PROJECT FINAL REPORT

Transforming growth factor beta proteins in the central nervous system

Research articles with the support of NFM OTKA NNF78219:

1. Cservenák M., Bodnár I., Usdin T.B., Palkovits M., Nagy G.M., Dobolyi A. (2010) Tuberoinfundibular peptide of 39 residues is activated during lactation and participates in the suckling-induced prolactin release. Endocrinology, 151, 5830-5840.
Impact factor: 4.752

2. Vincze C., Pál G., Wappler E.A., Szabó É.R., Nagy Z., Lovas G., Dobolyi A. (2010) Transforming growth factor beta isoforms in the intact rat brain and following experimentally induced focal ischemia. J. Comp. Neurol. 518, 3752-3770.
Impact factor: 3.718

3. Dobolyi A., Palkovits M., Usdin T.B. (2010) The TIP39-PTH2 receptor system: unique peptidergic cell groups in the brainstem and their interactions with central regulatory mechanisms. Prog. Neurobiol. 90, 29-59.
Impact factor: 9.140

4. Dobolyi A (2009) Central amylin expression and its induction in rat dams. J. Neurochem. 111, 1490-1500.
Impact factor: 3.999

Conference abstracts with the support of NFM OTKA NNF78219:

1 Pál, G.; Vincze, C.; Wappler, E.A.; Nagy, Z.; Lovas, G.; Dobolyi, A (2011) Spatial and temporal patterns of induction of transforming growth factor betas following middle cerebral artery occlusion in rat. 15th Congress of the European Federation of Neurological Societies, Budapest.

2 Pál, G.; Vincze, C.; Wappler, E. A.; Lovas, G.; Dobolyi, Á. (2011) The induction of TGFbetas in relation to immediate early genes following middle cerebral artery occlusion in rats. Front. Neurosci. Conference Abstract: 13th Conference of the Hungarian Neuroscience Society (MITT). doi: 10.3389/conf.fnins.2011.84.00012.

3 Vincze C., Pál G., Wappler E.A., Nardai S., Nagy Z., Lovas G., Dobolyi A. (2010) Transforming growth factor beta isoforms in intact rat brain and following middle cerebral artery occlusion. International IBRO Workshop, Pécs. Frontiers in Systems Neuroscience. Conference Abstract: IBRO International Workshop 2010. doi: 10.3389/conf.fnins.2010.10.00070.

4 Dobolyi A., Cservenák M., Bodnár I., Palkovits M., Nagy G.M., Usdin T.B. (2010) A novel neuromodulator system in the hypothalamic regulation of prolactin release. Society for Neuroscience, 40th Ann. Meet., San Diego, CA, USA. Abstract Viewer and Itinerary Planner. 793.11 /BBB12.

5 Dobolyi A. (2010) Central amylin expression and its potential involvement in maternal regulations. Invited lecture at the 7th International Symposia on the CGRP Family; CGRP, Adrenomedullin, Amylin, Intermedin and Calcitonin. Queenstown, New Zealand. Abstr. Vol. 1, p16.

6 Dobolyi A. (2010) Microarray reveals robust induction of amylin in the maternal preoptic area. The 4th International Conference on The Parental Brain; Neurobiology, Behaviour and the Next Generation. Edinburgh, Scotland, UK. Abstr. Vol. 1, p65.

7 Lovas G., Szuchet S., Darida M., Szilágyi N. (2010) Multiparametric non-linear statistical analysis of cardiovascular and respiratory alterations during extensive cerebral infarcts requiring neurointensive care, Society for Neuroscience, San Diego, CA. Abstract Viewer and Itinerary Planner.

8 Cservenák M., Bodnár I., Usdin T.B., Palkovits M., Nagy G.M., Dobolyi A. (2010) Intracerebroventricular injection of a parathyroid hormone 2 receptor antagonist abolishes suckling induced prolactin release. International IBRO Workshop, Pécs. Frontiers in Systems Neuroscience. Conference Abstract: IBRO International Workshop 2010. doi: 10.3389/conf.fnins.2010.10.00081.

9 Bagó A.G., Dimitrov E., Saunders R., Seress L., Palkovits M., Usdin T.B., Dobolyi A. (2010) Neurochemical investigations on parathyroid hormone 2 receptor-immunoreactive neurons in the human and macaque hypothalamus and brainstem. International IBRO Workshop, Pécs. Frontiers in Systems Neuroscience. Conference Abstract: IBRO International Workshop 2010. doi: 10.3389/conf.fnins.2010.10.00076.

10 Dobolyi A., Cservenák M., Bodnár I., Palkovits M., Nagy G.M., Usdin T.B. (2010) Anatomical and functional evidence for the involvement of tuberoinfundibular peptide of 39 residues in the regulation of suckling-induced prolactin release. The 7th International Congress of Neuroendocrinology, Rouen, France. No. O-16, p70.

11 Dobolyi A. (2009) Transzformáló növekedési faktor beta fehérjék mRNS expresszója és eloszlása patkány agyban. A Magyar Anatómus Társaság 15. Kongresszusa, Budapest.

12 Dobolyi A., Vincze C., Wappler E., Nardai S., Nagy Z., Lovas G. (2009) Distribution of mRNAs encoding transforming growth factor beta 1, 2 and 3 in the normal and ischemic rat brain. Soc. for Neurosci., 39th Ann. Meet., Chicago, USA. 737.11/N16.

Conference attendance with full or partial support of NFM OTKA NNF78219 (each participant had presentation as listed above):

39th Ann. Meeting of the Society for Neuroscience, Chicago, USA - Árpád Dobolyi
The 7th International Congress of Neuroendocrinology, Rouen, France - Árpád Dobolyi
International IBRO Workshop, Pécs. – Attila Bagó, Gábor Lovas, Csilla Vincze
40th Ann. Meeting of the Soc. for Neurosci., San Diego, USA - Árpád Dobolyi, Gábor Lovas
13th Conference of the Hungarian Neurosci. Society, Budapest – Tamás Varga, Gabriella Pál

Scientific report:

Transforming growth factor betas (TGF-βs) are multifunctional cytokines that affect cell proliferation, differentiation, and extracellular matrix formation in a variety of tissues (Burt and Law 1994). There are three separate genes encoding TGF-β1, -β2, and -β3, respectively (Lawrence 1996; Roberts 1998). In the framework of the project, first RT-PCR experiments were performed to demonstrate the presence of TGF-βs in the brain. The resulting cDNAs were also used to produce specific in situ hybridization probes for the different TGF-β subtypes (Dobolyi 2009b).

To understand the diverse functions of TGF-βs in the brain, we described the topographical distribution of mRNAs of the 3 subtypes of TGF-βs by in situ hybridization histochemistry in the rat (Dobolyi et al. 2009; Vincze et al. 2010b). TGF-β1, 2, and 3 all demonstrated relatively widespread mRNA expression in various brain areas. Nevertheless, the presence of their mRNAs was restricted to specific brain regions suggesting spatially regulated expression in the brain. In some brain areas, including brainstem motoneuron pools and the area postrema, the 3 subtypes had similar expression patterns. In most brain areas, however, their distributions were markedly different. In the cerebral cortex, TGF-βs were expressed in different layers. In the hippocampus, TGF-β1 was expressed in every region, TGF-β2 was abundantly expressed only in the dentate gyrus while TGF-β3 in the CA2 region and the dentate gyrus. In the cerebellum, TGF-β1 was expressed in all layers, TGF-β2 was abundantly expressed only in the Purkinje cell layer while TGF-β3 mRNA was not present in the cerebellum. In addition, many brain regions expressed only a single type of TGF-β. For example, the medial preoptic area, the paraventricular hypothalamic and central amygdaloid nuclei contained only TGF-β1 mRNA. The medial mamillary nucleus, the parafascicular thalamic nucleus and the choroid plexus expressed only TGF-β2 while the reticular thalamic nucleus, the superior colliculus, and the inferior olive contain exclusively TGF-β3 mRNA. These results demonstrated that TGF-βs are expressed in the rat brain and that the topographical distributions of TGF-βs are characteristic of the particular type of TGF-β suggesting that they play a role in specific brain functions. The paper reporting these data has been published (Vincze et al. 2010b). Therefore, we only present the distribution of TGF-β2 in some brain regions as an example (Fig. 1).

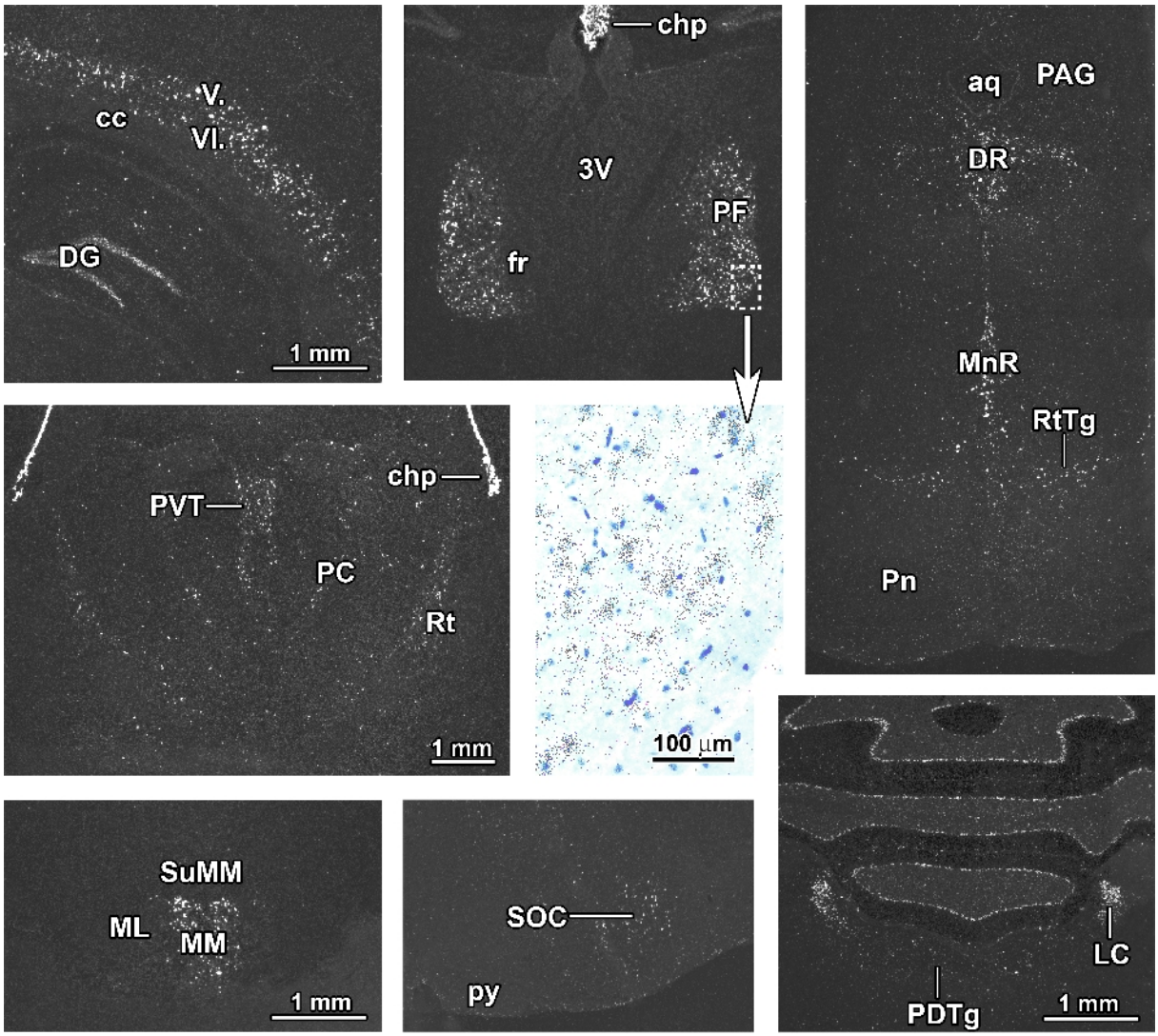

Fig. 1. Dark-field photomicrographs of in situ hybridization sections demonstrate the expression of mRNA of TGF-β2 in A: cerebral cortex and hippocampus, B: the parafascicular thalamic nucleus and the choroid plexus, C: midbrain raphe nuclei, D: midline thalamic nuclei and the choroid plexus, F: the medial subdivision of the medial mamillary nucleus, G: superior olive, H: locus coeruleus, pontine tegmental nuclei and the cerebellum. White dots represent the presence of TGF-β2 mRNA. The framed area in panel B is magnified in E in bright-field to show individual autoradiography grains above cell bodies. Abbreviations: aq - cerebral aqueduct, cc – corpus callosum, chp – choroid plexus, DG – dentate gyrus, DR – dorsal raphe nucleus, fr – fasciculus retroflexus, LC – locus coeruleus, ML – lateral subdivision of the medial mamillary nucleus, MM – medial subdivision of the medial mamillary nucleus, MnR – median rephe nucleus, PAG – periaqueductal gray, PC – paracentral thalamic nucleus, PDTg – posterodorsal tegmental nucleus, PF – parafascicular thalamic nucleus, Pn – pontine nuclei, PVT – paraventricular thalamic nucleus, py – pyramidal tract, Rt – reticular thalamic nucleus, RtTg – reticulotegmental nucleus, SuMM – supramamillary nucleus, SOC –superior olivary complex, V. – fifth layer of the cerebral cortex, VI. – sixth layer of the cerebral cortex, 3V – third ventricle.

Spatially restricted, distinct localizations of the individual subtypes of TGF-βs suggest their separate, specific functions. To further establish potential functions of TGF-βs, we made

attempts to identify the neuronal types that express of TGF-βs. TGF-β1 and TGF-β binding protein 1 are abundantly expressed in the preoptic area of the hypothalamus, which is a central element of the brain circuitry regulating maternal behaviors (Gray and Brooks 1984; Numan 1986; Lonstein et al. 1998; Stack and Numan 2000; Simerly 2002; Numan 2006). In the framework of the project, we published that the neurochemical characteristics of preoptic neurons change in mother rats as demonstrated by the dramatic induction of some neuropeptides including amylin and tuberoinfundibular peptide of 39 residues in the postpartum period (Dobolyi 2009a; Cservenak et al. 2010; Dobolyi et al. 2010b). The distribution of TGF-β1 is the same as that of amylin-expressing cell bodies (Fig. 2) and fiber terminals containing tuberoinfundibular peptide of 39 residues in the preoptic area (Dobolyi 2009a; Cservenak et al. 2010; Dobolyi et al. 2010b). We provided preliminary evidence that the expression level of TGF-β induced protein is elevated in the preoptic area of rat dams. Given the expected role of TGF-βs in the regulation of neuronal plasticity in endocrine regulations we suggest that TGF-β1 may play a role in maternal alterations in the preoptic area (Dobolyi et al. 2010a).

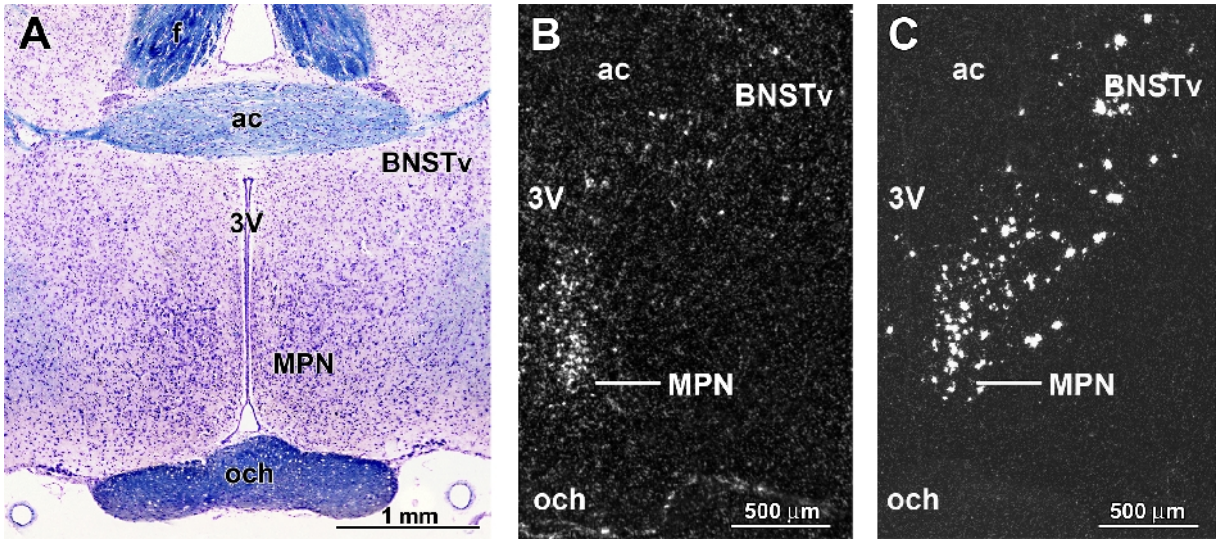

Fig. 2. Demonstration of the similarity between the distributions of TGF-β1 and maternally induced amylin in the preoptic area of the hypothalamus. A. A coronal section of the preoptic area is shown by Luxol-cresyl-violet staining. B: A dark-field photomicrograph of the preoptic area of a section hybridized with TGF-β1. C: A dark-field photomicrograph of the preoptic area of a section hybridized with amylin. Abbreviations: ac – anterior commissure, BNSTv – ventral subdivision of the bed nucleus of the stria terminalis, f – fornix, MPN – medial preoptic nucleus, och – optic chiasm, 3V – third ventricle.

To further identify brain regions and cell types that express TGF-βs, neuronal markers were identified in different hypothalamic, thalamic, hippocampal, and cortical brain regions. Since we had preliminary data using double in situ hybridization that TGF-β binding protein 2 mRNA is expressed in the perifornical / lateral hypothalamic area (Dobolyi and Palkovits 2008), we developed an antibody against this binding protein and performed double immunohistochemistry using this antibody and markers of neurons located in this hypothalamic area. We showed that TGF-β binding protein 2 immunoreactivity is present in orexin neurons but not in melanin-concentrating hormone-containing neurons in the hypothalamus (Fig. 3).

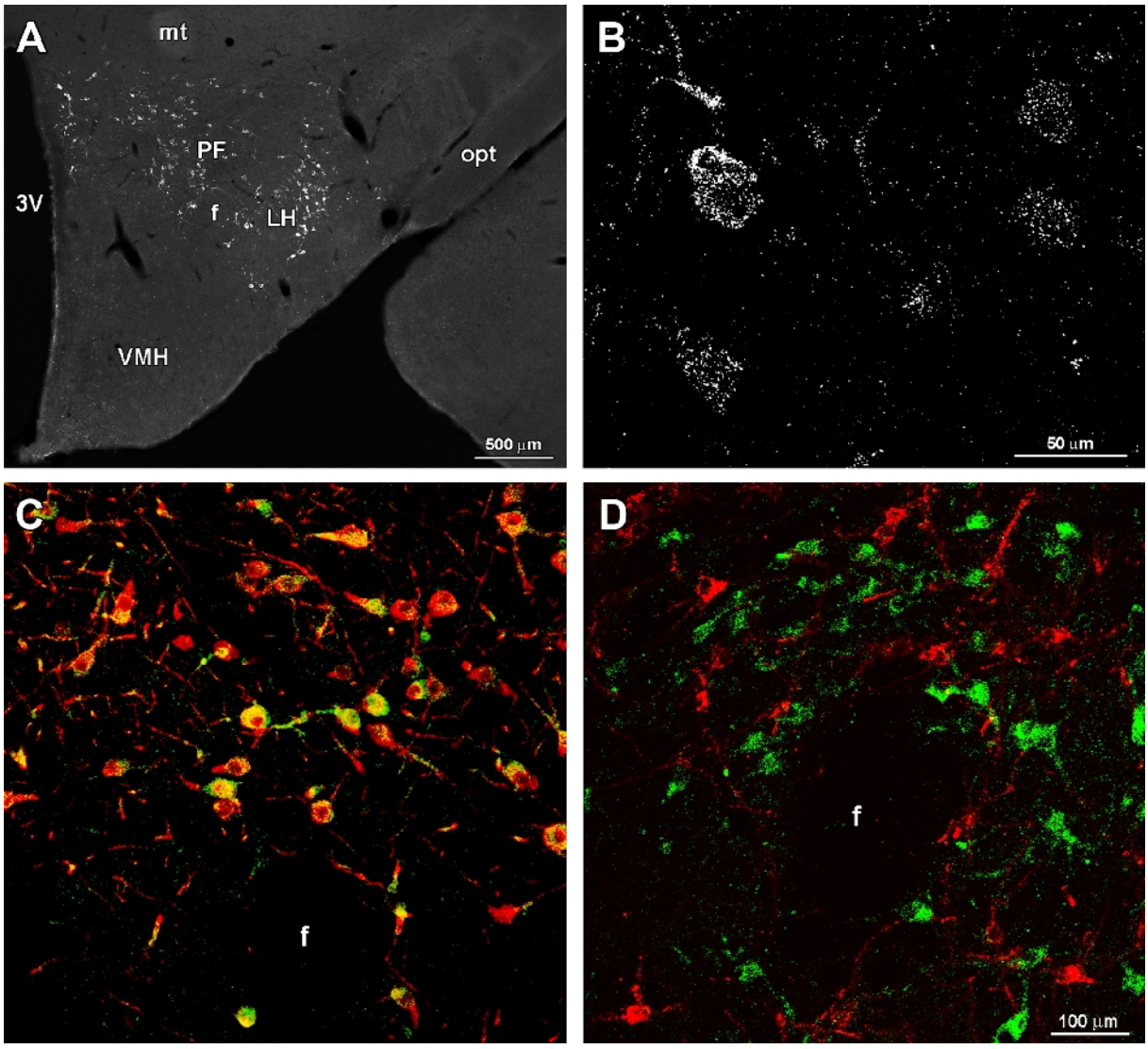

Fig. 3. TGF-β binding protein 2-immunoreactive neurons in the perifornical / dorsolateral hypothalamic area of a colchicines treated rat brain. A: The distribution of TGF-β binding protein 2 immunolabeling in the hypothalamus. B: A high magnification confocal photomicrograph indicates the accumulation of TGF-β binding protein 2 immunoreactivity into granules within cell bodies. C: TGF-β binding protein 2 immunoreactivity is co-localized with orexin A-immunoreactivity in neurons of the perifornical / dorsolateral hypothalamic area. The number of single labeled cells is very low compared to the number of double labeled ones. D: In contrast, melanin-concentrating hormone (MCH) immunoreactivity does not co-localize with TGF-β binding protein 2 immunoreactivity.

Among the potential neuronal functions of TGF-βs, their involvement in neuroprotection is the best established (Gross et al. 1993; Prehn et al. 1993; Ruocco et al. 1999; Zhu et al. 2002). TGF-β1 administered into the brain reduces the infarct size in experimental models of ischemia. To make a step forward towards understanding the role of endogenous TGF-β in neuroprotection, we induced focal brain ischemia with unilateral occlusion of the middle cerebral artery to establish expressional changes of the different subtypes of TGFbetas following an ischemic attack. Focal ischemia affected the expression patterns of TGF-βs. Within the infarct area, the TGF-β mRNA levels declined. In contrast, the expression of all 3

types of TGF-βs was induced outside the infarct area. There were, however, significant differences between the TGF-β subtypes in the spatial pattern of increased expression (Fig. 4). TGF-β1 showed elevated expression level throughout the penumbra. In contrast, mRNA levels of TGF-β2 and 3 were increased only in layers II., III., and V. of the cerebral cortex ipsilateral to the ischemic lesion. In these layers, however, the induced expressions were present even as far from the infarct area as the midline. In contrast, the contralateral hemisphere showed no labeling for TGF-β2 and 3 in layers II., and III. These results suggest that TGF-βs are involved in the response of the brain tissue to ischemic insult but the individual subtypes have different roles in the process (Vincze et al. 2010a).

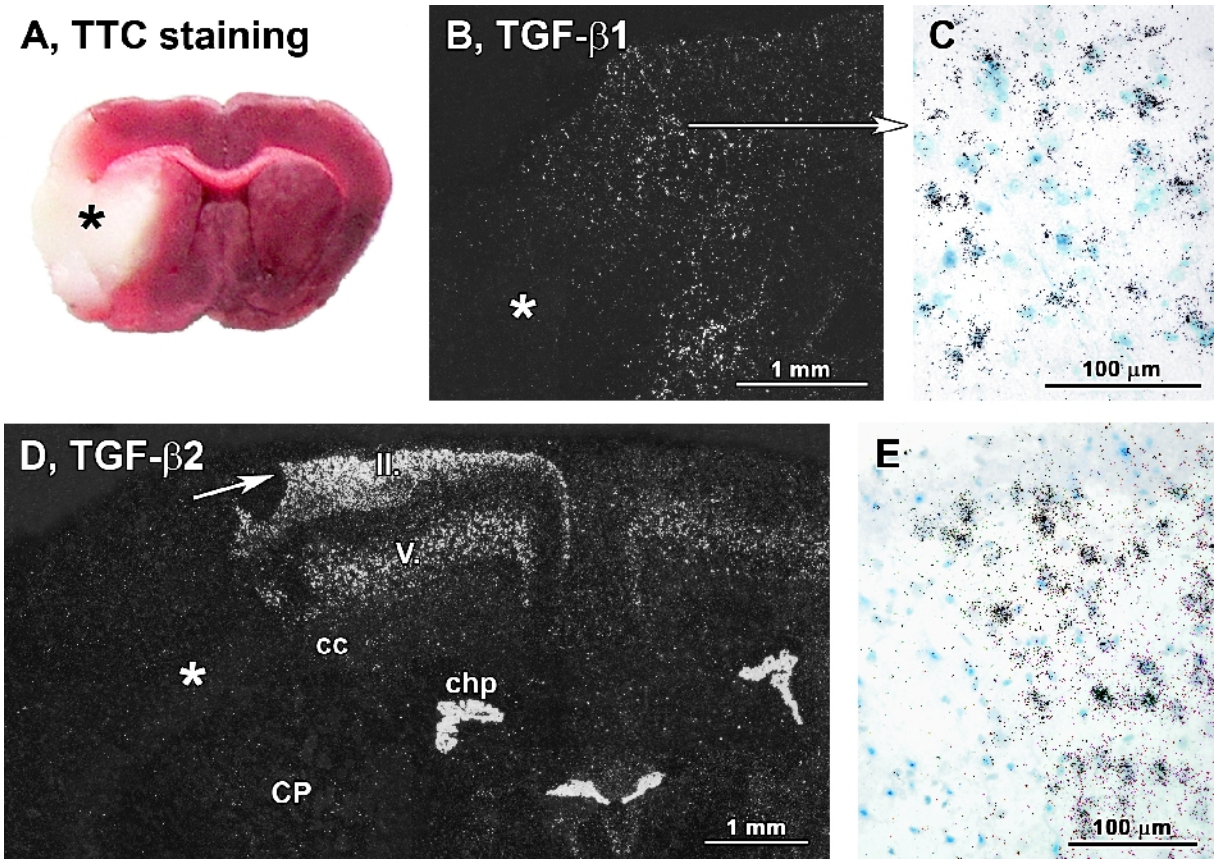

Fig. 4. Dark-field photomicrographs of in situ hybridization sections demonstrate the induction of mRNA of TGF-βs by middle cerebral artery occlusion (MCAO). A: TTC staining demonstrates the effect of ischemia on brain tissue. The infarct area is indicated by star symbol (*). As indicated in other panels, the infarct area does not contain mRNA of any of the TGF-βs. B: TGF-β1 expression is induced in the penumbra of the ischemic area. C, A bright-field photomicrograph of the brain area indicated by the white arrow in panel B demonstrates individual autoradiography grains above cell bodies expressing TGF-β1. D: TGF-β2 expression is induced in layers II., III., and V. of the ipsilateral cerebral cortex. The induction is particularly salient in layers II. and III. where no significant labeling is present in the contralateral hemisphere. Also see that different sublayers of layer V. contain TGF-β2. E: A bright-field photomicrograph of the brain areas indicated by the arrow in panel D shows individual autoradiography grains above cell bodies expressing TGF-β2.

Continuing this line of research, we also investigated the time course of the induction for each individual TGF-β subtype. The mRNA levels of TGF-βs was only slightly changed 3h after MCAO. TGF-β1, which we showed is expressed in the penumbral region of the infarct area

24 hours after the lesion, was present within the lesioned tissue 6 hours after the occlusion. In contrast, TGF-β2 and 3, which appear in specific layers of the cerebral cortex throughout the hemisphere ipsilateral to the lesion, are already present in these sites 6 hours after the lesion albeit with a lower level of expression than 24 hours after the lesion. The expression patterns 72h after the lesion were similar to that 24h after MCAO. These data were submitted as an abstract to the 15th Congress of the European Federation of Neurological Societies (Pál et al. 2011b), and will form part of a research article on the induction of TGF-βs following MCAO.

To characterize the mechanisms how the induction of the different types of TGF-βs occurs, immediate early gene expression was investigated following MCAO (Pál et al. 2011a). Interestingly, Fos expression appears in layers 2, 3 and 5 of the cerebral cortex exclusively in the hemisphere ipsilateral to the lesion (Fig. 4A). Since this distribution pattern is similar to the distribution of TGF-β2 and 3 induced by MCAO, we performed double labeling in a way that Fos immunohistochemistry was combined with radioactive in situ hybridization for each TGF-β subtype. A co-localization was found between Fos and TGF-β2 and 3 while we observed no TGF-β1 cells expressing Fos (Fig. 4). These data suggest that the immediate early gene Fos contributes to the induction of TGF-β2 and 3 (Pál et al. 2011a). To find out if regions of the cerebral cortex remote from the infarct area express Fos and TGF-β2 and 3 by means of neuronal connections, we performed transaction of the cortex medial to the expected infarct area and will establish if the induction of Fos and TGF-β2 and 3 occurs medial to the transactions, that is, if the stimulus for induction derived from the lesion can cross the transection.

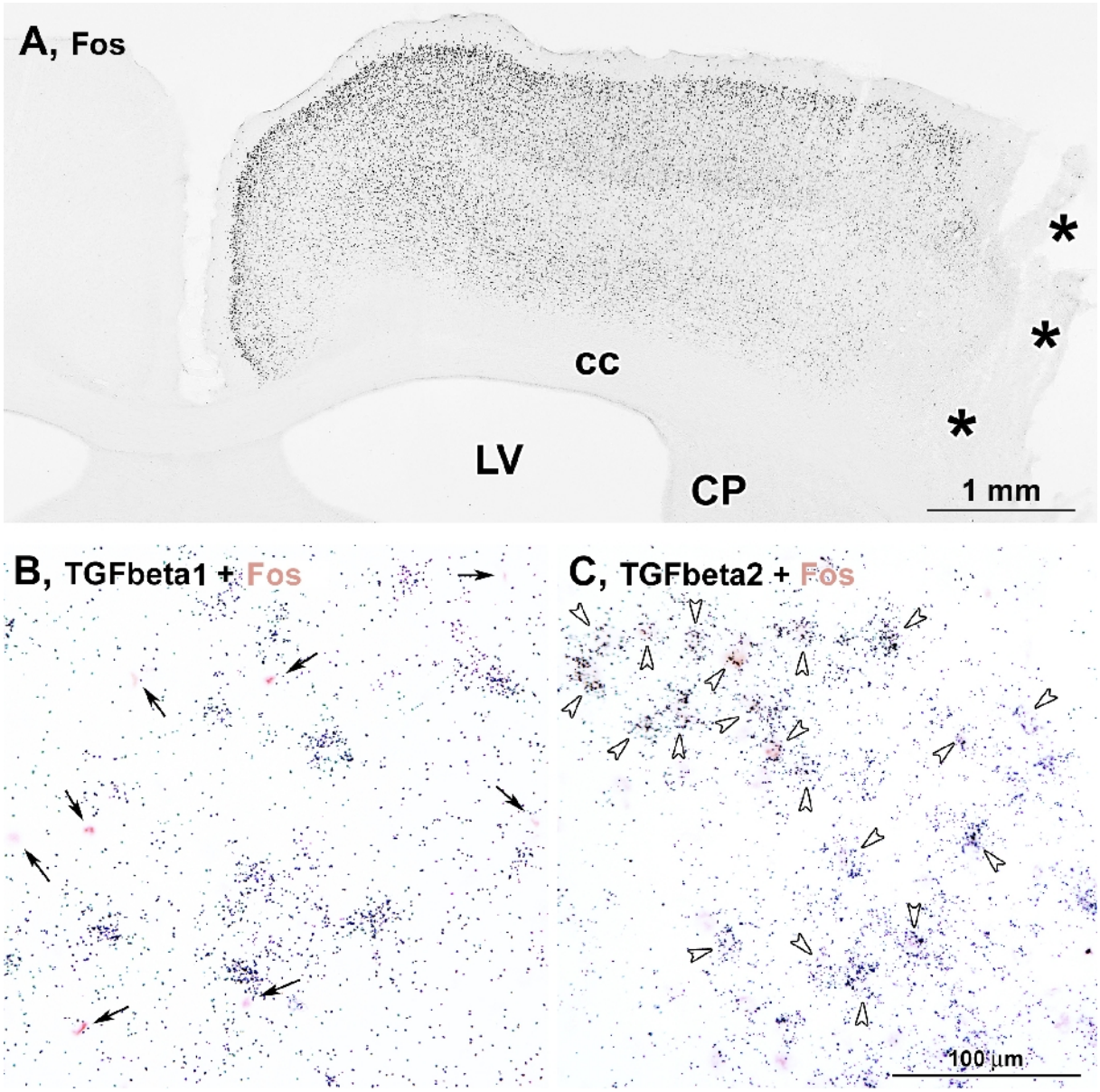

Fig. 4. Fos expression following MCAO. A: Fos-immunoreactive cell bodies are apparent ipsilateral but not contralateral to the lesion in the cerebral cortex. In addition, the density of Fos-ir neurons depends on the particular cortical layer: it is the highest in layer II. The lesion is indicated by *. The lesioned and consequently unfixed tissue is damaged and broken. B: TGF-β1 mRNA (black grains) does not co-localize with Fos-ir neurons. Black arrows indicate single labeled Fos-ir cell bodies. C: TGF-β2 mRNA (black grains) co-localizes with Fos-ir neurons. Double labeled neurons are indicated by arrowheads. Abbreviations: cc - corpus callosum, CP -caudate putamen, LV - lateral ventricle.

We also described the MCAO-induced expression pattern of another immediate early gene, the activating transcription factor 3 (ATF3). This protein is a member of the ATF/cyclic AMP response element-binding family of transcription factors, and is known to play a role in the regulation of cell cycle and apoptosis. Intriguingly, ATF3 is induced in the lesion site within 2 hours of MCAO and is present in the penumbral region 24 hours after the lesion (Fig. 5A). We performed double labeling in a way that ATF3 immunohistochemistry was combined with radioactive in situ hybridization for each TGF-β subtype (Pál et al. 2011a). TGF-β1 was found

to be co-localized with ATF3 (Fig. 5B) whereas TGF-β2 and 3 mRNA is not present in cells containing ATF3 (Pál et al. 2011a).

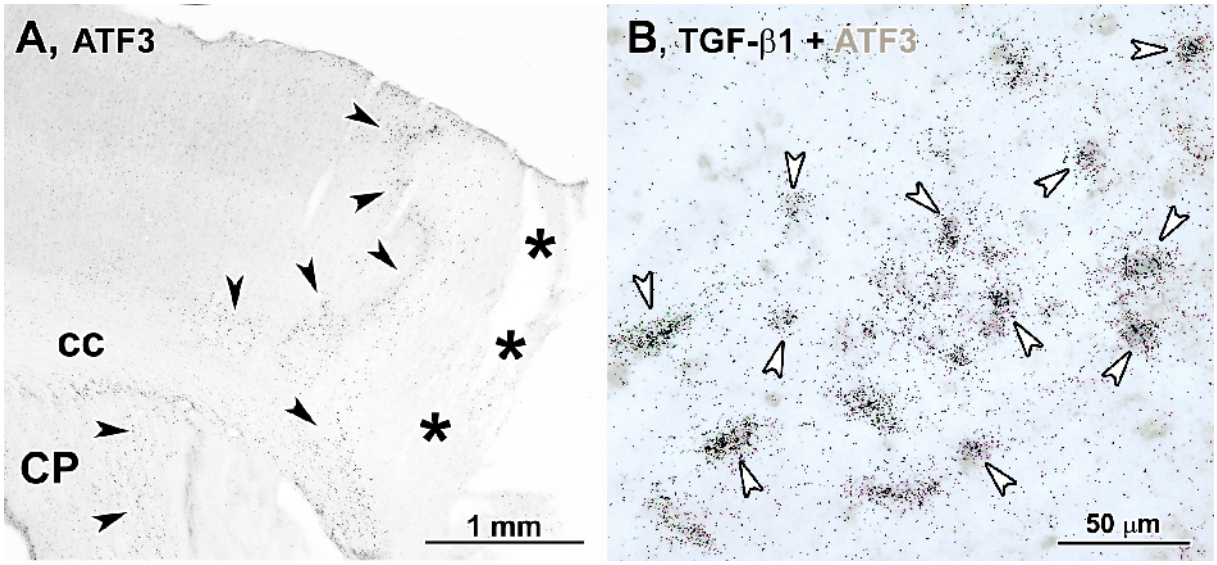

Fig. 5. ATF3 expression following MCAO. A: ATF3-immunoreactive cell bodies are abundant around the lesion site in the presumed prenumbra as indicated by arrowheads. B: TGF-β1 mRNA (black grains) co-localizes with ATF3-ir neurons (brown). Double labeled neurons are indicated by arrowheads. Abbreviations: cc - corpus callosum, CP - caudate putamen.

We also showed that neurons expressing immediate early genes (Fos and ATF3) did not contain Fluoro Jade C, a marker of neurodegeneration (Fig. 6). These observations suggest that endogenous TGF-β1 and TGF-β2 and 3 all participate in neuroprotection, although they are induced by different mechanisms following an ischemic attack. Some of these results have been reported in conference abstracts (Pál et al. 2011a). In addition, a manuscript is under preparation entitled: ´The mechanisms of the inductions of different TGF-βs following middle cerebral artery occlusion in rats´.

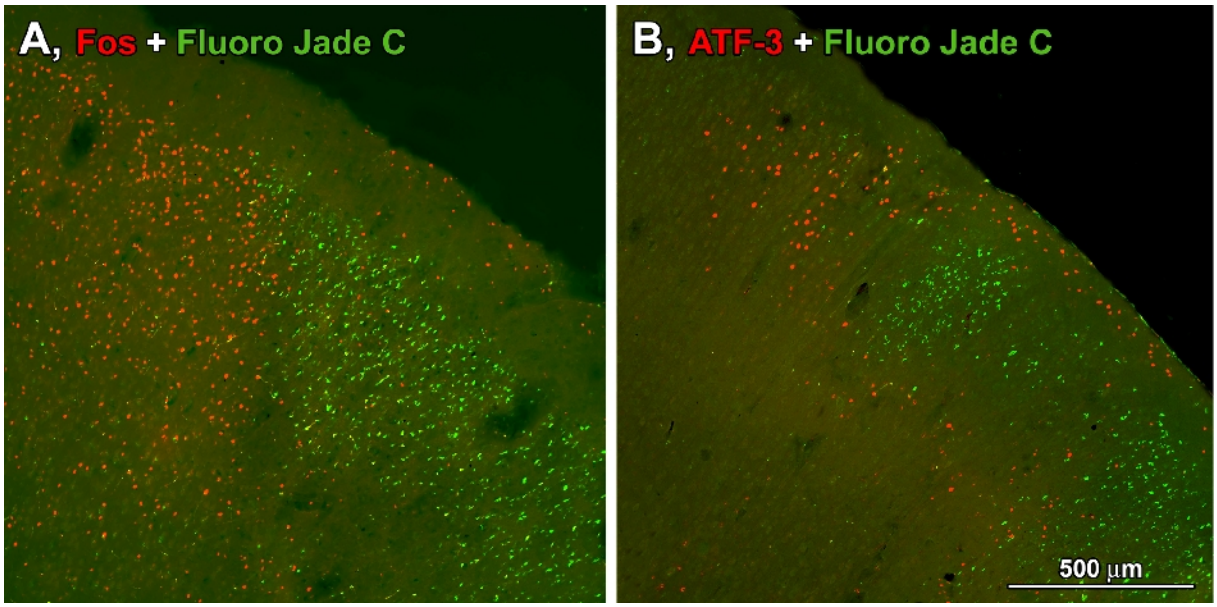

Fig. 6. Immediate early genes are expressed in non-degenerating neurons following MCAO. A: Fos expression following MCAO is not present in neurons labeled with Fluoro Jade C, a marker of degenerating neurons. B: ATF-3 immunoreactivity is not present in neurons labeled with Fluoro Jade C following MCAO.

The glial vs. neuronal character of cells expressing the particular TGF-β subtypes following MCAO has been examined. The co-expression of TGF-β with glial and neuronal markers was investigated by double in situ hybridization histochemistry experiments combining radioactive and non-radioactive in situ hybridization. These experiments were not successful. The lower sensitivity of the non-radioactive labeling as did not allow us to visualize the required markers. Therefore, we chose another approach and identified the types of cells expressing TGF-β using a combination of in situ hybridization with immunocytochemical methods (Pál et al. 2011b). NeuN, a neuronal marker, and glial fibrillary acidic protein, a glial marker, were labeled with immunocytochemistry combined with in situ hybridization histochemistry for TGF-βs (Pál et al. 2011b). We found that all types of TGF-βs were induced predominantly in neurons and not glial cells following MCAO (Fig. 7).

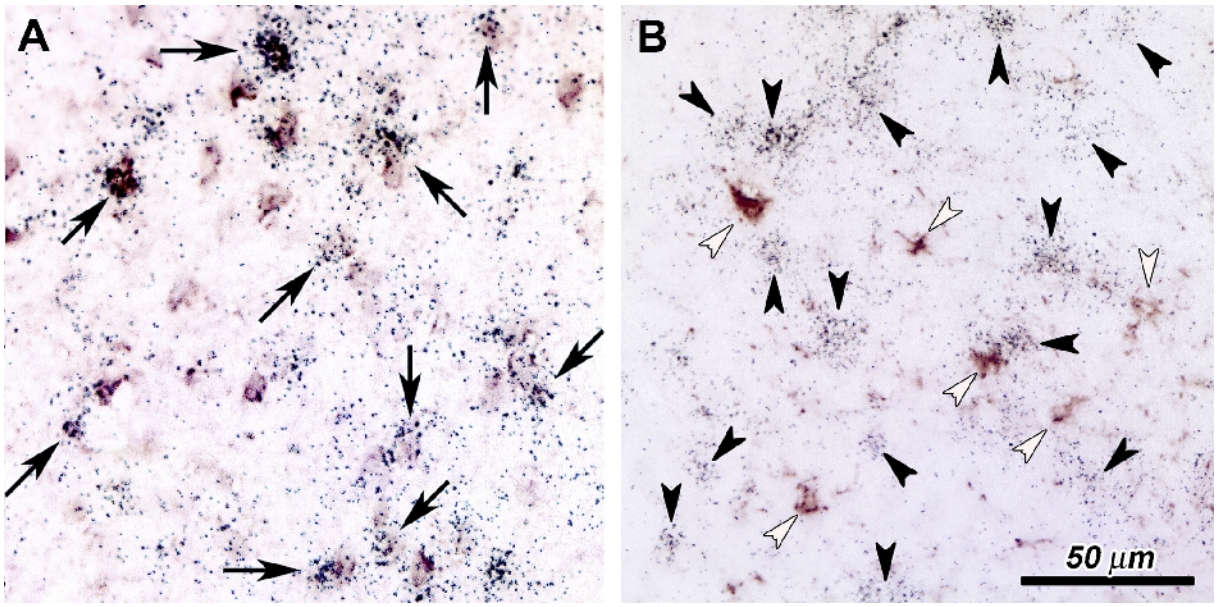

Fig. 7. MCAO induces TGF-β1 in neurons and not in glial cells. A: Black dots representing TGF- β1 mRNA are distributed above cells labeled with NeuN immunocytochemistry (brown cell bodies). Black arrows show double-labeled neurons. B: Black dots representing TGF- β1 mRNA are not distributed above cells labeled with GFAP immunocytochemistry (brown cell bodies). Black arrowheads show cells expressing TGF-β1 mRNA while white arrowheads point to glial cells immunolabeled with GFAP.

In addition to our studies in the rat, we developed in situ hybridization probes specific for mouse TGF-βs as well as TGF-β binding proteins. Two, non-overlapping probes were produced for each gene. These probes were used in additional in situ hybridization experiments in normal and transgenic mice. Probes against the same mRNA indeed provided identical expression patterns demonstrating their specificity. Analysis of the expression patterns in mice are under progress. In order to describe the distribution of mRNA of TGF-β subtypes during ontogeny we obtained mouse brains at different embryonic and postnatal ages. In situ hybridization was performed and the altering level of expression of TGF-βs in particular brain regions during ontogeny described.

Our subsequent goal was to examine the effect of the absence of TGF-β binding proteins on the TGF-β system. Transgenic approaches provided only a limited amount of information on the neural functions of TGF-βs because TGF-β knock-out mice die during ontogenic development for abnormalities and dysfunctions of peripheral organs (Kulkarni et al. 1993; Kaartinen et al. 1995; Sanford et al. 1997). An indirect approach to TGF-β function is knocking out TGF-β binding proteins. While TGF-β binding protein 2 knock-out mice die in early gestation (Shipley et al. 2000), mice lacking TGF-β binding protein 1, 3, and 4 hypomorphic mice containing only 2% of the normal level of TGF-β binding protein 4 mRNA survive until adulthood (Dabovic et al. 2002; Sterner-Kock et al. 2002; Drews et al. 2008). Although these transgenic mice possess various degrees of abnormalities in their peripheral organs, they are useful to study the neural functions of TGF-βs. The recently developed TGF-β binding protein 1 knock-out mice was the best candidate for our studies because these mice are viable and fertile, and demonstrate only mild peripheral abnormalities (Drews et al. 2008). In the framework of the project, we obtained transgenic mice from our our German collaborator, Prof. Dr. Ralf Weiskirchen, Aachen, Germany. The expression patterns of TGF-βs and their binding proteins were investigated in these mice. The mRNA expression patterns

of TGF-βs and TGF-β binding proteins in TGF-β binding protein 1 knock-out mice were compared to that in wild-type littermates. We expected disturbances in the mRNA distribution of TGF-βs and TGF-β binding proteins in the mouse line lacking TGF-β binding protein 1. However, no major difference in the expression pattern of the investigated genes was observed except for TGF-β binding protein 1, which was of course not present in the knock-out mice (Fig. 8). These data suggest that TGF-β binding protein 1 expression cannot be replaced by the expression of another subtype of TGF-β binding protein.

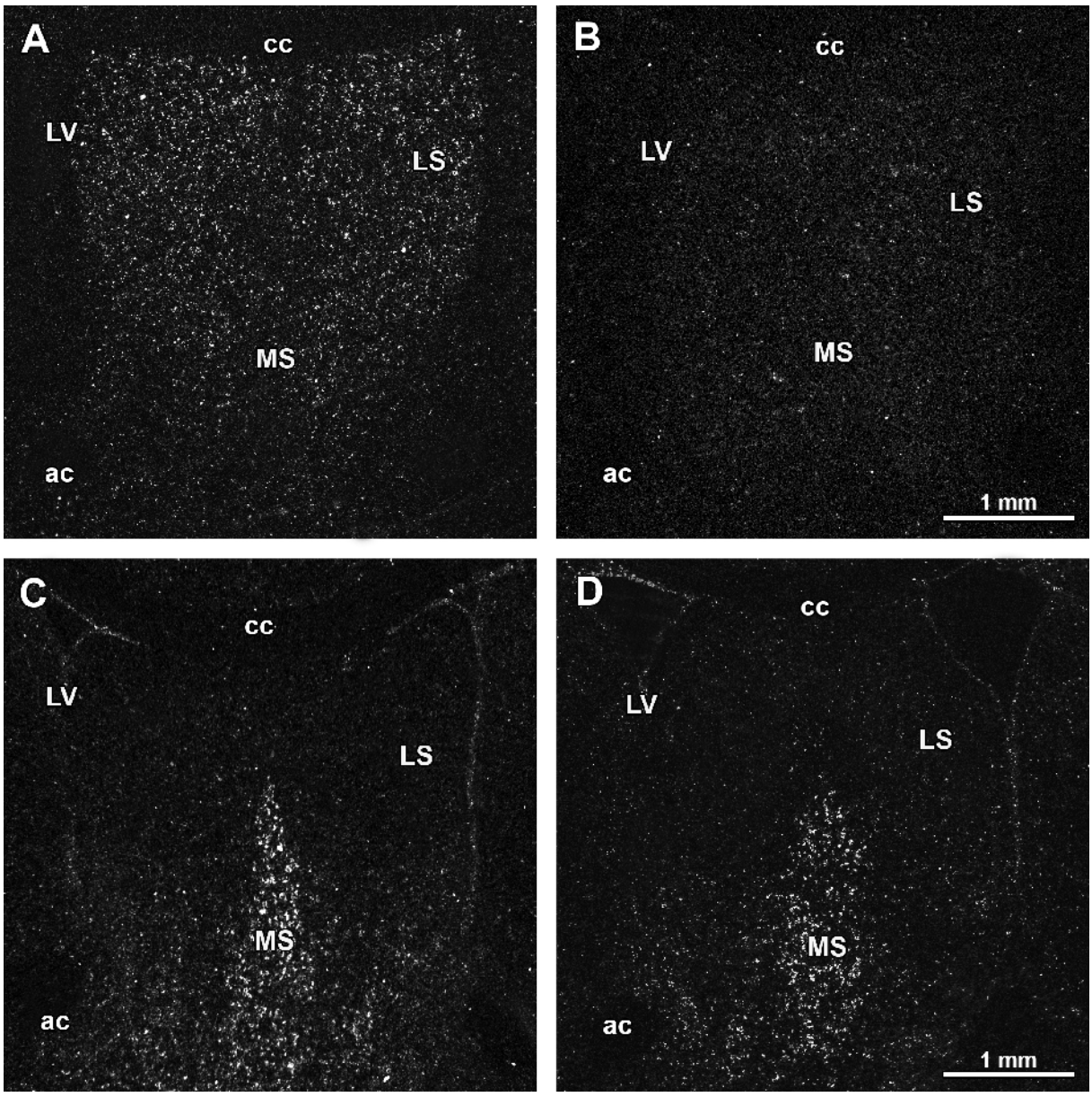

Fig. 8. TGF-β binding protein 3 expression in the septum is not perturbed in mice lacking TGF-β binding protein 1. A: TGF-β binding protein 1 expression in the septum in a wild-type mouse. The distribution is abundant in the lateral septal nucleus (LS). B: The in situ hybridization signal disappears in mice lacking TGF-β binding protein 1 as expected. C: TGF-β binding protein 3 expression in the septum in a wild-type mouse. The distribution is abundant in the medial septal nucleus (MS). D: TGF-β binding protein 3 expression in the septum of a mouse lacking TGF-β binding protein 1. TGF-β binding protein 3 does not appear

in the lateral septal nucleus that misses the expression of TGF-β binding protein 1. Additional abbreviations: ac – anterior commissure, cc – corpus callosum, LV – lateral ventricle.

Finally, we started to develop in situ hybridization probes specific to human TGF-βs in order to describe the presence and distribution of TGF-βs in the human brain. Human brain tissue samples have also been obtained for these experiments from the Human Brain Tissue Bank of the Neuromorphological and Neuroendocrine Research Laboratory of the Semmelweis University and the Hungarian Academy of Sciences. We also started to prepare for the investigation of the effect of cerebral infarcts on TGF-β in human. To characterize different human cerebral infarcts, a multiparametric non-linear statistical analysis of cardiovascular and respiratory alterations during extensive cerebral infarcts requiring neurointensive care was performed and reported in abstract form (Lovas et al. 2010).

Scientific originality and significance of the results

The critically important role that growth factors play in the development and function of the central nervous system has been studied by neuroscientists for decades. A great deal of information accumulated on the neural functions of neurotrophins including nerve growth factor, brain-derived neurotrophic factor, and other neurotraphic factors. Based on our results, which demonstrated the localized expression of TGF-βs and their binding proteins in specific types of neuronal cells, we concluded the innovative idea that TGF-βs also play an important role in the function of the central nervous system. Recent evidence provides initial information on the possible neural functions of TGF-βs. They are neuroprotective in hippocampal pyramidal cells (Dhandapani and Brann 2003), are potent survival factors for midbrain dopaminergic neurons (Markus 2007; Zhang et al. 2007), regulate neuronal differentiation (Garcia-Campmany and Marti 2007), increase neurogenesis in the dentate gyrus (Battista et al. 2006), modulate plasticity in hippocampal neurons (Fukushima et al. 2007), may participate in brain tumor formation (Aigner and Bogdahn 2008), and may directly influence autonomic (Fujikawa et al. 2007; Matsumura et al. 2007) and neuroendocrine functions (Bouret et al. 2004; Fevre-Montange et al. 2004). Further functional experiments were hindered by the lack of knowledge on the distribution of individual subtypes of TGF-βs and their localization in particular cell types.

In the adult rat brain, we first described the presence and distribution of TGF-βs using in situ hybridization histochemistry (Vincze et al. 2010b). The distribution pattern of the mRNA of TGF-βs resembles that of TGF-β immunoreactivities in most brain regions (Unsicker et al. 1991). However, TGF-β1 immunoreactivity was reported to be present constitutively only in meninges and the choroid plexus in the brain (Unsicker et al. 1991; Komuta et al. 2009) but we found a more widespread expression of the mRNA of this subtype. Furthermore, TGF-β2 and -3 immunoreactivities entirely overlapped and, in general, were found in large multipolar neurons (Unsicker et al. 1991), the level of TGF-β2 being considerably higher (Bottner et al. 2000). In contrast, we found significantly different expression patterns of TGF-β2 and -3 mRNAs. Insofar as the immunoreactivities were found in cell bodies rather than fibers, it is not likely that the differences stem from the transport of TGF-βs from the cell bodies. In turn, the better sensitivity and selectivity of in situ hybridization histochemistry could be the underlying reason of the reported differences.

Our results provided the anatomical basis of the already established actions of TGF-βs in the central nervous system thereby allowing the design of more specific experiments on the functions of individual TGF-β subtypes. In addition, our results on the distribution of TGF-βs

suggest the involvement of this system in functions previously not explored. Consequently, novel actions of TGF-βs were suggested based on the localization of TGF-βs in neuronal cell types previously not involved with this growth factor system. In particular, our results suggest that the TGF-β system may be involved in the maternal regulations governed by the preoptic area because TGF-β1 and TGF-β binding protein 1 have distributions similar to the neurons activated in the preoptic area in response to pup retrieval (Dobolyi and Palkovits 2008; Vincze et al. 2010b). Therefore, in the future, we will further investigate the role of the TGF-β system in the control of maternal adaptations.

Among the potential neuronal functions of TGF-βs, their involvement in neuroprotection is the best established (Flanders et al. 1998; Bottner et al. 2000; Dhandapani and Brann 2003). TGF-β1 administered into the brain reduces the infarct size in experimental models of ischemia (Gross et al. 1993; Prehn et al. 1993; Zhu et al. 2002) while antagonizing the endogenous action of TGF-β1 with the injection of a soluble TGF-β type II receptor, which binds TGF-β1 and prevents its biological actions, resulted in a dramatic increase in infarct area (Ruocco et al. 1999). In the framework of the current application, we established the induction of endogenous TGF-βs following focal brain ischemia. In addition, a subtype-specific expression pattern of TGF-βs was found suggesting the involvement of different TGF-βs in different aspects of neuroprotection. Establishing such roles of endogenous TGF-βs in neuroprotective mechanisms is particularly important because the brain is vulnerable to ischemic insults as most of the neurons cannot be replaced. Ischemic stroke alone is the fourth leading cause of death resulting in approximately 480,000 deaths each year in Europe. In addition, ischemic stroke is a leading cause of serious, long-term disability. Options for prevention and treatment are very limited, necessitating new approaches. Our results describing the morphological and neurochemical changes of the TGF-β system in animal models will provide the formation of exploring the TGF-βs system as drug targets creating a close link between basic scientific knowledge and potential medical applications to facilitate the development of drugs acting on the TGF-β system and the investigation of their effectiveness in alleviation of the consequences of stroke.

Collectively, the new knowledge gained in the framework of the proposal is expected to have a major positive impact on understanding the TGF-β system in the central nervous system and its utilization for therapeutic purposes.

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