

Expression of the *BDNF* gene in the developing visual system of the chick

Karl-Heinz Herzog^{1,*}, Karen Bailey² and Yves-Alain Barde¹

¹Max-Planck Institute for Psychiatry, Department of Neurobiochemistry, 82152 Planegg-Martinsried, Germany

²Present address: Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia

*Author for correspondence

SUMMARY

Using a sensitive and quantitative method, the mRNA levels of brain-derived neurotrophic factor (BDNF) were determined during the development of the chick visual system. Low copy numbers were detected, and BDNF was found to be expressed in the optic tectum already 2 days before the arrival of the first retinal ganglion cell axons, suggesting an early role of BDNF in tectal development. After the beginning of tectal innervation, BDNF mRNA levels markedly increased, and optic stalk transection at day 4 (which prevents subsequent tectal innervation) was found to reduce the contralateral tectal levels of BDNF mRNA. Comparable reductions were obtained after injection of tetrodotoxin into one eye, indicating that,

already during the earliest stages of target encounter in the CNS, the degree of *BDNF* gene expression is influenced by activity-dependent mechanisms. BDNF mRNA was also detected in the retina itself and at levels comparable to those found in the tectum. Together with previous findings indicating that BDNF prevents the death of cultured chick retinal ganglion cells, these results support the idea that the tightly controlled expression of the *BDNF* gene might be important in the co-ordinated development of the visual system.

Key words: neurogenesis, neurotrophins, optic tectum, retina, mRNA quantification

INTRODUCTION

Neurons require signals if they are to survive and coordinate their development with that of the territory that they innervate. These requirements can be conveniently demonstrated in neuronal populations that are separated from the target tissue to which they project. Manipulations like target ablation can be readily performed and, during development, they typically result in the elimination of the majority of the neurons that should have innervated the target (for review, see Oppenheim, 1991). Such is the case for example with the retinal ganglion cells of the chick, which disappear when their target, the optic tectum, is removed (Hughes and LaVelle, 1975). Little is known regarding the molecular nature of the signals involved in this target-dependent regulation, with the exception of studies performed with nerve growth factor (NGF) in the peripheral nervous system (PNS). In particular, the administration of NGF prevents neuronal death following target ablation, as well as the death of apparently superfluous neurons during normal development (Hamburger et al., 1981; Hamburger and Yip, 1984). Conversely, antibodies blocking the biological activity of NGF markedly increase the extent of neuronal death (Cohen, 1960; Levi-Montalcini and Booker, 1960; Johnson et al., 1980). Of particular relevance for the concept of a control of neuronal survival by target cells was the demonstration that NGF protein and mRNA levels could be measured in the targets of the neurons that need NGF for survival (Korsching and Thoenen, 1983; Heumann et al., 1984; Shelton and Reichardt, 1984; Davies et al., 1987). Both NGF

and its mRNA have been detected in very small amounts, and the levels determined could be correlated with the density of NGF-sensitive sympathetic or sensory innervation (Korsching and Thoenen, 1983; Harper and Davies, 1990). Finally, the use of transgenic mice expressing an NGF construct under the control of a keratin promoter has indicated that the resulting increased levels of NGF mRNA in the skin lead to an increase in the density of innervation and in the number of surviving neurons in the corresponding ganglia (Albers et al., 1994).

Compared with the peripheral nervous system, remarkably little is known about molecular mechanisms regulating neuronal survival and development in the central nervous system (CNS). This is because for a long time, NGF was the only molecule available for such studies and there are few NGF-responsive neurons in the CNS, which are difficult to investigate during development. In the present study, we examine mRNA levels of brain-derived neurotrophic factor (BDNF), a molecule that is structurally related to NGF (Leibrock et al., 1989). These levels were determined in the developing chick visual system using a quantitative and sensitive method. The chick retina and its major target, the optic tectum, are readily accessible allowing experimental manipulations to be performed, and the time course of the innervation of the optic tectum by retinal ganglion cell axons has been well studied. A previous study has also shown that, when retinal ganglion cells are placed in culture at a time when massive cell death begins to be observed in vivo, most of them will not survive unless BDNF is added to the culture medium (Rodríguez-Tébar et al., 1989).

MATERIALS AND METHODS

Tissue preparation

Fertilized eggs of white Leghorn chicken were incubated in a humidified incubator and staged according to Hamburger and Hamilton (1951). Tissues from embryonic chicken and of one adult hen were dissected and immediately frozen on dry ice and stored at -80°C .

Optic stalk transection

Egg shells were windowed during the second day of incubation and 2 days later, the right optic stalk was transected just behind the eyeball with a pair of microscissors. The windows were then sealed with a transparent tape, and the incubation continued until embryonic day (E) 7. Both tecta were then dissected and immediately frozen.

Injection of tetrodotoxin

E3 embryos were cultured in Petri dishes (Auerbach et al., 1974). Using a glass micropipette, 1 μl of a tetrodotoxin solution (100 μM) was injected into the right eye at E6 and embryos were allowed to develop until E7 (stage 31).

Cloning of the chick *BDNF* gene

A chick *BDNF* DNA fragment was obtained using PCR and primers (ATAATCTAGATGACCATCCTTTTCCTT sense, and ATAATCTAGACTATCTTCCCCTTAAAT antisense) corresponding to the amino- and carboxy-terminal sequences of mouse *BDNF* (Hofer et al., 1990), with the addition of *Xba*I sites. The amplified fragment was cloned into the Bluescript vector following digestion with *Xba*I, and the 531 fragment used as a probe to screen a chicken genomic library (Clontech, in λ EMBL 3). Two clones were isolated and sequenced. The nucleotide sequence obtained is identical to that published by Maisonpierre et al. (1992).

RNA isolation

For *BDNF* mRNA analysis, total RNA was purified from tissues by the cesium trifluoroacetate (CsTFA) centrifugation procedure (Okayama et al. 1987), taking advantage of this essentially one-step procedure, according to a modified protocol (Pharmacia/LKB). In brief, a known amount of recovery standard was added to a given amount of tissue and homogenized in 2 ml 5.5 M guanidinium thiocyanate containing 25 mM sodium citrate, 0.5% sodium lauryl sarcosine and 0.2 M β -mercaptoethanol. Insoluble particles were pelleted (7500 g, 20 minutes), and the supernatant loaded onto 2.2 ml CsTFA, 0.1 M EDTA, pH 7, density 1.51 g/ml. Density gradient centrifugation using a SW60 rotor (Beckman) was for 24 hours at 30,500 revs/minute. The RNA was then precipitated with ammonium acetate (see below) and used in the reverse transcription (RT)-PCR procedure.

Poly(A)⁺ preparation

Poly(A)⁺ RNA was prepared from total RNA obtained from tecta of E12 chicken according to the Stratagene protocol using the Poly(A)Quik™ mRNA purification kit. After denaturation at 65°C for 10 minutes, 500 μg of total RNA was loaded onto an oligo(dT) column. The flow through was collected, reloaded and recollected. After washing twice with a high salt buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5 M NaCl) and 3 times with a low salt buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1 M NaCl), the poly (A)⁺ was eluted with preheated (65°C) elution buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA). The recovery of both RNA fractions (flow through and eluted RNA) was quantified spectrophotometrically.

Northern blot analysis

For northern blot analysis, the amount of RNA corresponding to 1 mg tissue (E8 tectum), 3 μg poly(A)⁺ and 5 μg poly(A)⁻ (E12 tectum) were separated using 1.2% agarose gels containing 4.25% formaldehyde, transferred to nylon filters (Hybond N, Amersham) with $10\times$ SSC and hybridized with a chick *BDNF* cRNA probe. This antisense probe was transcribed in the presence of [α - ^{32}P]UTP (Amersham) and

encompasses 741 nucleotides of the coding, and 7 nucleotides of the 3' region of the chick *BDNF* gene. Hybridisation was carried out with 2.5×10^7 cpm/ml probe in 50% formamide, $7.5\times$ SSC, 50 mM sodium phosphate, pH 7.2, $5\times$ Denhardt's, 0.25% SDS, 250 mg/ml salmon sperm DNA and 5 mM EDTA at 65°C . Filters were washed in $2\times$ SSC, 0.5% SDS at room temperature, followed by two washes at 70°C with $0.1\times$ SSC, 0.5% SDS and exposed with Fuji X-ray film at -70°C using intensifying screens for 4 days.

mRNA quantification using reverse transcription and PCR

The number of copies of endogenous *BDNF* mRNA was determined using PCR preceded by reverse transcription of the mRNA and of a standard identical with the endogenous mRNA in the sequence to be amplified with the exception of a mutated base, according to the method of Becker-André and Hahlbrock, 1989 (see also Fig. 1). The recovery standard was prepared according to the protocol of Higuchi (1990) with 2 oligonucleotides flanking the region to be amplified and 2 oligonucleotides located in between with the purpose of creating a *Bam*HI restriction site (G \rightarrow C at position 352, +1 corresponding to A of the ATG initiation codon). The resulting product was ligated in a pBlue-script SK and transcribed by T7 RNA polymerase resulting in the sense transcript. The plasmid was digested by DNase I (Pharmacia) for 25 minutes and separated together with non-incorporated nucleotides from the in vitro transcript using Sephadex G-50 columns. The in vitro transcript was precipitated with 1/3 volume 8 M ammonium acetate, pH 5.4 and 3 volumes ethanol, the precipitate washed with ice-cold 70% ethanol and the amount determined spectrophotometrically. Complete digestion of the plasmid was checked by performing PCR without reverse transcriptase. At the time of homogenization, a known amount of this recovery standard (typically, 1 pg per mg tissue) was added to the tissue to be analysed and, after RNA extraction, RT-PCR was carried out for only 17 cycles to avoid the problem of primer competition between endogenous mRNA and standard, as well as heteroduplex formation. This allows only one RNA preparation to be analysed, and following hybridisation and densitometry, the copy number can be calculated based on the signal obtained with the standard. We directly checked that both standard and endogenous mRNA were amplified with identical efficiency in the concentration range used to determine *BDNF* mRNA by varying the amounts of standard (50 fg to 3 pg) using a given concentration of endogenous mRNA, as well as by measuring the levels of endogenous *BDNF* mRNA of one preparation using different dilutions (over a range of 10-fold). Clearly, reduction of cycle number to 17 markedly reduces the sensitivity of the method. However, we found that in spite of this, the signals can be comfortably detected by optimizing the enzymatic reactions. The most important variables were found to be the use of AMV as reverse transcriptase, its concentration, the temperature of the reaction and the use of recombinant Taq polymerase. In our routine, one-tube protocol, we added to RNA extracted from 1 mg tissue 12.5 pmol of both oligonucleotides (AGCAGT-CAAGTGCCCTTTG and GAGCCCACTATCTTCCCC), 6 μmol dNTP, 0.2 μl ampli-Taq-polymerase (1 u, Perkin-Elmer), 1 μg yeast t-RNA, 0.3 μl RNasin (12 u, Promega), 0.1 u AMV (Life Science) in 24 μl Taq-buffer. Reverse transcription was performed at 50°C for 10 minutes, followed by digestion (37°C , 45 minutes) with the frequent cutter *Hin*fI (to digest any genomic DNA contamination). This was followed by PCR for 17 cycles (94°C for 1 minute, 60° for 1 minute and 72° for 1.5 minutes). The resulting 463 bp product was digested using *Bam*HI (Pharmacia/LKB) (2.5 u, 60 minutes, 37°C), which cuts only the DNA resulting from the recovery standard, leading to a 397 bp and a 66 bp fragment. The DNA mixture was separated on a 8% polyacrylamide gel and electrophoresis was continued until only the two largest fragments remained in the gel, which was then denatured by boiling for 20 minutes. DNA was transferred onto nylon filters by electroblotting and immobilized by UV light. Hybridisation to digoxigenin-labeled random primed probe (the coding region of *BDNF* was labeled according to the protocol from Boehringer/Mannheim) was carried out at 42°C overnight in the presence of 35% formamide, 250

mM sodium phosphate, pH 7.2, 7% SDS, 250 mM NaCl and 5× Denhardt's. Filters were washed with 2× SSC, 0.5% SDS and twice with 0.1× SSC, 0.5% SDS at room temperature and incubated with alkaline phosphatase-conjugated antibodies directed against digoxigenin (Boehringer, Mannheim). Alkaline phosphatase converts the lumigen substrate to a light-emitting product, and the signal was detected with an X-ray film within 5 minutes exposure time. The lower band results from the recovery standard; the upper band corresponds to the amplified endogenous BDNF cDNA (Fig. 2). Both bands were analysed by laser densitometry and the quantity of endogenous mRNA determined on the basis of the size of the signal given by the known amount of standard.

RESULTS

Quantification of BDNF mRNA by reverse transcription and PCR

The low levels of BDNF mRNA detected in the developing

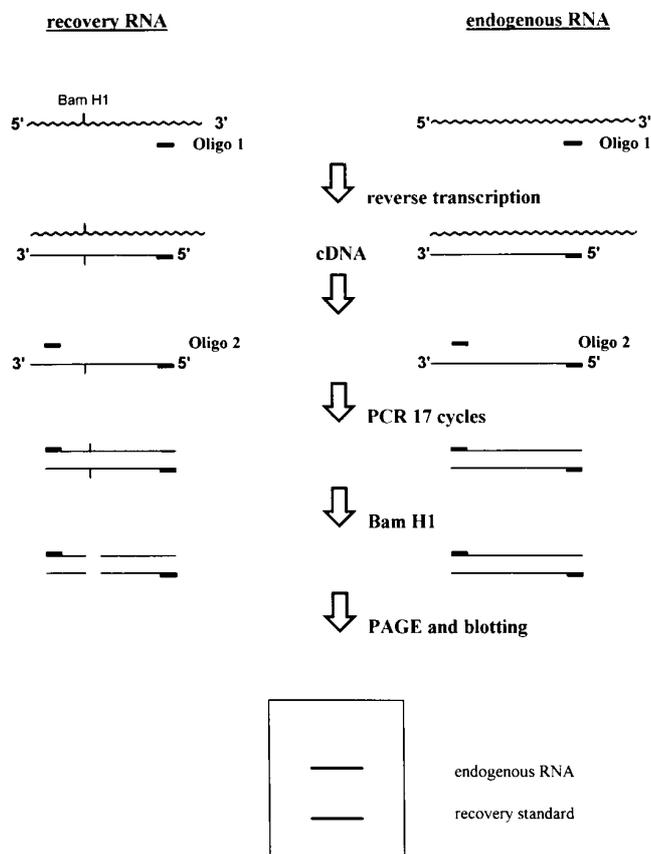


Fig. 1. Quantification method of BDNF mRNA. The recovery standard is an *in vitro* transcript of a cDNA comprising bases 26-741 of the coding region of BDNF and 7 additional nucleotides of the 3' untranslated region with one nucleotide exchanged resulting in a *Bam*HI cleavage site. A known amount of this standard is added to the tissue before RNA extraction. Following reverse transcription of the RNA, double-stranded DNA digestion, PCR is performed in one tube using Oligo 1 (which is also the primer for the reverse transcription) and Oligo 2. At the end of the PCR step, the product is cleaved using *Bam*HI, and cleaved (standard) and uncleaved (sample) DNA is separated by electrophoresis (continued until only the larger cleaved fragment is retained).

chick embryo (Hallböök et al., 1993) prompted us to use a PCR-based method using the mutated internal standard approach described by Becker-André and Hahlbrock (1989) (Figs 1, 2). This method was simplified to avoid primer competition between standard and the cDNA to be quantified by reducing the number of cycles (see Methods). The results obtained with this method were compared with those obtained after northern blot analysis using the same RNA preparations (Fig. 3). This analysis was performed using tissue (E8 tectum) where the BDNF mRNA reaches levels that can be quantified with northern blot analysis (in contrast to E6 tectum for example). The BDNF mRNA levels determined in 5 different preparations by both procedures were in close agreement (Fig. 4).

In order to exclude the possibility that the signal detected in our northern blot analysis at 4.5 kb is due to non-specific hybridisation of the riboprobe to ribosomal RNA, poly(A)⁺ mRNA was also analysed. This confirmed that the major BDNF transcript in E12 tectum is 4.5 kb in size, no signal being detected in the poly(A)⁻ fraction (data not shown). Thus, the reverse transcription PCR quantification method appears to be suitable to determine quantitatively the number of BDNF mRNA transcripts.

BDNF mRNA levels in the optic tectum and in the retina during development

In the optic tectum, BDNF mRNA could already be detected at E4, the earliest time point examined (see Fig. 5A). Subsequently, the mRNA levels decreased by a factor of 22-fold to reach a minimum at E6. This is the stage at which the first retinal axons reach the tectum (Crossland et al., 1975). There is a marked and steep increase thereafter until E11 and, during E11 and E17, the BDNF mRNA levels decrease by about 30%. It is during this period that 1.6×10^6 of retinal ganglion cells (40% of the highest number, determined at E11) are eliminated during normal development (Rager and Rager, 1978). In addition, BDNF mRNA is still clearly present in the adult tectum albeit at low levels (Fig. 5A).

Not only tectal-derived BDNF, but also retina-derived

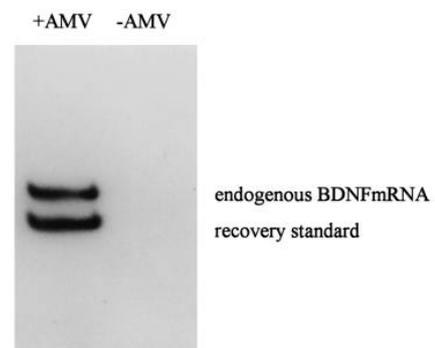


Fig. 2. Autoradiogram of the endogenous and standard BDNF DNA. RT-PCR was performed with RNA from 1 mg tissue containing 1 pg recovery standard, the products electrophoresed, blotted and detected with a digoxigenin-labelled BDNF probe. Lane 1 shows the product obtained in the presence of AMV reverse transcriptase, the lower band representing the recovery standard, the upper band originating from the endogenous BDNF mRNA. A control reaction (lane 2) was performed without reverse transcriptase.

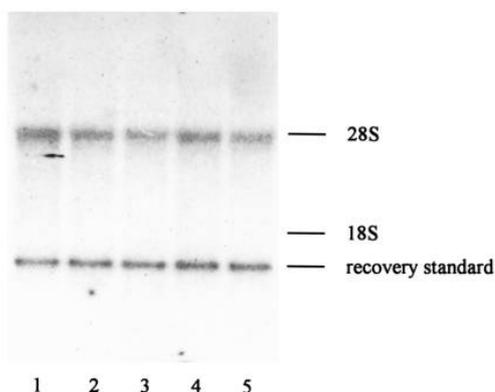


Fig. 3. Northern blot analysis with tectal RNA. E8 tectal RNA from five different samples (1-5), to which 1 pg/mg tissue recovery standard was added, was separated on 1.2% agarose gel, transferred to nylon filter and hybridised with a cRNA probe. The lower band from the recovery standard, the upper band which migrates with the 28S ribosomal RNA represents endogenous BDNF mRNA.

BDNF, could play a role in the developing visual system. For this reason, we examined the BDNF mRNA levels in the retina (Fig. 6). The *BDNF* gene is also clearly expressed in the neural retina, and there is a marked increase from E6 (the earliest time point investigated) until E11. The copy number was found to remain remarkably constant until E17. In the adult retina, it is reduced by half (Fig. 6).

BDNF mRNA tectal levels after optic stalk transection and blockade of electrical activity

The low expression levels in the tectum at E6, followed by a marked increase during the subsequent days correlate with the time during which the innervation by the retinal ganglion cell axons is established. To determine if this increase is influenced by the presence of the ingrowing retinal axons, innervation of the left tectum was prevented by transecting the right optic stalk at E4. At E7, these non-innervated tecta were compared to normal, innervated tecta. This particular time point was chosen because of the development of a transient ipsilateral projection from the retina that would complicate the analysis

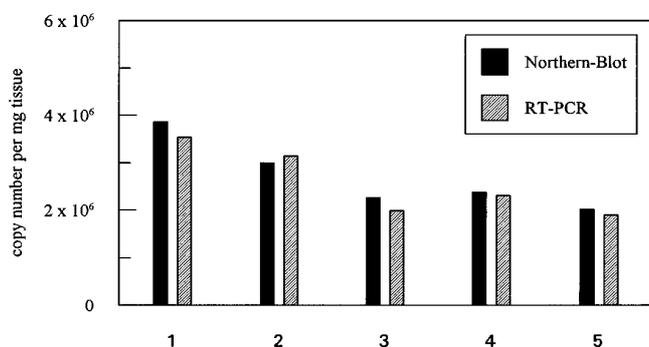


Fig. 4. Comparison of BDNF mRNA quantification using RT-PCR and northern blot analyses. The number of copies of BDNF mRNA from five different tissue samples were quantified either using Northern blot analysis (as in Fig. 2; filled bars) or RT-PCR (hatched bars). Both quantification methods give very similar results in the five samples analysed.

at later time points (Williams and McLoon, 1991). The results of these measurements (Fig. 5B) reveal that BDNF expression is reduced in the non-innervated tectum by 42.9%.

In order to determine if this decrease in BDNF mRNA levels resulting from denervation can be accounted for by mechanisms involving electrical activity, tetrodotoxin was applied into the right eye at E6 and BDNF mRNA levels of the left tectum determined at E7. This led to a reduction of BDNF mRNA levels in the tectum that is comparable in its extent to that observed after optic stalk transection (Fig. 5B).

DISCUSSION

The main objective of this study was to examine and quantify BDNF mRNA levels during the development of the chick visual system. We used a PCR-based quantification method to determine the number of BDNF mRNA molecules at critical ages during the development of both the retina and the tectum, and we found that the degree of BDNF expression in the tectum is influenced by the electrical activity of the retinal ganglion cells at the earliest stage of tectal innervation.

BDNF mRNA quantification using PCR

A previous northern blot study using 20 μ g poly(A)⁺ RNA isolated from whole embryos indicated that BDNF mRNA is present at very low levels during chick development, making quantification difficult and detection impossible at some ages like E18 (Hallböök et al., 1993). In view of this, as well as of the limited availability of tissue early in development, we used a very sensitive method based on reverse transcription of the endogenous mRNA and of an internal RNA standard followed by PCR. This method has the additional advantage that the results can readily be quantified using densitometric scanning, the signal-to-noise ratio being much more favourable than that obtained with northern blot analysis with total RNA, specially when the transcript of interest migrates at the level of the ribosomal RNAs (which is the case here with the main BDNF transcript, 4.5 kb, see Fig. 3; see also Hallböök et al., 1993). To obtain a similar amplification efficiency of the endogenous mRNA and of the recovery standard, we used the method described by Becker-André and Hahlbrock (1989) in which only one base differs in the sequences to be amplified, allowing the distinction to be made between the 2 amplification products by selective digestion at the end of the procedure. This method was simplified by using lower cycle numbers, thus avoiding primer competition between standard and endogenous mRNA and heteroduplex formation (see Becker-André and Hahlbrock, 1989). We were interested in determining not only relative, but also absolute numbers of BDNF mRNA transcripts. Indeed, neuronal survival and differentiation by neurotrophic factors like BDNF is likely to be regulated by the limited availability of such molecules, as suggested by previous experiments with NGF (see Introduction). These measurements not only revealed limited quantities of NGF protein, but also of NGF mRNA. The results obtained by the RT-PCR method were checked using quantification by northern blot analysis with total RNA preparations obtained from tissues expressing sufficient levels of BDNF mRNA, and were found to be in remarkable quantitative agreement (Fig. 4). It is interesting to note that at E6, when tectal innervation begins, the number of BDNF mRNA molecules (300,000 copies per mg of tissue) is close to that determined for NGF mRNA in the mouse whisker pad when

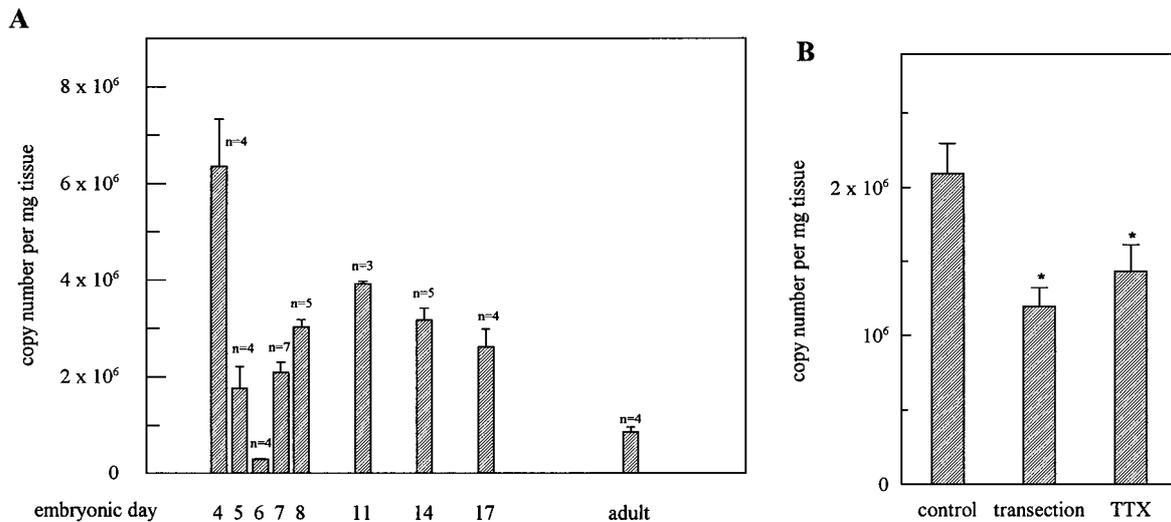


Fig. 5. BDNF mRNA copy number in the chick tectum during development (A) and after optic stalk transection or tetrodotoxin injection (B). (A) BDNF mRNA was quantified at different embryonic ages and in one adult sample using RT-PCR. *n* represents the number of separate measurements using different tissue preparations and error bars are \pm s.e.m. (B) The right optic stalk was severed at E4, or 1 μ l of a 100 μ M TTX solution was injected into the right eye at E6. At E7, the BDNF mRNA levels were determined in the left tectum, untreated tecta serving as the control. Error bars are \pm s.e.m. *different from control at $P < 0.05$ (Student's *t*-test).

innervation begins (about 150,000 of NGF mRNA per mg of tissue; Bandtlow et al., 1987; Davies et al., 1987). Also, in both cases, the number of copies increases by more than 10-fold over the following days when the bulk of the axons reach the target. We do not know the number of tectal cells, but it has been reported that the E11 chicken retina contains approximately 10^8 cells (Moscona and Moscona, 1979). In this tissue at E11, we determined 4×10^6 copies per mg tissue, i.e. approximately 1.2 transcripts per cell (in a 30 mg chick retina).

BDNF mRNA levels in the tectum and their regulation

Our study not only reveals that BDNF is expressed in the tectum, but that this expression is already evident at E4. This is before the first axons have reached the optic tectum and is in contrast with that observed with NGF in the target fields of the NGF-sensitive trigeminal neurons: expression of NGF is observed only after the arrival of the first trigeminal axons in their targets (at E10.5, Davies et al., 1987). What could be the meaning of the relatively high levels of BDNF mRNA in the tectum at E4? Neurons begin to differentiate between E3 and E4 in the chick tectum (Goldberg, 1974; Puelles and Bendala, 1978), and BDNF might regulate their phenotypic differentiation and promote neurite outgrowth. This possibility is supported by the observation (using *in situ* hybridisation) that these neurons express high levels of the putative BDNF receptor *trkB* (Biffo et al., 1994). In this context, it is interesting to note that with E4.5 dorsal root ganglion neurons (a stage at which these neurons do not require neurotrophins for their survival), BDNF has been shown to promote neurite outgrowth and differentiation (Wright et al., 1992). BDNF has also been shown to promote the phenotypic differentiation of non-committed neural crest cells along the sensory pathway (Sieber-Blum, 1991).

At E6, when the first retinal axons reach the tectum, the

BDNF mRNA expression has reached its lowest level to increase over the following days, suggesting a role for the ingrowing fibres in the regulation of BDNF. To check this possibility, the optic stalk was severed at E4, thus preventing tectal innervation. When measured at E7, the BDNF mRNA levels were found to be reduced to about 57% in the non-innervated tectum, compared to normal innervated tecta. To check whether this decrease occurs as a result of mechanisms involving electrically active retinal ganglion cells, unilateral injections of tetrodotoxin into one eye were performed at E6. When analysed at E7, the BDNF mRNA levels determined in the contralateral tectum also revealed a decrease (to about 68%, see Fig. 5B). In

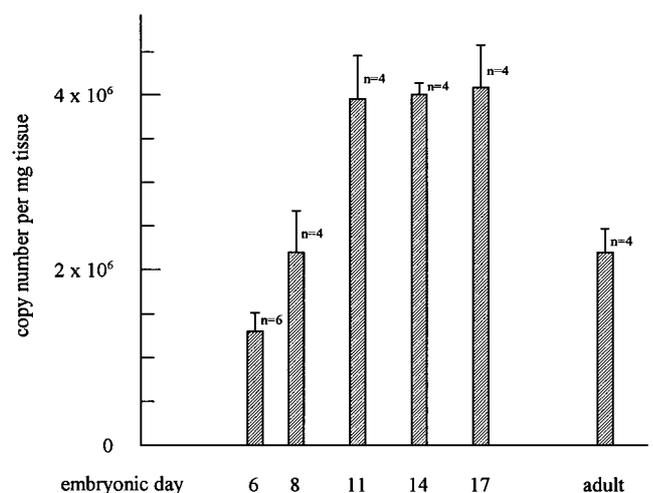


Fig. 6. BDNF expression in the embryonic retina. BDNF mRNA was quantified at different embryonic ages and in one adult sample using RT-PCR. *n* represents the number of separate measurements using different tissue preparations. Error bars are \pm s.e.m.

this context, it is interesting to note that, in higher vertebrates, retinal ganglion cells have been demonstrated to be spontaneously electrically active both in vivo (in the rat, Maffei and Galli-Resta, 1990) and in culture (Meister et al. 1991) and it is possible that one consequence of this activity is the regulation of the expression of genes such as BDNF in the target cells. What could be the role of BDNF on the developing retinal ganglion cell during the early stages of their development? A role of BDNF as a survival factor at this stage appears unlikely, since this is before the period of naturally occurring cell death of this cell population in the chick retina. Also, a previous in vitro study indicates that, at E6, the survival of retinal ganglion cells is not BDNF dependent (Rodríguez-Tébar et al., 1989), which is similar to peripheral sensory neurons early in development (Vogel and Davies, 1991). The regulated expression of BDNF at this stage is more likely to influence phenomena such as synapse formation, as the first immature synaptic contacts between ganglion cell axons and tectal cells are observed at E7 (McGraw and McLaughlin, 1980). It is worth noting in this context that BDNF has been observed in an in vitro system to increase the release of acetylcholine from spinal cord axons contacting skeletal muscle cells (Lohof et al., 1993).

While it is clear that the retinal axons influence the BDNF mRNA levels, a functional innervation appears to be only one of the mechanisms regulating the steady state levels of BDNF mRNA since denervation reduces the levels only by about 40%. Why the effects are not larger might be due to the predominance of other regulatory mechanisms or to the localisation of BDNF mRNA in cells that are not contacted by the retinal axons. While this study aimed at determining BDNF mRNA levels during the initial phase of the innervation of a CNS target, it is interesting to note that a partial fimbria-fornix transection, performed in rats several days after the beginning of target innervation, results in a 60% reduction in the BDNF mRNA levels in the hippocampus (Berzaghi et al., 1993). Pharmacological studies have demonstrated that cholinergic, glutamatergic, as well as GABAergic mechanisms are involved in the regulation of BDNF mRNA expression in the rodent hippocampus (Berzaghi et al., 1993; Dugich-Djordjevic et al., 1992; Zafra et al., 1990, 1991) and in the visual system of adult rats, a reduction of BDNF mRNA levels can be observed in the visual cortex after keeping the animals in darkness or treating one eye with tetrodotoxin (Castrén et al., 1992). Taken together with our data, these studies suggest that activity-dependent regulation of BDNF mRNA levels involving the release of classical neurotransmitters can be observed from the earliest stages of innervation in the CNS through to adulthood.

During the phase of massive retinal ganglion cell death in vivo between E11 and E17, and BDNF dependency for survival in vitro, we note that the BDNF mRNA levels in the tectum decrease by about 30%, and the reduced availability of BDNF during this developmental period might be one of the factors regulating the survival of retinal ganglion cells. Curiously in a recent study, we were unable to detect by in situ hybridisation *trkB* mRNA in the retinal ganglion cell layers of the E12 chick retina (Dechant et al., 1993). It is possible that, as with BDNF, the number of *trkB* transcripts is too low to be detected by in situ hybridisation.

BDNF expression in the retina

BDNF mRNA can be detected not only in the embryonic chick

tectum, but also in the retina. A marked increase is observed between E6 and E11, the expression levels being comparable with those determined in E11 tectum. Unfortunately, the cellular sites of BDNF mRNA expression could not be localised using in situ hybridisation, these levels being below our limit of detection. In the developing chick, the only structure where BDNF mRNA could be detected with confidence are the sensory epithelial cells of the ear (Biffo, personal communication). This confirms the findings of Hallböök et al., (1993) who did not detect BDNF mRNA by in situ hybridisation in the developing chick CNS, but did so in the developing otic vesicle. In the retina, it is unlikely that a small subpopulation of cells like the retinal ganglion cells are the exclusive sites of BDNF expression. There are about 4×10^6 such cells in the E11 retina and they would express about 30 transcripts per cell. In addition, after transection of the optic stalk (resulting in the elimination of virtually all ganglion cells), substantial levels of BDNF mRNA can still be detected at E17, which do not markedly differ from control retinae (data not shown). In the absence of cellular localisation, the role of BDNF mRNA can only be a matter of speculation. Either BDNF might play a role as an autocrine/paracrine factor for cells within the retina, or it might be taken up by the axon terminals of the isthmo-optic nucleus (ION), which reach the retina between E9 and E10 (for review see Clarke, 1992) and connect amacrine cells and displaced ganglion cells (Crossland and Hughes, 1978). 60% of the neurons of the ION are eliminated between E13 and E17 (Clarke and Cowan, 1976) and when their retinal target neurons are destroyed, almost all target-deprived neurons in the isthmo-optic nucleus degenerate (Catsicas and Clarke, 1987). It is conceivable that the survival of the ION depends on BDNF produced in the retina, specially in view of the observations that exogenous BDNF has been shown to be retrogradely transported from the retina to the ION and to support the survival of ION neurons (von Bartheld, 1993).

CONCLUSION

While this study indicates that the *BDNF* gene is expressed in the chick visual system, it also shows that the levels of expression are tightly regulated. The early expression of the *BDNF* gene in the tectum indicates that this factor is likely to play a role in the development of this structure, presumably not related to the control of neuronal survival. Retinal expression of the *BDNF* gene might be important for the development and maintenance of the innervation of the ION. Finally, the down-regulation of BDNF mRNA in the tectum following blockade of electrical activity suggests that the first retinal ganglion cells innervating the tectum are electrically active.

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