



Nerve growth factor is expressed and stored in central neurons of adult zebrafish

Pietro Cacialli, Claudia Gatta, Livia d'Angelo, Adele Leggieri, Antonio Palladino, Paolo de Girolamo, Elisabeth Pellegrini, Carla Lucini

► To cite this version:

Pietro Cacialli, Claudia Gatta, Livia d'Angelo, Adele Leggieri, Antonio Palladino, et al.. Nerve growth factor is expressed and stored in central neurons of adult zebrafish. *Journal of Anatomy*, 2019, 325 (1), pp.167-179. 10.1111/joa.12986 . hal-02091213

HAL Id: hal-02091213

<https://univ-rennes.hal.science/hal-02091213>

Submitted on 5 Jul 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

NERVE GROWTH FACTOR IS EXPRESSED AND STORED IN CENTRAL NEURONS OF
ADULT ZEBRAFISH

Pietro Cacialli^{1,4}, Claudia Gatta¹, Livia D'Angelo^{1,2}, Adele Leggieri¹, Antonio Palladino³, Paolo de
Girolamo¹, Elisabeth Pellegrini⁴, Carla Lucini¹

Dept. of Veterinary Medicine and Animal Productions, University of Naples Federico II, Naples,
Italy; 2. Stazione Zoologica Anton Dohrn, Napoli, Italy; 3. Centro Ricerche Interdipartimentali sui
Biomateriali, University of Naples Federico II, Naples, Italy; 4. Univ Rennes, Inserm, EHESP,
Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes,
France
1.

20 ABSTRACT

21

22 Nerve Growth Factor (NGF), a member of the neurotrophin family, was initially described as
23 neuronal survival and growth factor, but successively has emerged as an active mediator in many
24 essential functions in the central nervous system of mammals. NGF is synthesized as a precursor pro-
25 NGF and is cleaved intracellularly into mature NGF. However, recent evidence demonstrates proNGF
26 is not a simple inactive precursor, but is also secreted outside the cells and can exert multiple roles.
27 .Despite the vast literature present in mammals, studies devoted to NGF in the brain of other
28 vertebrate models are scarce. Zebrafish is a teleost fish widely known for developmental genetic
29 studies and is well established as model for translational neuroscience research. Genomic
30 organization of zebrafish and mouse NGF are highly similar, and zebrafish NGF protein has been
31 reported in a mature and two precursors forms. To add further knowledge on neurotrophic factors in
32 vertebrate brain models, we decided to determine the NGF mRNA and protein distribution in the
33 adult zebrafish brain and to characterize the phenotype of NGF positive cells.

34 NGF mRNA was visualized by *in situ* hybridization on whole mount brains. NGF protein distribution
35 was assessed on microtomic sections by using an antiserum against NGF, able to recognize proNGF
36 in adult zebrafish brain as demonstrated also in previous studies. To characterize NGF positive cells,
37 anti-NGF was employed on microtomic slides of aromatase B transgenic zebrafish (where radial
38 glial cells appeared fluorescent) and by means of double immunolabelling against NGF/PCNA
39 (proliferation marker) and NGF/MAP2 (neuronal marker).

40 NGF mRNA and protein were widely distributed in the brain of adult zebrafish and their pattern of
41 distribution of positive perikaryal was overlapping, both in males and females, with few slight
42 differences. Specifically, the immunoreactivity to the protein was observed in fibers over the entire
43 encephalon. MAP2 immunoreactivity was present in the majority of NGF positive cells, throughout
44 the zebrafish brain. PCNA and aromatase B cells were not positive to NGF, but they were closely

45 intermingled with NGF cells. In conclusion, our study demonstrated that mature neurons in the
46 zebrafish brain express NGF mRNA and store proNGF.

47

48 Key words: fish; neurotrophins; brain; encephalon; zebrafish neurons;

49

Accepted manuscript

50 INTRODUCTION

51

52 Nerve Growth Factor (NGF) is the first identified factor belonging, together with Brain Derived
53 Neurotrophic Factor (BDNF), Neurotrophin (NT) 3 and NT 4/5, the neurotrophin family. As all
54 members of the family, NGF is synthesized as a precursor form (pro-NGF) and either secreted outside
55 the cells, as pro- and mature NGF, or cleaved intracellularly to mature NGF. Active forms of pro-
56 and mature NGF are homodimers. To date, three types of NGF receptors are known: TrkA, p75 and
57 sortilin. TrkA, the high affinity receptor, is a member of receptor tyrosine kinases family which
58 includes receptor TrkB (for BDNF and NT 4/5) and TrkC (for NT 3). p75NTR is a member of the
59 tumor necrosis factor receptor (TNFR) superfamily and can transduce signals of all neurotrophins.
60 Sortilin is a member of the family of Vps10p-domain transmembrane receptors, and was earlier
61 characterized as a receptor for neurotensin. While TrkA mediates trophic signalling of the mature
62 form of NGF, p75NTR bifunctionally mediates: (a) a signal to neuronal survival, specially when
63 binding to mature NGF and acting together with TrkA; or (b) the induction of neuronal death when
64 forming a receptor complex with sortilin and mediating proNGF signal (for a review
65 see Niewiadomska et al., 2011).

66 NGF, initially described as neuronal survival and growth factor, encompasses roles regarding the
67 density of innervation, synthesis of neurotransmitters and neuropeptides and cell body size, axonal
68 sprouting and dendritic arborization (for a review see Minnone et al 2017).

69 The brain of fish possesses, beyond structural organization similar to all vertebrates, extremely high
70 adult neurogenesis and astonishing regenerative properties. These peculiarities make the fish brain
71 suitable for discovering regenerative mechanisms probably suppressed in mammals during evolutive
72 adaptation (Panula et al 2010; Cacialli et al 2018b). Among teleost fish, zebrafish (*Danio rerio*) is
73 widely used as model species for developmental genetic studies and functional mechanisms of
74 numerous genes responsible of human diseases (D'Angelo et al., 2016). In addition, zebrafish brain
75 has been largely utilized in numerous studies devoted to adult neurogenesis (Pellegrini et al 2007;

76 Diotel et al 2013; Coumailleau et al 2015; Than-Trong and Bally-Cuif 2015; Anand et al 2017) and
77 regenerative ability after injury (Cosacak et al 2015; Alunni and Bally-Cuif 2016; Cacialli et al 2018
78 a,b).

79 In fish species, homologs of mammalian neurotrophins have been identified, and an additional
80 member, named NT6/7, probably originated by a duplication of the ray-finned fish NGF (Dethleffsen
81 et al 2003). NT6/7 has been identified also in zebrafish (Nilsson et al., 1998). Genomic organization
82 of zebrafish and mouse NGF are highly similar, and zebrafish NGF protein has been reported in a
83 mature form of 194 aminoacid. (Dethleffsen et al 2003). In addition, two proNGF isoforms have been
84 described in zebrafish: isoform 1 (NP_001338647.1) and isoform 2 (NP_954680.2), respectively of
85 224 and 261 a.a.. (Dethleffsen et al 2003).

86 The distribution pattern of neurotrophins and their Trk receptors was described in the brain of adult
87 fish (Dalton et al 2009a,b, D'Angelo et al 2012, D'Angelo et al 2014a, D'Angelo et al 2014b, Cacialli
88 et al 2016, D'Angelo et al 2016, Gatta et al 2016). Furthermore, BDNF appeared involved in repairing
89 mechanisms of adult zebrafish after traumatic brain injury (Cacialli et al 2018 a,b; Lucini et al 2018).

90 In the present study, we evaluated NGF mRNA and protein distribution in the brain of zebrafish and
91 identified neurons as the NGF source cells. Thanks to the conserved adult neurogenesis in fish brain
92 and the potential involvement of neurotrophin in the regenerative ability, the identification of NGF
93 neurons in the adult brain of zebrafish could represent a useful tool to evaluate its involvement in the
94 regenerative process after injury or chemical/genetic induced degeneration.

95 MATERIALS AND METHODS

96 Animals and brain dissection

97 Animals used in this study were housed in zebrafish facilities (INRA LPGP, BIOSIT, Rennes, France,
98 agreement number: B 35-238-6) under standard conditions of photoperiod (14/10) and temperature
99 (28°C). This project was approved by the local animal care and Westerfield ethics committee (Comité
100 Rennais d'Ethique en matière d'Expérimentation Animale, Rennes, France), under the number EEA
101 B-35-040. Zebrafish did not receive medical treatment prior or during the experience. No deaths
102 occurred in the facilities before the euthanasia of animals used for *in situ* hybridization and
103 immunohistochemistry experiments. Fish were suppressed with an overdose of tricaine
104 methanesulfonate (MS-222).

106 *In situ* hybridization (ISH)

107 Oligonucleotide primers used to amplify and clone cDNA for the production of NGF ISH probes are:
108 forward 5'-ACATGTACCATGAGGAGCAC-3'; reverse 5'-GTCGCTGGTGTGTGGAAAAT-3'
109 (708 bp; NM_001351718.1). For preparation of NGF digoxigenin (DIG) labeled antisense riboprobe,
110 the vector pCRII-TOPO containing NGF was linearized by BamHI digestion and digoxigenin-labeled
111 riboprobe was prepared using *in vitro* transcription with T7 RNA polymerase. For sense riboprobe
112 the vector containing NGF was linearized by NotI and digoxigenin-labeled riboprobe was prepared
113 using *in vitro* transcription with SP6 RNA polymerase.

114 ISH was performed on whole mount brains as previously described by Adolf et al. (2006); Diotel et
115 al. (2015). Briefly, six zebrafish brains (males and females) were excised and fixed in
116 paraformaldehyde (PFA 4%) dissolved in phosphate-buffered saline (PBS), for 24 hours (h) at 4°C.
117 Then, the brains were dehydrated in a methanol/PBS concentration (25%; 50%; 75%; 100%) and
118 stored at -20°C. After rehydrating through methanol/PBS gradient series and washed in PBS, the
119 brains were incubated for 40 minutes (min) in PBS containing proteinase K (10 µg/ml) at room
120 temperature (RT). After post-fixation in 4% PFA for 20 min and washes in PBS, brains were then

121 prehybridized for 1 h and incubate overnight at 65°C in the hybridization buffer (pH 6) containing
122 the DIG-labeled probe. Then, brains were washed in SCC 2x/formamide 50% and SSC 0.2x, and pre-
123 incubated with blocking buffer for 3 h and then overnight with antidigoxigenin-AP, Fab fragments
124 (1:5,000; Roche, NJ; Cat# 11093274910, RRID: AB_514497) at 4°C. The next day the brain sections
125 were washed with PBS before staining with NBT/BCIP buffer (pH 9.5).

126 Whole mount stained brains were embedded in agar 2% and photographed with a Digital camera
127 equipped on Zeiss Stemi. Then, the embedded whole mount stained brains were transversally
128 sectioned with a razor blade or vibratome and the sections were mounted on the slide.

129 The specificity of the ISH labelling was demonstrated by using sense riboprobe, that showed absence
130 of any staining.

131

132 Immunohistochemistry (IHC)

133 Immunohistochemical procedures were performed following detailed suggestions reported by de
134 Girolamo and Lucini (2011). Six adult zebrafish brains (male and female) were fixed in Bouin's
135 solution for 24 h and processed for paraffin embedding. Transverse microtome sections were mounted
136 on poly-lysine slides. Sections were deparaffinised in xylene, rehydrated through graded ethanol,
137 treated with 3% H₂O₂ for 30 min and rinsed in PBS (pH 7.4) followed by antigen retrieval in sodium
138 citrate buffer (pH 6; 80 °C) for 30 min.

139 Single immunohistochemistry

140 After rinsing 2 times in 0.2% Triton PBS (PBT), non-specific binding was blocked by treating
141 sections with 1/5 normal goat serum (Vector, Burlingame, CA, USA, cod S-1000-20) for 30 min at
142 RT. Then, sections were incubated over night at RT in a humidified chamber with rabbit antibody
143 against NGF (Santa Cruz Biotechnology, CA, USA, cod sc-549. It recognizes N-terminus of the
144 mature chain of NGF of human origin) diluted 1/100 or 1/300, respectively depending on the
145 secondary antibody specified at following point a) or b). The next day, the sections were washed
146 several times in PBT and alternatively incubated with a) Alexa Fluor® goat anti-rabbit 488 (1:200;

147 Invitrogen Molecular probes, Eugene, OR, REF: A-11037; RRID: AB_10561549) for 2 h at RT in a
148 dark and humidified chamber or b) EnVision-horseradish anti-peroxidase (HRP)-system (Dako,
149 Santa Barbara, CA, cod. K4002). This system is based on a HRP labeled polymer conjugated with
150 goat anti-rabbit IgG.

151 Sections treated with Alexa Fluor® goat anti-rabbit 488, after three washes in PBT, were mounted
152 with the medium Vectashield (Vector) containing 4,6-diamino-2-phenylindole (DAPI), to visualize
153 cell nuclei. Sections treated with EnVision-HRP-system were immersed in a fresh solution of 10 µg
154 of 3,3'-diaminobenzidine tetrahydrochloride (Sigma–Aldrich Corporation, St. Louis, MO, USA, cod.
155 D5905) in 15 ml of a 0.5 mol, Tris buffer, pH 7.6, containing 1.5 ml of 0.03% H₂O₂. Then, sections
156 were dehydrated and mounted.

157 For immunohistochemistry on cyp19a1bGFP transgenic zebrafish (glial cell marker, Pellegrini et al.,
158 2007; Tong et al., 2008), NGF antibody was detected with Alexa Fluor® goat anti-rabbit 594 (1:200;
159 Invitrogen Molecular probes, Eugene, OR, USA, REF: A-11037; RRID: AB_10561549).

160 The specificity of IHC, assessed by substitution of NGF antiserum, secondary antibody fluorescent
161 dye -conjugated or the EnVision with PBS or normal serum, achieved no specific immunostaining.
162 Moreover, the incubation of NGF antiserum preincubated with its homologous antigen showed no
163 immunoreactivity, and NGF antiserum preincubated with its heterologous antigens did not modify
164 the normal pattern of immunostaining.

165

166 Double immunolabelling

167 Double immunocytochemical staining NGF/PCNA and NGF/MAP2 was performed as follows:
168 dewaxed and rehydrated consecutive sections were rinsed in PBS, and incubated for 48 h at RT.
169 PCNA (proliferative cell nuclear antigen), antibody at diluted 1:100, was used to detect proliferative
170 cells (Clone PC10; Dako, Glostrup, Denmark; REF: M0879; RRID: AB_2160651). This antibody is
171 a marker of proliferating cells in vertebrate species, including zebrafish (Pellegrini et al., 2007; Marz
172 et al., 2011; Cacialli et al., 2016; 2018). MAP2 (Microtubule-Associated Protein2) antibody, diluted

173 1:100, was used to detect neurons, (sc-74422 MAP-2 (A-8) Santa Cruz Biotechnology, Santa Cruz,
174 CA, USA). After rinsing in PBS, the sections were incubated for 2 h at RT with mixture of the
175 secondary antibodies directed against rabbit and mouse IgG. a) Alexa fluor® goat anti-mouse 594
176 (1:200; Invitrogen Molecular probes, Eugene, OR, REF: A-11005; RRID: AB_10561507; b) Alexa
177 Fluor® goat anti-rabbit 594 (1:200; Invitrogen Molecular probes, Eugene, OR, USA, REF: A-11037;
178 RRID: AB_10561549). Tissue sections were washed in PBS-Triton 0,2%, and slides were mounted
179 with the Vectashield medium containing DAPI for nuclei counterstaining (Vector Laboratories,
180 Burlingame, CA). Controls for double immunolabelling were performed by incubating the sections
181 with one of the two primary antisera and with the mismatched secondary antibodies.

182

183 Microscopy

184 The stained sections were photographed using a Nikon Eclipse 90i microscope, an epifluorescence
185 microscope Olympus equipped with a DP71 digital camera and Leica DM6B, SN: 449492.
186 The digital raw images were optimized for image resolution, contrast, evenness of illumination, and
187 background by using Adobe Photoshop CS5 (Adobe Systems, San Jose, CA, USA).

188

189

190 RESULTS

191

192 NGF mRNA (Fig 1A) and protein were widely distributed in the brain of adult zebrafish (Tab. 1). The
193 pattern of distribution of positive perikarya was overlapping, both in males and females, with few
194 slight differences (Table 1). Specifically, the immunoreactivity to the protein was observed in fibers
195 over the entire encephalon. Thus, regions characterized only by presence of fibers, such as the
196 glomerular layer of olfactory bulbs and deep and white zone of the optic tect, showed positivity to
197 the protein. Based on these general considerations, for sake of the simplicity, the term NGF in place
198 of NGF mRNA and NGF protein was used in the following description of results. The anatomical
199 terminology follows “Neuroanatomy of the zebrafish brain” by Wulliman et al. (1996).

200 *Telencephalon*

201 The olfactory bulbs showed moderate quantity of NGF (Fig 1C, D). The cells of both external and
202 internal cellular layer and fibers of the glomerular layer resulted positive.

203 In the whole telencephalon, more intense NGF positivity was seen in the ventral telencephalon (Fig
204 1C, D) and in the posterior zone dorsal telencephalic area (Fig 1B, C). Positive cells were distributed
205 in the medial (Fig 3B, B¹), dorsal (Fig 8B, D), lateral (Fig 2A¹, 3C, C¹, 10 B, D), central (Fig 11B,
206 D) and posterior part of dorsal part of telencephalic area. In the ventral telencephalic area, small round
207 cells were seen in the dorsal and ventral part.

208 *Diencephalon*

209 Numerous intensely stained cells were seen in the anterior parvocellular preoptic nucleus (Fig 1C,
210 2B¹), and in the posterior parvocellular preoptic nucleus (Fig 9 B, D). Weak signal was detected in
211 few cells of magnocellular preoptic nucleus. In dorsal and ventral habenular nucleus few positive
212 cells were detected. High density of NGF positive cells were observed in the ventro-medial and
213 ventro-lateral thalamic nuclei (Fig 9B, D). Few and weak positive cells were seen in the central
214 posterior thalamic nucleus. Numerous positive cells were detected in the posterior tuberal nucleus. In
215 the lateral and medial preglomerular nuclei, positive cells and some fibers were detected.

216 In the hypothalamus, the ventral zone of periventricular hypothalamus showed intense positivity in
217 the whole mount brain (Fig 1E) and numerous NGF positive cells were seen in histological sections
218 (Fig 2C¹, 5B-C¹). In the dorsal (Fig 1E) and caudal zone of periventricular hypothalamus moderate
219 positivity was seen. Large NGF positive cells belonging to the diffuse nucleus of the inferior lobe
220 were seen (Fig 6 A–B¹). Few NGF positive cells were also present in the mammillary body and in
221 the nucleus of the medial longitudinal fascicle

222 *Mesencephalon*

223 NGF positive fibers were present in the longitudinal tori and the optic tect, particularly in the deep
224 white zone and superficial white zone. Numerous small NGF positive cells appeared scattered in the
225 periventricular grey zone (Fig 4, B¹, B²).

226 In the tegmentum, NGF was observed in cells of central nucleus of semi-circular torus and superior
227 reticular formation.

228 *Rhombencephalon*

229 The cerebellar body was intensely reactive (Fig 1F). NGF positivity was observed in numerous large
230 cells of the Purkinje layer and in few cells of the molecular layer of valvula and body (Fig. 7A – C¹).
231 Few weak stained cells were observed in the granular eminence.

232 In the medulla oblongata, few large cells containing NGF and belonging to the inferior reticular
233 formation were seen.

234

235 *Characterization of NGF containing cells*

236 In order to identify the nature of NGF positive-cells in the brain of adult zebrafish, we carried out
237 immunohistochemical staining against NGF on slides of aromatase B transgenic fish (cyp19a1b
238 GFP), where radial glial cells appeared green fluorescent. Also, we performed double labelling using
239 antibodies against NGF/PCNA (proliferation marker) and NGF/MAP2 (neuronal marker). Aromatase
240 positive cells, distributed along ventricles, were closely intermingled with NGF positive cells along
241 telencephalic (Fig 8 B -D) and diencephalic (Fig 9B–D) ventricles. PCNA-positive cells were

242 positioned along the ventricular lining of the brain, and NGF was detected in the cytoplasm of cells
243 very close to PCNA labelled cells (Fig 10B-D). MAP2 immunoreactivity was present in the majority
244 of NGF positive cells, throughout the zebrafish brain (Fig 11B-D).

245

246

247

248

249

Accepted manuscript

250 DISCUSSION

251 This study documents the neuroanatomical distribution of NGF mRNA and protein in zebrafish.

252 The serum raised against NGF was previously characterized (Gatta et al 2016), where brain
253 homogenates of adult zebrafish showed only a band of 25 kDa, corresponding to the molecular weight
254 of proNGF isoform 1 (NP_001338647.1) (Gatta et al 2016). Accordingly, the presence of proNGF
255 was also reported in the brain of the teleost *N. furzeri*, by employing the same antiserum (D'Angelo
256 et al 2014). On the other hand, this antiserum detected the mature form of NGF in different organs
257 of fish, such as gut (Lucini et al 2003) and kidney (Arcamone et al., 2005). Taking into consideration
258 that the employed NGF antiserum is able to detect both pro- and mature NGF in fish tissues, our
259 results suggest that that only the proNGF form is present in the brain of zebrafish. Remarkably, in
260 mammalian brain, precursor and intermediate forms of NGF are expressed (Fahnestock et al 2001,
261 2004), and has been demonstrated to be actually the predominant form of NGF in central nervous
262 system (CNS) (Fahnestock et al., 2001), whereas mature NGF appears to be lacking.

263 The co-presence of pro-NGF and the neuronal marker MAP2 immunoreactivity throughout the brain
264 of adult zebrafish demonstrates that pro-NGF containing cells are neurons. This result was further
265 confirmed by the absence of the glial marker aromatase B and proliferative marker PCNA.
266 Consistently with our observations, in the teleost fish *N. furzeri*, NGF was morphologically detected
267 in neurons widespread throughout all brain regions (D'Angelo et al., 2014). Only some glial cells
268 lining the mesencephalic and rhombencephalic ventricles of *N. furzeri* seemed to express NGF. At
269 opposite, in goldfish NGF was almost totally localized in radial glia cells lining the ventricles
270 (Benowitz and Shashoua, 1979). Species specific characteristics could explain the different results
271 achieved in zebrafish, *N. furzeri* and goldfish. In agreement with our results, in the rat brain, NGF
272 production was reported in neurons, predominantly localized in GABAergic neurons of the cortex,
273 hippocampus, striatum and basal forebrain (Lauterborn et al 1993, 1995; Pascual et al 1998; Bizon et
274 al 1999; Sofroniew et al 2001; Biane et al 2014). NGF was also reported in neuronal populations of
275 adult monkey brain (Hayashi et al 1993; Zhang et al 2007). However, oligodendroglial progenitors

276 derived from human embryonic stem cells are a source of NGF (Zhang et al 2006; Althaus et al 2000).
277 Remarkably in adult zebrafish, another member of neurotrophin family, BDNF, was also expressed
278 in neuronal populations of the whole brain (Cacialli et al 2016) and of telencephalon after injury
279 (Cacialli et al 2018).

280 NGF expression was comparable between zebrafish and *N. furzeri* (D'Angelo et al 2014), despite
281 some slight differences in the neuroanatomy, whereas substantial differences in NGF cell localization
282 and distribution were seen between goldfish and zebrafish as previously described. In rat brain, NGF
283 levels resulted consistent in all regions with the highest presence in cortical areas (Hoener et al., 1996;
284 Sakamoto et al., 1998). Specifically, the pattern of distribution of both NGF mRNA (Shelton and
285 Reichardt, 1986) and protein (Nishio et al., 1992) was described throughout the rat brain, with the
286 highest intensity in the neocortex, the hippocampal pyramidal layer and striatum (Gall and Isackson,
287 1989, Rylett and Williams, 1994). Notably, observations in adult zebrafish brain do not support the
288 fact that the forebrain is the NGF prevalent containing region.

289 In our study, both NGF mRNA and protein were detected in the perikaryon, and only proNGF protein
290 was distributed along neuronal prolongations. Although the cellular co-presence of NGF mRNA and
291 protein was not investigated, the overlapping pattern of distribution of NGF mRNA and protein
292 throughout the zebrafish brain suggests that NGF expression and translation take place in mature
293 neurons. The presence of NGF protein along neuronal prolongments could be retrogradely
294 transported, according to the classical view on neurotrophins considered as target-derived
295 retrogradely transported substances, but also anterogradely transported, accordingly to a vast
296 literature. The idea that NGF may be produced locally was suggested since two decades ago
297 (Lauterborn et al 1991, Conner and Varon 1992) and activity-dependent release of NGF and its effect
298 on synaptic plasticity was postulated (Blöchl and Thoenen, 1995, 1996; Wu et al., 2004). Finally,
299 Guo and collaborators (2012) demonstrated, by immunohistochemical, ELISA and
300 electrophysiological analyses, anterograde delivery of NGF in hippocamposeptal system of mice.

301 ProNGF in the CNS is released in the extracellular space (Bruno and Cuello 2006) and induces
302 activation of the apoptotic machinery with subsequent death of different neuronal populations, mostly
303 after injury and neurodegenerative disorders (for a review see Costa et al 2018).

304 The present results demonstrate that also in zebrafish brain NGF is synthesized in perykaria, however
305 future studies are necessary to test whether proNGF is anterogradely transported and released in the
306 extracellular space, at terminal ending of NGF positive fibers.

307 In conclusion, our study demonstrated that mature neurons of the zebrafish brain express NGF mRNA
308 and store proNGF. Experimental studies reported proNGF as inhibition factor of the proliferation
309 of neural stem cells isolated from postnatal mouse hippocampus, causing cell cycle arrest in the
310 G0/G1 phase (Guo et al 2013). Thus, it is tempting to speculate that proNGF in the zebrafish brain,
311 where cell proliferation is considerably high and persist along the entire lifespan, could represent a
312 key negative regulator factor of this process.

313

314 Author's contribution

315 PC conceived and planned the experimentation, acquired and analyzed data; CG, AL, AP acquired
316 and analyzed data; LDA, PdG, EP critically revised the manuscript; CL analyzed data and wrote the
317 paper.

318

REFERENCES

- Adolf B, Chapouton P, Lam CS, et al. (2006) Conserved and acquired features of adult neurogenesis in the zebrafish telencephalon. *Developmental Biology*, **295**, 278-293.
- Althaus HH, Richter-Landsberg C (2000) Glial cells as targets and producers of neurotrophins. *International Review of Cytology - a Survey of Cell Biology, Vol. 197*, **197**, 203-277.
- Alunni A, Bally-Cuif L (2016) A comparative view of regenerative neurogenesis in vertebrates. *Development*, **143**, 741-753.
- Anand SK, Mondal AC (2017) Cellular and molecular attributes of neural stem cell niches in adult zebrafish brain. *Developmental Neurobiology*, **77**, 1188-1205.
- Arcamone N, Lucini C, Borzacchiello G, Castaldo L, Gargiulo G, De Girolamo P (2005) Distribution of NGF and NT-3-like protein immunoreactivity in the teleost kidney. *Microscopy Research and Technique*, **66**, 17-24.
- Biane J, Conner JM, Tuszynski MH (2014) Nerve growth factor is primarily produced by GABAergic neurons of the adult rat cortex. *Frontiers in Cellular Neuroscience*, **8**.
- Bizon JL, Lauterborn JC, Gall CM (1999) Subpopulations of striatal interneurons can be distinguished on the basis of neurotrophic factor expression. *Journal of Comparative Neurology*, **408**, 283-298.
- Bloch A, Thoenen H (1995) Characterization of Nerve Growth -Factor (NGF) release from hippocampal-neurons- evidence for a constitutive and unconventional sodium-dependent regulated pathway. *European Journal of Neuroscience*, **7**, 1220-1228.
- Bloch A, Thoenen H (1996) Localization of cellular storage compartments and sites of constitutive and activity-dependent release of nerve growth factor (NGF) in primary cultures of hippocampal neurons. *Molecular and Cellular Neuroscience*, **7**, 173-190.
- Bruno MA, Cuello AC (2006) Activity-dependent release of precursor nerve growth factor, conversion to mature nerve growth factor, and its degradation by a protease cascade. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 6735-6740.
- Cacialli P, D'Angelo L, Kah O, et al. (2018a) Neuronal expression of brain derived neurotrophic factor in the injured telencephalon of adult zebrafish. *Journal of Comparative Neurology*, **526**, 569-582.
- Cacialli P, Gueguen MM, Coumailleau P, et al. (2016) BDNF Expression in Larval and Adult Zebrafish Brain: Distribution and Cell Identification. *Plos One*, **11**.
- Cacialli P, Palladino A, Lucini C (2018b) Role of brain-derived neurotrophic factor during the

353 regenerative response after traumatic brain injury in adult zebrafish. *Neural Regeneration*
354 *Research*, **13**, 941-944.

355 Conner JM, Varon S (1992) Distribution of nerve Growth factor-like immunoreactive neurons in the
356 adult-rat brain following colchicine treatment. *Journal of Comparative Neurology*, **326**, 347-
357 362.

358 Cosacak MI, Papadimitriou C, Kizil C (2015) Regeneration, Plasticity, and Induced Molecular
359 Programs in Adult Zebrafish Brain. *Biomed Research International*.

360 Costa CJ, Willis DE (2018) To the end of the line: Axonal mRNA transport and local translation in
361 health and neurodegenerative disease. *Developmental Neurobiology*, **78**, 209-220.

362 Coumailleau P, Pellegrini E, Adrio F, et al. (2015) Aromatase, estrogen receptors and brain
363 development in fish and amphibians. *Biochimica Et Biophysica Acta-Gene Regulatory*
364 *Mechanisms*, **1849**, 152-162.

365 D'Angelo L, Avallone L, Cellerino A, et al. (2016a) Neurotrophin-4 in the brain of adult
366 *Nothobranchius furzeri*. *Annals of Anatomy-Anatomischer Anzeiger*, **207**, 47-54.

367 D'Angelo L, Castaldo L, Cellerino A, de Girolamo P, Lucini C (2014a) Nerve growth factor in the
368 adult brain of a teleostean model for aging research: *Nothobranchius furzeri*. *Annals of*
369 *Anatomy-Anatomischer Anzeiger*, **196**, 183-191.

370 D'Angelo L, de Girolamo P, Cellerino A, Tozzini ET, Castaldo L, Lucini C (2012) Neurotrophin Trk
371 receptors in the brain of a teleost fish, *Nothobranchius furzeri*. *Microscopy Research and*
372 *Technique*, **75**, 81-88.

373 D'Angelo L, De Girolamo P, Lucini C, et al. (2014b) Brain-Derived Neurotrophic Factor: mRNA
374 Expression and Protein Distribution in the Brain of the Teleost *Nothobranchius furzeri*.
375 *Journal of Comparative Neurology*, **522**, 1004-1030.

376 D'Angelo L, Lossi L, Merighi A, de Girolamo P (2016b) Anatomical features for the adequate choice
377 of experimental animal models in biomedicine: I. Fishes. *Annals of Anatomy-Anatomischer*
378 *Anzeiger*, **205**, 75-84.

379 Dalton VS, Borich SM, Murphy P, Roberts BL (2009a) Brain-Derived Neurotrophic Factor mRNA
380 Expression in the Brain of the Teleost Fish, *Anguilla anguilla*, the European Eel. *Brain*
381 *Behavior and Evolution*, **73**, 43-58.

382 Dalton VS, Roberts BL, Borich SM (2009b) Brain derived neurotrophic factor and trk B mRNA
383 expression in the brain of a brain stem-spinal cord regenerating model, the European eel, after
384 spinal cord injury. *Neuroscience Letters*, **461**, 275-279.

385 de Girolamo P, Lucini C (2011) Neuropeptide localization in nonmammalian vertebrates. *Methods in*
386 *molecular biology (Clifton, N.J.)*, **789**, 37-56.

387 Dethleffsen K, Heinrich G, Lauth M, Knapik EW, Meyer M (2003) Insert-containing neurotrophins
388 in teleost fish and their relationship to nerve growth factor. *Molecular and Cellular*
389 *Neuroscience*, **24**, 380-394.

390 Diotel N, Vaillant C, Gabbero C, et al. (2013) Effects of estradiol in adult neurogenesis and brain
391 repair in zebrafish. *Hormones and Behavior*, **63**, 193-207.

392 Diotel N, Viales RR, Armant O, et al. (2015) Comprehensive Expression Map of Transcription
393 Regulators in the Adult Zebrafish Telencephalon Reveals Distinct Neurogenic Niches.
394 *Journal of Comparative Neurology*, **523**, 1202-1221.

395 Fahnestock M, Michalski B, Xu B, Coughlin MD (2001) The precursor pro-nerve growth factor is
396 the predominant form of nerve growth factor in brain and is increased in Alzheimer's disease.
397 *Molecular and Cellular Neuroscience*, **18**, 210-220.

398 Fahnestock M, Yu G, Michalski B, et al. (2004) The nerve growth factor precursor proNGF exhibits
399 neurotrophic activity but is less active than mature nerve growth factor. *Journal of*
400 *Neurochemistry*, **89**, 581-592.

401 Gall CM, Isackson PJ (1989) Limbic seizures increase neuronal production of messenger-RNA for
402 nerve growth-factor. *Science*, **245**, 758-761.

403 Gatta C, Altamura G, Avallone L, et al. (2016) Neurotrophins and Their Trk-Receptors in the
404 Cerebellum of Zebrafish. *Journal of Morphology*, **277**, 725-736.

405 Guo L, Yeh ML, Carlson VCC, Johnson-Venkatesh EM, Yeh HH (2012) Nerve Growth Factor in the
406 Hippocamposeptal System: Evidence for Activity-Dependent Anterograde Delivery and
407 Modulation of Synaptic Activity. *Journal of Neuroscience*, **32**, 7701-7710.

408 Guo JJ, Wang JN, Liang CR, Yan J, Wang Yr, Liu GX, Jiang ZZ, Zhang LY, Wang XB, Wang YJ,
409 Zhou XF, Liao H (2013) proNGF inhibits proliferation and oligodendrogenesis of postnatal
410 hippocampal neural stem/progenitor cells through p75NTR in vitro. *Stem Cell Research*, **11**,
411 874-887.

412 Hayashi M, Yamashita A, Shimizu K, Sogawa K, Fujii Y (1993) Expression of the gene for nerve
413 growth-factor (NGF) in the monkey central nervous system. *Brain Research*, **618**, 142-148.

414 Hoener MC, Hewitt E, Conner JM, Costello JW, Varon S (1996) Nerve growth factor (NGF) content
415 in adult rat brain tissues is several-fold higher than generally reported and is largely associated
416 with sedimentable fractions. *Brain Research*, **728**, 47-56.

417 Lauterborn JC, Bizon JL, Tran TMD, Gall CM (1995) NGF messenger-RNA is expressed by
418 gabaergic but not cholinergic neurons in rat basal forebrain. *Journal of Comparative*
419 *Neurology*, **360**, 454-462.

420 Lauterborn JC, Isackson PJ, Gall CM (1991) NERVE GROWTH-FACTOR MESSENGER RNA-

421 CONTAINING CELLS ARE DISTRIBUTED WITHIN REGIONS OF CHOLINERGIC
 422 NEURONS IN THE RAT BASAL FOREBRAIN. *Journal of Comparative Neurology*, **306**,
 423 439-446.

424 Lauterborn JC, Tran TMD, Isackson PJ, Gall CM (1993) NERVE GROWTH-FACTOR
 425 MESSENGER-RNA IS EXPRESSED BY GABAERGIC NEURONS IN RAT
 426 HIPPOCAMPUS. *Neuroreport*, **5**, 273-276.

427 Lucini C, D'Angelo L, Cacialli P, Palladino A, de Girolamo P (2018) BDNF, Brain, and Regeneration:
 428 Insights from Zebrafish. *International Journal of Molecular Sciences*, **19**.

429 Lucini C, Maruccio L, Arcamone N, Lamanna C, Castaldo L (2003) Neurotrophin-like
 430 immunoreactivity in the gut of teleost species. *Neuroscience Letters*, **345**, 33-36.

431 Marz M, Schmidt R, Rastegar S, Strahle U (2011) Regenerative Response Following Stab Injury in
 432 the Adult Zebrafish Telencephalon. *Developmental Dynamics*, **240**, 2221-2231.

433 Minnone G, De Benedetti F, Bracci-Laudiero L (2017) NGF and Its Receptors in the Regulation of
 434 Inflammatory Response. *International Journal of Molecular Sciences*, **18**.

435 Niewiadomska G, Mietelska-Porowska A, Mazurkiewicz M (2011) The cholinergic system, nerve
 436 growth factor and the cytoskeleton. *Behavioural Brain Research*, **221**, 515-526.

437 Nilsson AS, Fainzilber M, Falck P, Ibanez CF (1998) Neurotrophin-7: a novel member of the
 438 neurotrophin family from the zebrafish. *Febs Letters*, **424**, 285-290.

439 Nishio T, Akiguchi I, Furukawa S (1992) Detailed distribution of nerve growth-factor in rat-brain
 440 determined by a highly sensitive enzyme-immunoassay. *Experimental Neurology*, **116**, 76-
 441 84.

442 Panula P, Chen YC, Priyadarshini M, et al. (2010) The comparative neuroanatomy and
 443 neurochemistry of zebrafish CNS systems of relevance to human neuropsychiatric diseases.
 444 *Neurobiology of Disease*, **40**, 46-57.

445 Pascual M, Rocamora N, Acsady L, Freund TF, Soriano E (1998) Expression of nerve growth factor
 446 and neurotrophin-3 mRNAs in hippocampal interneurons: Morphological characterization,
 447 levels of expression, and colocalization of nerve growth factor and neurotrophin-3. *Journal*
 448 *of Comparative Neurology*, **395**, 73-90.

449 Pellegrini E, Mouriec K, Anglade I, et al. (2007) Identification of aromatase-positive radial glial cells
 450 as progenitor cells in the ventricular layer of the forebrain in zebrafish. *Journal of*
 451 *Comparative Neurology*, **501**, 150-167.

452 Rylett RJ, Williams LR (1994) ROLE OF NEUROTROPHINS IN CHOLINERGIC-NEURON
 453 FUNCTION IN THE ADULT AND AGED CNS. *Trends in Neurosciences*, **17**, 486-490.

454 Sakamoto H, Kuzuya H, Tamaru M, et al. (1998) Developmental changes in the NGF content in the

455 brain of young, growing, low-birth-weight rats. *Neurochemical Research*, **23**, 115-120.

456 Shelton DL, Reichardt LF (1986) Studies on the expression of the beta-nerve growth-factor (NGF)
457 gene in the central nervous system level and regional distribution of NGF messenger RNA
458 suggest that NGF functions as a trophic factor for several distinct populations of neurons.
459 *Proceedings of the National Academy of Sciences of the United States of America*, **83**, 2714-
460 2718.

461 Sofroniew MV, Howe CL, Mobley WC (2001) Nerve growth factor signaling, neuroprotection, and
462 neural repair. *Annual Review of Neuroscience*, **24**, 1217-1281.

463 Than-Trong E, Bally-Cuif L (2015) Radial glia and neural progenitors in the adult zebrafish central
464 nervous system. *Glia*, **63**, 1406-1428.

465 Wu YJ, Kruttgen A, Moller JC, et al. (2004) Nerve growth factor, brain-derived neurotrophic factor,
466 and neurotrophin-3 are sorted to dense-core vesicles and released via the regulated pathway
467 in primary rat cortical neurons. *Journal of Neuroscience Research*, **75**, 825-834.

468 Wullman MF, Rupp B, Reichert H (1996) Neuroanatomy of the Zebrafish Brain. A topological atlas.
469 Birkhauser Basel, Birkhauser Verlag.

470 Zhang HT, Li LY, Zou XL, et al. (2007) Immunohistochemical distribution of NGF, BDNF, NT-3,
471 and NT-4 in adult rhesus monkey brains. *Journal of Histochemistry & Cytochemistry*, **55**, 1-
472 19.

473 Zhang YH, Vasko MR, Nicol GD (2006) Intracellular sphingosine 1-phosphate mediates the
474 increased excitability produced by nerve growth factor in rat sensory neurons. *Journal of*
475 *Physiology-London*, **575**, 101-113.

LEGENDS

Fig.1 NGF mRNA in whole mount-brain of adult zebrafish.

NGF mRNA distribution in whole mount brain (A); telencephalon (B); preoptic area and olfactory bulbs (C, D); hypothalamus (E), cerebellum (F). CCe: cerebellum; DIL: diffuse nucleus of the inferior lobe; Hv: ventral zone of periventricular hypothalamus; Hc: caudal zone of periventricular hypothalamus; Hd: dorsal zone of periventricular hypothalamus; Mo: medulla oblongata; OB: olfactory bulbs; PPa: preoptic area; Tel: telencephalon; TeO: optic tect; Scale bar: (A) 500 μ m; (B, C, E, F) 250 μ m; D: 150 μ m.

Fig.2 NGF mRNA in cross-section of adult zebrafish brain.

(A – C) Representative section taken from the zebrafish atlas (Wullimann et al., 1996). NGF positive cells are represented by blue dots. (A¹ – C¹) NGF positive cells in the dorsal part of telencephalon (A¹), in the anterior parvocellular preoptic nucleus (B¹) and along the ventral zone of periventricular hypothalamus (C¹). Hv: ventral zone of periventricular hypothalamus; PPa: anterior parvocellular preoptic nucleus; Tel: telencephalon. Scale bar: 50 μ m.

Fig.3 NGF protein in the telencephalon.

(A) Representative section taken from the zebrafish atlas (Wullimann et al., 1996). NGF positive cells are represented by green dots. (B - C) Cells positive to NGF in the medial (B) and lateral (C) part of dorsal telencephalic area. (B¹, C¹) NGF positive cells co-marked with DAPI. Dl: lateral zone of dorsal telencephalic area; Dm: medial zone of dorsal telencephalic area. Scale bar: (C, C') 100 μ m; (B, B') 50 μ m.

Fig.4 NGF protein in the forebrain and midbrain.

503 (A, B) Representative sections taken from the zebrafish atlas (Wullimann et al., 1996). NGF positive
504 cells are represented by green dots. Cells positive to NGF in the posterior zone of dorsal telencephalic
505 area (A¹) and in periventricular gray zone of optic tect (B¹). (A², B²) NGF positive cells co-marked
506 with DAPI. TelV: telencephalic ventricle; Scale bar: 50 µm.

507

508 Fig.5 NGF protein in the hypothalamus.

509 (A) Representative section taken from the zebrafish atlas (Wullimann et al., 1996). NGF positive cells
510 are represented by green dots. (B – C) NGF protein (green) along the ventral hypothalamus at low
511 (B) and high (C) magnification. (C¹) NGF positive cells co-marked with DAPI. Hv: ventral zone of
512 periventricular hypothalamus. Scale bar: (B) 100 µm; (C, C¹) 50 µm.

513

514 Fig.6 NGF protein in the hypothalamus.

515 NGF positive cells in the diffuse nucleus of inferior lobe (DIL) at low (A) and high (A¹, B)
516 magnification. In B¹ NGF positive cells co-marked with DAPI. Scale bar: (A) 120 µm; (A¹) 30 µm;
517 (B, B¹) 20 µm.

518

519 Fig.7 NGF protein in the cerebellum.

520 (A) Representative section taken from the zebrafish atlas (Wullimann et al., 1996). NGF positive cells
521 are represented by green dots. (B, C) NGF positive cells in the Purkinje layer of cerebellum. In C'
522 NGF positive cells co-marked with DAPI. CCe: cerebellar body; Scale bar: 50 µm.

523

524 Fig.8 NGF positive cells are close to aromatase B cells along telencephalic ventricle.

525 (A) Representative section of dorsal and ventral telencephalon taken from the zebrafish atlas
526 (Wullimann et al., 1996). NGF positive cells are represented by red dots and Aromatase B is
527 represented by black dots with thin lines indicating radial glia cytoplasmic processes. (B – F) Cross
528 sections of dorsal telencephalic area. Double staining for NGF (red) (B), Aromatase-B (green) (C)

529 and merge with DAPI (D). High magnification of NGF and aromatase B cells, closely intermingled
530 (E, F). Scale bar: (B – D) 40 μm ; (E) 20 μm ; (F) 10 μm .

531

532 Fig.9 NGF positive cells are close to aromatase B cells along diencephalic ventricle.

533 (A) Representative section of diencephalon taken from the zebrafish atlas (Wullimann et al., 1996).

534 NGF positive cells are represented by red dots and Aromatase B is represented by black dots with

535 thin lines indicating radial glia cytoplasmic processes. (B – D) Double staining for NGF (red) (B),

536 Aromatase-B (green) (C) and merge with DAPI (D) on cross-section of area surrounding diencephalic

537 ventricle. (E -F) high magnifications of B and C images. Scale bar: (B – D) 50 μm ; (E _F) 20 μm .

538

539 Fig.10 NGF positive cells are intermingled with PCNA positive cells.

540 (A) Representative section of dorsal and ventral telencephalon taken from the zebrafish atlas

541 (Wullimann et al., 1996) NGF positive cells are represented by green dots and PCNA positive cells

542 by red dots. (B – D) Double staining for NGF (green) (B), PCNA(red) (C), merge with DAPI and

543 high-magnification of a zoom area (D) on cross-sections through the telencephalon. Scale bar: 50 μm ,

544 and particular of the region in (D) 30 μm .

545

546 Fig.11 NGF immunoreactivity is colocalized with MAP2 in cells of central zone of dorsal

547 telencephalic area.

548 Representative section dorsal and ventral telencephalon taken from the zebrafish atlas (Wullimann et

549 al., 1996). NGF positive cells are represented by red dots and MAP2 positive cells by green dots. (B

550 – D) Double staining for NGF (red) (B), MAP2 (green) (C) and merge (D) on cross-sections through

551 the telencephalon. Scale bar: 20 μm

552

553 Supplementary material

554 Negative controls performed by NGF antiserum preincubated with its homologous antigen did not
555 show any reactivity. Scale bar: (A) 100 μm ; (B) 50 μm
556

Accepted manuscript

557
558
559

Table 1. Distribution of NGF mRNA and protein in the brain of adult zebrafish. The scheme was done following qualitative and not quantitative criteria.

Brain region	NGF mRNA	NGF protein
Olfactory bulbs		
Glomerular layer		++
External cellular layer	+	+
Internal cellular layer	+	+
Dorsal telencephalic area		
Medial zone of dorsal telencephalic area	+	++
Dorsal zone of dorsal telencephalic area	+	+
Lateral zone of dorsal telencephalic area	+	+
Central zone of dorsal telencephalic area	++	++
Posterior zone of dorsal telencephalic area	++	+
Ventral telencephalic area		
Ventral-dorsal part	+	++
Ventral-central part	+	++
Preoptic area		
Magnocellular preoptic nucleus	+	+
Parvocellular preoptic nucleus, anterior part	++	++
Parvocellular preoptic nucleus, posterior part	+	++
Epithalamus		
Dorsal habenular nucleus	+	+
Ventral habenular nucleus	+	+
Dorsal thalamus		
Central posterior thalamic nucleus	+	++
Ventral thalamus		
Ventromedial thalamic nucleus	+	++
Ventrolateral thalamic nucleus	+	+
Posterior Tuberculum		
Posterior tuberal nucleus	++	++
Lateral preglomerular nucleus	+	+
Medial preglomerular nucleus	+	++
Hypothalamus		
Diffuse nucleus of the inferior lobe	+	++
Ventral zone of periventricular hypothalamus	++	++
Dorsal zone of periventricular hypothalamus	+	+
Caudal zone of periventricular hypothalamus	+	+
Mammillary body	+	+
Synencephalon		
Nucleus of the medial longitudinal fascicle	+	+
Optic Tectum		
Periventricular grey zone of optic tectum	++	++
Deep white zone		++
Central zone	+	+
Superficial white grey zone	+	++
Longitudinal torus	+	+
Torus semicircularis		
Central nucleus of semicircular torus	+	+
Tegmentum		
Superior reticular formation	+	++
Cerebellum		
Molecular layer	+	+
Purkinje cell layer	++	++
Medulla oblongata		
Inferior reticular formation	+	+

560
561

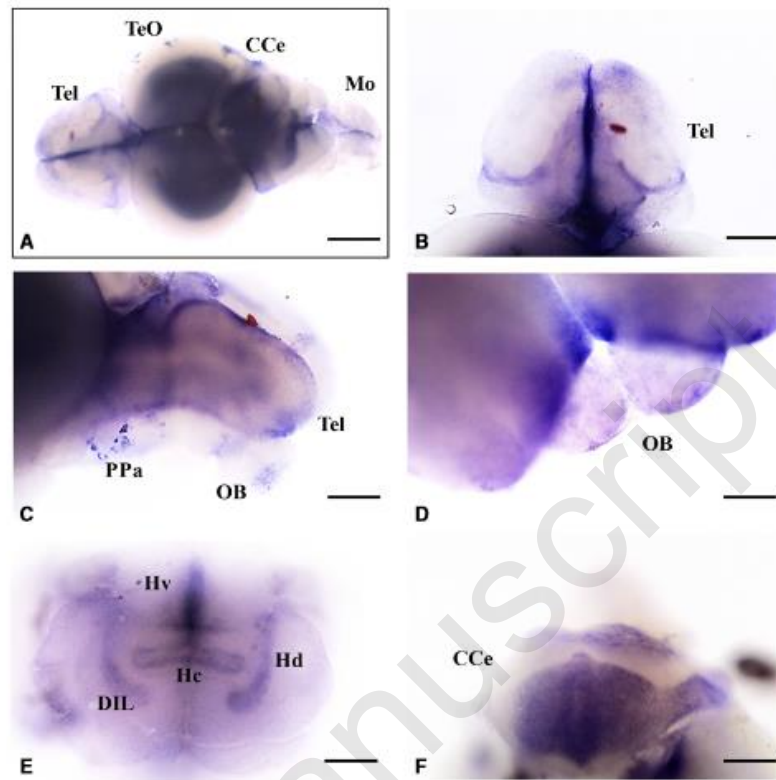


Fig. 1 Nerve growth factor (NGF) mRNA in whole-mount brain of adult zebrafish. NGF mRNA distribution in (A) whole-mount brain; (B) telencephalon; (C, D) preoptic area and olfactory bulbs; (E) hypothalamus; and (F) cerebellum. CCe, cerebellum; DIL, diffuse nucleus of the inferior lobe; Hv, ventral zone of periventricular hypothalamus; Hc, caudal zone of periventricular hypothalamus; Hd, dorsal zone of periventricular hypothalamus; Mo, medulla oblongata; OB, olfactory bulbs; PPa, preoptic area; Tel, telencephalon; TeO, optic tect; Scale bars: 500 μ m (A); 250 μ m (B, C, E, F); 150 μ m (D).

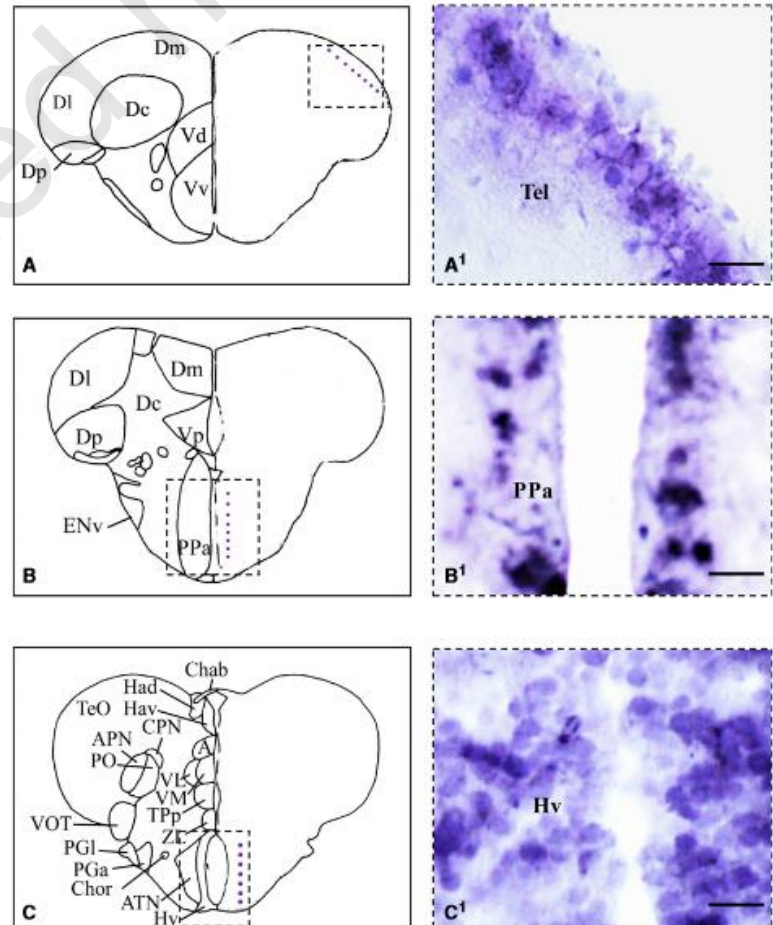


Fig. 2 Nerve growth factor (NGF) mRNA in cross-section of adult zebrafish brain. (A–C) Representative section taken from the zebrafish atlas (Wullman et al., 1996). NGF-positive cells are represented by blue dots. (A'–C') NGF-positive cells in the dorsal part of the telencephalon (A'), in the anterior parvocellular preoptic nucleus (B') and along the ventral zone of the periventricular hypothalamus (C'). Hv, ventral zone of periventricular hypothalamus; PPa, anterior parvocellular preoptic nucleus; Tel, telencephalon. Scale bar: 50 μ m.

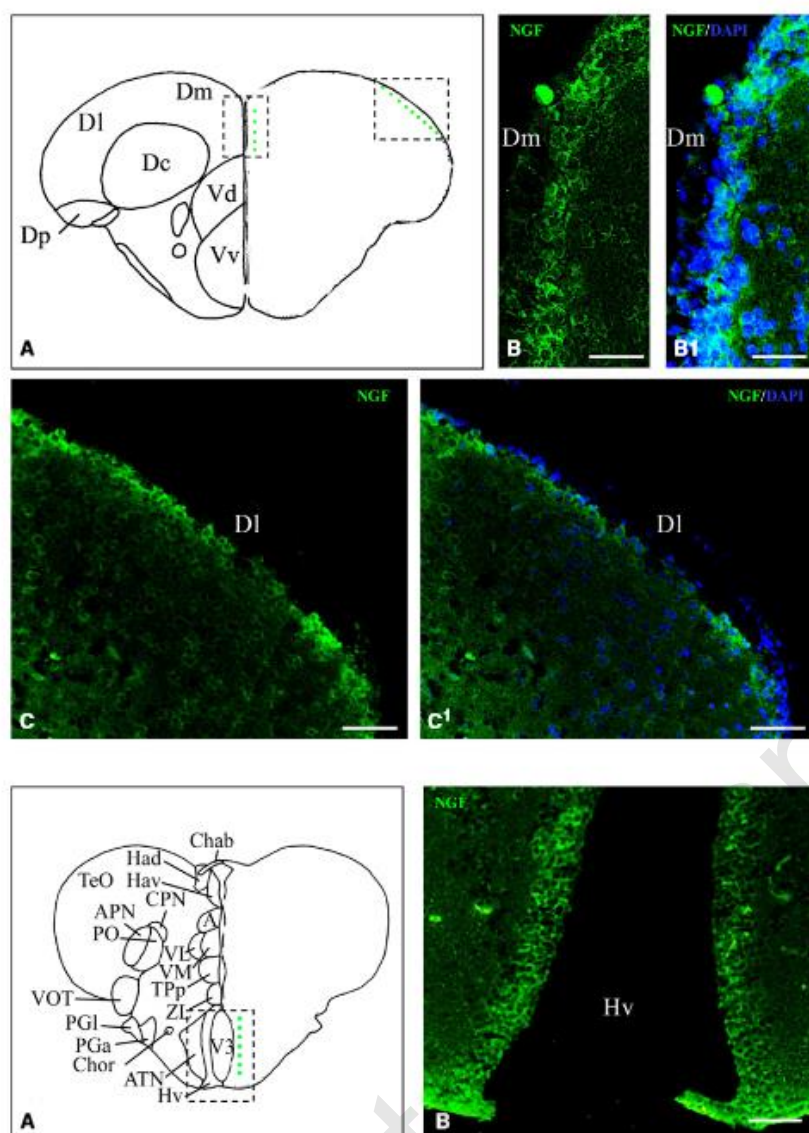


Fig. 3 Nerve growth factor (NGF) protein in the telencephalon. (A) Representative section taken from the zebrafish atlas (Wullman et al., 1996). NGF-positive cells are represented by green dots. (B, C) Cells positive to NGF in the medial (B) and lateral (C) part of the dorsal telencephalic area. (B¹, C¹) NGF-positive cells co-marked with 4,6-diamino-2-phenylindole (DAPI). Dl, lateral zone of dorsal telencephalic area; Dm, medial zone of dorsal telencephalic area. Scale bars: 100 μ m (C, C¹); 50 μ m (B, B¹).

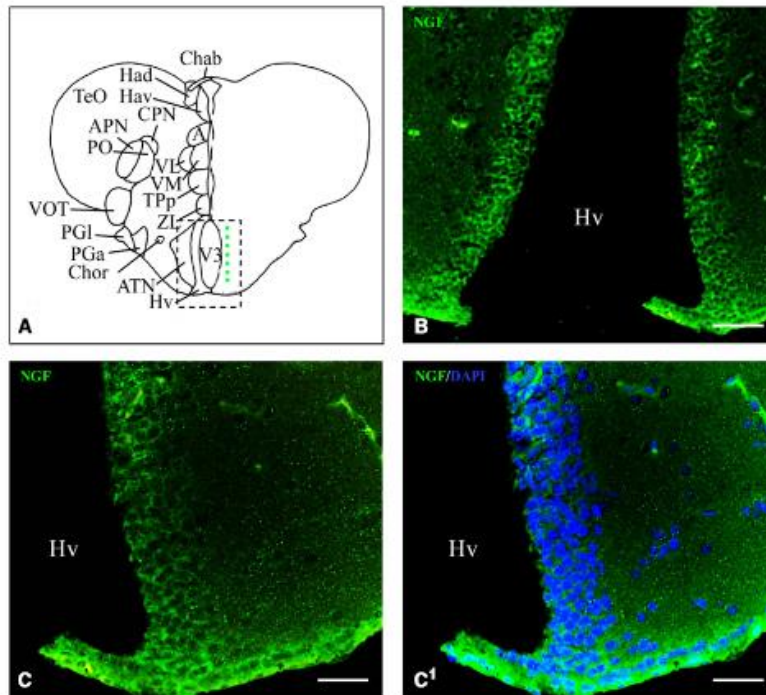


Fig. 4 Nerve growth factor (NGF) protein in the hypothalamus. (A) Representative section taken from the zebrafish atlas (Wullman et al., 1996). NGF-positive cells are represented by green dots. (B, C) NGF protein (green) along the ventral hypothalamus at low (B) and high (C) magnification. (C') NGF-positive cells co-marked with 4,6-diamino-2-phenylindole (DAPI). Hv, ventral zone of periventricular hypothalamus. Scale bars: 100 μ m (B); 50 μ m (C, C').

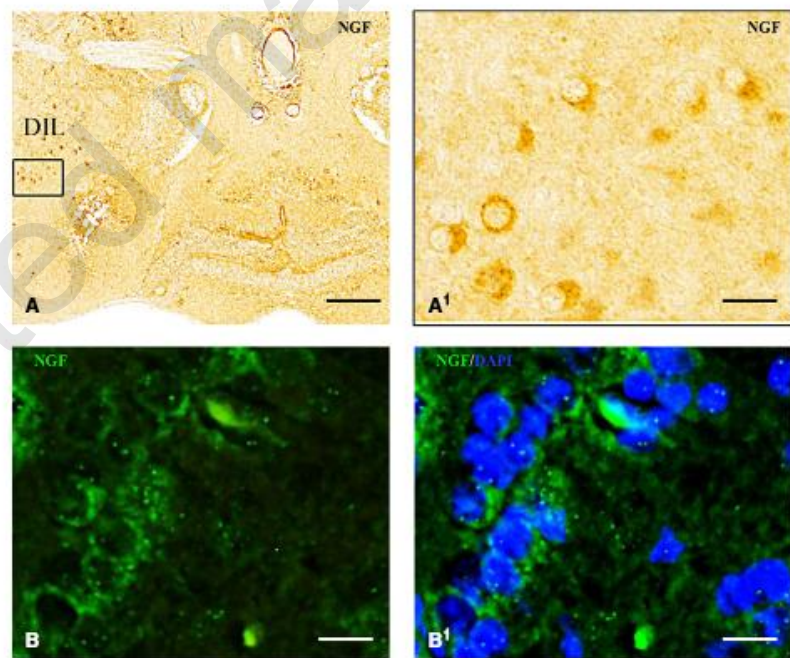


Fig. 5 Nerve growth factor (NGF) protein in the hypothalamus. NGF-positive cells in the diffuse nucleus of inferior lobe (DIL) at low (A) and high (A', B) magnification. (B') NGF-positive cells co-marked with 4,6-diamino-2-phenylindole (DAPI). Scale bars: 120 μ m (A); 30 μ m (A'); 20 μ m (B, B').

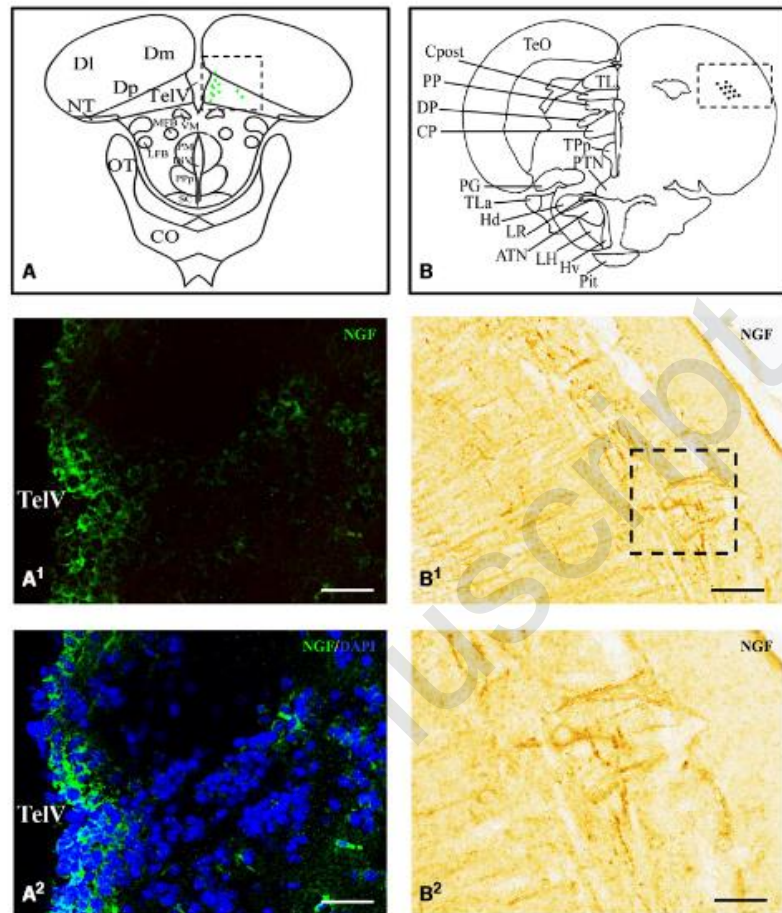


Fig. 6 Nerve growth factor (NGF) protein in the forebrain and midbrain. (A, B) Representative sections taken from the zebrafish atlas (Wulliman et al., 1996). NGF-positive cells are represented by green dots. Cells positive to NGF in the posterior zone of the dorsal telencephalic area (A¹) and in the periventricular gray zone of optic tect (B¹). (A², B²) NGF-positive cells co-marked with 4,6-diamino-2-phenylindole (DAPI). TelV, telencephalic ventricle. Scale bar: 50 μm.

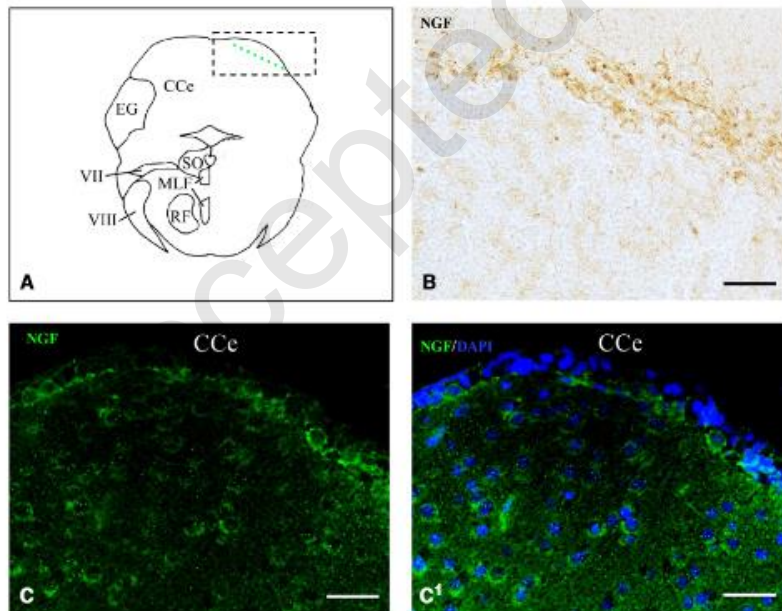


Fig. 7 Nerve growth factor (NGF) protein in the cerebellum. (A) Representative section taken from the zebrafish atlas (Wulliman et al., 1996). NGF-positive cells are represented by green dots. (B, C) NGF-positive cells in the Purkinje layer of cerebellum. (C¹) NGF-positive cells co-marked with 4,6-diamino-2-phenylindole (DAPI). CCE, cerebellar body. Scale bar: 50 μm.

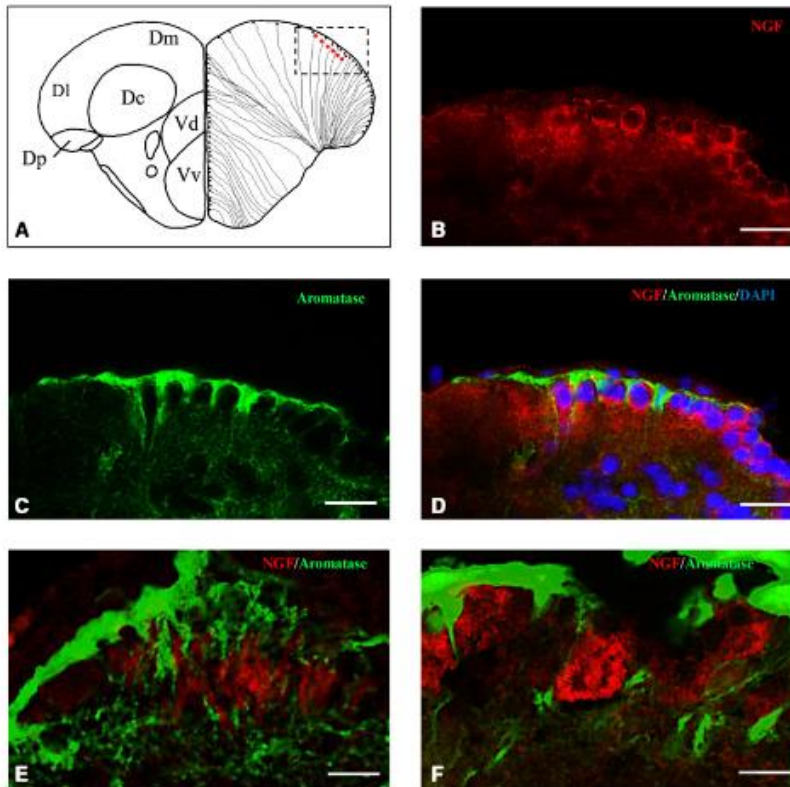


Fig. 8 Nerve growth factor (NGF)-positive cells are close to aromatase B cells along telencephalic ventricle. (A) Representative section of dorsal and ventral telencephalon taken from the zebrafish atlas (Wulliman et al., 1996). NGF-positive cells are represented by red dots, and aromatase B is represented by black dots with thin lines indicating radial glia cytoplasmic processes. (B–F) Cross-sections of the dorsal telencephalic area. Double-staining for (A) NGF (red), (C) aromatase B (green), and merge with (D) 4,6-diamino-2-phenylindole (DAPI). High magnification of NGF and aromatase B cells, closely intermingled (E, F). Scale bars: 40 μ m (B–D); 20 μ m (E); 10 μ m (F).

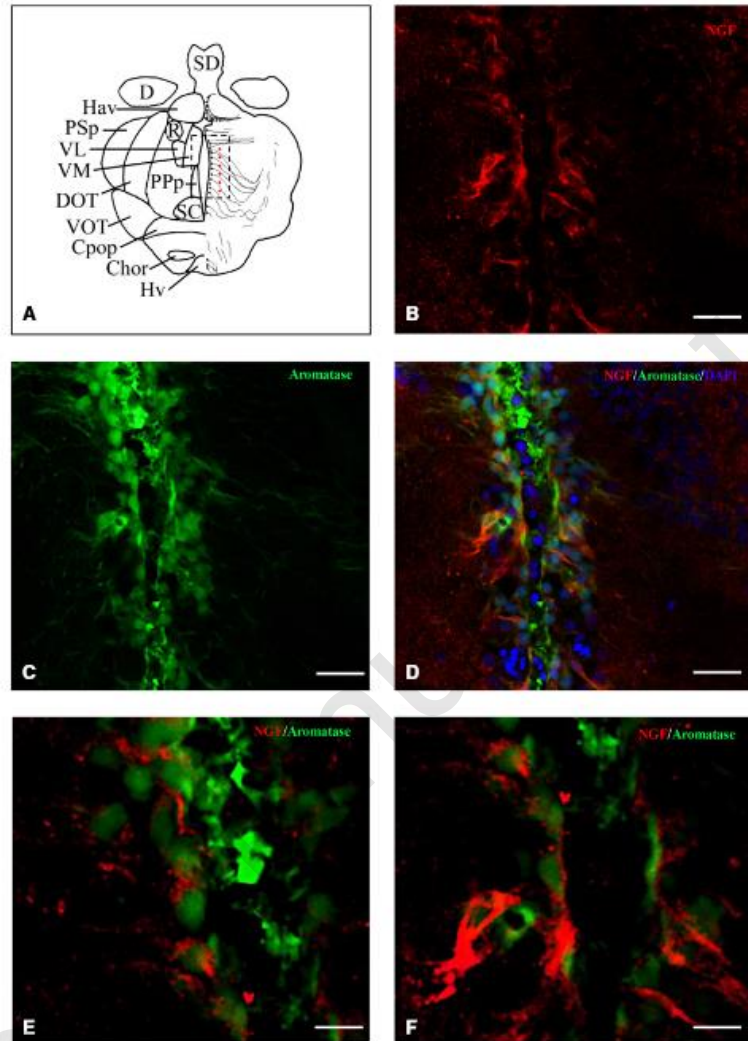


Fig. 9 Nerve growth factor (NGF)-positive cells are close to aromatase B cells along the diencephalic ventricle. (A) Representative section of the diencephalon taken from the zebrafish atlas (Wullman et al., 1996). NGF-positive cells are represented by red dots, and aromatase B is represented by black dots with thin lines indicating radial glia cytoplasmic processes. (B–D) Double-staining for (B) NGF (red), (C) aromatase B (green), and merge with (D) 4,6-diamino-2-phenylindole (DAPI) on cross-section of the area surrounding diencephalic ventricle. (E, F) High magnifications of (B) and (C) images. Scale bars: 50 μ m (B–D); 20 μ m (E, F).

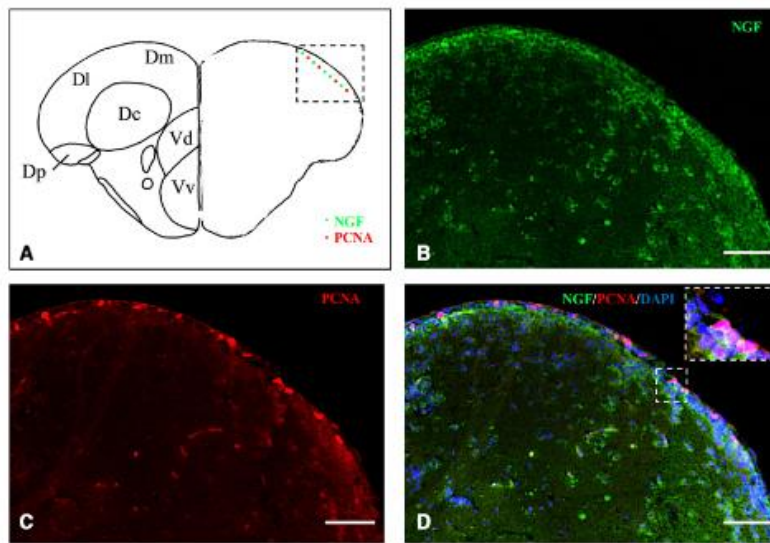


Fig. 10 Nerve growth factor (NGF)-positive cells are intermingled with proliferative cell nuclear antigen (PCNA)-positive cells. (A) Representative section of dorsal and ventral telencephalon taken from the zebrafish atlas (Wulliman et al., 1996). NGF-positive cells are represented by green dots and PCNA-positive cells by red dots. (B-D) Double-staining for (B) NGF (green), (C) PCNA (red), and merge with (D) 4,6-diamino-2-phenylindole (DAPI) and high-magnification of a zoom area on cross-sections through the telencephalon. Scale bar: 50 μ m, and particular of the region in (D) 30 μ m.

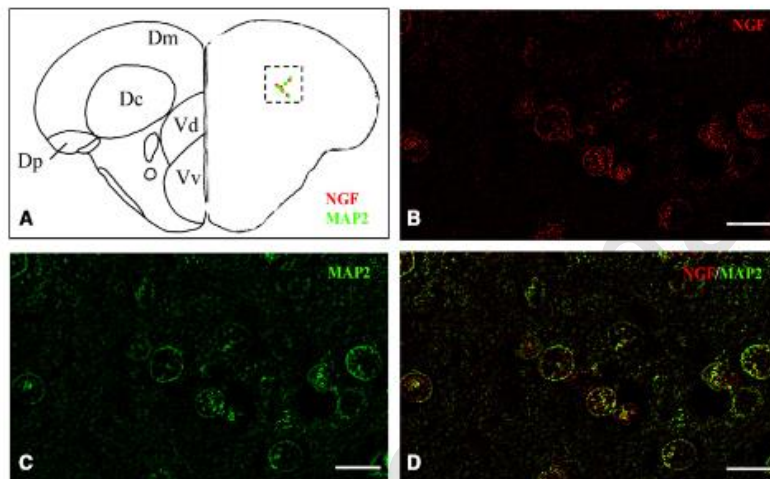


Fig. 11 Nerve growth factor (NGF) immunoreactivity is co-localized with microtubule-associated protein2 (MAP2) in cells of the central zone of the dorsal telencephalic area. Representative section of dorsal and ventral telencephalon taken from the zebrafish atlas (Wulliman et al., 1996). NGF-positive cells are represented by red dots, and MAP2-positive cells by green dots. (B-D) Double-staining for (B) NGF (red), (C) MAP2 (green), and merge (D) on cross-sections through the telencephalon. Scale bar: 20 μ m.