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BDNF and trkB mRNA expression in the hippocampus and temporal cortex during the human lifespan

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Abstract

Brain-derived neurotrophic factor (BDNF) and its receptor tyrosine kinase B (trkB) influence neuronal survival, differentiation, synaptogenesis, and maintenance. Using in situ hybridization we examined the spatial and temporal expression of mRNAs encoding these proteins during diverse stages of life in the human hippocampus and inferior temporal cortex. We examined six postnatal time points: neonatal (1–3 months), infant (4–12 months), adolescent (14–18 years), young adult (20–24 years), adult (34–43 years), and aged (68–86 years). Within the hippocampus, levels of BDNF mRNA did not change significantly with age. However, levels of both the full-length form of trkB ($trkB^{TK+}$) mRNA and the truncated form of trkB ($trkB^{TK-}$) decreased over the life span ($p < 0.05$). In the temporal cortex, BDNF and $trkB^{TK+}$ mRNA levels were highest in neonates and decreased with age ($r = -0.4$ and $r = -0.7$, respectively, both $p < 0.05$). In contrast, $TrkB^{TK-}$ mRNA levels remained constant across the life span in the temporal cortex. The peak in both BDNF and $trkB^{TK+}$ mRNA expression in the neonate temporal cortex differs from that previously described for the frontal cortex where both mRNAs peak in expression during young adulthood. The increase in BDNF and $trkB^{TK+}$ mRNA in the temporal cortex of the neonate suggests that neurotrophin signaling is important in the early development of the temporal cortex. In addition, since BDNF and both forms of its high affinity receptor are expressed throughout the development, maturation, and aging of the human hippocampus and surrounding neocortex they are likely to play roles not only in early growth but also in maintenance of neurons throughout life.

Keywords: Neurotrophin; Development; Neonate; Aging; In situ hybridization

1. Results and discussion

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family of growth factors which binds with high affinity to the trkB tyrosine kinase receptor. TrkB exists in a full-length form ($trkB^{TK+}$) containing a catalytic tyrosine kinase domain, and in a truncated form ($trkB^{TK-}$). Both BDNF and trkB mRNA and protein are widely distributed in the central nervous system, and are highly expressed in the mammalian hippocampus and cerebral cortex (Hofer et al., 1990; Ernfors et al., 1990a,b; Wetmore et al., 1990; Phillips et al., 1990; Huntly et al., 1992; Hayashi et al., 2000; Romanczyk et al., 2002; Weickert et al., 2003; Silhol et al., 2005). In addition to promoting the survival of neurons (Gosh et al., 1994; Lindholm et al., 1996; Lowenstein and Arsenault, 1996), BDNF appears to mediate activity-dependent synaptic plasticity, even in the mature nervous system (Kang and Shuman, 1995; Korte et al., 1995, 1996; Patterson et al., 1996; McAllister et al., 1999) and can enhance synaptic efficacy (Levine et al., 1995; Messaoudi et al., 1998). BDNF expression is increased in the hippocampus during learning-related events and by long-term potentiation (LTP), the most widely used paradigm to study events underlying neuronal plasticity (Korte et al., 1995, 1996; Ma et al., 1998; Kovalchuk et al., 2002). BDNF expression is induced in the rat hippocampus during contextual learning (Hall et al., 2000) and

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in the primate temporal cortex during declarative memory formation (Tokuyama et al., 2000).

In contrast, mice lacking the BDNF gene show abnormal LTP (Korte et al., 1995) and forebrain-restricted BDNF mutant mice show learning deficits (Gorski et al., 2003), as do mice lacking the trkB receptor (Minichiello et al., 1999). Moreover, a decrease in BDNF expression occurs in the hippocampus during aging (Hayashi et al., 1997; Murer et al., 2001) and in Alzheimer's disease (Phillips et al., 1991; Murray et al., 1994; Connor et al., 1997; Connor and Dragunow, 1998; Ferrer et al., 1999). BDNF has also been implicated in the neurobiology of schizophrenia (Weickert et al., 2003; Hashimoto et al., 2005), bipolar disorder (Nakata et al., 2003), and depression (Duman et al., 1999; Chen et al., 2001; Shirayama et al., 2002; Molnar et al., 2003).

Given the integral role of BDNF and trkB in the normal development and plasticity of the brain, in specific learning and memory processes, and in aging and neurobiological disorders, it is imperative that we understand the normal distribution and temporal profile of BDNF and trkB expression in the human brain. Previous studies in rat and primate hippocampus found higher levels of BDNF and trkB expression early in the postnatal period and then relatively stable levels throughout life (Friedman et al., 1991; Lapchak et al., 1993; Ringstedt et al., 1993; Freyer et al., 1996; Hayashi et al., 1999; Silhol et al., 2005). In contrast, in the human prefrontal cortex both BDNF (Webster et al., 2002b) and trkB^{TK+} (Romanczyk et al., 2002) mRNA levels peak in expression during young adulthood, coincident with the structural and functional maturation of the frontal cortex. The human hippocampus and medial temporal cortex may mature earlier than the frontal cortex, and so BDNF and trkB mRNA expression may show a distinct developmental profile in these brain regions. Understanding the normal profile of BDNF and trkB expression in the hippocampus and temporal cortex may further our understanding of how this system is implicated in the etiology and neuropathology of various neurobiological disorders.

1.1. Anatomical distribution of BDNF and trkB mRNA transcripts

BDNF mRNA hybridization signal was evident throughout the gray matter of the hippocampal formation and temporal cortex (Fig. 1). The autoradiographic films showed a robust signal over the granule cells of the dentate gyrus (arrow, Fig. 1A). Inspection of the dipped sections revealed that the majority of granule cells were labeled, as were almost all the pyramidal cells of the CA subfields. In BA20, BDNF mRNA hybridization was also apparent over the majority of pyramidal neurons with a slight increase in intensity of signal over the middle and deep layers as previously described in the frontal cortex (Weickert et al., 2003). In the younger age groups there was also a slight increase in intensity over layer II.

The trkB^{TK+} mRNA signal was more intense over the neurons of the CA subfields than over the dentate granule cells of the hippocampus (arrow Fig. 1D). In BA20, the trkB^{TK+} mRNA signal was highest in layers II and IV. Inspection of dipped sections revealed that trkB^{TK+} mRNA silver grains were located over both pyramidal and non-pyramidal neurons as previously described in the frontal cortex (Romanczyk et al., 2002). In contrast, trkB^{TK-} mRNA was homogeneously distributed over the gray and white matter of the hippocampal formation and BA20 and was expressed at relatively lower levels than BDNF or trkB^{TK+} mRNA. No laminar pattern was apparent in the cortex. Inspection of dipped sections demonstrated silver grains over small darkly stained glial cells in all cortical layers and in the white matter as previously described in the frontal cortex (Romanczyk et al., 2002). However, not every glial cell profile appeared to be labeled.

1.2. Quantitative age-related differences in the hippocampus

ANOVAs to determine the effect of age on BDNF mRNA density in each individual subregion of the hippocampus revealed no significant effect of age in any of the regions (Fig. 2A). Nevertheless, in the dentate gyrus there is a 29% increase in BDNF mRNA density between the neonate and adolescent group and then a 29% decrease in density between the adolescent group and the adults. Thus, while not statistically significant the highest mean value for BDNF mRNA expression in the dentate occurs during the adolescent–young adult period. BDNF mRNA levels appear to be expressed at constant levels in the other hippocampal regions at the time points examined.

TrkB^{TK+} mRNA density tended to decrease with advancing age across the subregions of the hippocampus, particularly in the dentate gyrus, CA1, and subiculum (Fig. 2B). ANOVAs to determine if trkB^{TK+} mRNA density varied according to age group in the hippocampal subregions did not reveal any significant effect of age, however, a correlation analysis revealed that in the dentate gyrus, there was a significant correlation between trkB^{TK+} mRNA density and age from the infant to aged groups ($r = -0.39$; $p = 0.045$) with a 37% reduction in density from infancy to aged. In the subiculum, trkB^{TK+} mRNA density correlated with age from the neonatal to the aged group ($r = -0.44$; $p = 0.01$).

TrkB^{TK-} mRNA levels also decreased across the life span in the hippocampus. ANOVAs to determine if trkB^{TK-} mRNA density varied according to age group revealed a significant effect of age group in CA3 ($F = 2.6$; df = 5,25; $p = 0.05$) and in subiculum ($F = 3.4$; df = 5,26; $p = 0.02$, Fig. 2C). Post hoc least significant differences (LSD) tests revealed that in CA3 the trkB^{TK-} mRNA level in the adult group was significantly lower than in the infant ($p = 0.04$) and adolescent groups ($p = 0.03$) and the aged group was significantly lower than in infants ($p = 0.02$), adolescents ($p = 0.01$) and young adults ($p = 0.03$). In the subiculum, the adult group was significantly lower than

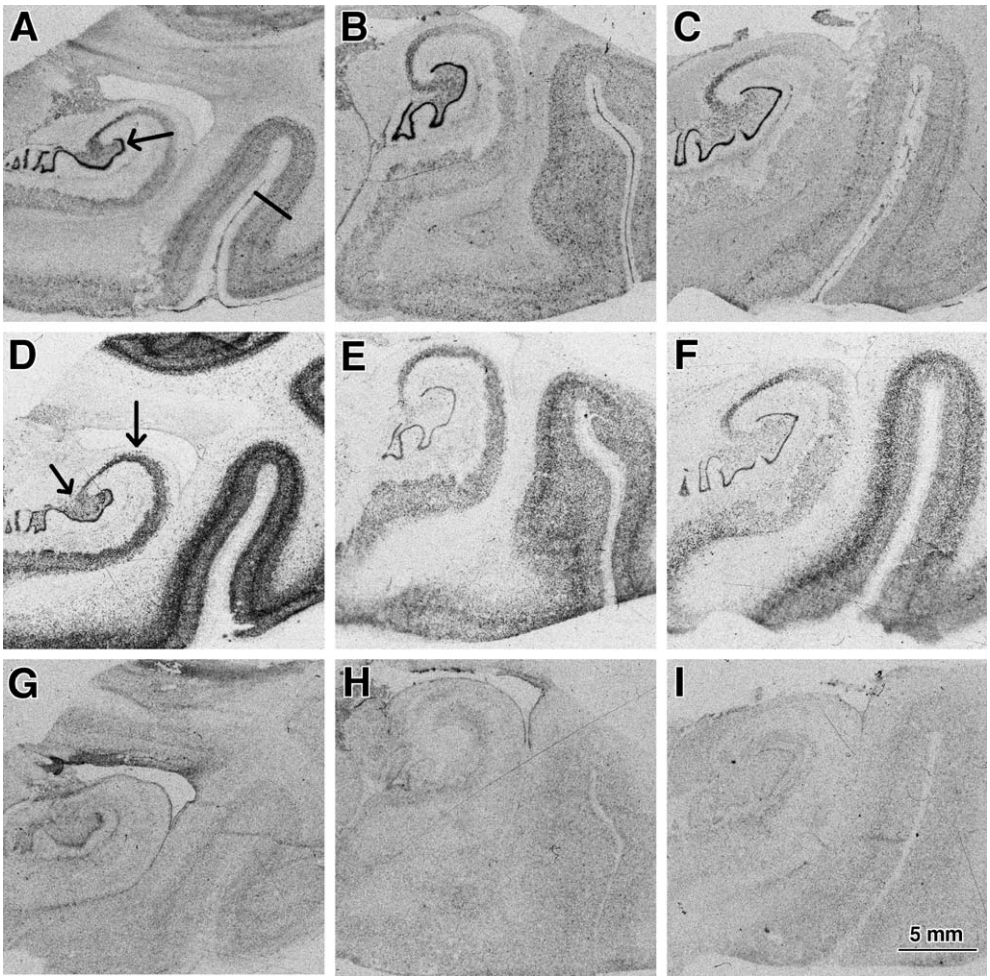

Fig. 1. Autoradiographs of BDNF (A, B, and C), Trk^{TK+} (D, E, and F) and Trk^{TK-} (G, H, and I) mRNA hybridization in the hippocampal formation from representative cases (neonates A, D, and G; young adults B, E, and H; aged C, F, and I). Arrow in (A) indicates BDNF mRNA signal in the dentate gyrus granule cells. Arrows in (D) indicate Trk^{TK+} mRNA signal in CA4 and CA3. The line, perpendicular to the pial surface in (A), represents the approximate location of the measurements taken across the inferior temporal cortex.

the neonate ($p = 0.004$), adolescent ($p = 0.03$), and young adult ($p = 0.04$) groups and the aged group was significantly lower than the neonate ($p = 0.005$) and adolescent ($p = 0.03$) groups. Thus, $trkB^{TK-}$ mRNA levels appear to be highest in the neonates and decline over time to lowest levels in the aged group. Indeed, correlation analysis showed a statistically significant negative correlation between age and $trkB^{TK-}$ mRNA levels in CA4 ($r = -0.45$; $p = 0.009$), CA3 ($r = -0.477$; $p = 0.007$), and subiculum ($r = -0.39$; $p = 0.03$) across all age groups and a trend toward statistical significance in the dentate gyrus ($r = -0.34$; $p = 0.057$).

1.3. Quantitative age-related differences in the temporal cortex

Fig. 3A shows the profile of mean BDNF mRNA density for the five age groups as a function of cortical width. The BDNF mRNA levels were higher in the neonatal group and showed a decrease of 33% between the neonate and adult groups. A correlation analysis across all ages showed a significant negative effect of increasing age on BDNF mRNA levels ($r = -0.397$; $p = 0.02$). Thus, BDNF mRNA levels appear to gradually decline over the postnatal period. However, an ANOVA with BDNF mRNA AUC data comparing the full cortical width of temporal cortex across age groups was not significant ($F = 1.94$; $df = 5,27$; $p = 0.12$).

In order to determine if BDNF mRNA levels differed in any particular cortical layer as a function of age, six separate one-way ANOVAs were conducted, one for each cortical layer using age group as an independent between group factor and BDNF density as the dependent variable. There was a significant effect of age in layer III only ($F = 2.6$; $df = 5,27$; $p = 0.047$). Post hoc LSD testing revealed that in layer III the neonates expressed more BDNF mRNA than the adolescent, adult, and aged groups ($p < 0.04$, 0.006, and 0.004, respectively).

$TrkB^{TK+}$ mRNA density was also highest in the neonatal group (Fig. 3B). The profile plot of mean $trkB^{TK+}$ mRNA density for the five age groups as a function of cortical width shows elevated levels occurring early in life in

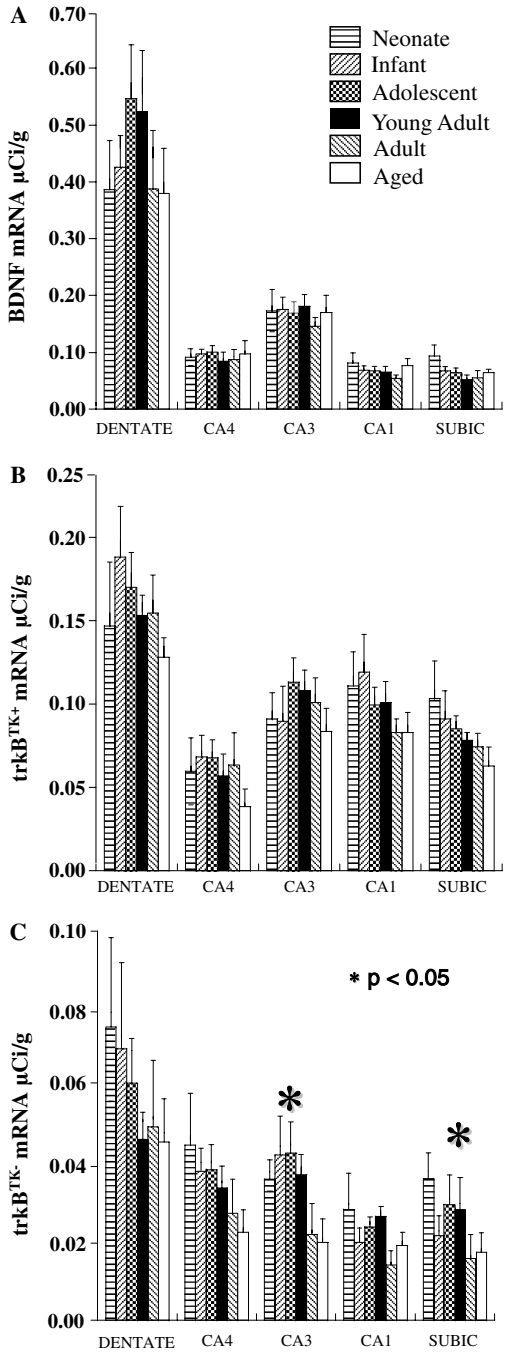

Fig. 2. Graphs demonstrating mean (A) BDNF mRNA, (B) TrkB^{TK+} mRNA, and (C) TrkB^{TK-} mRNA (μCi/g) in the hippocampal subfields, for the six age groups.

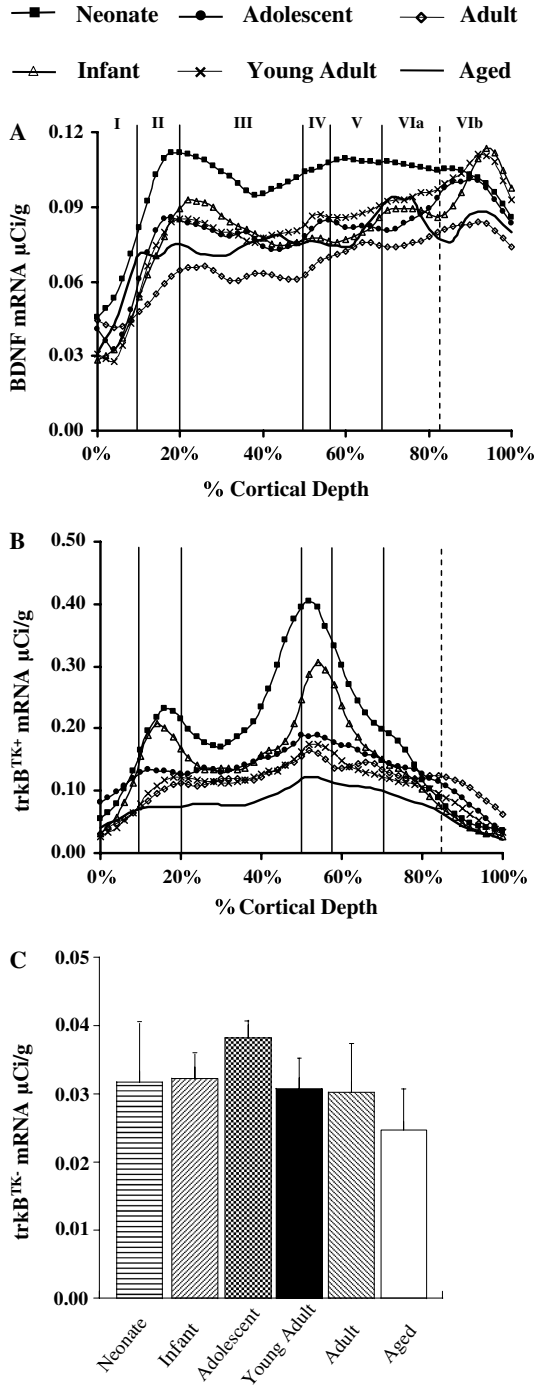

Fig. 3. Profiles showing the mean (A) BDNF mRNA and (B) TrkB^{TK+} mRNA levels (μCi/g) for the six age groups across the width of the temporal cortex and (C) a graph summarizing the mean TrkB^{TK-} mRNA (μCi/g) of the temporal cortex for the six age groups.

layers II and IV. ANOVA with AUC data revealed a significant effect of age group ($F = 7.9$; df = 5,26; $p < 0.0001$). Post hoc LSD tests demonstrated that the neonate group has significantly higher trkB^{TK+} mRNA levels as compared to all other groups ($p < 0.01$) while the aged group has significantly lower levels as compared to all other groups ($p < 0.02$). A correlation analysis across all subjects showed a very significant relationship between age and cortical trkB^{TK+} mRNA levels ($r = -0.68$; $p < 0.0001$) indicating that as humans age, less full-length trkB mRNA is expressed in the temporal cortex. In order to determine in which cortical layers trkB^{TK+} mRNA differed as a function of age, six separate one-way ANOVAs were conducted. There was a significant effect of age in all layers except layer I. Post hoc LSD testing revealed that in layers II and IV the neonate and infant groups expressed significantly more trkB^{TK+} than in all other groups ($p < 0.002$). In addition, the adolescent and young adult

groups expressed significantly more than the aged group ($p < 0.02$). In layer III, the neonatal group expressed more than all other groups ($p < 0.007$) and the aged group expressed significantly less than all groups ($p < 0.04$) except the adults. In layer V, the neonate group expressed significantly more trkB^{TK+} mRNA than all other groups ($p < 0.02$) except the infants, and the aged group expressed significantly less than the infant, neonate, and adolescent groups ($p < 0.005$). In layer VIA, the neonates and adolescents expressed significantly more than the aged group ($p < 0.04$) and in layer VIB the adults expressed more than all groups except the adolescents ($p < 0.02$).

In contrast to the decrease in trkB^{TK+} mRNA levels occurring across the human lifespan in the temporal cortex, trkB^{TK-} mRNA appears to be expressed at constant levels across the ages examined. ANOVA comparing the mean trkB^{TK-} mRNA levels for the five age groups revealed no significant differences ($F = 0.8$; df = 5,27; $p = 0.5$; Fig. 3C) and there was no correlation between age and cortical trkB^{TK-} mRNA levels ($r = -0.23$).

1.4. Effect of descriptive variables

The postmortem pH values ranged from 5.62 to 6.76. The six age groups did not differ significantly in pH levels ($F = 0.37$, df = 5,28, $p = 0.87$). The PMI values ranged from 13.5 to 66.5 h. The PMIs for the age groups also did not differ significantly ($F = 1.22$, df = 5,28, $p = 0.32$).

In the hippocampus, BDNF mRNA was significantly positively correlated with PMI in CA1 and also with pH in CA3. TrkB^{TK+} mRNA was negatively correlated with PMI in CA4 and positively correlated with pH in dentate, CA4, CA3, and subiculum. TrkB^{TK-} mRNA was positively correlated with pH in dentate, CA4, and CA3.

In the temporal cortex there were no significant correlations between BDNF mRNA levels and PMI or pH. TrkB^{TK+} mRNA was negatively correlated with PMI in layer VI and positively correlated with pH in layers II–VI. TrkB^{TK-} mRNA levels were also negatively correlated with PMI in the cortex. ANCOVA with PMI and pH as a covariate did not alter the statistical significance in any of the regions for BDNF or either trkB transcript.

There was no effect of gender on BDNF or trkB mRNA levels in the neonates or infants (the only groups with both males and females). There was no effect of hemisphere on BDNF or trkB mRNA levels.

1.5. Conclusion

In the hippocampus, levels of BDNF mRNA did not change significantly with age. However, levels of the full-length form of the trkB (trkB^{TK+}) receptor and the truncated form (trkB^{TK-}) decreased over the life span. In the temporal cortex, levels of BDNF and the full-length trkB receptor were highest in the neonates and decreased with age while trkB^{TK-} mRNA remain constant across the life span. This is in contrast to cyclophilin mRNA, used as a control, which shows slight variation across development in the hippocampus but does not change in the surrounding temporal cortex (Law et al., 2003a,b). The developmental changes that we detect in the different forms of the trkB transcripts may be due to unequal degradation rates of a particular transcript due to individual variations in postmortem interval, agonal events surrounding death or experimental variation. However, we find that the same neurotrophin or neurotrophin receptor transcript is expressed at different levels in different brain regions of the same individuals, on the same tissue slice, depending on developmental age. This argues against variations in transcript-specific degradation rates as a primary determinate of neurotrophin or neurotrophin receptor mRNA levels as one would expect the unequal degradation rates to influence the different brain regions equally. It is also possible that uncontrolled pre-mortem factors that are known to influence BDNF mRNA levels, like the amount of the brain stimulation or exercise prior to death, could increase the variance in BDNF mRNA levels within a particular group and mask any changes in mRNA expression that occurs across the life span.

While there may be significant obstacles to the study of gene expression in human postmortem brain tissue, we have successfully minimized the potential postmortem variability and confounders in this cohort by collecting all specimens in a standardized manner from one collection site and by monitoring the pH of the tissue (Johnston et al., 1997; Webster et al., 2002a; Li et al., 2004; Altar et al., 2005). Furthermore, by screening all brain tissue from all specimens with cyclophilin and actin in situ hybridization before their inclusion in a cohort, we ensure that only those specimens meeting all inclusion criteria, including robust control RNA signal, are included in the cohort. The cohort studied here has been appropriately matched for PMI, cause of death (Romanczyk et al., 2002) and pH, factors that potentially effect gene expression. Moreover, covarying for PMI and pH, were appropriate, did not differentially affect the statistical outcome. Furthermore, multiple probes have been examined in this cohort and each mRNA examined shows a distinct developmental pattern using the same in situ hybridization protocol on tissue sections from the same or overlapping populations of cases. Previous studies have examined the age-related expression of mRNAs for BDNF in frontal and occipital cortex (Webster et al., 2002b), trkB^{TK+} and trkB^{TK-} (Romanczyk et al., 2002), and trkC in the frontal cortex (Beltaifa et al., 2005), NMDA receptor subunits NR1 and NR2 in the hippocampus, parahippocampal and perirhinal cortex (Law et al., 2003a,b), and the glucocorticoid receptor in hippocampus, frontal cortex, temporal cortex, and occipital cortex (Perlman et al., 2006). These studies all show unique patterns of mRNA expression across the life span that taken together suggest that the pattern of results achieved in the current study is not a function of any systematic variation in tissue quality or processing, or methodology employed.

In this study, we show that there is differential expression of BDNF and trkB mRNA in both the hippocampus and temporal cortex over the human lifespan. In the hippocampus, we find that BDNF mRNA is expressed at relatively constant levels over the human lifespan whereas trkB^{TK+} is higher earlier in postnatal life in the dentate gyrus and subiculum. Previous studies in the hippocampus of rat and monkey showed an increase in both BDNF and trkB^{TK+} very early in the postnatal period (Friedman et al., 1991; Lapchak et al., 1993; Freyer et al., 1996; Hayashi et al., 1999) followed by relatively constant levels through adulthood. The significance of this increased level of trkB^{TK+} early in life in the hippocampus is not known, but the relative temporal conservation of mRNA expression across species suggests that it may have a unique role in hippocampal circuitry in the newborn. Recent studies using exon-specific BDNF mRNA probes have shown that the developmental regulation of BDNF may be more complex than what can be realized with a pan probe (Sathanoori et al., 2004). Since we used a pan BDNF probe generated from a cDNA fragment containing the common exon (exon 5) we would not have detected differential developmental expression of hippocampal BDNF from different promoter usage (Timmusk et al., 1993; Timmusk and Metis, 1994). Nonetheless, our results showing constant levels of the BDNF exon that codes for the BDNF protein throughout life suggest that maintaining high levels of BDNF throughout life may be necessary for hippocampal function.

The relatively constant expression of BDNF mRNA that we find in the CA subfields of the hippocampus across the life span may reflect the role of BDNF in synaptic plasticity that is essential for learning and memory throughout life. BDNF/trkB signaling mediates activity-dependent synaptic plasticity, even in the mature nervous system (Kang and Shuman, 1995; Korte et al., 1995, 1996; Patterson et al., 1996; McAllister et al., 1999) and is involved in several processes that may enhance specific learning and memory processes (Levine et al., 1995; Li et al., 1998; Messaoudi et al., 1998). BDNF expression is induced in the hippocampus during learning and memory tasks (Hall et al., 2000; Mizuno et al., 2000) and mice lacking the BDNF gene show abnormal long-term potentiation (Korte et al., 1995). BDNF and trkB forebrain-restricted null mutant mice both show deficits in hippocampus-dependent learning tasks (Minichiello et al., 1999; Gorski et al., 2003). While BDNF and trkB mRNA levels do not appear to decline during aging in the rat hippocampus (Lapchak et al., 1993; Freyer et al., 1996; Silhol et al., 2005), there is evidence that BDNF mRNA levels decrease in the hippocampus of the aged monkey (Hayashi et al., 1997). We did not find a significant decrease in BDNF mRNA levels in the aged human hippocampus. However, trkB^{TK+} mRNA levels did appear to decline in several of the hippocampal subfields in the aged human hippocampus, most notably in the subiculum. Thus, although BDNF may be in adequate supply in the aged hippocampus, a decline in the trkB receptor would nevertheless compromise hippocampal function and may contribute to the decline in congnitive function that accompanies normal senescence. BDNF and TrkB are both reduced in the hippocampus of individuals with Alzheimer's disease (Phillips et al., 1991; Murray et al., 1994; Connor et al., 1997; Ferrer et al., 1999), although it is unclear as to whether the decline is a primary or secondary pathological event (Connor et al., 1997).

In contrast to BDNF, trkB^{TK-} mRNA levels show a marked decline with advancing age across most hippocampal subfields. TrkB^{TK-} mRNA signal is detected over glial cells in the gray matter regions which is consistent with other reports (Frisen et al., 1993: Rudge et al., 1994; Biffo et al., 1995; Roback et al., 1995). The trkB^{TK-} receptor on astrocytes can bind BDNF with high affinity (Biffo et al., 1995; Freyer et al., 1997; Rubio, 1997), internalize the BDNF and then the astrocytes can re-excrete the BDNF (Rubio, 1997). TrkB^{TK-} thereby acts to sequester and/or redirect the availability of neurotrophins (Biffo et al., 1995; Rubio, 1997). While it has been shown that hippocampal-dependent learning and memory functions decline with normal aging (Rapp and Gallagher, 1996; Barnes et al., 1997; Gallagher and Rapp, 1997; Rapp and Amaral, 1992; Rapp et al., 1996), it is apparent that this decline is not due to a loss of hippocampal neurons (Rapp and Gallagher, 1996; reviewed by Morrison and Hof, 1997; Gallagher and Rapp, 1997) but may be due to a decline in the functional integrity of the cells, e.g., LTP is adversely affected by the aging process (Barnes, 1979; Barnes and McNaughton, 1985; deToledo-Morrell et al., 1988). Moreover, it is interesting to note that astrocytes also do not decline with advancing age but rather increase in number and reactivity (de Vellis, 2002). The increase in astrocytic proliferation and reactivity has been interpreted as a consequence of an increasing need for neuronal protection in the aging brain. Thus, the converse decrease in trkB^{TK-} mRNA that occurs with advancing age may be a consequence of the decrease in functional integrity of these astrocytes and may contribute to a decrease in neuronal plasticity and to a decline in the maintenance of the hippocampal circuitry that underlies learning and memory.

In the temporal cortex BDNF and trkB^{TK+} mRNA levels are highest during the neonatal period and decline with advancing age. A similar profile has been described for the rodent cortex (Ringstedt et al., 1993; Linnarsson et al., 1997; Katoh-Semba et al., 1998) and for the temporal cortex of the monkey (Hayashi et al., 1997; Ohira et al., 1999). In contrast, trkB^{TK-} mRNA is expressed at constant levels throughout the life span of the human temporal cortex. The lack of change in levels of trkB^{TK-} together with the decrease in trkB^{TK+} mRNA suggests that the ratio of trkB^{TK-} to trkB^{TK+} may change in the temporal cortex as individuals age, thus potentially altering the availability of, and neuronal response to, neurotrophins late in human life. TrkB^{TK-} in non-neuronal cells inhibits BDNF-induced neurite outgrowth in vitro (Freyer et al., 1997). Consequently, in regions where the ratio of trkB^{TK-} to

trkB^{TK+} increases with age, neuronal plasticity is likely to decrease. The inferior temporal cortex (BA 20) is a visual association area, possibly homologous to area TE in the monkey, which is a final stage in the processing of visual information and is essential for processing of visual memory (Mishkin et al., 1983; Desimone and Ungerleider, 1989). A decrease in neuronal plasticity within BA20 with age is likely to have an effect on visual memory processing.

In contrast to the temporal cortex, in the human prefrontal cortex, both BDNF (Webster et al., 2002b) and trkB^{TK+} (Romanczyk et al., 2002) mRNA levels peak in expression at a time during young adulthood that is coincident with the maturation of the frontal cortex, both structurally and functionally. Moreover, in the primary visual cortex of the rat, BDNF mRNA levels increase dramatically between birth and opening of the eyes (Bozzi et al., 1995; Shoups et al., 1995), and altering normal visual experience will influence levels of BDNF mRNA in the visual cortex (Castren et al., 1992; Bozzi et al., 1995; Shoups et al., 1995; Capsoni et al., 1999). It is not surprising then, that in humans, who are born with their eyes open, the level of BDNF mRNA in the visual cortex is already high at birth and does not appear to decline with age (Webster et al., 2002a,b). Thus, the peak in expression levels of both BDNF and trkB^{TK+} mRNA that we find in the temporal cortex in the neonatal period would suggest that the temporal cortex matures later than the primary visual cortex but earlier than the prefrontal cortex. There is evidence to suggest that the association cortices mature later than primary sensory cortex (Yakovlev and Lecours, 1967; Huttenlocher, 1990, 1994; Sowell et al., 1999), however, there may also be differential molecular expression across the life span within the various association areas. Maturation of the sophisticated cognitive functions of the prefrontal cortex may continue to require modification of the molecular elements, including neurotrophic expression, into much later stages of life than in the temporal association cortex.

The profiles for BDNF, trkB^{TK+} and trkB^{TK-} mRNA expression are quite unique for each anatomical area that we have studied so far. The areas include the frontal (Webster et al., 2002b; Romanczyk et al., 2002), and occipital cortex (Webster et al., 2002b), and now the temporal cortex and hippocampal subfields. Disrupting these unique expression patterns at specific time points could interrupt the normal structural and functional development of an area and lead to very different disorders, depending on the timing and area involved.

2. Experimental procedures

2.1. Processing of the tissue

Postmortem samples of the medial temporal lobe were collected by the Clinical Brain Disorders Branch at NIMH (Bethesda) through the Medical Examiner's Office of the District of Columbia, as previously described (Kleinman et al., 1995). The samples were obtained from 34 subjects ranging in age from 5 weeks to 86 years. The brains were divided into six groups based on age, and included neonates (1–3 months), infants (4–12 months), adolescents (14–18 years), young adults (20–24 years), adults (34–43 years), and elderly (68–86 years). Demographic data on age, brain pH and postmortem interval (PMI) for each subject are listed in Table 1. The individuals used in this study overlap with the subjects described in our previous studies (Romanczyk et al., 2002; Webster et al., 2002a,b; Law et al., 2003a,b).

All subjects were free of neurologic or psychiatric symptoms. Neuropathological examination, including Bielschowsky's silver stain of multiple areas, ruled out those cases with gross pathological or microscopic lesions such as neuritic pathology or vascular disease. Brain pH was measured as described previously (Johnston et al., 1997; Romanczyk et al., 2002). Because brain pH is correlated with the expression level of many transcripts (Webster et al., 2002a; Li et al., 2004; Altar et al., 2005) the mean pH of each age group was matched. RNA integrity was determined for each case by cyclophilin mRNA in situ hybridization of adjacent sections. These data, previously published (Law et al., 2003a), indicated that while less cyclophilin mRNA was found in the neonates as compared to the other groups, the neonates nevertheless expressed more transcripts of interest than the other groups (Law et al., 2003a,b). Moreover, normalizing to the cyclophilin levels did not change the statistical significance of the results indicating that with this cohort and this technique we have the power to detect developmental changes in gene expression in this region.

2.2. Riboprobe design

The complete coding sequence of the human BDNF cDNA (M161176) (Maisonpierre et al., 1991) in bluescript vector was generously provided by George D. Yancopoulos at Regeneron Pharmaceuticals Inc. (Tarrytown, NY). An Apa 1 fragment (511 bp) of this full-length human BDNF cDNA was subcloned into Bluescript (SK−) vector. The insert corresponded to base pairs 704–1214 (Accession No. M61176). Antisense riboprobes and sense strand RNAs were generated from linearized plasmid using either a T7 or T3 polymerase and an in vitro transcription kit as recommended by the manufacturer (Promega). ^{35}S antisense and sense riboprobes were labeled to a specific activity of $1–2 \times 10^9$ cpm/μg by addition of radiolabeled UTP and were purified by ethanol precipitation.

For the trkB^{TK+} cDNA clone, a 216 base pair template corresponding to the region containing the 3′ end of the transmembrane domain ending just 5′ to the tyrosine kinase domain (base pairs 1753–1969, Accession No. U12140) was amplified. The ^{35}S antisense and sense riboprobes were labeled to a specific activity of 2.6×10^9 cpm/μg. The trkB^{TK-} cDNA template was a 431-bp insert corresponding to the region just after the transmembrane domain, which lacks the kinase domain (base pairs 907–1093, Accession No. X75958). The forward and reverse primer sequences for amplifying trkB^{TK+} or trkB^{TK-} are detailed in Romanczyk et al. (2002). The transcripts recognized by our BDNF, trkB^{TK+}, and trkB^{TK-} riboprobes were previously described (Romanczyk et al., 2002; Weickert et al., 2003).

2.3. In situ hybridization histochemistry

Frozen blocks from the medial temporal lobe were sectioned in the coronal plane at 14 μm thickness, mounted on gelatin subbed slides and stored at −70 °C. The in situ hybridization histochemistry (ISHH) was performed according to the protocol of Whitfield et al. (1990), on a total of six slides/case (two slides/probe). After hybridization and washing steps, sections were exposed to autoradiographic film (Bio-Max, Kodak) for 2 weeks (BDNF), 5 days (trkB^{TK+}) or 12 days (trkB^{TK-}). The sections were then dipped in photographic emulsion (Kodak, NTB2) for 17 weeks (BDNF), 9 weeks (trkB^{TK+}) or 12 weeks (trkB^{TK-}) and then developed and lightly counterstained with thionin. All sections for each probe were assayed together to eliminate interassay variability.

2.4. Image analysis

Calibrated densitometric analysis (NIH Image 1.33) of autoradiograms was conducted, blind to age, on regions of the hippocampal formation delineated microscopically from the Nissl-stained sections according to

Table 1
Demographic details of subjects

	Subject #	Gender	Race	Age	PMI (h)	Hemisphere	Brain Weight (g)	pH
Neonates	001	F	AA	18 W	20	R	560	6.35
	002	F	AA	9 W 5 D	56	L	500	6.76
	003	F	AA	16 W	18.5	R	550	6.69
	005	M	AA	13 W 2 D	59.5	L	750	6.34
	006	F	AA	5 W 1 D	64	R	510	6.3
Means	$N = 5$			12 W	43.6		574	6.49
Infants	008	F	AA	20 W 4 D	40.5	L	875	6.26
	009	F	AA	37 W 2 D	60	R	900	6.5
	010	M	AA	50 W 5 D	37.5	R	1040	6.4
	011	F	AA	25 W 2 D	31.5	R	800	6.74
	012	M	AA	21 W 4 D	38	L	750	6.69
Means	$N = 5$			31 W	41.5		873	6.52
Adolescents	013	M	AA	18 Y	56.5	R	1375	6.3
	014	M	AA	18 Y	36.5	R	1210	6.64
	015	M	AA	18 Y	21.5	R	1250	6.66
	017	M	AA	18 Y	14.5	R	1430	6.57
	018	M	AA	14 Y	17	L	1430	6.27
	019	M	AA	15 Y	21	R	1510	6.41
	020	M	AA	18 Y	21.5	L	1440	6.68
	021	M	AA	17 Y	59.5	L	1450	5.62
Means	$N = 8$			17 Y	31		1387	6.4
Young adults	022	M	AA	24 Y	43.5	R	1350	6.69
	023	M	AA	22 Y	38	R	1300	5.85
	024	M	AA	20 Y	32.5	L	1370	6.39
	025	M	AA	21 Y	28.5	L	1410	6.18
	026	M	AA	24 Y	21.5	R	1450	6.5
	027	M	AA	23 Y	16.5	L	1430	6.77
Means	$N = 6$			22 Y	30.08		1385	6.40
Adults	028	M	AA	43 Y	24.5	R	1480	6.44
	029	M	AA	35 Y	55.5	R	1250	6.37
	030	M	AA	34 Y	13.5	L	1340	6.31
	031	M	AA	43 Y	23	L	1500	6.71
Means	$N = 4$			38 Y	29.13		1392.5	6.46
Aged	032	M	AA	86 Y	45	L	1250	6.26
	033	M	AA	71 Y	40.5	R	1450	6.25
	034	M	AA	74 Y	42	R	1400	6.39
	035	M	AA	83 Y	66.5	L	1200	5.88
	036	F	Asian	78 Y	23	R	1220	6.53
	037	M	AA	68 Y	58	R	1220	6.61
Means	$N = 6$			76 Y	45.83		1290	6.32

F, female; M, male; AA, african american; W, weeks; D, days; Y, years; PMI, postmortem interval; R, right; L, left.

the criteria of Amaral and Insausti (1990). The regions analyzed included the granule cells of the dentate gyrus, CA_4, CA_3, CA_1, and subiculum. Three areas of interest were analyzed per region.

Brodmann's area 20 (BA20) of the temporal cortex was delineated microscopically from the Nissl-stained sections. For each section, three lines (230 μm in width) were drawn perpendicular to the pial surface on the lateral bank of the collateral sulcus and traversed the entire cortical gray matter, as indicated in Fig. 1A. Continuous optical density measurements were recorded along the length of these lines. The density of BDNF and $trkB^{TK+}$ (μCi/g) mRNA as a function of cortical depth was computed from optical density data using the radioactive standards. The percent cortical depth occupied by individual cortical lamina was calculated from Nissl-stained sections. The percentage of full cortical width corresponding to the individual lamina was as follows: I (1–10%), II (11–20%), III (21–48%), IV (49–58%), V (59–70%), VIA (71–84%), and VIB (85–100%). Because $trkB^{TK-}$ mRNA density did not vary across cortical layers, three ROIs were taken from the cortex of each case and averaged.

2.5. Statistical analyses

Group differences in demographic variables [pH and postmortem interval (PMI)] were tested with a one-way analysis of variance (ANOVA). Correlation analyses were also performed between mRNA density values and demographic variables. In the hippocampus, mRNA levels in the different age groups were compared in each hippocampal region using an ANOVA. For BA 20, a plot of BDNF and $TrkB^{TK+}$ mRNA density as a function of cortical width was generated for each subject based on the mean of the three measurements made per subject. From this plot, a single

measure of mRNA density for each subject was generated by calculating the area under the curve (AUC). AUC calculations were done with Number Cruncher Statistical Systems program (NCSS, Kaysville, Utah, 1999). AUC measurements for the different age groups were compared using ANOVA. For trk^{TK-} the mRNA density from the ROI measurements were compared across different age groups with an ANOVA. Correlation analysis were also performed between the mRNA density values and age for each transcript in each brain region.

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