



Distinct Effects of BDNF and NT-3 on the Dendrites and Presynaptic Boutons of Developing Olfactory Bulb GABAergic Interneurons In Vitro

Vanesa Nieto-Estévez^{1,2} · Çağla Defterali^{1,2} · Carlos Vicario^{1,2}

Received: 23 September 2020 / Accepted: 11 December 2020 / Published online: 4 January 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC part of Springer Nature 2021

Abstract

Brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT-3) are known to regulate neuronal morphology and the formation of neural circuits, yet the neuronal targets of each neurotrophin are still to be defined. To address how these neurotrophins regulate the morphological and synaptic differentiation of developing olfactory bulb (OB) GABAergic interneurons, we analyzed the effect of BDNF and NT-3 on GABA⁺-neurons and on different subtypes of these neurons: tyrosine hydroxylase (TH⁺); calretinin (Calr⁺); calbindin (Calb⁺); and parvalbumin (PVA⁺). These cells were generated from cultured embryonic mouse olfactory bulb neural stem cells (eOBNSCs) and after 14 days in vitro (DIV), when the neurons expressed TrkB and/or TrkC receptors, BDNF and NT-3 did not significantly change the number of neurons. However, long-term BDNF treatment did produce a longer total dendrite length and/or more dendritic branches in all the interneuron populations studied, except for PVA⁺-neurons. Similarly, BDNF caused an increase in the cell body perimeter in all the interneuron populations analyzed, except for PVA⁺-neurons. GABA⁺- and TH⁺-neurons were also studied at 21 DIV, when BDNF produced significantly longer neurites with no clear change in their number. Notably, these neurons developed synaptophysin⁺ boutons at 21 DIV, the size of which augmented significantly following exposure to either BDNF or NT-3. Our results show that in conditions that maintain neuronal survival, BDNF but not NT-3 promotes the morphological differentiation of developing OB interneurons in a cell-type-specific manner. In addition, our findings suggest that BDNF and NT-3 may promote synapse maturation by enhancing the size of synaptic boutons.

Keywords Neural stem cells · BDNF · NT-3 · Olfactory bulb interneurons · Dendrite morphology · Synaptic boutons

Introduction

The neurotrophins BDNF (Brain-Derived Neurotrophic Factor) and NT-3 (Neurotrophin 3) play critical roles in the development of neural circuits, controlling neuronal survival, dendrite and axon growth, and synapse formation (Park and Poo 2013; Vicario-Abejón et al. 2002, 1998; Vilar and Mira 2016). In vitro studies revealed that BDNF and NT-3 promote the differentiation of neurons isolated from neural stem cells (NSCs) and progenitor cells (Ahmed et al.

1995; Vicario-Abejón et al. 2000). In vivo studies using Knock-out (KO) mice showed that the lack of BDNF or its tropomyosin receptor kinase B (TrkB) receptor resulted in a loss of specific neuronal populations in various brain regions, including the OB (Galvão et al. 2008; Jones et al. 1994; Waterhouse et al. 2012; Bath et al. 2008; Sairanen et al. 2005; Bergami et al. 2013; Berghuis et al. 2006). BDNF also stimulated the growth of GABAergic axons and dendrites, and the expression of glutamic acid decarboxylase (GAD) in dissociated hippocampal neurons and hippocampal slices (Vicario-Abejón et al. 1998; Yamada et al. 2002; Marty et al. 2000). Consistent with these findings, the lack of *TrkB* or *TrkC* reduced axonal arborization in the mouse hippocampus (HP) (Martinez et al. 1998). Notably, each neurotrophin may have specific effects on different neuronal populations and each individual neurotrophin may elicit neuron specific effects within a particular brain region (McAllister et al. 1997; Vicario-Abejón et al. 1998; Gascon et al. 2005; Zagrebelsky et al. 2018).

✉ Carlos Vicario
cvicario@cajal.csic.es

¹ Instituto Cajal-Consejo Superior de Investigaciones Científicas (IC-CSIC), Avenida Doctor Arce 37, 28002 Madrid, Spain

² Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED-ISCIH), Madrid, Spain

In the OB, deletion of BDNF diminished the dendritic complexity of PVA⁺-neurons (Berghuis et al. 2006), whereas neurons derived from eOBNSCs acquired more complex morphologies and synapsin-I-positive boutons when grown in the presence of BDNF (Vergaño-Vera et al. 2006). BDNF has also been shown to promote the dendritic growth of mitral and tufted OB neurons in vitro (Imamura and Greer 2009), and this neurotrophin alters the morphology and number of spines on granule neurons (Matsutani and Yamamoto 2004; McDole et al. 2015). However, BDNF does not significantly enhance the survival of adult generated granule neurons in the OB (McDole et al. 2020). As opposed to its growth-promoting effects, during development, pro-BDNF and BDNF can influence axon and spine pruning, two mechanisms that refine and stabilize neuronal connections (An et al. 2008; Johnson et al. 2007; Orefice et al. 2016, 2013; Singh et al. 2008; Choo et al. 2017). Thus, further studies are necessary to understand the role of BDNF and NT-3 during the morphological differentiation and synaptic differentiation of OB neurons.

Neurotrophins also regulate the formation, as well as the structural and functional maturation of synapses (Park and Poo 2013; Vicario-Abejón et al. 2002; Jacobi et al. 2009; Gottmann et al. 2009; Zagrebelsky and Korte 2014). The first evidence that neurotrophins affect synapse formation and maturation independently of neuronal survival was obtained from cultured hippocampal neurons and from the HP of *TrkB* and *TrkC* KO mice (Martinez et al. 1998; Vicario-Abejón et al. 1998). The synaptic terminals of neurotrophin-treated neurons contained more total and docked synaptic vesicles (SVs), larger SVs and a thicker postsynaptic density (Collin et al. 2001; Vicario-Abejón et al. 1998). In addition, BDNF increased the number of SVs and dendritic spines in pyramidal neurons of postnatal hippocampal slices (Tyler and Pozzo-Miller 2001) and tectal neurons in vivo (Sanchez et al. 2006). Accordingly, mice lacking *TrkB* and *TrkC* had fewer hippocampal synaptic contacts, and the former also had fewer SVs (Martinez et al. 1998). Similarly, neurons from the HP of E18.5 *Bdnf* KO mice had fewer vesicular glutamate transporter (vGLUT) boutons (Singh et al. 2006), and comparable results were found in the cerebellum and cortex of *TrkB* KO mice (Sanchez-Huertas and Rico 2011; Rico et al. 2002). Indeed, BDNF-*TrkB* signaling is necessary for NMDAR clustering in hippocampal synapses of cultured neurons (Elmariah 2004) and in the OB, BDNF can enhance the excitability of mitral neurons (Mast and Fadool 2012).

BDNF and NT-3 are expressed weakly in the embryonic and neonatal OB, although the expression of BDNF is stronger than that of NT-3. Both neurotrophins bind preferentially to *TrkB* and *TrkC* receptors, respectively, which are detected at different levels in the different layers of the neonatal and adult OB. In contrast, transcripts for the high affinity NGF receptor, *TrkA*, have not been detected in the

olfactory bulb (Fryer et al. 1996; Mackay-Sim and Chuah 2000; Nef et al. 2001; Vicario-Abejón et al. 2002). The constitutive presence of neurotrophins before or during the initiation of pre- and postsynaptic contact could regulate neuron development (Vicario-Abejón et al. 2002).

Here, we detected *TrkB* and *TrkC* immunoreactivity in the embryonic OB and in cultured eOBNSC-derived neurons. Thus, we analyzed the effect of chronic BDNF and NT-3 treatment on the morphological differentiation of eOBNSC-derived GABA⁺ interneurons in vitro, including TH⁺, Calr⁺, Calb⁺, and PVA⁺ neurons. These cells represent major subtypes of GABA⁺ neurons in the OB in vivo and indeed, no cell type (TH⁺, Calr⁺, Calb⁺, and PVA⁺) co-expresses two or more of these markers (Parrish-Aungst et al., 2007; Hurtado-Chong et al., 2009; Curto et al., 2014). The effects of these neurotrophins on the number of synaptophysin⁺ cells and of synaptophysin⁺ boutons was also assessed. As such, the data show that BDNF (but not NT-3) regulates dendrite morphology of developing OB interneurons in a cell-type specific manner, and that both BDNF and NT-3 increase the size of synaptophysin⁺ boutons.

Materials and Methods

Animals

All animal care and handling was carried out in accordance with European Union guidelines (directive 2010/63/EU) and Spanish legislation (Law 32/2007 and RD 53/2013), and the protocols were approved by the Ethical Committee of the Consejo Superior de Investigaciones Científicas (CSIC) and of the Comunidad de Madrid. Animals were administered food and water ad libitum, and the environmental conditions were strictly controlled: 12-h light/dark cycle, temperature 22 °C, and humidity 44%. All efforts were made to ameliorate the animal's suffering.

Neural Stem Cell Cultures

Embryonic OBNSCs were obtained from embryonic day 13.5 (E13.5) CD1 embryos as previously described (Nieto-Estévez et al. 2013). Briefly, pregnant mice were sacrificed by cervical dislocation and their embryos were decapitated. The OB was then extracted from the embryos, and the cells obtained by mechanical dissociation were plated at a density of 35,000 cells/cm² and grown as neurospheres by daily addition of fibroblast growth factor-2 (FGF-2, 20 ng/ml: PeproTech) and epidermal growth factor (EGF, 20 ng/ml: PeproTech) in Dulbecco's modified Eagle medium (DMEM)/nutrient mixture F12 (F12), supplemented with insulin, apotransferrin, putrescine, progesterone and sodium selenite (N2: DMEM/F12/N2). Every 3–4 days, the neurospheres

were split by mechanical procedures and plated at 5000 cells/cm² in DMEM/F12/N2 with FGF-2 and EGF (Nieto-Estévez et al. 2013). To induce differentiation, the cells were seeded on polyornithine and fibronectin-coated glass or thermanox coverslips at a density of 125,000 cells/cm² in DMEM/F12/N2/B27 (Gibco) plus 5% Fetal Bovine Serum (FBS, Sigma). BDNF (Peprotech 450-02) or NT-3 (R&D Systems 267-N3 and Peprotech 450-03) were added at 20 ng/ml every 3 days throughout the duration of the culture, as previously reported (Vicario-Abejón et al. 1995, 2000). After 6 days, the medium was partially changed with DMEM/N2/B27 plus 5% FBS. The presence of FBS in the cultures promotes NSC differentiation and favors neuronal survival (Vicario-Abejón et al. 2000). The cells were then fixed with 4% paraformaldehyde (PFA) for 25 min after 14 or 21 DIV. Although these differentiated eOBNSC cultures are composed of neurons and glial cells, this study focused on the interneuron populations. All the experiments were performed on eOBNSCs between passages 3–10, when the majority of cells exhibited a stable karyotype (Vergaño-Vera et al. 2009).

Immunostaining of Cryostat Sections and Adherent Cell Cultures

Pregnant mice were anaesthetized by intraperitoneal injection of ketamine/xylazine and the E14.5 embryos were intraventricularly perfused with 0.9% NaCl followed by 4% PFA. Their heads were post-fixed, cryoprotected and frozen, and then, coronal cryostat serial Sects. (15 µm) were obtained and kept at –80 °C until they were used for immunohistochemistry.

After treating the cultured cells and the sections with 0.1–0.2% Triton X-100/10% NGS (normal goat serum)/PBS, they were incubated overnight at 4 °C with primary antibodies raised against: Calb (mouse antibody, 1:2000, Swant Cat. No. 300, RRID:AB_10000347), Calr (rabbit antiserum, 1:1500, Swant Cat. No. 7699/4, RRID:AB_2313763), GABA (rabbit antiserum, 1:2000; Sigma Cat. No. A2052, RRID:AB_477652), MAP2ab (mouse antibody, 1:250; Sigma-Aldrich Cat. No. M1406, RRID:AB_477171 and chicken antibody, 1:5000; Abcam Cat. No. ab5392, RRID:AB_2138153), PVA (mouse antibody, 1:1000, Swant Cat. No. 235, RRID:AB_10000343), Synaptophysin (rabbit antiserum, 1:4; ZYMED Laboratories and ThermoFisher Cat. No. 180130, RRID:AB_10836766), TH (rabbit antiserum, 1:100; Millipore Cat. No. AB152, RRID:AB_390204), TrkB (rabbit antiserum, 1:50; Santa Cruz Biotechnology Cat. No. sc-12-G, RRID:AB_632558), or TrkC (rabbit antiserum, 1:50; Santa Cruz Biotechnology Cat. No. sc-117, RRID:AB_632560). The cells and sections were then incubated with Alexa fluor 488 or 594 or 647 conjugated secondary antibodies (1:500; Invitrogen) and

finally, with 4',6-diamidino-2-phenylindole (DAPI: Vector Laboratories, Burlingame, CA) before they were mounted in Mowiol solution (Calbiochem, San Diego, CA). Controls were performed to confirm the specificity of the primary and secondary antibodies.

Cell Counts and Morphological Analysis

To determine the number of cells in the adherent culture expressing a specific antigen, 10 random fields per coverslip were counted using a 20× objective and fluorescence filters, counting cells in a total of 30–40 microscope fields per each condition (control, BDNF or NT-3). The results were expressed as the mean (\pm SEM) number of cells from 3–4 cultures. To quantify synaptophysin⁺ neurons, only those cells with synaptophysin⁺ boutons (a feature of mature neurons) (Vicario-Abejón et al. 1998) were scored. Therefore, a neuron with diffuse staining of the cell body but no bouton or punctate staining was not considered synaptophysin⁺.

To analyze the effect of the neurotrophins on neuronal morphology, we counted the number of primary and secondary neurites, and measured the total neurite length and cell body perimeter in 40× images of 20–30 GABA⁺ neurons, 12–31 TH⁺ neurons, 16–18 Calr⁺ neurons, 12–18 Calb⁺ neurons and 13 PVA⁺ neurons per condition and time point, using ImageJ software. These morphological analyses were performed blind to the treatment. The images presented in Figs. 2–8 were taken under a fluorescent microscope and some pictures were composed of 2–4 images stitched together using the ImageJ software. We chose to present fewer cells in the pictures to better assess the effects of the neurotrophins on neuronal morphology.

We also determined the total number of synaptic boutons per neuron and per dendrite length, and the bouton size from 63× images (digital zoom, \times 3.5; numerical aperture, 1.4), taken every 0.76 µm (11 optical sections per field) on a confocal microscope. ImageJ was used to measure the size of individual boutons and the average bouton size per neuron was calculated, expressing the results as the mean \pm SEM of 130–150 boutons from 13–15 neurons in 3–4 cultures.

Statistical Analysis

To determine if the data followed a parametric distribution and with equal variances, a Barlett's test was used, and the results from the three conditions studied were analyzed by one-way ANOVA with a Tukey's post hoc test. When the variances were not equal, a non-parametric Kruskal–Wallis test was used with a post hoc Dunn's test (Table 1). Statistical significance was set at $*P < 0.05$ and GraphPad Prism 8.0 was used for all statistical analyses.

Table 1 Summary of the statistics analyses performed

Figure	Panel	Test	p-value	Experimental Group	p-value Tukey/Dunn
Fig. 2	F	Kruskal–Wallis test + Post test	<i>< 0.0001</i>	Control vs BDNF	<i>0.0001</i>
				Control vs NT3	0.7827
				BDNF vs NT3	<i>< 0.0001</i>
	G	Kruskal–Wallis test + Post test	<i>0.0004</i>	Control vs BDNF	<i>0.0238</i>
				Control vs NT3	0.4702
				BDNF vs NT3	<i>0.0004</i>
	H Branches	Kruskal–Wallis test + Post test	<i>0.0185</i>	Control vs BDNF	0.1868
				Control vs NT3	0.8599
				BDNF vs NT3	<i>0.0188</i>
	H Total	Kruskal–Wallis test + Post test	<i>0.0185</i>	Control vs BDNF	0.0638
				Control vs NT3	0.8325
				BDNF vs NT3	<i>0.0281</i>
I	Kruskal–Wallis test + Post test	<i>0.0443</i>	Control vs BDNF	0.3710	
			Control vs NT3	0.8545	
			BDNF vs NT3	<i>0.0433</i>	
J	One-Way ANOVA + Post test	<i>0.0487</i>	Control vs BDNF	0.1751	
			Control vs NT3	0.7337	
			BDNF vs NT3	0.0531	
Fig. 3	F	Kruskal–Wallis test + Post test	<i>0.0070</i>	Control vs BDNF	<i>0.0050</i>
				Control vs NT3	0.4662
				BDNF vs NT3	0.2538
	G	Kruskal–Wallis test + Post test	<i>0.0007</i>	Control vs BDNF	<i>0.0024</i>
				Control vs NT3	<i>> 0.9999</i>
				BDNF vs NT3	0.0034
J	One-Way ANOVA + Post test	<i>< 0.0001</i>	Control vs BDNF	<i>0.0009</i>	
			Control vs NT3	0.7168	
			BDNF vs NT3	<i>< 0.0001</i>	
Fig. 4	F	One-Way ANOVA + Post test	<i>0.0069</i>	Control vs BDNF	<i>0.0048</i>
				Control vs NT3	0.1480
				BDNF vs NT3	0.5257
	G	Kruskal–Wallis test + Post test	0.1728	Control vs BDNF	0.1867
				Control vs NT3	0.7768
				BDNF vs NT3	<i>> 0.9999</i>
	H	Kruskal–Wallis test + Post test	0.0577	Control vs BDNF	0.1469
				Control vs NT3	<i>> 0.9999</i>
				BDNF vs NT3	0.1291
I	Kruskal–Wallis test + Post test	<i>0.0075</i>	Control vs BDNF	0.0992	
			Control vs NT3	<i>> 0.9999</i>	
			BDNF vs NT3	<i>0.0096</i>	
Fig. 5	G	Kruskal–Wallis test + Post test	<i>0.0011</i>	Control vs BDNF	0.2066
				Control vs NT3	0.2491
				BDNF vs NT3	0.0008
	H	Kruskal–Wallis test + Post test	0.1072	Control vs BDNF	<i>> 0.9999</i>
				Control vs NT3	0.2388
				BDNF vs NT3	0.1409
J	Kruskal–Wallis test + Post test	<i>0.0004</i>	Control vs BDNF	<i>0.0066</i>	
			Control vs NT3	<i>> 0.9999</i>	
			BDNF vs NT3	<i>0.0016</i>	

Table 1 (continued)

Figure	Panel	Test	p-value	Experimental Group	p-value Tukey/Dunn
Fig. 6	D	One-Way ANOVA + Post test	0.0589	Control vs BDNF	<i>0.0474</i>
				Control vs NT3	0.5408
				BDNF vs NT3	0.3408
	F Primary	Kruskal–Wallis test + Post test	<i>0.0118</i>	Control vs BDNF	> 0.9999
				Control vs NT3	0.0684
				BDNF vs NT3	<i>0.0163</i>
	F Branches	Kruskal–Wallis test + Post test	<i>0.0007</i>	Control vs BDNF	0.1788
				Control vs NT3	0.1961
				BDNF vs NT3	<i>0.0004</i>
Fig. 7	D	One-Way ANOVA + Post test	< <i>0.0001</i>	Control vs BDNF	<i>0.0008</i>
				Control vs NT3	0.0542
				BDNF vs NT3	< <i>0.0001</i>
	E	Kruskal–Wallis test + Post test	<i>0.0229</i>	Control vs BDNF	0.1856
				Control vs NT3	0.8078
				BDNF vs NT3	<i>0.0214</i>
	F	One-Way ANOVA + Post test	0.0512	Control vs BDNF	0.9458
				Control vs NT3	0.0504
				BDNF vs NT3	0.1283
	H	Kruskal–Wallis test + Post test	0.0667	Control vs BDNF	0.1052
				Control vs NT3	> 0.9999
				BDNF vs NT3	0.1598
Fig. 9	H	Kruskal–Wallis test + Post test	< <i>0.0001</i>	Control vs BDNF	< <i>0.0001</i>
				Control vs NT3	< <i>0.0001</i>
				BDNF vs NT3	<i>0.0315</i>
	I	Kruskal–Wallis test + Post test	< <i>0.0001</i>	Control vs BDNF	< <i>0.0001</i>
				Control vs NT3	< <i>0.0001</i>
				BDNF vs NT3	<i>0.0243</i>

Significant changes ($P < 0.05$) are written in italics

Results

Expression of TrkB and TrkC in the OB and in Neurons Derived From eOBNSCs

We first analyzed the expression of the TrkB and TrkC neurotrophin receptors (Park and Poo 2013; Vicario-Abejón et al. 2002) by immunostaining cryostat sections of the embryonic OB and neurons derived from eOBNSCs. Both receptors were widely expressed in the embryonic OB layers containing early post-mitotic neurons (Méndez-Gómez et al. 2011). TrkB was more abundant in the mitral cell layer (MCL), while it was expressed more weakly in the ventricular zone/subventricular zone (VZ/SVZ) and the intermediate zone (IZ; Fig. 1a). TrkC expression was also detected in these layers and in the surrounding area corresponding to the primordial glomerulus (Fig. 1b). In agreement

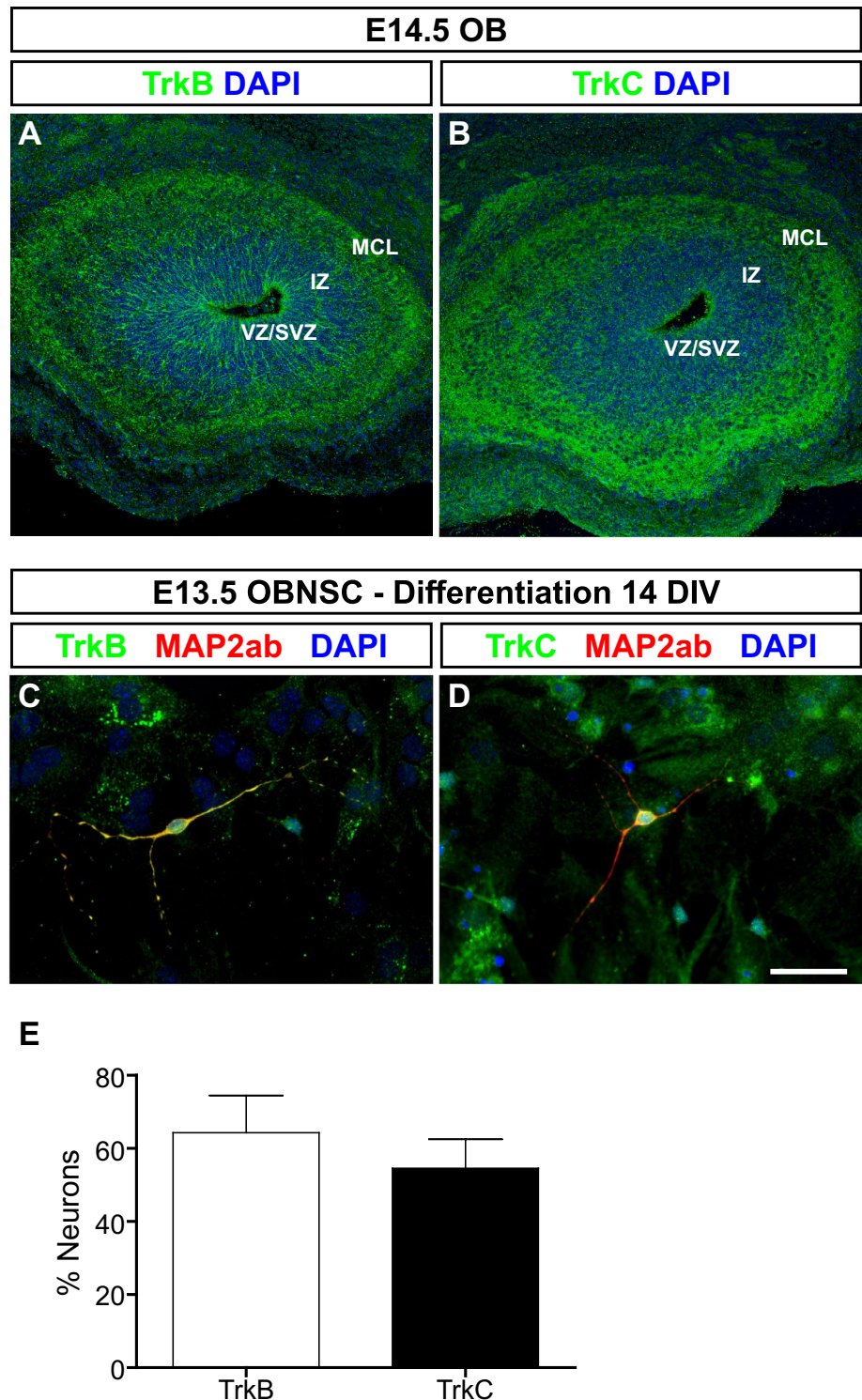
with this relatively abundant TrkB and TrkC expression in the embryonic OB, we found that 64.3% and 54.6% of MAP2ab⁺-neurons differentiated from eOBNSCs expressed TrkB⁺ and TrkC⁺, respectively (Fig. 1 c–e).

Since the formation of OB interneurons begins at early stages of mouse OB development (Vergaño-Vera et al. 2006; Batista-Brito et al. 2008; Lledo et al. 2008), and we detected both TrkB and TrkC in the OB and in neurons derived from eOBNSCs, we analyzed the effect of BDNF and NT-3 on the morphology of interneurons derived from eOBNSCs, and on the number and size of synaptic boutons.

The Effect of BDNF and NT-3 on the Morphological Differentiation of OB GABAergic Neurons

GABAergic cells form the largest population of interneurons in the OB (Vergaño-Vera et al. 2006; Díaz-Guerra

Fig. 1 The expression of TrkB and TrkC in OB sections, and in neurons derived from eOBNSCs. The images show coronal OB sections from E14.5 embryos (**a**, **b**) and eOBNSC-derived neurons (MAP2ab⁺, **c**, **d**) immunostained with antibodies against TrkB and TrkC, and counterstained with DAPI. Both receptors are expressed in the outer IZ and MCL layers, which contain early differentiating neurons. The graph (**e**) indicates the percentage of MAP2ab⁺ neurons that express TrkB or TrkC. The results are the mean \pm SEM of 4 cultures: MCL, mitral cell layer; IZ, intermediate zone; VZ/SVZ, ventricular zone/subventricular zone. Scale bar (D) = **a–b**, 114.3 μ m; **c–d**, 37.3 μ m



et al. 2013; Ravi et al. 2017) and thus, we first assessed the effect of BDNF and NT-3 on the number and morphology of eOBNSC-derived GABA⁺ neurons after 14 days in vitro (DIV; Fig. 2). Cells were immunostained with antibodies against MAP2ab (a general neuronal marker that labels cell bodies and dendrites) and GABA (Fig. 2a–c), and

the number and morphology of MAP2ab⁺ and GABA⁺ neurons were assessed. After 14 DIV, the total number of MAP2ab⁺ and of GABA⁺ neurons was similar in all the conditions studied (Fig. 2d), and the proportion of GABA⁺ neurons in the cultures was also similar in the control and in the presence of either BDNF or NT-3 (control

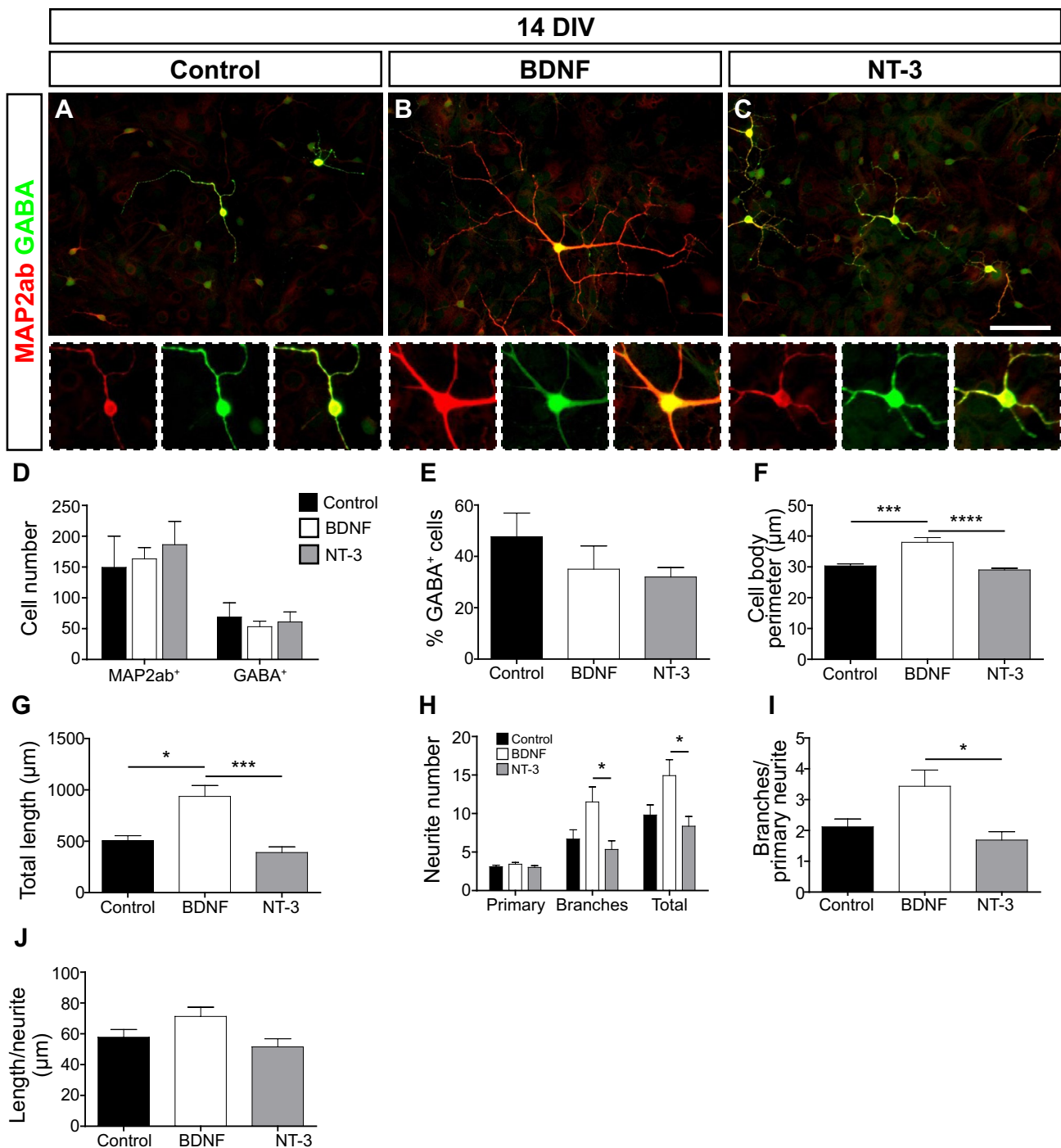


Fig. 2 The effect of BDNF on the number and morphology of GABA⁺ neurons derived from eOBNSCs after 14 DIV. The images show representative neurons from Control cultures (a), BDNF-treated cultures (b) or NT-3 treated cultures (c), immunostained with anti-MAP2ab and anti-GABA antibodies. The colocalization of MAP2ab and GABA can be better assessed in higher magnification images (bottom). The graphs show the average number of MAP2ab⁺ and GABA⁺ cells per 10 microscope fields acquired with a 20× objective (d), the percentage of GABA⁺ neurons (e) and the effects on

the morphological parameters of GABA⁺ neurons generated in each of the three conditions (f–j). The results are the mean ± SEM of 3–4 cultures per condition (d, e). The morphological analysis (f–j) was performed on 20–30 GABA⁺ neurons per condition. BDNF treatment significantly increased the cell body perimeter, total neurite length and number of neurite branches but not the ratio length/neurite: *P < 0.05, ***P < 0.001, ****P < 0.0001 (Kruskal–Wallis test followed by post hoc Dunn’s test). Scale bar (C) = 77.0 μm; high magnification images, 38.5 μm

$47.7 \pm 9.2\%$, BDNF $35.0 \pm 9.1\%$ and NT-3 $32.0 \pm 3.7\%$: Fig. 2E).

However, GABAergic neurons exposed to BDNF were larger than both control neurons and those exposed to NT-3, with a 25% and 31% increase in cell body perimeter after exposure to BDNF, respectively ($P < 0.001$ and $P < 0.0001$: Fig. 2f, Table 1), as well as an 85% and 142% or 2.42-fold increase in total neurite length ($P < 0.05$ and $P < 0.001$; Fig. 2g, Table 1). Moreover, GABA⁺ cells exposed to BDNF had more neurite branches and total neurites than cells exposed to NT-3 (115% or 2.15-fold and 79%, $P < 0.05$: Fig. 2h, Table 1), and more branches per primary neurite (104% or 2.04-fold, $P < 0.05$; Fig. 2i, Table 1). Interestingly, the ratio of the total length per neurite did not change significantly in the presence of BDNF, suggesting that the increase in the total length was mainly due to the increase in neurite branches promoted by BDNF.

After 21 DIV, the total number of MAP2ab⁺ and GABA⁺ neurons (Fig. 3a–c) remained similar in all three conditions (Fig. 3d), with no significant differences in the proportion of GABA⁺ neurons in the cultures treated with BDNF or NT-3 (control $36.3 \pm 10.5\%$, BDNF $24.4 \pm 11.4\%$ and NT-3 $25.0 \pm 12.4\%$: Fig. 3e). Similar to the results obtained after 14 DIV, GABA⁺ neurons treated with BDNF had a larger cell body perimeter than the control neurons (13.2%, $P < 0.01$: Fig. 3f, Table 1). Moreover, the neurons exposed to BDNF had an 82% and a 96% longer total neurite length than control and NT-3 treated neurons ($P < 0.01$: Fig. 3g, Table 1), and 52.3% and 71% longer neurites, respectively ($P < 0.001$ and $P < 0.0001$: Fig. 3j, Table 1). However, BDNF did not cause significant changes in neurite number (Fig. 3h) or in the number of branches per neurite (Fig. 3i). These findings support the idea that the increase in total length promoted by BDNF at 21 DIV could be mainly due to the maintenance and elongation of pre-existing neurites rather than to the growth of more neurites.

The Effect of BDNF and NT-3 on the Morphological Differentiation of OB GABAergic Interneuron Subtypes

There are multiple subtypes of GABAergic interneurons in the OB that could potentially respond distinctly to BDNF and NT-3 (Batista-Brito et al. 2008; Díaz-Guerra et al. 2013; Lledo et al. 2008; Vergaño-Vera et al. 2006; Wen et al. 2019; Yang 2008; Curto et al. 2014; Ravi et al. 2017). Hence, we tested the effect of exogenous BDNF and NT-3 on TH⁺, Calr⁺, Calb⁺ and PVA⁺ neurons generated from OBNSCs. As described above, no cell type in the OB co-expresses two or more of these markers (Parrish-Aungst et al. 2007; Hurtado-Chong et al. 2009; Curto et al. 2014). OB TH⁺ neurons represent a subpopulation of GABAergic interneurons located in the OB glomeruli (Hurtado-Chong et al. 2009;

Díaz-Guerra et al. 2013; Bonzano et al. 2016; Pignatelli and Belluzzi 2017). Here, we analyzed the effect of BDNF and NT-3 on the number and morphology of eOBNSC-derived TH⁺ neurons after immunostaining with antibodies against MAP2ab and TH (Fig. 4a–c). As expected, eOBNSC-derived TH⁺ cells also expressed GAD (data not shown), the rate-limiting enzyme in GABA synthesis (Vergaño-Vera et al. 2006).

After 14 DIV, the total number of MAP2ab⁺ and TH⁺ neurons (Fig. 4a–c), and the percentage of TH⁺ neurons was similar in all conditions (Fig. 4d–e). TH⁺ neurons in cultures treated with BDNF were bigger and more complex than in Control and in NT-3-treated cultures, as reflected by the 17.7% increase in cell body perimeter compared to Controls ($P < 0.01$; Fig. 4f, Table 1) and the 93% increase in total branches per primary neurite compared to NT-3 conditions ($P < 0.01$; Fig. 4i, Table 1). BDNF-treated neurons had an apparent higher total neurite length (Fig. 4g) and neurite number than Control and NT-3 treated neurons (Fig. 4h) but the increases were not statistically significant. The ratio of total length per neurite was similar in the three conditions.

At 21 DIV, cultures treated with BDNF or NT-3 showed no significant differences either in the number of MAP2ab⁺ and TH⁺ cells or in the percentage of TH⁺ cells compared to Controls (Fig. 5a–e). However, BDNF-treated TH⁺ cells presented a 195% or 2.95-fold increase in total neurite length compared to NT-3-treated cells ($P < 0.001$; Fig. 5g, Table 1). Furthermore, the length per neurite of BDNF-treated TH⁺ neurons was 84% and 114% or 2.14-fold larger than that of the Control cultures and NT-3 cultures, respectively ($P < 0.01$; Fig. 5j, Table 1). No significant changes were observed in other analyzed parameters (Fig. 5f,h,i).

Since the morphological changes produced by BDNF in GABA⁺ neurons and in the TH⁺ GABAergic neuron subpopulation were already observed by 14 DIV, the effects of BDNF and NT-3 on Calr⁺, Calb⁺ and PVA⁺ neurons were only assessed after 14 DIV. Colocalization of these markers with GABA or GAD immunoreactive neurons (data not shown) demonstrated that the three eOBNSC-neuronal subtypes were GABAergic. BDNF-treated Calr⁺ neurons (Fig. 6a–c) had a 15% larger cell body perimeter than Control neurons ($P < 0.05$: Fig. 6d, Table 1), and 9% more primary neurites and 37% more neurite branches than NT-3 treated neurons ($P < 0.05$ and $P < 0.001$: Fig. 6f, Table 1). No significant differences were observed in other parameters analyzed (Fig. 6e,g,h). Similarly, BDNF-treated Calb⁺ neurons (Fig. 7a–c) possessed a 15% and a 28% larger cell body perimeter than control and NT-3 treated neurons, respectively ($P < 0.001$ and $P < 0.0001$: Fig. 7d, Table 1), as well as a 112% or 2.12-fold % longer total neurite length than NT-3 treated neurons ($P < 0.05$: Fig. 7e, Table 1). The apparently larger branches/primary neurites ratio (Fig. 7g) and length per neurite (Fig. 7h) on exposure to BDNF was

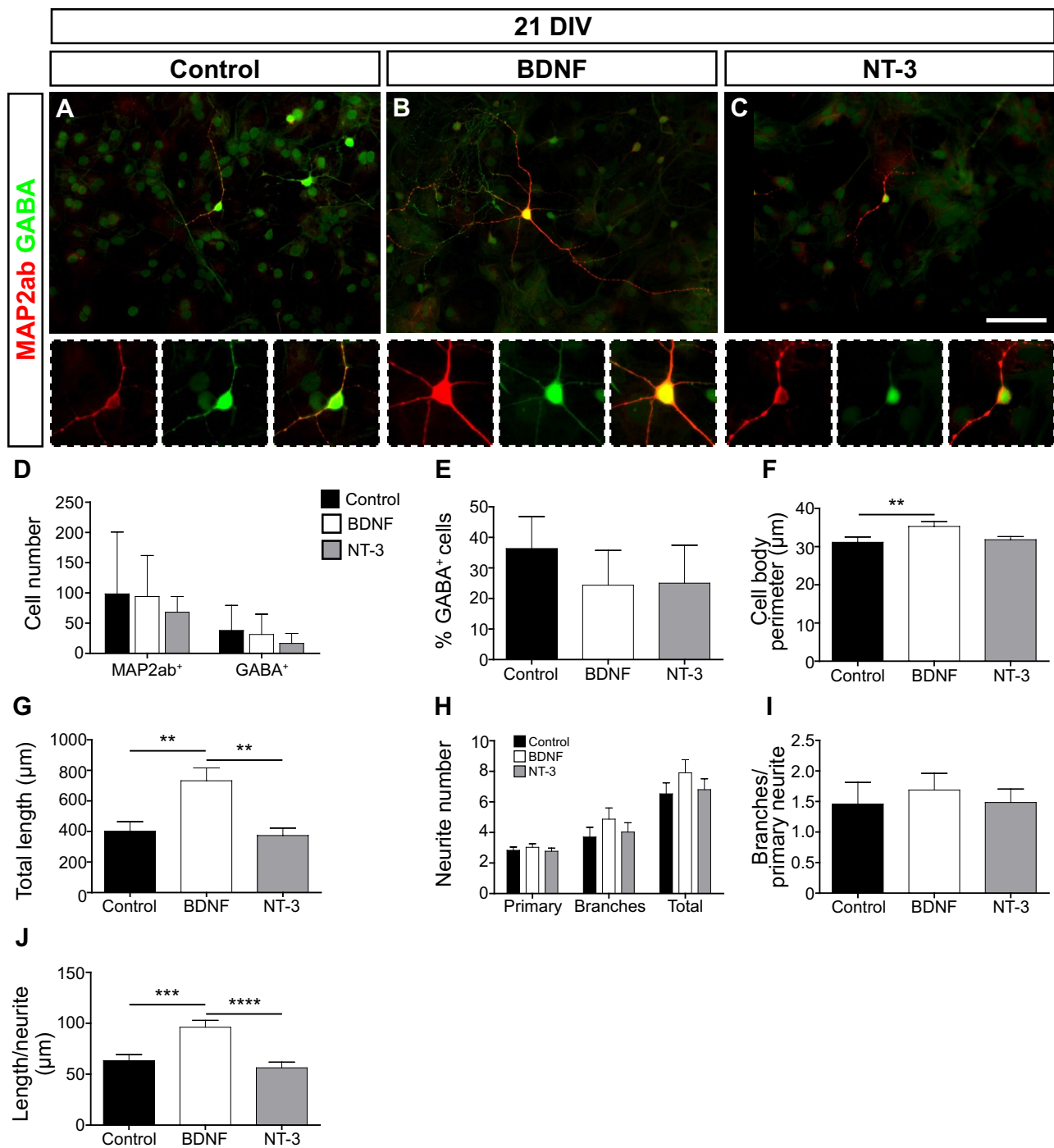


Fig. 3 The effect of BDNF on the number and morphology of GABA⁺ neurons derived from eOBNSCs after 21 DIV. The images show representative neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with anti-MAP2ab and anti-GABA antibodies. The colocalization of MAP2ab and GABA can be better assessed in the higher magnification images (bottom). The graphs show the average number of MAP2ab⁺ and GABA⁺ cells per 10 microscope fields acquired with a 20× objective (d), the percentage of GABA⁺ neurons (e) and the effect on the morphological

parameters analyzed in GABA⁺ neurons generated in each of the three conditions (f–j). The results are the mean ± SEM of 4 cultures per condition (d, e). The morphological analysis (f–j) was performed in 30 GABA⁺ neurons per condition. BDNF treatment significantly increased the cell body perimeter, total neurite length and the ratio length/neurite but not the number of neurites: **P < 0.01, ***P < 0.001, ****P < 0.0001 (F and G, Kruskal–Wallis test followed by post hoc Dunn’s test; J, one-way ANOVA with Tukey’s post hoc test). Scale bar (C) = 77.0 μm; high magnification images, 38.5 μm

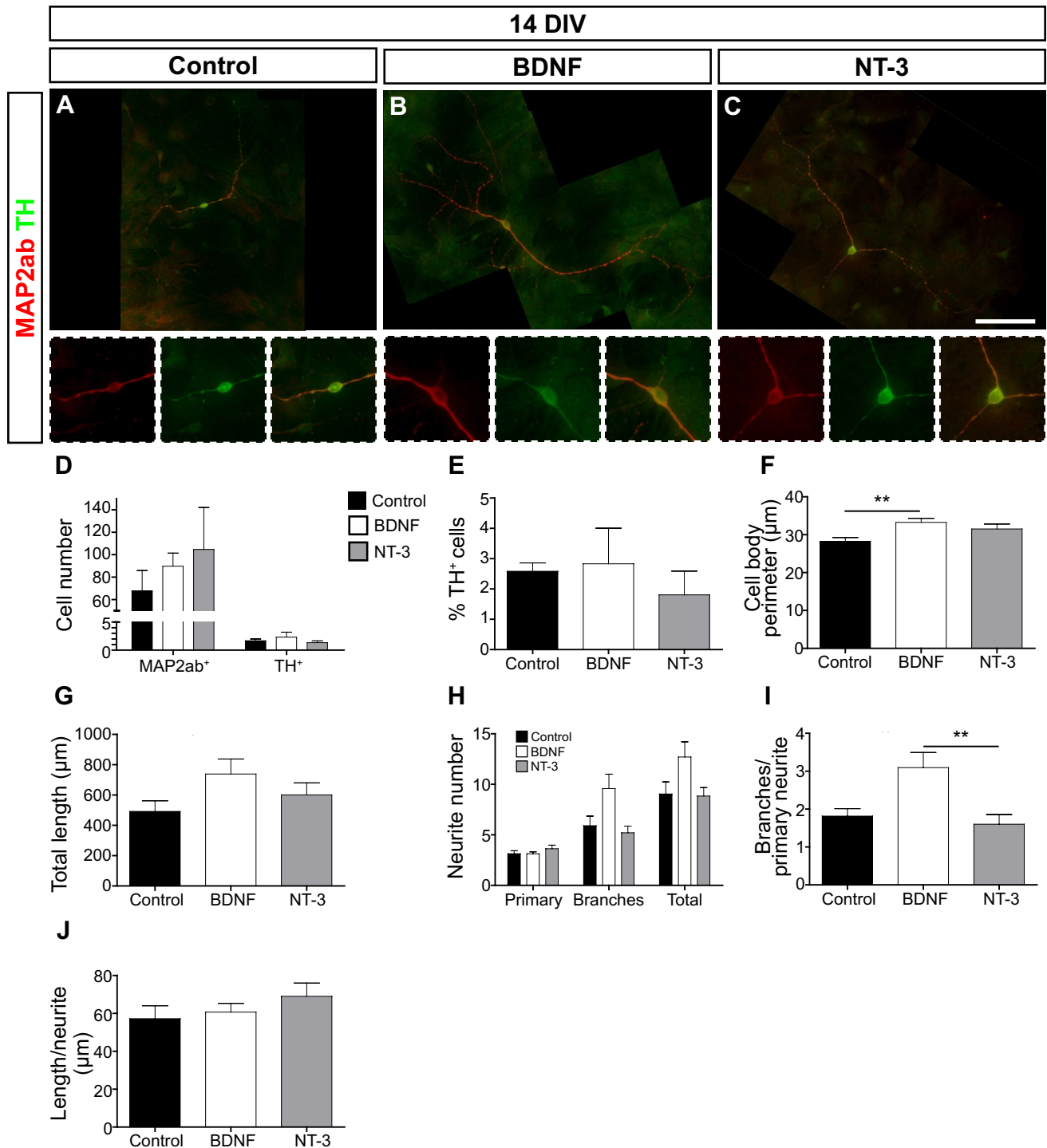


Fig. 4 The effect of BDNF on the number and morphology of tyrosine hydroxylase (TH⁺) neurons derived from eOBNSCs after 14 DIV. The images show representative neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with antibodies against MAP2ab and TH. The colocalization of MAP2ab and TH can be better assessed in the higher magnification images (bottom). The low magnification pictures are composed of 2–4 images and stitched together using ImageJ software. The graphs show the average number of MAP2ab⁺ and TH⁺ cells per 10 microscope fields acquired with a 20× objective (d), the percentage of TH⁺ neu-

rons (e) and the effect on the morphological parameters of the TH⁺ neurons generated in the three conditions (f–j). The results are the mean ± SEM of 3 cultures per condition (d, e). The morphological analysis was performed on 19–31 TH⁺ neurons per condition. BDNF treatment significantly increased the cell body perimeter and the ratio branches/primary neurite but not the ratio length/neurite: **P < 0.01 (F, one-way ANOVA with Tukey's post hoc test; I, Kruskal–Wallis test followed by post hoc Dunn's test). TH, tyrosine hydroxylase. Scale bar (C) = 77.0 μm; high magnification images, 38.5 μm

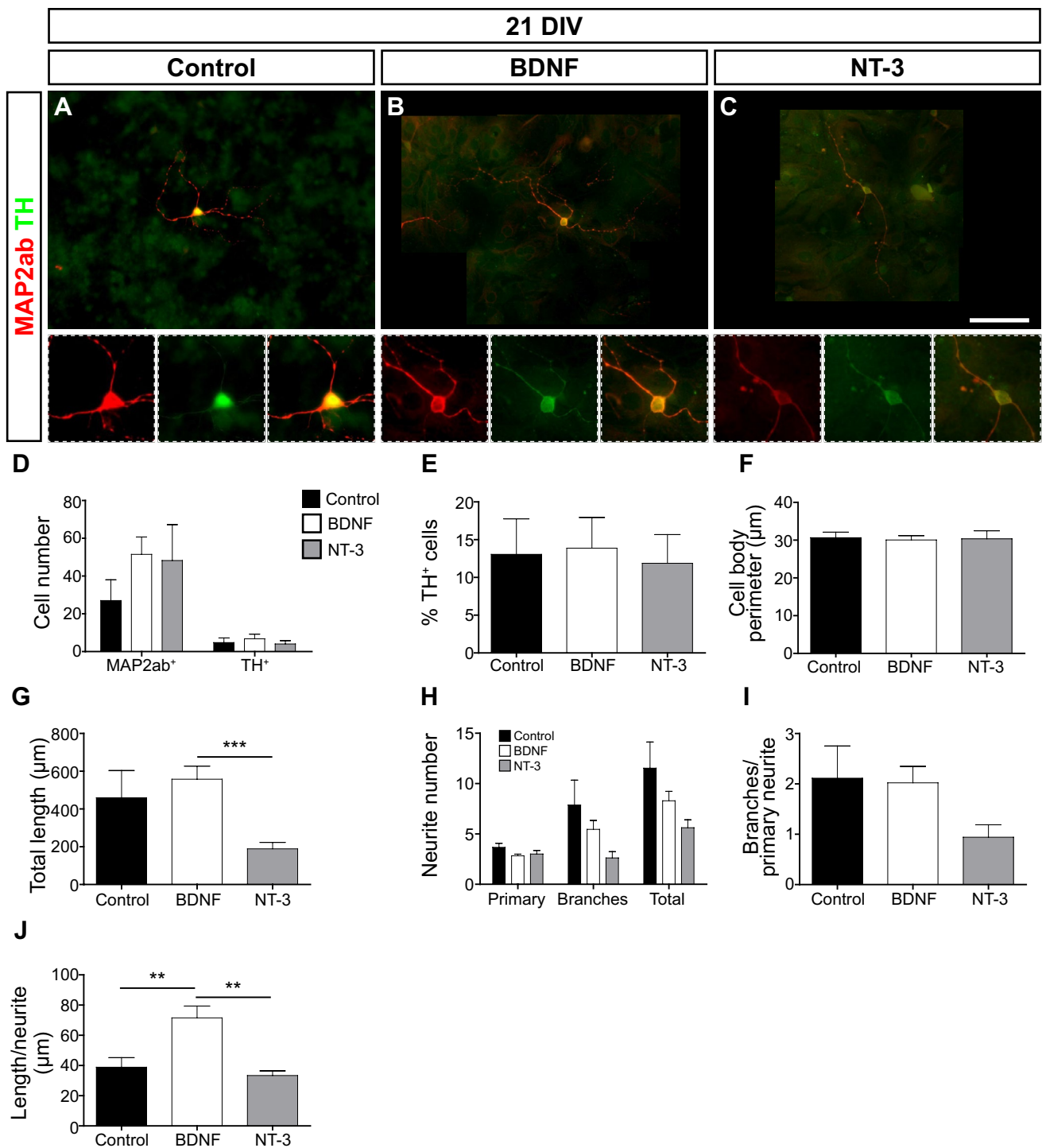


Fig. 5 The effect of BDNF on the number and morphology of TH⁺ neurons derived from eOBNSCs after 21 DIV. The images show representative neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with anti-MAP2ab and anti-TH. Antibody colocalization of MAP2ab and TH can be better assessed in the higher magnification images (bottom). The low magnification pictures are composed of 2–4 images and stitched together using ImageJ software. The graphs show the average number of MAP2ab⁺ and TH⁺ cells per 10 microscope fields acquired with a 20× objective (d), the

percentage of TH⁺ neurons (e) and the effect on the morphological parameters of the TH⁺ neurons generated in the three conditions (f–j). The results are the mean ± SEM of 3 cultures per condition (d, e). The morphological analysis was performed on 13–24 TH⁺ neurons per condition (f–j). BDNF treatment significantly increased total neurite length and the ratio length/neurite but not the number of neurites: **P < 0.01, ***P < 0.001 (Kruskal–Wallis test followed by post hoc Dunn’s test). TH, tyrosine hydroxylase. Scale bar (C) = 77.0 μm; high magnification images, 38.5 μm

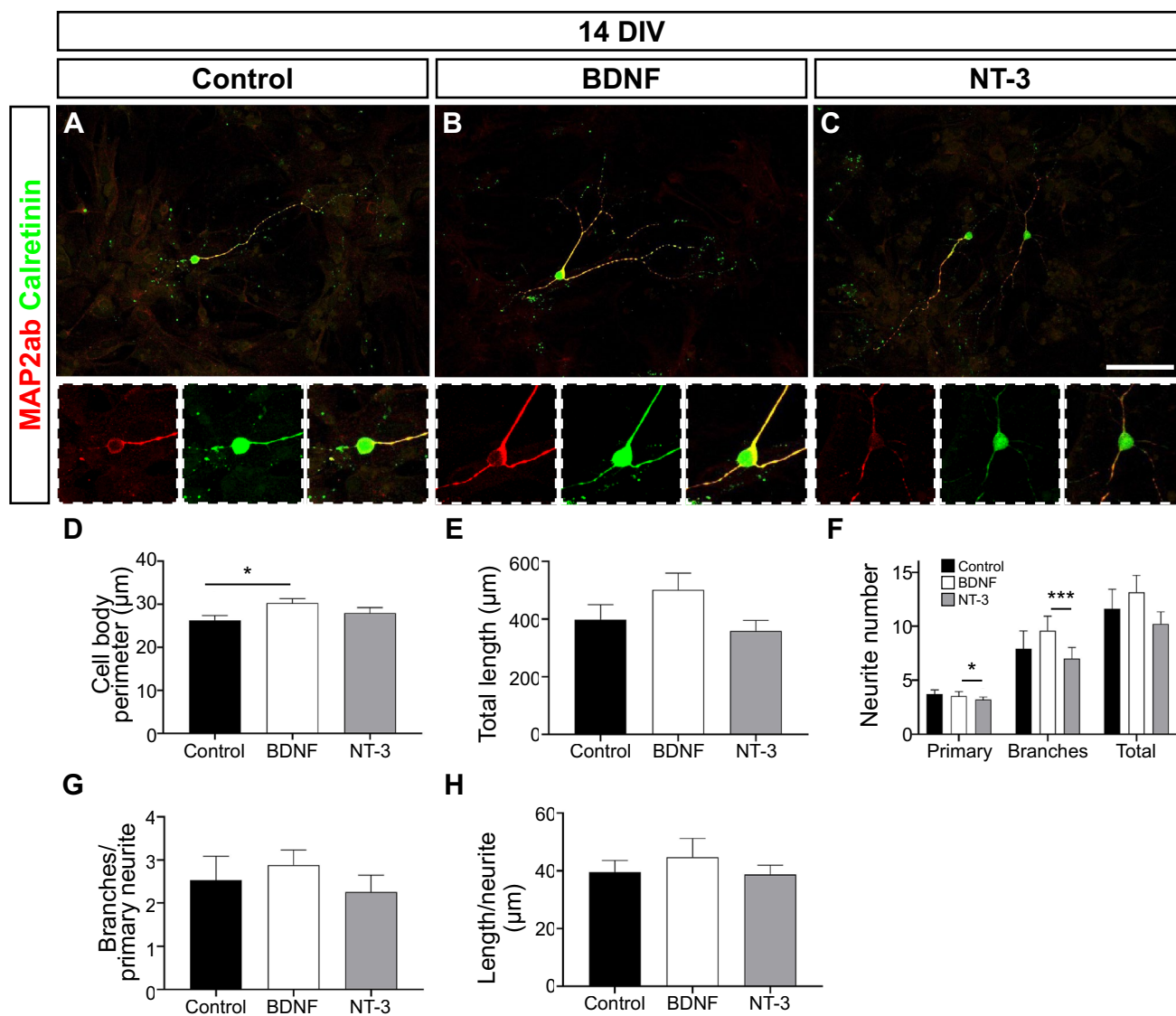


Fig. 6 The effect of BDNF on the morphology of Calretinin (Calr)⁺ neurons derived from eOBNSCs after 14 DIV. The images show representative neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with anti-MAP2ab and anti-Calr antibodies. The colocalization of MAP2ab and Calr can be better assessed in the higher magnification images (bottom). The graphs show the effects on the morphological parameters of 16–18 Calr ⁺

neurons generated in each of the three conditions. BDNF treatment increased the cell body perimeter, and the number of primary neurites and neurite branches: * $P < 0.05$, *** $P < 0.001$, (D, one-way ANOVA with Tukey's post hoc test; F, Kruskal–Wallis test followed by post hoc Dunn's test). Scale bar (C) = 77.0 μm ; high magnification images, 38.5 μm

not statistically significant. In contrast to the morphological effects produced by BDNF on GABA^+ , TH^+ , Calr^+ and Calb^+ neurons, we did not observe any significant changes in the morphology of PVA^+ neurons grown in the presence of BDNF or NT-3 (Fig. 8).

The Effect of BDNF and NT-3 on the Presynaptic Differentiation of Neurons Derived from eOBNSCs

To analyze the effect of BDNF and NT-3 on the maturation of neurons derived from eOBNSCs, cells were maintained

for 21 DIV, then immunostained with antibodies against MAP2ab and synaptophysin (Fig. 9a–c), a presynaptic protein that accumulates in boutons (Vergaño-Vera et al. 2006; Vicario-Abejón et al. 1998). Importantly, only those neurons with synaptophysin⁺ puncta or boutons but not those with diffuse staining in the cell body were considered synaptophysin⁺ neurons, explaining the relative small number and proportion (0.3–5.7%) of synaptophysin⁺ neurons. Similar numbers of MAP2ab⁺ and synaptophysin⁺ neurons were found in the three conditions (Fig. 9d,e), and although exposure to BDNF produced more synaptophysin⁺ boutons

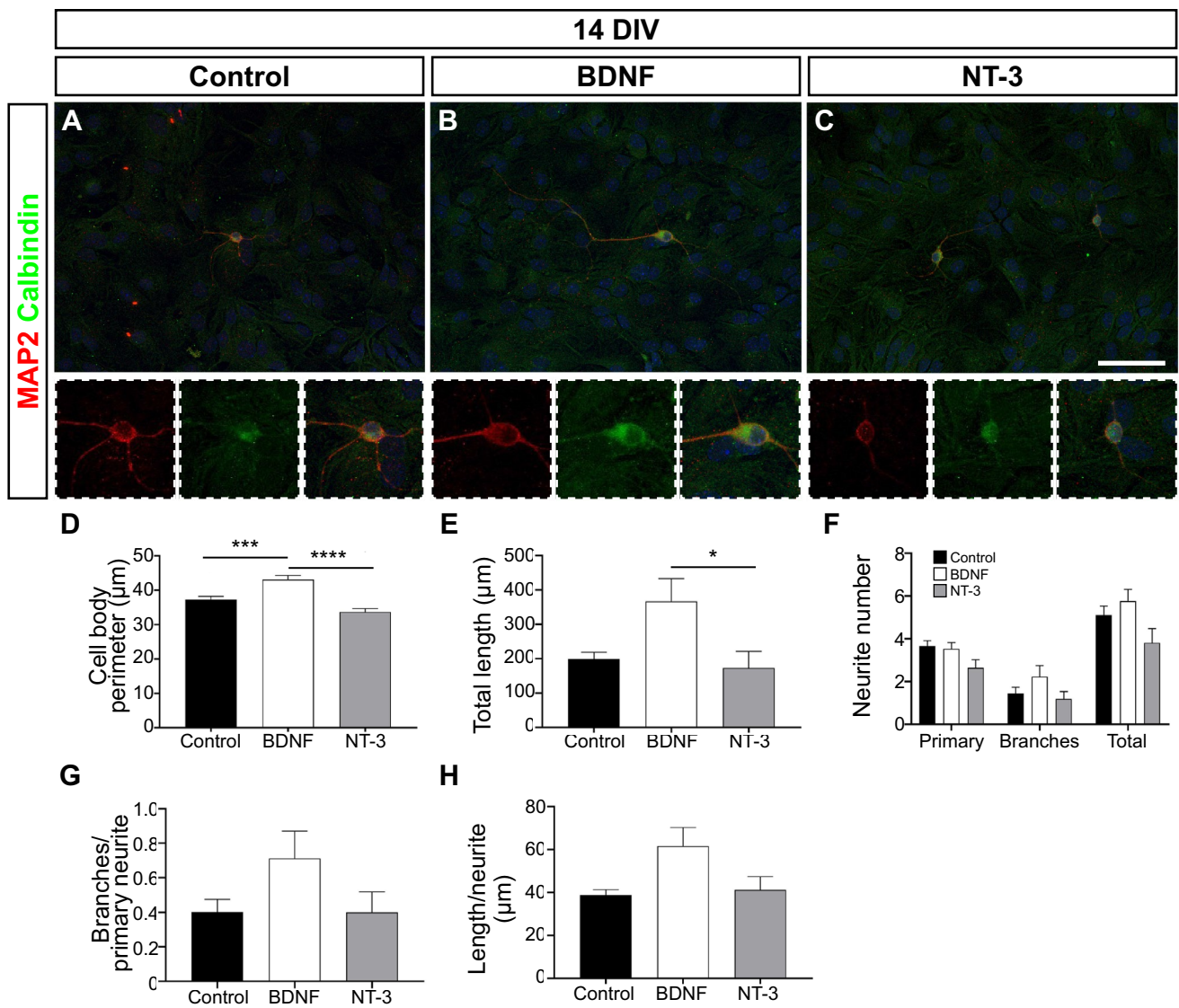


Fig. 7 The effect of BDNF on the morphology of Calbindin (Calb)⁺ neurons derived from eOBNSCs after 14 DIV. The images show representative neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with antibodies against MAP2ab and Calb. The colocalization of MAP2ab and Calb can be better assessed in the higher magnification images (bottom). The graphs

show the effects on the morphological parameters of 12–18 Calb⁺ neurons in each of the three conditions. Exposure to BDNF increased the cell body perimeter and the total neurite length: * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$ (D, one-way ANOVA with Tukey's post hoc test; E, Kruskal–Wallis test followed by post hoc Dunn's test). Scale bar (C) = 77.0 μm ; high magnification images, 38.5 μm

than in the control cultures, the 2.2-fold increase detected was not statistically significant (Fig. 9f). Similarly, we found no significant differences in the density of boutons per dendrite length (Fig. 9g). However, the synaptic boutons of BDNF-treated neurons were larger than those of Control neurons, with a 43% increase in their perimeter ($P < 0.0001$: Fig. 9h, Table 1) and a 100% or twofold increase in bouton area ($P < 0.0001$: Fig. 9i, Table 1).

Synaptic boutons of NT-3 treated neurons were also bigger than those in Control neurons, with significant increases in the perimeter ($P < 0.0001$: Fig. 9h, Table 1) and in the bouton area ($P < 0.0001$: Fig. 9i, Table 1). However, the

boutons of BDNF-treated neurons had both a larger perimeter and a larger area than those exposed to NT-3 ($P < 0.05$: Fig. 9h, Table 1). Hence, BDNF and NT-3 appear to have an effect specifically on boutons, which were located either on neurons making synapses with the MAP2ab⁺ cells (green boutons) or they were presynapses of MAP2ab cells (yellow boutons). Nevertheless, it was not possible to unambiguously detect and quantify the postsynaptic protein PSD95 by immunofluorescence in these cells (data not shown).

In summary, the data presented here show that BDNF affects the morphology (cell body size, number of primary neurites and neurite branches, and neurite length)

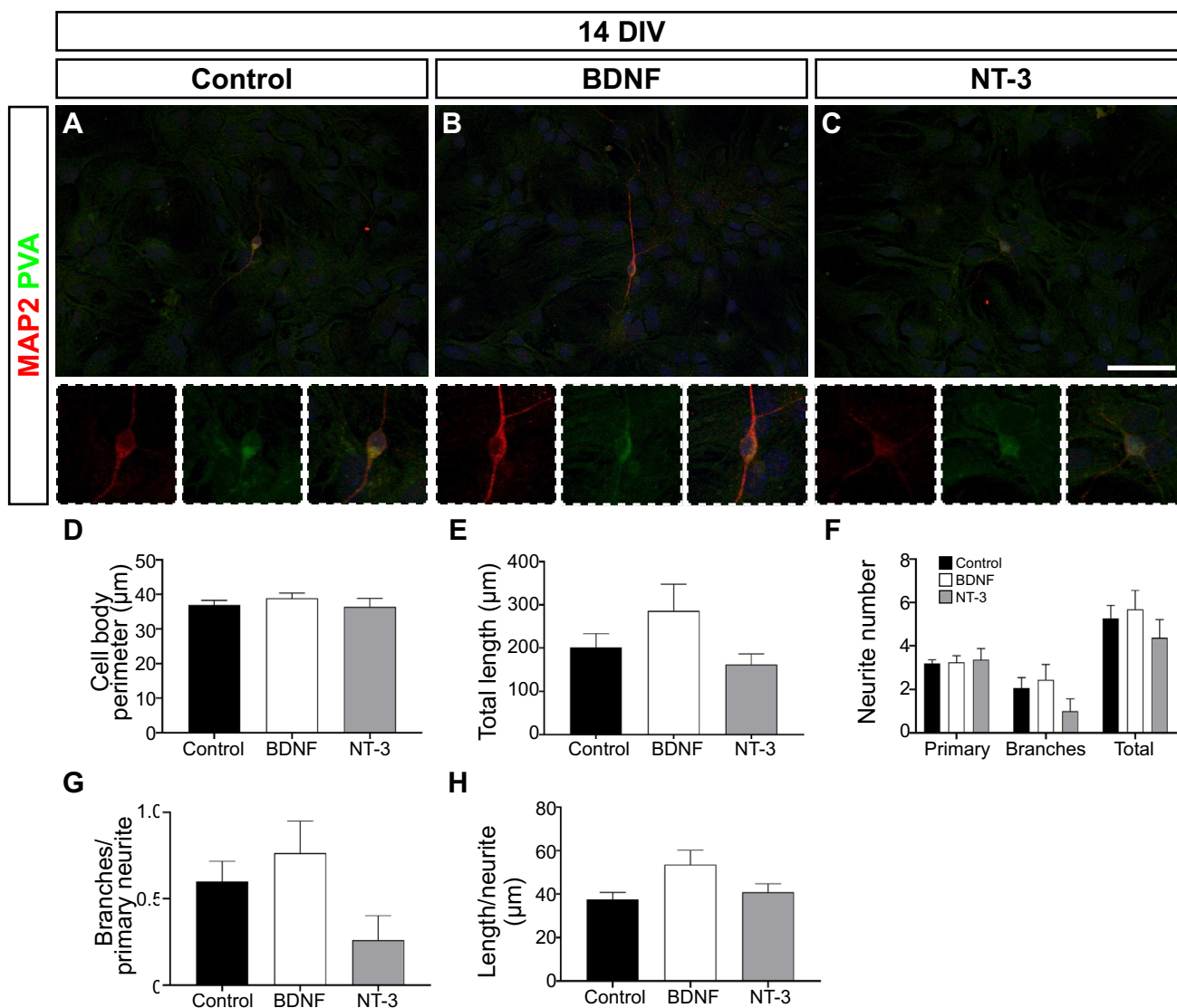


Fig. 8 BDNF did not significantly affect the morphology of Parvalbumin (PVA)⁺ neurons derived from eOBNSCs after 14 DIV. The images show representative neurons from Control (a), BDNF-treated (b), or NT-3 treated cultures (c), immunostained with antibodies against MAP2ab and PVA. The colocalization of MAP2ab and PVA can be better assessed in the higher magnification images (bottom).

The graphs show the effects on the morphological parameters analyzed in 13 PVA⁺ neurons generated in each of the three conditions. The changes produced by BDNF on cell morphology did not reach statistical significance. Scale bar (C)=77.0 μm ; high magnification images, 38.5 μm

of eOBNSC-derived GABAergic neurons in a cell-type specific manner. Relative to control conditions or the neurons exposed to NT-3, the effects of BDNF were stronger on GABA⁺ neurons and in their subtypes TH⁺, Calr⁺ and Calb⁺ neurons than on PVA⁺ cells, in which no significant changes were detected. Our time course study indicated that BDNF initially promoted an increase in total neurite length in GABA⁺ neurons, mostly due to the growth of neurite branches (14 DIV), and it subsequently favored the elongation of pre-existing neurites (21 DIV). Notably, both BDNF and NT-3 increased the size of synaptophysin⁺ synaptic boutons.

Discussion

BDNF and NT-3 are involved in neuronal differentiation and maturation during embryonic brain development and adult neurogenesis, promoting axonal and dendritic growth and synapse formation (Park and Poo 2013; Vicario-Abejón et al. 2002; Gottmann et al. 2009; Zagrebelsky and Korte 2014). However, their specific actions depend on the cell type and stage of development (McAllister et al. 1997; Petridis and El Maarouf 2011; Galvão et al. 2008; Vicario-Abejón et al. 1998; Gascon et al. 2005; Zagrebelsky et al. 2018).

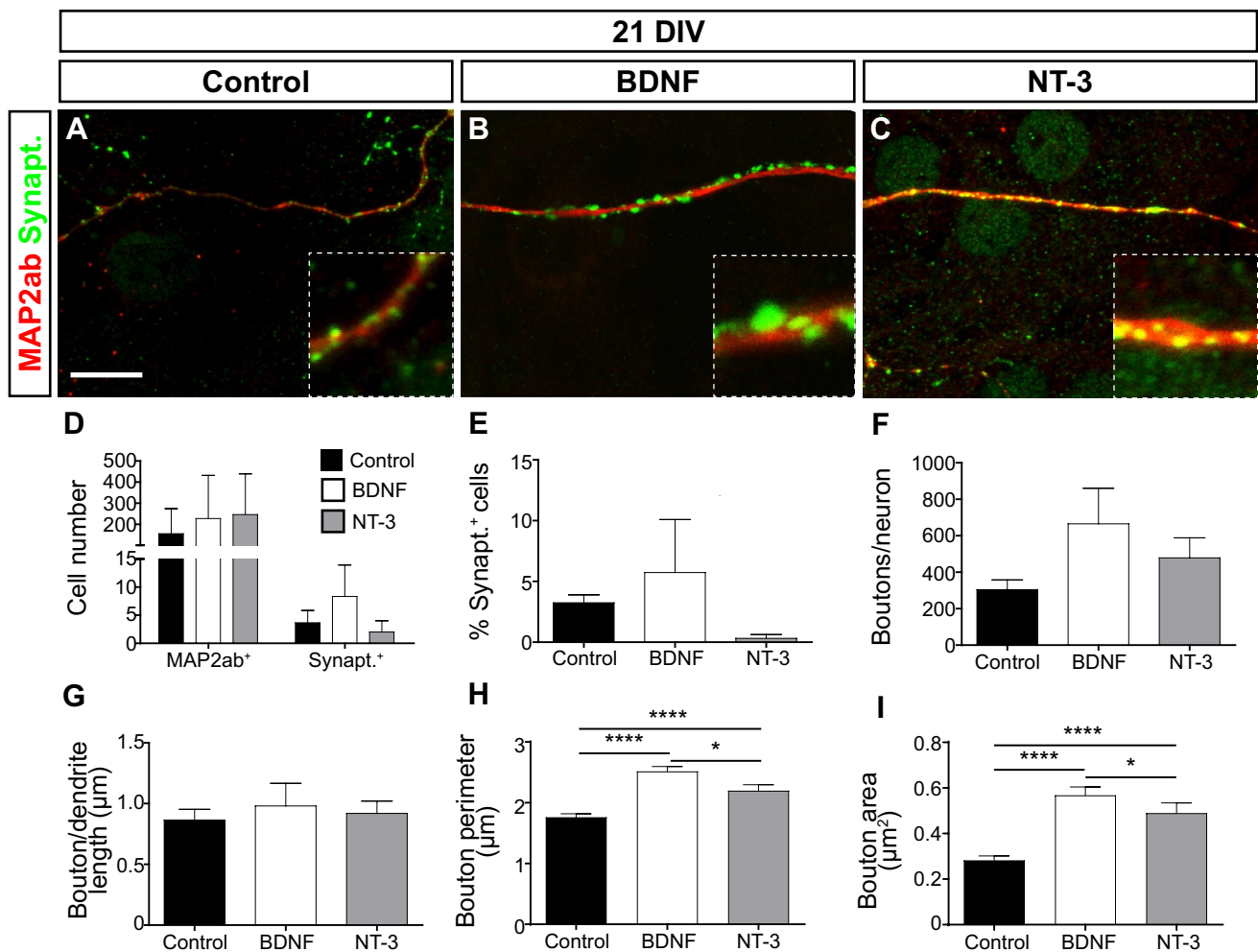


Fig. 9 BDNF and NT-3 increase the size of presynaptic boutons in neurons derived from eOBNSCs after 21 DIV. The images show representative processes of neurons from Control (a), BDNF-treated (b) or NT-3 treated cultures (c), immunostained with antibodies against MAP2ab and Synaptophysin (Synapt.). The graphs show the number of MAP2ab⁺ and Synapt⁺ cells (d), the percentage of Synapt⁺ neurons (e), the number of boutons per neuron (f), the density of the synaptic boutons (g), and the average perimeter (h) and area (i) of synaptic

boutons per neuron generated in the three conditions. The results are the mean \pm SEM of 3 cultures analyzed in triplicate (d, e). BDNF and NT-3 (although to a lesser degree) augmented the perimeter and area of presynaptic boutons. The analysis of synaptic boutons was performed on 130–150 boutons from 13–15 neurons from 3–4 cultures (f–i): * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$ (one-way ANOVA with Tukey's post hoc test). Scale bar (A) = 10.37 μm ; insets = 2.6 μm

Here we report that chronic BDNF treatment regulates the dendrite morphology of eOBNSC-derived GABAergic interneurons in a cell-type specific manner and that this neurotrophin may stimulate synapse formation and maturation by regulating the size of synaptic boutons. By contrast, NT-3 has no significant effects on the morphological differentiation of eOBNSC-derived GABAergic interneurons although it does affect synaptic boutons. Neither BDNF nor NT-3 significantly alter the number of neurons generated in vitro, indicating that they do not affect neuronal survival. Indeed, in our experimental conditions neuronal survival appears to depend on the duration of the culture (21 vs 14 DIV) but not on the presence

of neurotrophins. Hence, BDNF and NT-3 appear to act specifically on both dendrites and presynaptic boutons.

The Role of BDNF in the Morphological Differentiation of OB GABAergic Interneurons

The generation of neurons from eOBNSCs is a useful model to study the differentiation and maturation of specific neuronal types and the molecules involved in these processes (Vergaño-Vera et al. 2009, 2015). Here, we show that GABAergic neurons are the main neuronal type produced from eOBNSCs. Surprisingly, a very small proportion of glutamatergic cells was obtained from eOBNSCs (between

0% to 1.5% of Tbr1⁺ or Tbr2⁺ cells), while these neurons are produced abundantly in the mouse in vivo between embryonic days 11 and 13 (E11 and E13) (Díaz-Guerra et al. 2013; Weinandy et al. 2011). This limited production of glutamatergic neurons in vitro could be due to the action or absence of intrinsic mechanisms controlling neuronal fate in eOBNSCs, and/or to special conditions that may be required to maintain eOBNSC-derived glutamatergic neurons in culture.

Our data highlight the influence of BDNF (but not NT-3) on the differentiation of eOBNSC-derived neurons. They show that BDNF affects the morphology (cell body size, number of primary neurites and neurite branches, as well as neurite length) of eOBNSC-derived GABAergic neurons in a cell-type specific manner. Compared to the control conditions or NT-3, BDNF had a stronger effect on GABA⁺, TH⁺, Calr⁺ and Calb⁺ neurons than on PVA⁺ neurons, in which no significant changes were detected. This distinction was somewhat unexpected as it was reported previously that BDNF promoted the growth of PVA⁺ neuron's dendrites in rat E19 OB neuron cultures. Moreover, BDNF deletion caused a reduction in PVA expression and it altered the dendritic arborization of these neurons in the neonatal-postnatal mouse OB (Berghuis et al. 2006). One possible explanation to reconcile these findings could be that in our eOBNSC culture system, differentiating neurons are in an immature stage compared to those neurons derived from the E19 rat OB and from postnatal OB neurons in vivo. Accordingly, early differentiation of OB PVA neurons might not necessarily require BDNF whereas this neurotrophin may be critical for the morphological and neurochemical differentiation of these neurons during late gestation and in the neonate. In addition, it may be possible that PVA neurons generated in our eOBNSC cultures are of a different subtype to those PVA neurons located in the external plexiform layer, which are dependent on BDNF for their maintenance and differentiation (Berghuis et al. 2006). Although the TrkC receptor is expressed by a similar proportion of eOBNSC-derived neurons as TrkB, the absence of significant effects of NT-3 on the parameters tested suggest that the signaling downstream of TrkC is not fully active in growing OB GABAergic neurites.

Collectively, our findings suggest the existence of cell-type-specific mechanisms that regulate neuron's morphological differentiation via BDNF. In this sense, opposite effects of BDNF and NT-3 have been described in different layers of the cerebral cortex: BDNF stimulates dendrite growth in layer 4 where it is inhibited by NT-3, and with the reverse effect seen in layer 6 (McAllister et al. 1997). Furthermore, BDNF promotes the growth of hippocampal GABAergic neuron's dendrites and axon, whereas NT-3 only affects dendrite growth (Vicario-Abejón et al. 1998). Furthermore, BDNF depletion impairs the dendritic architecture of both

hippocampal interneurons and granule neurons, while having only a mild effect on pyramidal neurons (Zagrebelsky et al. 2018).

Since the large majority of GABAergic interneurons in the OB either lack or have a short axon (Lledo et al. 2008; Weinandy et al. 2011), we suggest that BDNF regulates the sculpting of dendrite morphology in OB GABAergic neurons, which could be a mechanism to efficiently establish and maintain neuronal connections in vivo. Neuronal circuit development relies on regulating dendrite, spine, axon and synapse growth, as well as on neurite elimination (Park and Poo 2013; Shen and Scheiffele 2010; Zagrebelsky and Korte 2014). Here, BDNF produces both neuritogenesis and neurite elongation, as best illustrated by comparing its effects in GABA⁺ neurons at 14 DIV and 21 DIV. Indeed, this simple time course study indicates that BDNF initially promotes an increase in total neurite length of GABA⁺ neurons, mostly through the growth of neurite branches (14 DIV), and it subsequently favors the elongation of pre-existing neurites (21 DIV). Whether these effects of BDNF are exclusively mediated by TrkB (Vicario-Abejón et al. 1998; Zagrebelsky et al. 2018) or by sequential activation of p^{75NTR} and TrkB (Gascon et al. 2005) is not known.

BDNF and NT-3 Increase the Size of Presynaptic Boutons in Neurons Derived From eOBNSCs

Neurotrophins have also been associated with the regulation of morphological and functional synapse maturation (Collin et al. 2001; Martinez et al. 1998; Tyler and Pozzo-Miller 2001; Vicario-Abejón et al. 1998; Sanchez-Huertas and Rico 2011; Zagrebelsky and Korte 2014). Indeed, eOBNSC-derived neurons exposed to BDNF and NT-3 have larger presynaptic boutons (even larger on exposure to BDNF) without significantly affecting their density per neuron or dendrite length. These findings indicate that these neurotrophins may have a major role in promoting the maturation of the presynaptic terminal in OB neurons. The impact of NT-3 on presynaptic boutons but not on neurites indicates that NT-3/TrkC signaling is spatially regulated in OB developing neurons.

Several studies have reported that neurotrophins may influence synapse formation and maturation through various mechanisms. On the one hand, they may stimulate axon and dendrite growth, with neurons developing more synaptic contacts (Alsina et al. 2001; Cohen-Cory and Fraser 1995; Sanchez et al. 2006; Vicario-Abejón et al. 2002). On the other hand, neurotrophins may promote synapse formation and stabilization, increasing the number of synapses and the synapse density in addition to regulating axon arborization (Alsina et al. 2001; Martinez et al. 1998; Rico et al. 2002; Vicario-Abejón et al. 2002; Sanchez et al. 2006). Our results show an additional mechanism by which BDNF and NT-3

can promote synapse maturation, specifically increasing presynaptic bouton size, which could enhance presynaptic activity. Accordingly, these boutons may contain more proteins involved in the formation and mobilization of presynaptic vesicles, and in neurotransmitter release (Vicario-Abejón et al. 2002). Indeed, hippocampal neurons treated with BDNF or with a plasmid overexpressing BDNF had more total and docked SVs and synaptophysin⁺ puncta capable of releasing neurotransmitters (Tyler and Pozzo-Miller 2001; Collin et al. 2001; Rauti et al. 2020), a phenomenon that may be related to larger spines and postsynaptic NMDA receptor clusters (Elmariah 2004; Orefice et al. 2013). These actions could reflect the potential of BDNF and/or NT-3 to establish synaptic repair strategies to avoid synapse loss during neurodegeneration (Caffino et al. 2020; Lu et al. 2013).

Taken together, our results indicate that BDNF plays a major role in regulating the dendritic morphology of developing OB GABAergic interneurons and that BDNF's actions are cell-type dependent. Furthermore, BDNF and NT-3 increase the size of presynaptic boutons, which could be a critical event during synapse formation and maturation.

Acknowledgements We thank M.J. Román (Instituto Cajal-CSIC, Madrid, Spain) for her technical support and Dr. Mark Sefton (BiomedRed, Madrid, Spain) for English editing.

Funding This study was funded by grants from MINECO (Grant Numbers: SAF2013-47596-R, SAF2016-80419-R and CIBERNED CB06/05/0065), the Comunidad de Madrid (Grant Number S2011/BMD-2336) and Fundación Ramón Areces (Grant Number CIVP18A3941) to C.V.

Compliance with Ethical Standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical Approval All applicable international, national, and institutional guidelines for the care and use of animals were followed: European Union guidelines (directive 2010/63/EU) and Spanish legislation (Law 32/2007 and RD 53/2013), and the protocols were approved by the Ethical Committee of the Consejo Superior de Investigaciones Científicas (CSIC) and of the Comunidad de Madrid, Spain.

References

- Ahmed S, Reynolds BA, Weiss S (1995) BDNF enhances the differentiation but not the survival of CNS stem cell-derived neuronal precursors. *J Neurosci* 15:5765–5778
- Alsina B, Vu T, Cohen-Cory S (2001) Visualizing synapse formation in arborizing optic axons in vivo: dynamics and modulation by BDNF. *Nat Neurosci* 4:1093–1101
- An JJ, Gharami K, Liao GY, Woo NH, Lau AG, Vanevski F, Torre ER, Jones KR, Feng Y, Lu B, Xu B (2008) Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell* 134:175–187
- Bath KG, Mandairon N, Jing D, Rajagopal R, Kapoor R, Chen ZY, Khan T, Proenca CC, Kraemer R, Cleland TA, Hempstead BL, Chao MV, Lee FS (2008) Variant brain-derived neurotrophic factor (Val66Met) alters adult olfactory bulb neurogenesis and spontaneous olfactory discrimination. *J Neurosci* 28:2383–2393
- Batista-Brito R, Close J, Machold R, Fishell G (2008) The distinct temporal origins of olfactory bulb interneuron subtypes. *J Neurosci* 28:3966–3975
- Bergami M, Vignoli B, Motori E, Pifferi S, Zuccaro E, Menini A, Canossa M (2013) TrkB signaling directs the incorporation of newly generated periglomerular cells in the adult olfactory bulb. *J Neurosci* 33:11464–11478
- Berghuis P, Agerman K, Dobszay MB, Minichiello L, Harkany T, Ernfors P (2006) Brain-derived neurotrophic factor selectively regulates dendritogenesis of parvalbumin-containing interneurons in the main olfactory bulb through the PLCgamma pathway. *J Neurobiol* 66:1437–1451
- Bonzano S, Bovetti S, Gendusa C, Peretto P, De MS (2016) Adult Born Olfactory Bulb Dopaminergic Interneurons: Molecular Determinants and Experience-Dependent Plasticity. *Front Neurosci* 10:189
- Caffino L, Mottarlini F, Fumagalli F (2020) Born to Protect: Leveraging BDNF Against Cognitive Deficit in Alzheimer's Disease. *CNS Drugs* 34:281–297
- Choo M, Miyazaki T, Yamazaki M, Kawamura M, Nakazawa T, Zhang J, Tanimura A, Uesaka N, Watanabe M, Sakimura K, Kano M (2017) Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. *Nat Commun* 8:195
- Cohen-Cory S, Fraser SE (1995) Effects of brain-derived neurotrophic factor on optic axon branching and remodelling in vivo. *Nature* 378:192–196
- Collin C, Vicario-Abejón C, Rubio ME, Wenthold RJ, McKay RD, Segal M (2001) Neurotrophins act at presynaptic terminals to activate synapses among cultured hippocampal neurons. *Eur J Neurosci* 13:1273–1282
- Curto GG, Nieto-Estévez V, Hurtado-Chong A, Valero J, Gomez C, Alonso JR, Weruaga E, Vicario-Abejón C (2014) Pax6 is essential for the maintenance and multi-lineage differentiation of neural stem cells, and for neuronal incorporation into the adult olfactory bulb. *Stem Cells Dev* 23:2813–2830
- Díaz-Guerra E, Pignatelli J, Nieto-Estévez V, Vicario-Abejón C (2013) Transcriptional regulation of olfactory bulb neurogenesis. *Anat Rec* 296:1364–1382
- Elmariah SB (2004) Postsynaptic TrkB-Mediated Signaling Modulates Excitatory and Inhibitory Neurotransmitter Receptor Clustering at Hippocampal Synapses. *J Neurosci* 24:2380–2393
- Fryer RH, Kaplan DR, Feinstein SC, Radeke MJ, Grayson DR, Kromer LF (1996) Developmental and mature expression of full-length and truncated TrkB receptors in the rat forebrain. *J Comp Neurol* 374:21–40
- Galvão RP, Garcia-Verdugo JM, Alvarez-Buylla A (2008) Brain-derived neurotrophic factor signaling does not stimulate subventricular zone neurogenesis in adult mice and rats. *J Neurosci* 28:13368–13383
- Gascon E, Vutskits L, Zhang H, Barral-Moran MJ, Kiss PJ, Mas C, Kiss JZ (2005) Sequential activation of p75 and TrkB is involved in dendritic development of subventricular zone-derived neuronal progenitors in vitro. *Eur J Neurosci* 21:69–80
- Gottmann K, Mittmann T, Lessmann V (2009) BDNF signaling in the formation, maturation and plasticity of glutamatergic and GABAergic synapses. *Exp Brain Res* 199:203–234
- Hurtado-Chong A, Yusta-Boyo MJ, Vergaño-Vera E, Bulfone A, de Pablo F, Vicario-Abejón C (2009) IGF-I promotes neuronal migration and positioning in the olfactory bulb and the exit of neuroblasts from the subventricular zone. *Eur J Neurosci* 30:742–755

- Imamura F, Greer CA (2009) Dendritic branching of olfactory bulb mitral and tufted cells: regulation by TrkB. *PLoS ONE* 4:e6729
- Jacobi S, Soriano J, Segal M, Moses E (2009) BDNF and NT-3 increase excitatory input connectivity in rat hippocampal cultures. *Eur J Neurosci* 30:998–1010
- Johnson EM, Craig ET, Yeh HH (2007) TrkB is necessary for pruning at the climbing fibre-Purkinje cell synapse in the developing murine cerebellum. *J Physiol* 582:629–646
- Jones KR, Farinas I, Backus C, Reichardt LF (1994) Targeted disruption of the BDNF gene perturbs brain and sensory neuron development but not motor neuron development. *Cell* 76:989–999
- Lledo PM, Merkle FT, Alvarez-Buylla A (2008) Origin and function of olfactory bulb interneuron diversity. *Trends Neurosci* 31:392–400
- Lu B, Nagappan G, Guan X, Nathan PJ, Wren P (2013) BDNF-based synaptic repair as a disease-modifying strategy for neurodegenerative diseases. *Nat Rev Neurosci* 14:401–416
- Mackay-Sim A, Chuah MI (2000) Neurotrophic factors in the primary olfactory pathway. *Prog Neurobiol* 62:527–559
- Martinez A, Alcantara S, Borrell V, Del Rio JA, Blasi J, Otal R, Campos N, Boronat A, Barbacid M, Silos-Santiago I, Soriano E (1998) TrkB and TrkC signaling are required for maturation and synaptogenesis of hippocampal connections. *J Neurosci* 18:7336–7350
- Marty S, Wehrle R, Sotelo C (2000) Neuronal activity and brain-derived neurotrophic factor regulate the density of inhibitory synapses in organotypic slice cultures of postnatal hippocampus. *J Neurosci* 20:8087–8095
- Mast TG, Fadoo DA (2012) Mature and precursor brain-derived neurotrophic factor have individual roles in the mouse olfactory bulb. *PLoS ONE* 7:e31978
- Matsutani S, Yamamoto N (2004) Brain-derived neurotrophic factor induces rapid morphological changes in dendritic spines of olfactory bulb granule cells in cultured slices through the modulation of glutamatergic signaling. *Neuroscience* 123:695–702
- McAllister AK, Katz LC, Lo DC (1997) Opposing roles for endogenous BDNF and NT-3 in regulating cortical dendritic growth. *Neuron* 18:767–778
- McDole B, Isgor C, Pare C, Guthrie K (2015) BDNF over-expression increases olfactory bulb granule cell dendritic spine density in vivo. *Neuroscience* 304:146–160
- McDole B, Berger R, Guthrie K (2020) Genetic Increases in Olfactory Bulb BDNF Do Not Enhance Survival of Adult-Born Granule Cells. *Chem Senses* 45:3–13
- Méndez-Gómez HR, Vergaño-Vera E, Abad JL, Bulfone A, Moratalla R, de Pablo F, Vicario-Abejón C (2011) The T-box brain 1 (Tbr1) transcription factor inhibits astrocyte formation in the olfactory bulb and regulates neural stem cell fate. *Mol Cell Neurosci* 46:108–121
- Nef S, Lush ME, Shipman TE, Parada LF (2001) Neurotrophins are not required for normal embryonic development of olfactory neurons. *Dev Biol* 234:80–92
- Nieto-Estévez V, Pignatelli J, Arauzo-Bravo MJ, Hurtado-Chong A, Vicario-Abejón C (2013) A global transcriptome analysis reveals molecular hallmarks of neural stem cell death, survival, and differentiation in response to partial FGF-2 and EGF deprivation. *PLoS ONE* 8:e53594
- Orefice LL, Waterhouse EG, Partridge JG, Lalchandani RR, Vicini S, Xu B (2013) Distinct roles for somatically and dendritically synthesized brain-derived neurotrophic factor in morphogenesis of dendritic spines. *J Neurosci* 33:11618–11632
- Orefice LL, Shih CC, Xu H, Waterhouse EG, Xu B (2016) Control of spine maturation and pruning through proBDNF synthesized and released in dendrites. *Mol Cell Neurosci* 71:66–79
- Park H, Poo MM (2013) Neurotrophin regulation of neural circuit development and function. *Nat Rev Neurosci* 14:7–23
- Parrish-Aungst S, Shipley MT, Erdelyi F, Szabo G, Puche AC (2007) Quantitative analysis of neuronal diversity in the mouse olfactory bulb. *J Comp Neurol* 501:825–836
- Petridis AK, El Maarouf A (2011) Brain-derived neurotrophic factor levels influence the balance of migration and differentiation of subventricular zone cells, but not guidance to the olfactory bulb. *J Clin Neurosci* 18:265–270
- Pignatelli A, Belluzzi O (2017) Dopaminergic Neurons in the Main Olfactory Bulb: An Overview from an Electrophysiological Perspective. *Front Neuroanat* 11:7
- Rauti R, Cellot G, D'Andrea P, Colliva A, Scaini D, Tongiorgi E, Ballerini L (2020) BDNF impact on synaptic dynamics: extra or intracellular long-term release differently regulates cultured hippocampal synapses. *Mol Brain* 13:43
- Ravi N, Sanchez-Guardado L, Lois C, Kelsch W (2017) Determination of the connectivity of newborn neurons in mammalian olfactory circuits. *Cell Mol Life Sci* 74:849–867
- Rico B, Xu B, Reichardt LF (2002) TrkB receptor signaling is required for establishment of GABAergic synapses in the cerebellum. *Nat Neurosci* 5:225–233
- Sairanen M, Lucas G, Ernfors P, Castren M, Castren E (2005) Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *J Neurosci* 25:1089–1094
- Sanchez AL, Matthews BJ, Meynard MM, Hu B, Javed S, Cohen Cory S (2006) BDNF increases synapse density in dendrites of developing tectal neurons in vivo. *Development* 133:2477–2486
- Sanchez-Huertas C, Rico B (2011) CREB-Dependent Regulation of GAD65 Transcription by BDNF/TrkB in Cortical Interneurons. *Cereb Cortex* 21:777–788
- Shen K, Scheiffele P (2010) Genetics and cell biology of building specific synaptic connectivity. *Annu Rev Neurosci* 33:473–507
- Singh B, Henneberger C, Betances D, Arevalo MA, Rodriguez-Tebar A, Meier JC, Grantyn R (2006) Altered balance of glutamatergic/GABAergic synaptic input and associated changes in dendrite morphology after BDNF expression in BDNF-deficient hippocampal neurons. *J Neurosci* 26:7189–7200
- Singh KK, Park KJ, Hong EJ, Kramer BM, Greenberg ME, Kaplan DR, Miller FD (2008) Developmental axon pruning mediated by BDNF-p75NTR-dependent axon degeneration. *Nat Neurosci* 11:649–658
- Tyler WJ, Pozzo-Miller LD (2001) BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *J Neurosci* 21:4249–4258
- Vergaño-Vera E, Yusta-Boyo MJ, de Castro F, Bernad A, de Pablo F, Vicario-Abejón C (2006) Generation of GABAergic and dopaminergic interneurons from endogenous embryonic olfactory bulb precursor cells. *Development* 133:4367–4379
- Vergaño-Vera E, Méndez-Gómez HR, Hurtado-Chong A, Cigudosa JC, Vicario-Abejón C (2009) Fibroblast growth factor-2 increases the expression of neurogenic genes and promotes the migration and differentiation of neurons derived from transplanted neural stem/progenitor cells. *Neuroscience* 162:39–54
- Vergaño-Vera E, Díaz-Guerra E, Rodríguez-Traver E, Méndez-Gómez HR, Solís O, Pignatelli J, Pickel J, Lee SH, Moratalla R, Vicario-Abejón C (2015) Nurr1 blocks the mitogenic effect of FGF-2 and EGF, inducing olfactory bulb neural stem cells to adopt dopaminergic and dopaminergic-GABAergic neuronal phenotypes. *Dev Neurobiol* 75:823–841
- Vicario-Abejón C, Johe KK, Hazel TG, Collazo D, McKay RDG (1995) Functions of basic-fibroblast growth factor and neurotrophins in the differentiation of hippocampal neurons. *Neuron* 15:105–114
- Vicario-Abejón C, Collin C, McKay RD, Segal M (1998) Neurotrophins induce formation of functional excitatory and inhibitory

- synapses between cultured hippocampal neurons. *J Neurosci* 18:7256–7271
- Vicario-Abejón C, Collin C, Tsoulfas P, McKay RD (2000) Hippocampal stem cells differentiate into excitatory and inhibitory neurons. *Eur J Neurosci* 12:677–688
- Vicario-Abejón C, Owens D, McKay R, Segal M (2002) Role of neurotrophins in central synapse formation and stabilization. *Nat Rev Neurosci* 3:965–974
- Vilar M, Mira H (2016) Regulation of Neurogenesis by Neurotrophins during Adulthood: Expected and Unexpected Roles. *Front Neurosci* 10:26
- Waterhouse EG, An JJ, Orefice LL, Baydyuk M, Liao GY, Zheng K, Lu B, Xu B (2012) BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. *J Neurosci* 32:14318–14330
- Weinandy F, Ninkovic J, Götz M (2011) Restrictions in time and space—new insights into generation of specific neuronal subtypes in the adult mammalian brain. *Eur J Neurosci* 33:1045–1054
- Wen Y, Zhang Z, Li Z, Liu G, Tao G, Song X, Xu Z, Shang Z, Guo T, Su Z, Chen H, You Y, Li J, Yang Z (2019) The PROK2/PROKR2 signaling pathway is required for the migration of most olfactory bulb interneurons. *J Comp Neurol* 527:2931–2947
- Yamada MK, Nakanishi K, Ohba S, Nakamura T, Ikegaya Y, Nishiyama N, Matsuki N (2002) Brain-derived neurotrophic factor promotes the maturation of GABAergic mechanisms in cultured hippocampal neurons. *J Neurosci* 22:7580–7595
- Yang Z (2008) Postnatal subventricular zone progenitors give rise not only to granular and periglomerular interneurons but also to interneurons in the external plexiform layer of the rat olfactory bulb. *J Comp Neurol* 506:347–358
- Zagrebelsky M, Korte M (2014) Form follows function: BDNF and its involvement in sculpting the function and structure of synapses. *Neuropharmacology* 76:628–638
- Zagrebelsky M, Godecke N, Remus A, Korte M (2018) Cell type-specific effects of BDNF in modulating dendritic architecture of hippocampal neurons. *Brain Struct Funct* 223:3689–3709

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.