −2.0 mm (cathode). In the NAc, coordinates were: AP + 2.33 mm, ML ± 1.0 mm, DV −4.75 mm (anode); AP + 0.23 mm, ML ± 1.0 mm, DV −4.75 mm (cathode). Five unipolar square wave pulses (125 μA, 50 ms, 1 Hz) were given using a current-controlled stimulator/isolator (DS8000; WPI, Sarasota, FL). Mice were distributed over 5 experimental groups and balanced for weight: untreated ($n = 13$), HC-control ($n = 13$), HC-TrkB ($n = 13$), NAc-control ($n = 13$), NAc-TrkB ($n = 13$).

2.4. Behavior

Mice were allowed to recover for 2 weeks before behavioral testing. The sucrose intake test (SIT) and open field (OF) test were performed 2 weeks following electroporation, the elevated zero maze (EZM) test after 2.5 weeks and the FST after 3 weeks. Testing took place during the dark phase of the light/dark cycle between 7:00 AM and 7:00 PM. Mice were automatically tracked during behavioral testing in the OF, EZM and FST via a video camera connected to a video tracking system (Ethovision Pro, Noldus, the Netherlands). Outcome measures were calculated using Ethovision Pro software. In order to ensure that electroporating a vector does not induce behavioral changes itself, we electroporated the hippocampus of additional animals with a pVAX-LacZ vector expressing the reporter protein β-galactosidase. This was not the case as no differences in any of the behavioral measures were found between untreated animals, saline electroporated animals and animals electroporated with pVAX-LacZ (data not shown).

2.4.1. Open field test

As certain behavioral tasks like the FST or EZM can be affected by changes in locomotor activity, the OF was used to assess locomotor

activity. As a measure of anxiety-like behavior, thigmotaxis or wall-hugging was also analyzed in the OF by the total time spent near the walls and corners of the arena [31]. The test was conducted in a square Plexiglas base (100 × 100 cm) with a black floor and 40 cm high transparent Plexiglas walls and it was divided into 4 equal arenas (50 × 50 cm each) separated by 40 cm high black Plexiglas walls. The illumination of the room was reduced to 20 lux on the floor of the apparatus. Four mice were placed in the center of each arena and the total distance moved was measured over a period of 30 min.

2.4.2. Elevated zero maze

The EZM is used to measure anxiety-like behavior and is made of black plastic (transparent for infrared light). It consists of a circular runway (50 cm in diameter, 6 cm path width, 70 cm above floor level), which is divided equally into two opposite open and two opposite parts enclosed with 25 cm high side walls. A 2 mm high rim surrounds the open parts to prevent falls. A mouse was placed into one of the open parts facing a closed part and allowed to explore the maze over a period of 5 min. The number of open arm entries and the time spent in the open arms were measured under low light conditions (1–2 lux) via an infrared video camera connected to the video tracking system.

2.4.3. Sucrose intake test

The SIT was used to assess anhedonia, one of the core symptoms of depression. For the SIT, the mice were habituated to sucrose by replacement of the normal drinking water with water containing 1% sucrose for 24 h. After the habituation period, animals had access to normal drinking water and chow for one night. Subsequently, the SIT started at the beginning of the dark phase by depriving the animals from water and chow for 6 h. Immediately afterwards, the mice were allowed to drink from a bottle with 1% sucrose solution for 1 h. Before and after the test the bottles were weighed to determine sucrose consumption. Sucrose consumption was expressed as ml sucrose/g body weight to control for intake differences due to possible differences in body weight. No differences in water consumption over 24 h were found (data not shown), ensuring no changes in overall drinking rates in the SIT.

2.4.4. Forced swim test

Animals were tested in the FST, a well-known test for screening antidepressants in rodents, which was modified according to Detke et al. [32]. Four cylindrical glass tanks (50 cm height × 19 cm diameter) are used in this test, filled to a depth of 20 cm with 32 °C water. The animals were tested by placing them in the water for 5 min. The movements were recorded from above using a video camera. Immobility was defined as follows: making no movements or only making those movements necessary to keep the nose above the water. The outcome measures for this test include immobility, latency to immobility and total distance moved.

2.5. Blood sampling and corticosterone radioimmunoassay

Blood samples (50 μl) were taken 4.5 weeks after electroporation between 11:00 AM and 3:00 PM from the vena saphena using heparinized blood collection tubes (Microvette®CB300, Sarstedt, Germany). All blood samples were kept on ice and subsequently centrifuged at 3.000×*g* for 10 min at 4 °C after which plasma was stored at −80 °C. ImmuChem™ Double Antibody Corticosterone ^{125}I RIA Kit (MP Biomedicals, Orangeburg, NY, USA) was used according to the manufacturer's instructions. For each sample, 5 μl serum was diluted 1:100 in steroid diluent. Per 50 μl of diluted sample, 50 μl of corticosterone–^{125}I and 100 μl anti-corticosterone was added, and samples were incubated at room temperature for 2 h. Afterwards, 250 μl precipitant solution was added and tubes were centrifuged at 2.300 rpm for 15 min. Supernatant was aspirated and the precipitate was counted in a Wizard Gamma Counter 2470 (Perkin Elmer, Waltham, MA, USA).

2.6. Perfusion and tissue preparation

The day after blood sampling, animals were deeply anesthetized with an overdose of sodiumpentobarbital (60 mg/kg; CEVA, Libourne, France) and intracardially perfused with ice-cold Somogyi fixative (4% paraformaldehyde, 15% picric acid, and 0.05% glutaraldehyde in 0.1 M phosphate buffer, pH 7.6). Brains were dissected, post-fixed in the same fixative without glutaraldehyde for 2 h and washed overnight in 0.1 M phosphate buffer. Next, brains were immersed in sucrose solution at 4 °C during two overnight steps in 10% and 20% sucrose (in 0.1 M phosphate buffer). Brains were snap-frozen with CO_2 and stored at −80 °C. Coronal sections of 30 μm were cut at −25 °C and stored at −80 °C.

2.7. Immunohistochemistry

After removal from the freezer, sections were washed three times for 10 min at room temperature in Tris-buffered saline with Tween-20 (TBS-T), Tris-buffered saline (TBS) and TBS-T. Sections were then incubated overnight at 4 °C in TBS-T containing the following primary antibodies: rabbit anti-TrkB (#4606Cell Signaling Technology, Beverly, MA; 1:100), mouse anti-FLAG (#F3165 Sigma–Aldrich, St. Louis, MO; 1:200), mouse anti-NeuN (#MAB377B Millipore; 1:50), mouse anti-GFAP-biotin (#ab79203 Abcam, Cambridge, United Kingdom; 1:400), mouse anti-S100β (#S2532 Sigma–Aldrich; 1:500), goat anti-doublecortin (#sc-8066 Santa Cruz, Santa Cruz, CA; 1:200). After washing in TBS-T/TBS/TBS-T, sections were incubated for 2 h at 4 °C with the following secondary antibodies: donkey anti-rabbit Alexa-488 (#A-21206 Invitrogen; 1:100), donkey anti-mouse Alexa-488 (#A-21202 Invitrogen; 1:100), donkey anti-mouse Alexa-594 (#A-21203 Invitrogen; 1:100), Alexa-350 streptavidine conjugate (#S-11249 Invitrogen, 1:500), donkey anti-goat Alexa-594 (#A-11058 Invitrogen; 1:100). After washing in TBS/TBS/TBS, nuclear counterstaining was performed by 30 min incubation at room temperature with Hoechst in TBS (#33342 Sigma-Aldrich; 1:500). TUNEL staining was done using the TUNEL reaction buffer kit (Roche, Basel, Switzerland) and streptavidine-Alexa-Fluor-594 (1:2.000 in TBS-T, Invitrogen).

2.8. Western blot

In an additional experiment, mice were electroporated with saline or pEF/BOS-TrkB in the DG or in the NAc-shell (n = 6 per group) and sacrificed 4.5 weeks after electroporation by decapitation. The hippocampus or NAc was isolated and snap-frozen in liquid nitrogen [33]. Samples were homogenized in 2 ml ice-cold lysis buffer (100 mM Tris-HCl (pH 8.0), 200 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.05% triton vol/vol, 1 tablet complete protease inhibitor mix (Roche) and 1 tablet phosphostop (Roche) per 10 ml buffer), centrifuged for 20 min at 4 °C and 16.000×*g*, and the supernatant was stored at −80 °C. Brain homogenates in sample buffer were boiled for 5 min and then separated on a 10–14% (depending on the target protein) SDS–PAGE gel (30 μg per sample). Following electrophoresis, proteins were transferred to a nitrocellulose membrane (Bio-Rad Laboratories, Hercules, CA) which was previously blocked with blocking buffer (50% Odyssey blocking buffer in phosphate-buffered saline, Li-Cor, Lincoln, NE) for one hour at room temperature. Next, the membranes were incubated overnight at 4 °C with the following primary antibodies in blocking buffer: mouse anti-CREB (#9104 Cell Signaling Technology, Beverly, MA; 1:3.000), rabbit anti-p-CREB (#9198 Cell Signaling Technology; 1:100), rab-

bit anti-BDNF (#sc-20981 Santa Cruz, Santa Cruz, CA; 1:600), rabbit anti-GSK-3β (#9315 Cell Signaling Technology; 1:1.000), rabbit anti-p-GSK-3β (#9336 Cell Signaling Technology; 1:1.000) and mouse anti-β-catenin (#20079 BD Transduction Laboratories, Franklin Lakes, NJ; 1:2.000), with anti-β-actin (#sc81178 Santa Cruz; 1:1.000 mouse) or mouse anti-GAPDH (#10R-G109A Fitzgerald, Huissen, the Netherlands; 1:2,000,00) for normalization. After washing with phosphate-buffered saline-0.1%Tween (PBS-T), membranes were incubated for 1 h at room temperature with the following secondary antibodies in blocking buffer: goat anti-rabbit IRDye800 (#926-32211 Li-Cor; 1:5.000) and donkey anti-mouse IRDye680 (#926-32222 Li-Cor; 1:5.000). After washing in PBS-T, fluorescent bands were visualized using an Odyssey Infrared Imaging System (Li-Cor) and intensities of specific bands were quantified using ImageJ (http://rsbweb.nih.gov/ij/). We distinguished between mBDNF (14kDa) and glycosylated proBDNF (18 kDa) as described previously [34,35].

2.9. Statistics

Data were analyzed by one-way ANOVA for each target area separately including the untreated control group. Post-hoc Bonferroni t-tests were performed in case of statistical significance. Western blot data were analyzed by Student t-test in the two brain structures separately. Significance was set at $p < 0.05$. SPSS 16.0 (SPSS Inc., Chicago, IL) was used for statistical analysis. All data are presented as means + SEM.

3. Results

3.1. Efficient gene transfer in the hippocampus and NAc via micro-electroporation

Electroporation of pEF/BOS-TrkB resulted in successful overexpression of TrkB in the DG as well as in the NAc-shell that was still detectable 4.5 weeks later at the end of behavioral experiments (Fig. 1a). Exogenous TrkB overexpression was confirmed by double-labeling with FLAG (Fig. 1b). $TrkB^+$ cells in the DG and the NAc-shell were minimally positive for NeuN, a neuronal marker (Fig. 2a). Overexpressing TrkB massively increased glial fibrillary acidic protein (GFAP) staining in the DG, but not in the NAc (Fig. 2b). In addition, transfected cells in the DG co-labelled with GFAP, a marker for astrocytes and young neuronal progenitor cells. Doublestaining for TrkB and S100β, an alternative marker for astrocytes, confirmed that targeted cells in the DG were astrocytes (Fig. 2c). $TrkB^+$ cells in the NAc co-labelled with doublecortin (DCX), a marker for later stages of neuronal differentiation (Fig. 2d). In addition, TrkB overexpression decreased DCX^+ cells in the DG, while it induced the formation of DCX^+ cells in the NAc. Therefore, targeted cells in the DG ($GFAP^+$, $S100\beta^+$ and DCX^-) were astrocytes while cells targeted in the NAc ($GFAP^-$ and DCX^+) were young neuronal progenitor cells.

3.2. TrkB overexpression in the NAc decreases despair

Anhedonia in mice can be assessed by measuring sucrose intake in the SIT [36]. Electroporation of the hippocampus or the NAc did not affect sucrose intake (hipp: $F_{2,34}$=1.10, n.s.; NAc: $F_{2,34}$=0.17, n.s.; Fig. 3a). In addition, we used the FST to measure behavioral despair [37]. Electroporation of the hippocampus did not affect immobility ($F_{2,36} = 2.13$, n.s.; Fig. 3b), latency to immobility ($F_{2,36} = 0.69$, n.s.; Fig. 3c) or the total distance moved ($F_{2,36} = 1.30$, n.s.; Fig. 3d). However, electroporation of the NAc affected all three measures (immobility: $F_{2,36} = 6.19$, $p < 0.01$; latency to immobility: $F_{2,36} = 5.15$, $p < 0.05$; distance moved: $F_{2,36} = 5.50$, $p < 0.01$). Post-hoc analysis revealed that TrkB overexpression in the NAc increased the latency to immobility ($p < 0.05$) compared to NAc-saline control animals ($p < 0.05$). Along this line, TrkB overexpression in the NAc decreased immobility ($p < 0.01$), increased the distance swum ($p < 0.01$) and increased the latency to immobility ($p < 0.05$) compared to untreated control animals. Electroporation of the hippocampus or NAc did not alter locomotor activity as measured by the total distance moved in the OF (hipp: $F_{2,36} = 0.17$, n.s.; NAc: $F_{2,36} = 0.92$, n.s.; Fig. 4a).

3.3. TrkB overexpression in the NAc decreases corticosterone levels

Anxiety-like behavior was measured in the open field and EZM [38]. Electroporation in the hippocampus or the NAc did not alter thigmotaxis (wall-hugging) in the open field (hippocampus: $F_{2,36} = 0.11$, n.s.; NAc: $F_{2,36} = 0.333$, n.s.; Fig. 4b) or the number of open arm entries (hippocampus: $F_{2,35} = 0.92$, n.s.; NAc: $F_{2,36} = 0.86$, n.s.; Fig. 4c) or the time spent in the open arms of the EZM (hippocampus: $F_{2,35} = 1.07$, n.s.; NAc: $F_{2,36} = 1.52$, n.s.; Fig. 4d). As the hippocampus is an important regulator of HPA-axis activity and glucocorticoid levels, we measured basal plasma corticosterone levels. Electroporation in the NAc but not in the hippocampus altered plasma corticosterone levels (hippocampus: $F_{2,36} = 0.66$, n.s.; NAc: $F_{2,35} = 4.17$, $p < 0.05$). Post-hoc analysis suggested that overexpression of TrkB in the NAc significantly decreased corticosterone levels compared to the respective saline condition ($p < 0.05$), which suggests a reduction of HPA-axis activity following TrkB overexpression (Fig. 4e).

3.4. TrkB overexpression induces CREB phosphorylation in the hippocampus but not in the NAc

In order to explain the behavioral effects of TrkB overexpression in the DG and the NAc-shell, we investigated hippocampal and striatal protein levels of the downstream signaling molecules CREB, GSK-3β and β-catenin. Animals were electroporated with saline or TrkB in the NAc-shell or DG, and protein levels in these brain areas were analyzed 4.5 weeks later by western blot. TrkB overexpression in the hippocampus ($p < 0.05$), but not in the NAc, significantly increased CREB phosphorylation (Fig. 5a). The increased p-CREB/CREB ratio following TrkB overexpression in the hippocampus was caused by an increase in absolute p-CREB levels ($0.05 < p < 0.1$) without affecting absolute CREB levels. GSK-3β phosphorylation and β-catenin protein levels were unaffected by TrkB overexpression in the NAc or the hippocampus (Fig. 5b–c). Since BDNF is an important target gene of CREB and a ligand for TrkB, we also looked at protein levels of mature BDNF (mBDNF) and its precursor proBDNF. No changes in mBDNF, proBDNF or proBDNF/mBDNF ratio were found following overexpression of TrkB in either of the two brain structures (Fig. 5d).

4. Discussion

In this paper we showed that overexpressing TrkB in the NAc-shell increased the latency to immobility in the FST, while simultaneously decreasing basal corticosterone levels in these animals. No effects were found on anxiety-like behavior or on general locomotor activity. Electroporation of plasmid DNA by itself did not affect any of the behavioral measures as no differences were found between untreated animals and animals electroporated with saline or a reporter plasmid in the hippocampus.

In the hippocampus, TrkB overexpression did not induce antidepressant responses. This is in line with studies showing that transgenic mice lacking TrkB or overexpressing truncated TrkB in the forebrain and hippocampus do not display increased depressive-like behavior [15,39]. As mentioned in the introduction

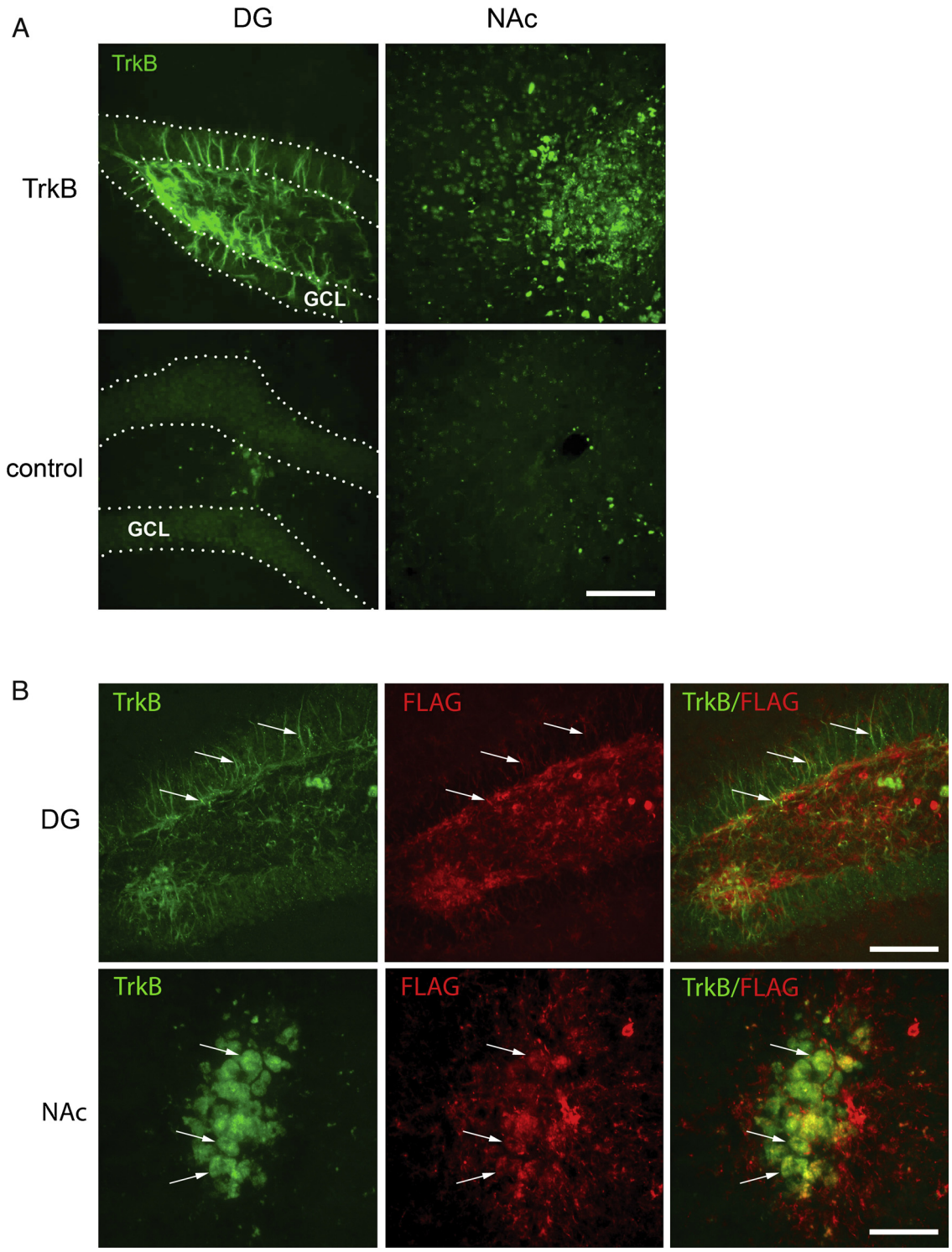

Fig. 1. Immunohistochemical stainings showing successful overexpression of TrkB with the vector pEF/BOS-TrkB.TK(+) encoding rat full length TrkB with a 5′ FLAG-tag. Animals were electroporated with saline (control) or the TrkB plasmid in the DG of the hippocampus or in the NAc. (a) Successful overexpression of TrkB is visible in both target areas 4.5 weeks after electroporation by immunostaining for TrkB. (b) Immunohistochemical stainings show overlap between TrkB and FLAG expression in the HC and in the NAc. Arrows indicate co-labeling of TrkB and FLAG staining. Scale bar represents 200 μm in (a) and in the upper panel of (b), and 100 μm in the lower panel of (b). DG = dentate gyrus, GCL = granular cell layer, NAc = nucleus accumbens.

though, TrkB in the hippocampus and cerebral cortex was shown to increase the latency to immobility in the forced swim test (FST) [12,13]. This discrepancy with our data can be explained by several factors. Firstly, TrkB overexpression in those studies resulted in activation of the PLCγ signaling cascade without affecting phosphorylation of the signaling molecules Shc, mitogen-activated protein kinase (MAPK) or Akt, which are mechanistically linked to CREB. Therefore, the antidepressant effect of TrkB reported in these studies may be linked to a specific activation of PLCγ signaling, while we found increased CREB signaling. Secondly, in the studies of Koponen et al., transgenic mice were used with a general upregulation of TrkB throughout the brain. We only targeted the DG of

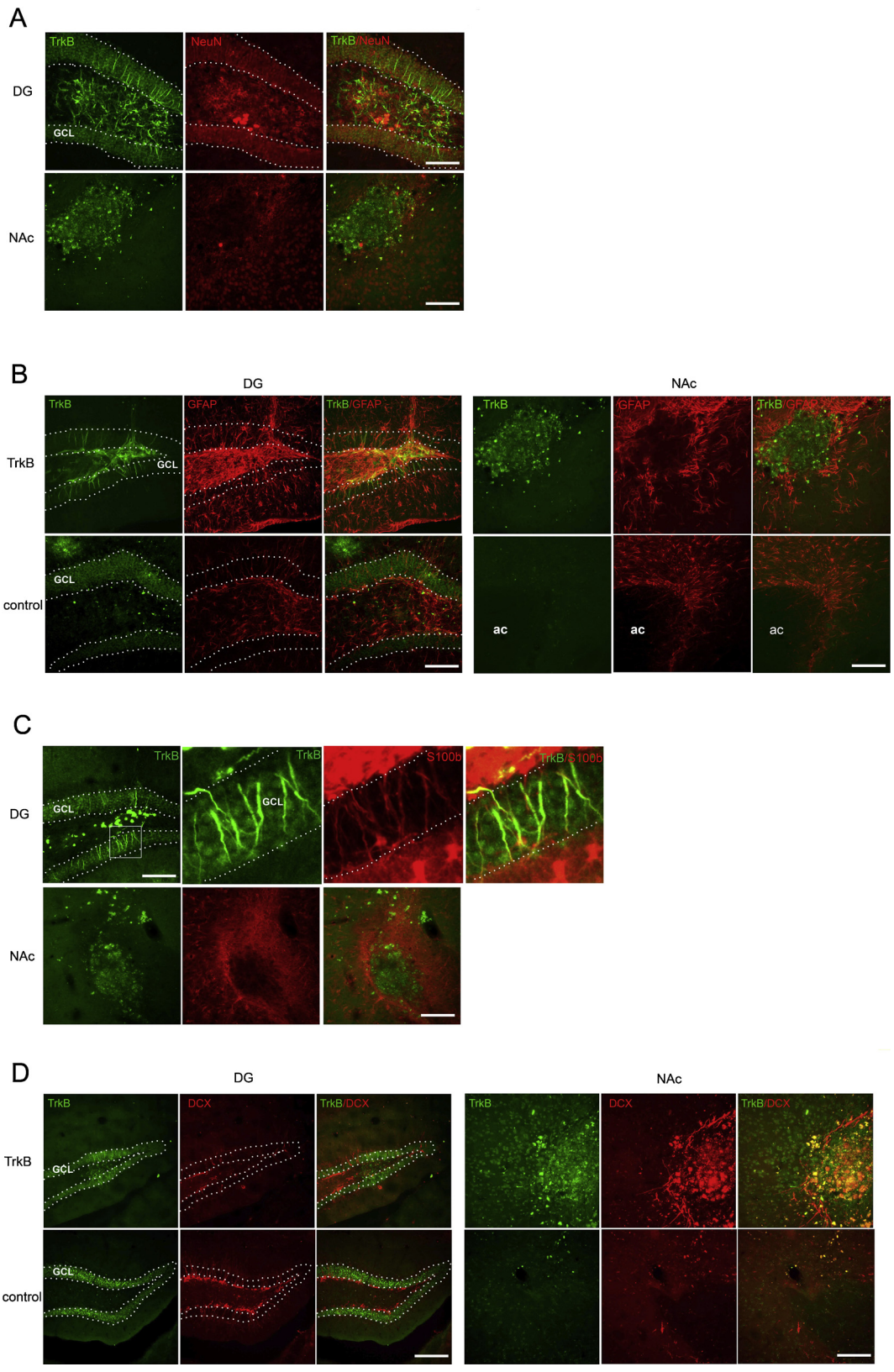

Fig. 2. Immunohistochemical stainings used to identify transfected cell types in the DG and in the NAc. These photomicrographs were taken from animals that were sacrificed 4.5 weeks after electroporation with TrkB plasmid or saline. Double immunohistochemical staining was done for TrkB and either NeuN (adult neuronal marker), GFAP (marker for adult astrocytes as well as young neuronal progenitor cells), S100β (another marker for astrocytes) or doublecortin (DCX, a marker for neuronal progenitor cells). (a) No overlap was detected with NeuN in the DG or the NAc indicating that targeted cells were not adult neurons. (b) A significant increase in GFAP which partially overlapped with exogenous TrkB was seen in the DG but not in the NAc. (c) Targeted cells in the DG were also positive for S100β, indicating that these cells were astrocytes and not young neuronal progenitor cells. (d) Interestingly, TrkB overexpression in the DG reduced the amount of DCX^+ cells, while in the NAc it increased DCX labeling which overlapped with TrkB. Scale bars represent 200 μm. ac = anterior commissura, DG = dentate gyrus, GCL = granular cell layer, NAc = nucleus accumbens.

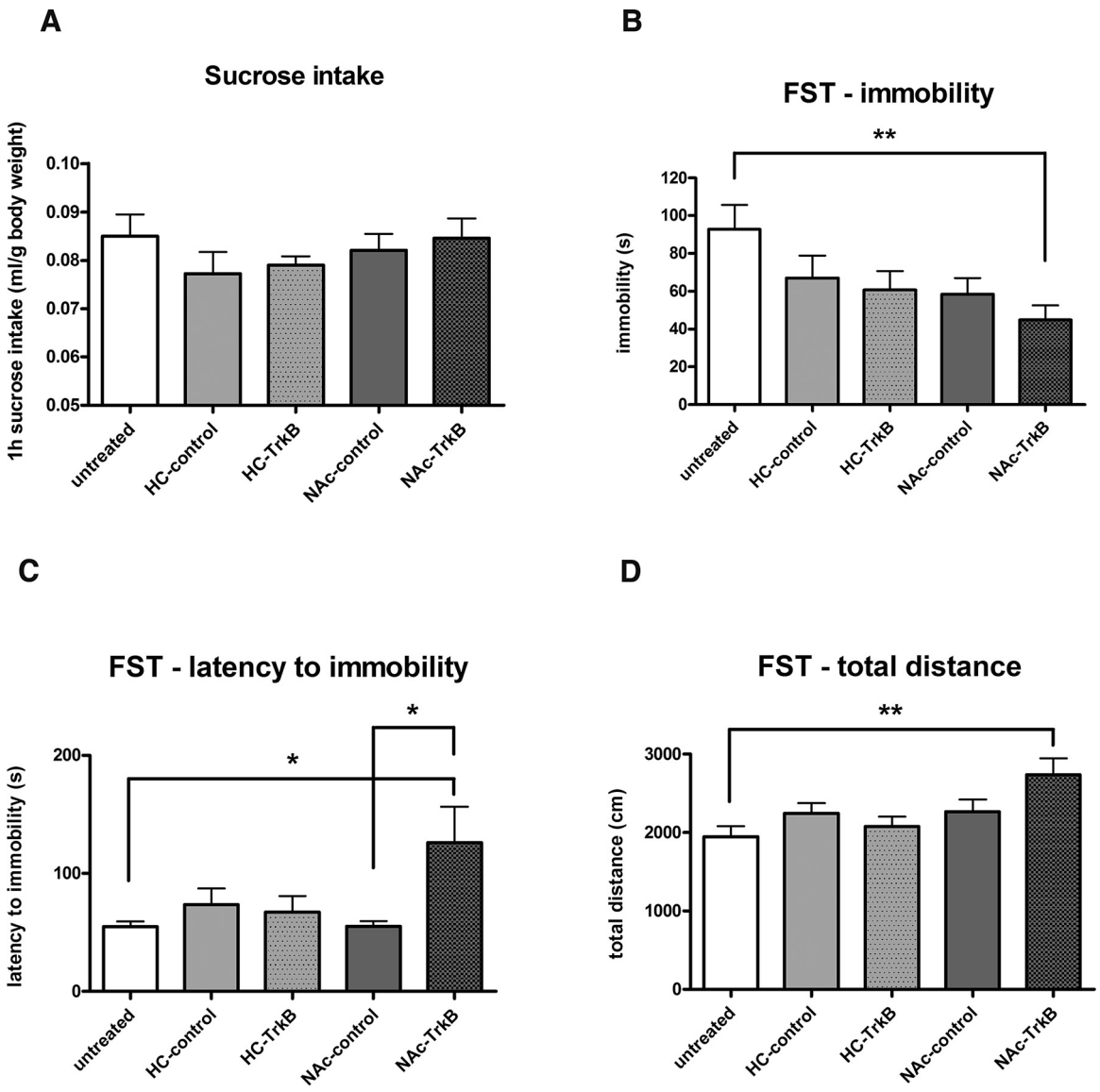

Fig. 3. Effects of TrkB overexpression in the hippocampus and the NAc on depressive-like behavior. Animals were electroporated with saline or with a plasmid expressing TrkB in the hippocampus or the NAc (all groups n = 13). (a) Two weeks after electroporation, animals were deprived of food and water for 6 h, after which the consumption of a 1% sucrose solution was measured over 1 h. Decreased consumption of this sucrose solution in the SIT is thought to reflect anhedonia, an important hallmark of depression. Behavioral despair was monitored 3 weeks after electroporation by measuring immobility (b), latency to immobility (c), and the total distance swum **(d)** during a 5 min trial in the FST. Data are shown as mean value + SEM. Untreated = no electroporation, HC-control = saline electroporation in the hippocampus, HC-TrkB = TrkB electroporation in the hippocampus, NAc-control = saline electroporation in the nucleus accumbens, NAc-TrkB = TrkB electroporation in the nucleus accumbens. $^{*}p < 0.05$; $^{**}p < 0.01$.

the hippocampus, which may therefore be insufficient to produce a strong antidepressant effect. Thirdly, strain differences can also explain differences in behavioral phenotype. TrkB overexpression in the NAc increased the latency to immobility compared to NAc-saline controls, suggesting that TrkB has antidepressant properties in the NAc. Together with equal total immobility and equal total distance swum, this indicates a behavioral shift in the FST towards a longer initial active phase followed by a longer inactive phase. As the NAc plays a critical role in motivated behavior, TrkB overexpression in the NAc likely has an effect on motivation in the FST. Increased motivation by overexpressing TrkB in the NAc likely contributes to the antidepressant effect, observed here by the increased latency to immobility. In contrast, viral-induced overexpression of truncated TrkB in the NAc of rats also produces antidepressant effects in the FST [25]. Likewise, ANA-12, a selective TrkB antagonist which primarily blocks TrkB in the striatum following systemic administration, exerts antidepressant effects in the FST and tail suspension test in mice [40]. These data suggests opposite functioning of BDNF-TrkB signaling in the NAc and hippocampus with regard to depressive-like behavior. Surprisingly, we found that TrkB overexpression in the NAc induced antidepressant effects without effects in the hippocampus, which suggests that increasing or decreasing TrkB activity in a specific brain region does not necessarily result in opposite behavioral responses.

TrkB overexpression in the NAc decreased basal corticosterone levels, which could explain its antidepressant effect as long-lasting increased glucocorticoid levels can damage the hippocampus thus causing depressive-like states [16,17,19]. Interestingly, TrkB in the NAc but not the hippocampus increased DCX immunostaining. The NAc acts intermediary between the hippocampus and amygdala to regulate the paraventricular nucleus, thus inhibiting HPA-axis activity. Increased DCX staining in the NAc could therefore increase its inhibiting effect on the HPA-axis and thus decrease corticosterone levels. Also, antidepressant effects correlate with lower

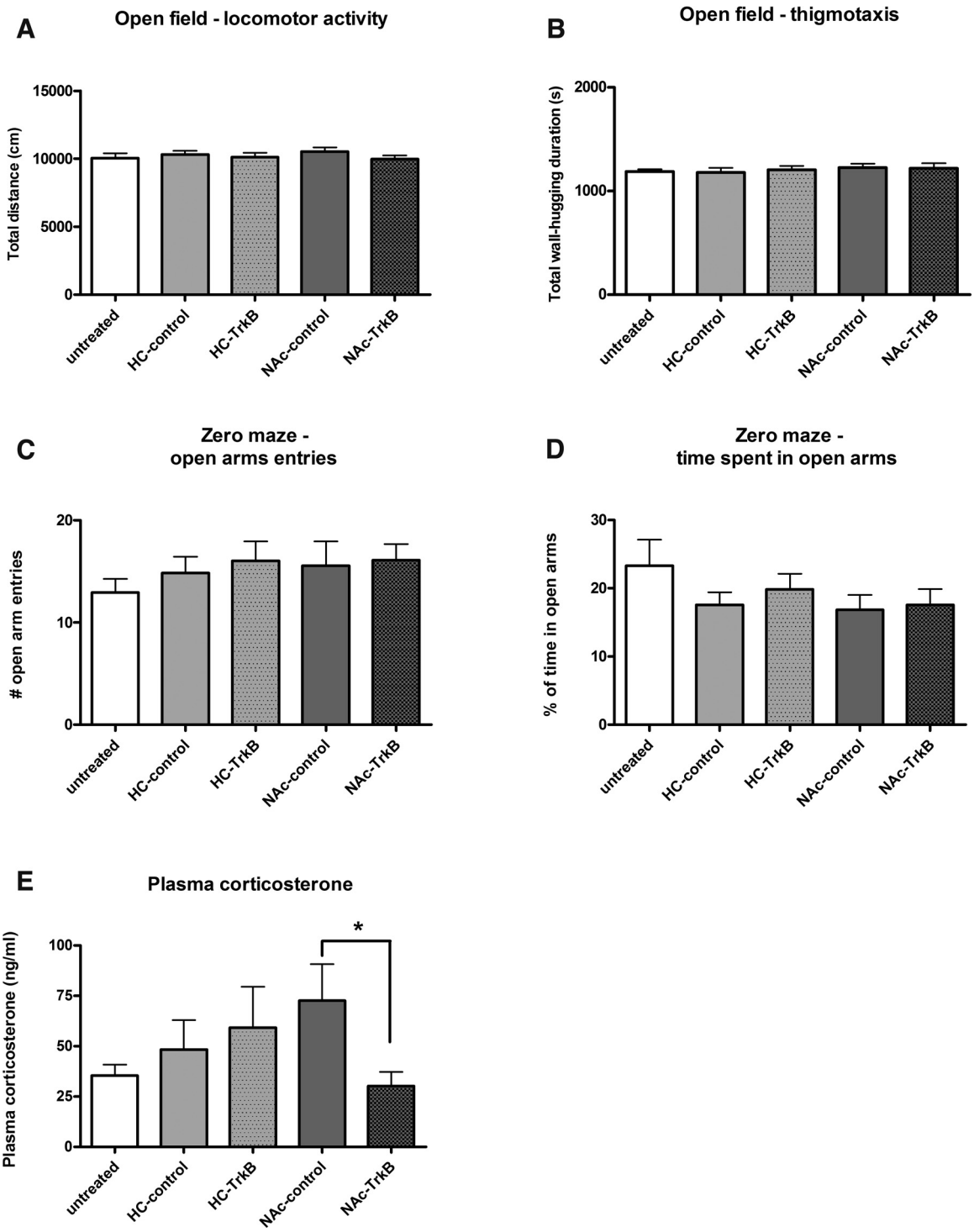

Fig. 4. Effects of TrkB overexpression in the hippocampus and the NAc on locomotor activity, anxiety-like behavior and basal plasma corticosterone levels. Animals were electroporated with saline or with a plasmid expressing TrkB in the hippocampus or the NAc (all groups $n = 13$). Two weeks after electroporation, the total distance covered in the OF was measured to assess locomotor activity (a), and we analyzed thigmotaxis in the OF (b), the number of open arms entries (c) and the time spent in the open arms of the EZM (d). Basal corticosterone levels in the plasma were measured by radioimmunoassay at the end of behavioral experiments (e). Data are shown as mean value + SEM. Untreated = no electroporation, HC-control = saline electroporation in the hippocampus, HC-TrkB = TrkB electroporation in the hippocampus, NAc-control = saline electroporation in the nucleus accumbens, NAc-TrkB = TrkB electroporation in the nucleus accumbens. $^{*}p < 0.05$.

corticosterone levels. Given the antidepressant effect we found in the NAc, but not the hippocampus, it is not surprising that we only find effects on corticosterone in the NAc treated animals. No changes in anxiety-like behavior were recorded following manipulation of the NAc, which makes sense as fear and anxiety are rather linked to the amygdala and the ventral hippocampus [41,42]. Other work has indeed shown that overexpressing TrkB in the VTA-NAc of rats did not alter anxiety-like behavior in the elevated plus maze (EPM) [25]. Also, loss-of-function studies with transgenic mice deficient of TrkB in the neocortex and hippocampus did not display changes in anxiety-like behavior [39]. Likewise, we found that electroporation of the dorsal hippocampus did not alter

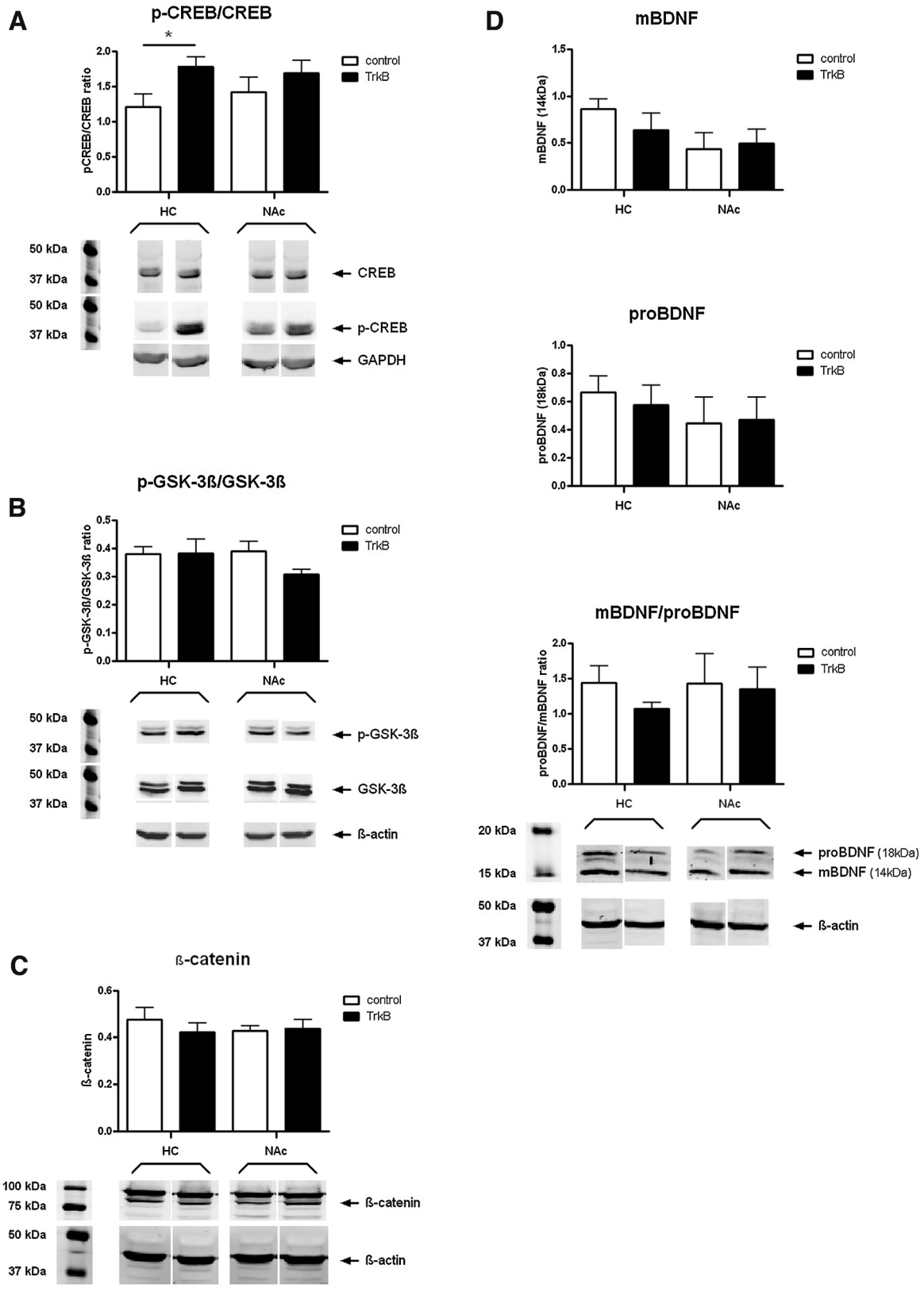

Fig. 5. Western blot analysis of downstream TrkB signaling molecules following TrkB overexpression in the hippocampus and the NAc. In an additional experiment, animals were electroporated with saline or with a plasmid expressing TrkB in the hippocampus or the NAc ($n = 6$ per group). Animals were sacrificed after 4.5 weeks and hippocampus and NAc were quickly dissected. Protein levels of mature BDNF, proBDNF (glycosylated, 18 kDa), p-CREB, total CREB, p-GSK-3β, total GSK-3β and total β-catenin were analyzed by western blotting. Electroporation of TrkB in the hippocampus but not in the NAc significantly increased CREB phosphorylation (a) without affecting protein levels of its target gene BDNF (d). GSK-3β/β-catenin signaling was unaffected in both structures (b–c). Data are shown as mean value + SEM. control = saline electroporation, TrkB = TrkB electroporation, HC = hippocampus, NAc = nucleus accumbens. $^{*}p < 0.05$.

measures of anxiety-like behavior in the EZM. In another study however, TrkB overexpression in the hippocampus and cortex decreased anxiety-like behavior in the EPM in mice [14]. Interestingly, TrkB.T1-deficient mice showed increased anxiety-related behavior in the EPM [43]. In this case however, the mice carried an overall mutation throughout the entire brain. Together with our data, this indicates that the involvement of TrkB in anxiety-like behaviors probably depends on the specific region being studied, as well as on the TrkB manipulation being applied and the behavioral tests that are used. For example, anxiety is predominantly linked to the ventral hippocampus and amygdala rather than the dorsal hippocampus. It may therefore come as no surprise that transgenic mice carrying a general mutation throughout the entire brain show different behaviors than animals with manipulations restricted to a limited brain area. When studying TrkB function, a plethora of methods are available: TrkB agonists/antagonists, local viral-mediated TrkB overexpression or knock-down, transgenic models, and so forth. These approaches may affect TrkB signaling to varying degrees and may therefore produce different behavioral effects. In addition, stimulating a protein, TrkB in this case, does not necessarily produce opposite behavioral effects as inhibiting the protein's function. Finally, anxiety-like behavior in animals can be studied with different tests. The elevated zero maze or the elevated plus maze are commonly used, but other tests exist, such as the open field or novelty-suppressed feeding. These tests involve different behavioral aspects and may therefore focus on slightly different types of anxiety. For example, novelty-induced feeding relies more on learned past experiences (emotional memory), appetitive conditions, exploratory drive and risk avoidance; while stress-induce hyperthermia is clearly a more physiological measure of anxiety.

TrkB overexpression in the DG decreased the amount of DCX^+ cells, while it increased DCX-staining in the NAc. The former finding is not surprising as TrkB overexpression in neuroblastoma cells is associated with their de-differentiation [44]. Interestingly, TrkB overexpression in hippocampal astrocytes increased the expression of GFAP, which indicates an increase in adult astrocytes. Whether our decrease in DCX^+ progenitor cells in the DG, which did not produce any behavioral consequences, can be explained by their de-differentiation or their differentiation into adult astrocytes needs to be determined in future studies. Progenitor cells from the subventricular zone migrate along the ventral migratory stream across the NAc into the basal forebrain [45]. BDNF, an effector gene of TrkB has been shown to be necessary for survival and differentiation of human inducible pluripotent stem cells into neurons [46]. TrkB overexpression in the NAc, either directly or indirectly by enhancing BDNF expression, may therefore stimulate proliferation and/or increase neuronal differentiation along this migratory stream, which may explain the increase in DCX^+ neuronal cells in the NAc. This increase in newborn neuronal cells in the NAc may be partly responsible for the antidepressant effects in the FST.

Downstream transcription factors of TrkB including CREB and β-catenin may explain how BDNF influences behavior. In contrast to the hippocampus, CREB signaling in the NAc has been reported to induce depressive-like behavior by regulating different target genes [27,47–50]. β-catenin, a transcription factor downstream of WNT/Dishevelled/GSK-3β signaling that also can be cross-activated via TrkB/PI-3K/GSK3β signaling, has antidepressant properties in the NAc and hippocampus [51]. In line with our behavioral data, CREB was not phosphorylated in the NAc. This may be due to a possible dilution of phosphorylated CREB in our samples although this is not likely given the effect we found in the hippocampus, where the samples were more diluted because of the relatively bigger size of the dissected tissue. GSK-3β phosphorylation and β-catenin levels were also unaffected in both brain regions so it remains to be elucidated what signaling cascade may mediate a TrkB-induced antidepressant effect in the NAc and why a specific signaling cascade is activated following TrkB manipulation. PLCγ1 signaling for example, which results in activation of the transcription factors NFAT and NF-κB, might be involved as genes transcribed by these transcription factors regulate synaptic plasticity and memory. An explanation may be found in the specific cell types that are targeted and that preferentially activate certain signaling cascades. It is interesting to note that TrkB overexpression in the hippocampus induced CREB phosphorylation without producing antidepressant effects. This was unexpected as the antidepressant effects of CREB in the hippocampus are well established [52]. These apparently contradicting findings may be explained by the manipulation of specific cell types as we targeted astrocytes in the hippocampus while other studies usually report a neuronal target. Future studies are needed to unravel the role of TrkB-related transcription factors in the NAc and the hippocampus in animal models for depression. In addition, the exact role of different cell types in this context warrants further investigation.

Declaration of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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