Journal of Chemical Neuroanatomy 41 (2011) 219–226

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Review

Development by environment interactions controlling tryptophan hydroxylase expression

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ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 19 April 2011
Accepted 6 May 2011
Available online 25 May 2011

Keywords:
Tryptophan hydroxylase
Tph1
Tph2
Serotonin
Dorsal raphe nucleus

ABSTRACT

Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of serotonin (5-hydroxytryptamine; 5-HT). Two isoforms of tryptophan hydroxylase, derived from different genes, *tph1* and *tph2*, have been identified. The *tph1* isoform is expressed in peripheral tissues, whereas *tph2* is brain and neuron-specific. Recent studies suggest that *tph2* expression and brain serotonin turnover are upregulated in depressed suicide patients, and drug-free depressed patients, respectively. Increased *tph2* expression could result from genetic influences, early life developmental influences, adverse experience during adulthood, or interactions among these factors. Studies in rodents support the hypothesis that interactions between early life developmental influences and adverse experience during adulthood play an important role in determining *tph2* expression. In this review, we highlight the evidence for the effects of adverse early life experience and stressful experience during adulthood on both *tph1* and *tph2* expression.

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1. Introduction

Tryptophan hydroxylase is the rate-limiting enzyme in the biosynthesis of serotonin (5-hydroxytryptamine; 5-HT). Two isoforms have been identified, which are derived from different genes, tryptophan hydroxylase 1 (*tph1*) and tryptophan hydroxylase 2 (*tph2*). *Tph1* is expressed predominantly in peripheral tissues, while *tph2* is expressed in serotonergic neurons within the central nervous system. Recent studies suggest that *tph2* expression within the dorsal raphe nucleus (DR), an important source of serotonergic innervation of limbic forebrain structures, is elevated in depressed suicide patients (Bach-Mizrachi et al., 2006, 2008; Boldrini et al., 2005; Bonkale et al., 2006; Underwood et al., 2004, 2010, 1999) as well as in DR projection regions (Perroud et al., 2010). Expression of *tph2* mRNA in projection regions of the DR is thought to reflect transport of *tph2* transcripts from the brainstem raphe nuclei to the nerve terminal, allowing local serotonin synthesis at the synapse (Perroud et al., 2010). These findings are consistent with the observation that brain serotonin turnover is elevated in depressed patients and returns to baseline following successful antidepressant treatment (Barton et al., 2008). Finally, genetic linkage studies support an association between *tph2*

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0891-0618/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchemneu.2011.05.002

genetic polymorphisms and depression (Zhang et al., 2005). Together, these findings suggest that elevated *tph2* expression and activity may be a biomarker or endophenotype of depression.

Elevated *tph2* expression in depressed suicide victims could result from genetic influences, adverse early life experience, chronic stress or major life events during adulthood, or medication. However, a picture is emerging of interactions among these factors being important determinants of *tph2* regulation. Genetic regulation of *tph2* expression has been reviewed in a number of excellent review articles (see especially, Matthes et al., 2010; Popova and Kulikov, 2010). Briefly, in a number of studies genetic polymorphisms in *tph2* have been associated with major depressive disorder (MDD; Haghighi et al., 2008; Tsai et al., 2009; Zhang et al., 2005; Zill et al., 2004a), bipolar disorder (Cichon et al., 2008; Harvey et al., 2004; Roche and McKeon, 2009; Van Den Bogaert et al., 2006), and suicidal behavior (Ke et al., 2006; Lopez de Lara et al., 2007; Zill et al., 2004b). In this review, we focus on development (D) × environment (E) interactions determining *tph2* expression and the potential consequences of altered basal *tph2* expression during adulthood and altered vulnerability to stress-induced changes in *tph2* expression during adulthood.

2. Developmental influences on *tph1* and *tph2* expression

Whereas the predominant isoform of tryptophan hydroxylase expressed in the brain during adulthood is *tph2*, some evidence suggests that both *tph1* and *tph2* are expressed in the brainstem raphe complex during development (Nakamura et al., 2006). Studies using reverse transcriptase polymerase chain reaction (RT-PCR) in mice suggest that *tph1* expression peaks at weaning (postnatal day (P) 21) and then is nearly absent by adulthood (Nakamura et al., 2006). Meanwhile, *tph2* expression is relatively constant from P7 through adulthood. Consequently, developmental influences on serotonergic signaling may alter adult phenotypes through actions on either *tph1* or *tph2* expression during the critical neonatal period. Nakamura et al. (2006) argue that at weaning *tph1* is a major determinant of serotonergic signaling due to a higher affinity for tryptophan and a higher enzymatic activity. However, more recent studies by Gutknecht et al. (2009), using quantitative RT-PCR, *in situ* hybridization and immunohistochemistry, found no detectable *tph1* mRNA or protein expression during the postnatal period in mouse or human brain, including P21 in mice. This raises the possibility that the *tph1* expression in the studies by Nakamura et al. (2006) was present at very low levels, that are only readily detectable by RT-PCR, or that environmental influences during the neonatal period (housing conditions, diet, etc.) resulted in increased *tph1* expression in these mice. A further possibility is that studies using brain microdissection and RT-PCR techniques detect *tph1* expression from non-neuronal sources. For example, *tph1* expression has been characterized in peripheral vasculature (Linder et al., 2008), including arterial smooth muscle cells (Ni et al., 2008). The presence or absence of *tph1* in the vasculature of the brainstem raphe complex has not been specifically addressed. In addition, *tph1* is expressed by T cells (O'Connell et al., 2006), and upon activation, T cells can synthesize and release serotonin. Importantly, *tph1* expression in T cells is dynamically regulated and is upregulated approximately 30-fold following T cell activation (Leon-Ponte et al., 2007). Thus, a portion of *tph1* expression, measured by brain microdissection and RT-PCR techniques, could represent *tph1* expression in non-neuronal sources, including T cells. In contrast, T cells do not express *tph2* (Leon-Ponte et al., 2007; O'Connell et al., 2006). As stress-related stimuli can activate T cells (Merlot et al., 2004; Satoh et al., 2006; Schmidt et al., 2010), it is possible that stress-induced increases in *tph1* expression in T cells can account for some previously reported stress-induced changes in *tph1* expression in studies using brain microdissection and RT-PCR (see below for further discussion). Clearly, the cellular localization and regulation of *tph1* expression during development requires further studies, taking into account the potential contribution of non-neuronal tissues to *tph1* expression when using brain microdissection and RT-PCR techniques. Regardless of the source of *tph1* expression that has been described in the brainstem raphe complex, genetic studies suggest that polymorphisms in the *tph1* gene are associated with depression (Gizatullin et al., 2006; Nash et al., 2005); thus, further studies of D × E interactions in the control of *tph1* expression are warranted.

Few studies have investigated factors controlling either *tph1* or *tph2* expression during the neonatal period. However, a study by Sidor et al. (2010) found that treatment of neonatal mice with the bacterial cell wall component, lipopolysaccharide (LPS), increased *tph2* mRNA expression within the dorsolateral part of the dorsal raphe nucleus/ventrolateral periaqueductal gray region (DRVL/VLPAG) at P14, but not at P17, 21, or 28. This effect was specific for the DRVL/VLPAG region and was not seen in other subregions of the DR. Interestingly, however, the LPS-induced increase in *tph2* expression was observed in the same subset of serotonergic neurons that have been shown to be activated by LPS in adult mice (Hollis et al., 2006). To our knowledge, no studies have yet examined the control of *tph1* expression by environmental factors during the neonatal period, and this remains an important objective for future studies as altered *tph1* expression during this critical period of development may alter the adult phenotype. This is of particular importance because, as will be described below, *tph1* expression appears to be more stress-sensitive than *tph2*, at least during adulthood.

Studies by Lowry and colleagues (Gardner et al., 2009) suggest that early life experience during the neonatal period can influence basal *tph2* expression during adulthood. In these studies, neonatal rats were exposed to one of three conditions, i.e. neonatal handling, maternal separation, or animal facility rearing control conditions, during P2–P14. Neonatal handling involves separation of pups from the dam for 15 min each day, whereas maternal separation involves separation of pups from the dam for 180 min each day. Neonatal handling has been associated with decreased hypothalamic–pituitary–adrenal (HPA) axis responses to stress and decreased anxiety in adulthood (Ladd et al., 2000; Meaney et al., 1996). In contrast, maternal separation has been associated with increased HPA axis responses to stress and increased anxiety-state, anhedonia, increased ethanol preference and impairment of sexual behavior in males during adulthood (Huot et al., 2001; Kalinichev et al., 2002; Ladd et al., 1996; Plotsky and Meaney, 1993; Rhees et al., 2001; Wigger and Neumann, 1999).

As adults, rats previously exposed to neonatal handling during development had decreased *tph2* expression in subdivisions of the DR, measured using *in situ* hybridization histochemistry, relative to animal facility reared control rats (Gardner et al., 2009). Specifically, rats exposed to neonatal handling as pups had decreased *tph2* mRNA expression, relative to animal facility reared controls, in the DRVL/VLPAG region, bilaterally (cf. Fig. 1A and C).

When *tph2* expression was considered at specific rostrocaudal levels of specific subdivisions of the DR, additional differences between neonatal handled and control rats emerged. Rats exposed to neonatal handling had decreased *tph2* mRNA expression, relative to animal facility reared controls, specifically in the mid-rostrocaudal portion of the dorsal raphe nucleus, dorsal part (DRD; approximately −8.25 mm bregma; cf. Fig. 1A and C). Interestingly, this portion of the DR is an important component of a stress- and anxiety-related neuronal circuit (Commons et al., 2003; Hale and Lowry, 2010; Lowry et al., 2008; Lowry and Hale, 2010), and increased activity of DRD serotonergic neurons is thought to result in increases in anxiety state (Lowry et al., 2005, 2008; Lowry

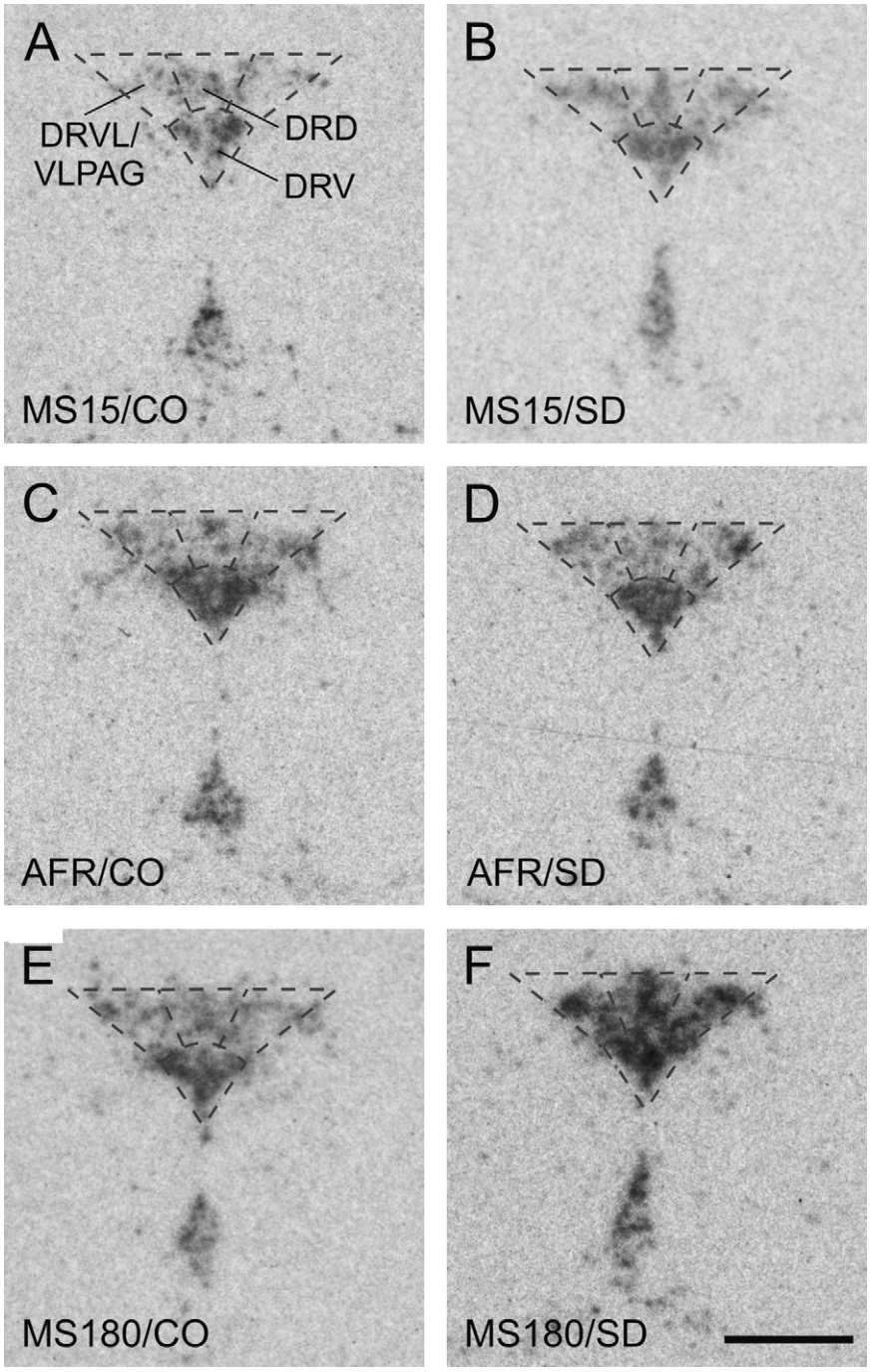

Fig. 1. Early life experience and adverse experience in adulthood interact to alter *tph2* mRNA expression in subregions of the dorsal raphe nucleus of rats (Adapted with permission, from Fig. 5 of Gardner et al., 2009). Autoradiographic images illustrate *tph2* mRNA expression at approximately −8.084 mm bregma from a single rat from each treatment group including rats exposed to novel cage control (CO) and social defeat (SD) conditions among three early life experience groups, including (1) neonatal handling (15 min of maternal separation from postnatal day P2 to P14; MS15), (2) animal facility rearing (handling pups twice per week during routine cage cleaning, AFR), and (3) maternal separation (180 min of maternal separation from P2 to P14; MS180). The following treatment groups are illustrated, (A) MS15/CO, (B) MS15/SD, (C) AFR/CO, (D) AFR/SD, (E) MS180/CO and (F) MS180/SD. Abbreviations: DRD, dorsal raphe nucleus, dorsal part; DRV, dorsal raphe nucleus, ventral part; DRVL/VLPAG, dorsal raphe nucleus, ventrolateral part/ventrolateral periaqueductal gray. Scale bar, 1 mm. For full experimental details, see Gardner et al. (2009).

and Hale, 2010; Maier and Watkins, 2005). The DRD is activated by a number of stress- and anxiety-related stimuli, including anxiogenic drugs (Abrams et al., 2005), the anxiety-related neuropeptide, urocortin 2 (Ucn 2; Amat et al., 2004; Hale et al., 2010; Staub et al., 2005, 2006), social defeat (Gardner et al., 2005) and uncontrollable stress (Amat et al., 2005; Grahn et al., 1999). In addition, alcohol-dependent depressed suicide patients show a 46% increase in TPH expression in the DRD compared with controls (Bonkale et al., 2006). Thus, rats exposed to neonatal handling, which have a decreased anxiety state as adults, have decreased *tph2* expression in the mid-rostrocaudal DR, a region associated with responses to anxiogenic drugs and anxiogenic stimuli.

As adults, rats previously exposed to maternal separation during development had increased *tph2* expression in subdivisions of the DR, measured using *in situ* hybridization histochemistry, relative to animal facility reared control rats (Gardner et al., 2009). Rats exposed to maternal separation had increased *tph2* mRNA expression, relative to animal facility reared controls, in the caudal

portion of the dorsal raphe nucleus (DRC, including its dorsal and ventral parts; approximately −8.42 and −8.50 mm bregma). Like the DRD, the DRC appears to be part of a stress- and anxiety-related neuronal circuit, and is activated by anxiogenic drugs (Abrams et al., 2005) and Ucn 2 (Hale et al., 2010; Staub et al., 2005, 2006). Meanwhile, injection of corticotropin-releasing factor (CRF) in the DRC, but not the rostral part of the DR, mimics the behavioral effects of inescapable stress (Hammack et al., 2002), including escape deficits and potentiation of fear conditioning in a model of learned helplessness. In addition, exposure to unpredictable acoustic stimulation *in vivo*, or application of CRF to rat brain slices *in vitro*, increases TPH activity selectively in the DRC (Evans et al., 2009). Depressed suicide patients have elevated *tph2* expression that is also restricted to the DRC (Bach-Mizrachi et al., 2008). As both the number and density of tryptophan hydroxylase-positive neurons is higher in depressed suicide patients, it is possible that depressed patients have increased *tph2* expression that is dependent on adverse early life experience, independent of stressful life events during adulthood. This might confer a vulnerability to stress-related psychiatric disorders. Indeed, children exposed to adverse experience have increased risk of developing depressive or anxiety disorders as adults (Heim and Nemeroff, 2001; McEwen, 2003; Ressler et al., 2010).

3. Environmental influences on *tph1* and *tph2* expression

As illustrated in Fig. 2, TPH1 immunoreactivity is evident in the pineal gland of adult male rats, but not in DR serotonergic neurons; conversely, TPH2 immunoreactivity is evident in the DR, but not in the pineal gland. A number of studies have characterized the distribution of *tph1* and *tph2* in adult rodents and humans (Gutknecht et al., 2009; Liang et al., 2004; Malek et al., 2005; Nakamura et al., 2006; Patel et al., 2004; Perroud et al., 2010; Sakowski et al., 2006; Sugden, 2003; Sugden et al., 2009; Zill et al., 2005, 2009). Generally, *tph2* is believed to be the neuron-specific isoform, and *tph1* is believed to be expressed in peripheral tissues, including the pineal gland, enterochromaffin cells, etc. However, a number of studies, using RT-PCR and *in situ* hybridization histochemistry techniques, have reported low levels of *tph1* expression in the brainstem raphe complex of rodents (Abumaria et al., 2008; Gundlah et al., 2005; Nakamura et al., 2006). However, as recently pointed out by Gutknecht et al. (2009), the probe that was used to detect *tph1* using *in situ* hybridization histochemical techniques (Abumaria et al., 2008) is directed against a nucleotide sequence that includes an extensive stretch of the coding region of *tph1*, including highly conserved functional domains that are highly homologous (85% identity) to *tph2* at the amino acid level, corresponding to 72% sequence identity in the two isoforms. Therefore, it is possible that the *in situ* hybridization histochemistry using this riboprobe results in cross-hybridization to *tph2*. This possibility is highlighted by studies by Malek et al. (2005), in which *in situ* hybridization using a riboprobe directed against a portion of the coding region of *tph1* detected a low level of mRNA expression in the midbrain raphe complex, whereas *in situ* hybridization using a selective oligonucleotide probe did not. In some studies, low levels of *tph1* mRNA have been detected in microdissected tissues including the midbrain raphe complex using RT-PCR (Abumaria et al., 2008; Nakamura et al., 2006), whereas in other studies, they have not (Gutknecht et al., 2009). As described briefly above, it remains possible that studies detecting low levels of *tph1* in microdissections of the midbrain raphe complex are detecting *tph1* that is expressed in non-neuronal cells, including T cells, as these studies did not perfuse the brain tissue in order to remove blood components prior to collecting brain tissue. In one study comparing *tph1* and *tph2* expression in microdissected tissues from the midbrain raphe complex in perfused and non-perfused brain (Gutknecht et al., 2009), there was no detectable *tph1* expression in either perfused or non-perfused tissue using RT-PCR techniques. Similar procedures should be used when evaluating stress-induced increases of *tph1* in microdissected brain tissues to evaluate whether or not stress-induced increases in *tph1* are dependent on expression in neurons or blood components such as T cells (Leon-Ponte et al., 2007). Conclusive demonstration of *tph1* expression specifically in serotonergic neurons will require further studies, including use of nucleotide probes for *in situ* hybridization that are directed toward non-conserved regions of *tph1* mRNA, such as the 3′ untranslated region, and ensuring that low levels of *tph1* expression detected using RT-PCR in microdissected brain tissues are not due to the presence of blood components that are known to express *tph1*, such as T cells.

Tryptophan hydroxylase protein in rats is expressed in a circadian pattern in forebrain structures, including the suprachiasmatic nucleus and intergeniculate leaflet, brain structures that are important for control of circadian function (Malek et al., 2004). In the median raphe nucleus and in the lateral wings of the DR TPH protein concentrations peak approximately 6 h into the dark phase of a 12 h light: 12 h dark light cycle, a pattern that persists in total darkness. In contrast, TPH protein in terminal regions peaks before the onset of the dark phase and declines thereafter, with peak concentrations occurring approximately 18 h following the peak TPH concentration in the midbrain raphe nuclei. The peak in TPH in terminal regions precedes the increase in serotonin release that occurs at the onset of the dark phase (Rueter et al., 1997) and the associated increase in behavioral arousal. *Tph2* mRNA expression throughout the DR and median raphe nuclei also varies in a diurnal pattern. Studies by Malek et al. (2005) have shown *tph2* mRNA expression in the midbrain raphe nuclei peaks approximately 2 h prior to the onset of the dark phase, or 8 h prior to the peak of midbrain TPH protein concentrations. In further studies using adrenalectomy and corticosterone replacement (Malek et al., 2007), the authors demonstrated that the diurnal pattern of *tph2* mRNA expression is dependent on glucocorticoids. *Tph2* mRNA expression increases in the late afternoon/early evening, coinciding with the increase in plasma corticosterone concentrations.

As far as we are aware, no rodent or primate studies to date have compared *tph2* expression in males and females. However, it is clear that female sex hormones play an important role in *tph2* expression. Daily, systemic treatment of ovariectomized female rats with diarylpropionitrile (DPN), a selective estrogen receptor beta (ERβ) agonist, increases *tph2* expression selectively in the mid-rostrocaudal DRD and the DRC (Donner and Handa, 2009). Local treatment with DPN, using stereotaxically implanted wax pellets containing the drug, increased *tph2* expression in the same regions. Additional studies using local treatment with 17-β-estradiol using stereotaxically implanted wax pellets (Donner and Handa, 2009) or systemic treatment with estrogen (Hiroi et al., 2006), found increased *tph2* expression in the mid-rostrocaudal DRD and DRC, respectively. These studies in rats are consistent with studies in primates showing that systemic treatment of ovariectomized female macaques with estrogen, progesterone, or estrogen plus progesterone for 1 month increases *tph2* expression, as measured using *in situ* hybridization histochemistry (Sanchez et al., 2005), or RT-PCR using microdissected raphe nuclei or pools of serotonergic neurons obtained using laser capture microdissection techniques (Bethea and Reddy, 2008). Studies in primates (Bethea et al., 2000) and guinea pigs (Lu et al., 1999) demonstrate that similar treatments also increase TPH protein. Together, these studies provide strong support for the hypothesis that female sex hormones are strong determinants of *tph2* expression, particularly in the mid-rostrocaudal and caudal parts of the DR, regions that give rise to projections to forebrain

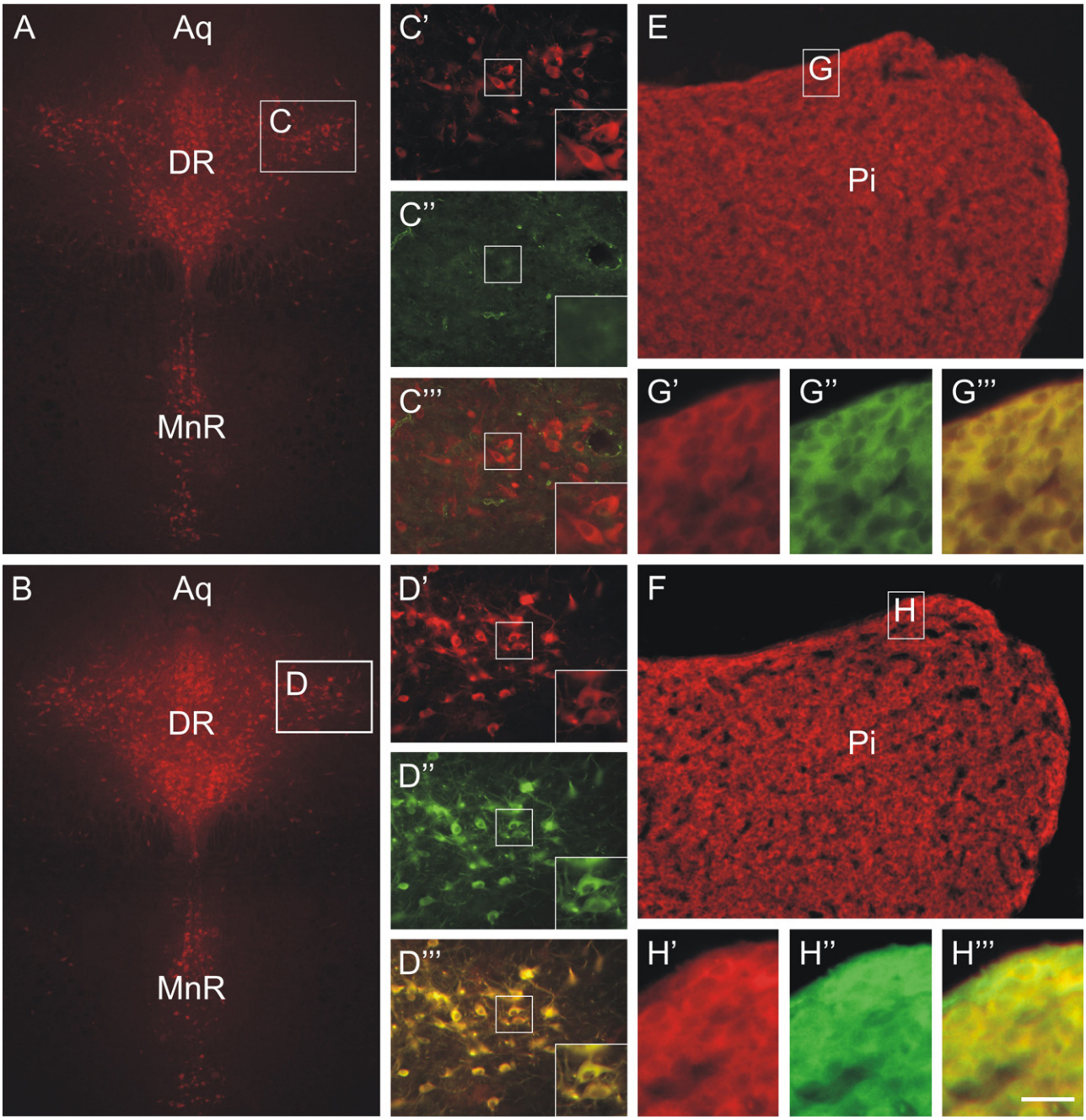

Fig. 2. Tryptophan hydroxylase 2- (TPH2-), but not tryptophan hydroxylase 1- (TPH1-) like immunoreactivity (ir) is present in cell soma and fibers in the dorsal raphe nucleus (DR) and median raphe nucleus (MnR), while TPH1- but not TPH2-like immunoreactivity is present in the pineal gland (Adapted, with permission, from Figs. 3 and 4 of Hale et al., 2011). (A and B) Low power photomicrographs from two adjacent brain sections (30 μm) of the same rat showing TPH-ir in the DR and MnR at approximately −8.18 mm bregma using antisera supplied by Sigma–Aldrich (sheep anti-TPH; Cat. No. T8575, Lot No. 096K1026, St. Louis, MO, USA) that recognizes both TPH1 and TPH2 (Hale et al., 2011). Regions indicated by white boxes in A and B are shown at higher magnification in C and D, respectively. (C) High magnification photomicrographs showing TPH-ir (C′), TPH1-ir (C″; using selective rabbit anti-TPH1 antiserum kindly supplied by Professor Donald Kuhn), and the merged image (C‴). Regions indicated by white boxes in C panels are shown at higher magnification in insets in the lower right corner of each image. These data suggest that TPH1-ir is not expressed in cell soma or fibers in the DR. (D) High magnification photomicrographs showing TPH-ir (D′), TPH2-ir (D″; using selective rabbit anti-TPH2 antiserum also kindly supplied by Professor Donald Kuhn), and the merged image (D‴). Dual labeled TPH and TPH2 cell soma and fibers appear yellow. Regions indicated by white boxes in D panels are shown at higher magnification in insets in the lower right corner of each image. These data suggest that TPH2 is expressed in cell soma and fibers in the rat DR. (E and F) Low power photomicrographs from two adjacent sections (30 μm) of the same rat showing TPH-ir in the pineal gland using antisera supplied by Sigma–Aldrich. White boxes in E and F are shown at higher magnification in G and H, respectively. (G) High magnification photomicrographs showing TPH-ir (G′), TPH1-ir (G″; using selective rabbit anti-TPH1 antiserum), and the merged image (G‴). Dual immunostained TPH and TPH1 cells (presumed pinealocytes) appear yellow. These data suggest that TPH1-ir is expressed in cells in the pineal gland. (H) High magnification photomicrographs showing TPH-ir (H′), TPH2-ir (H″; using selective rabbit anti-TPH2 antiserum), and the merged image (H‴). These data suggest that TPH2 is not expressed in cells in the pineal gland. Abbreviations: Aq, cerebral aqueduct; DR, dorsal raphe nucleus; MnR, median raphe nucleus; Pi, pineal gland. Scale bar, 250 μm (A and B), 100 μm (C–F), 50 μm (insets), 25 μm (G and H). For full experimental details, see Hale et al. (2011).

limbic structures controlling cognitive function and mood (Hale and Lowry, 2010; Lowry et al., 2008).

A number of studies suggest that *tph2* mRNA expression is relatively insensitive to exposure to acute, and to some chronic, stress-related stimuli including social defeat (Gardner et al., 2009), chronic restraint stress (Abumaria et al., 2008), and chronic social stress (Abumaria et al., 2006). As detailed topographical analysis of the midbrain raphe complex was not conducted in several of these studies, it may be the case that there were increases in *tph2* mRNA expression in specific subregions of the raphe nuclei, but these were undetected. Consistent with this hypothesis, chronic mild stress in adult male mice increases *tph2* expression in the mid-rostrocaudal DRD and in the DRC, but not other regions of the DR (McEuen et al., 2008). Alternatively, genetic or developmental factors may alter the vulnerability to stress-induced elevations of *tph2* expression (Gardner et al., 2009).

Multiple studies have found that *tph1* mRNA expression, in marked contrast to *tph2* mRNA expression, is upregulated following exposure to chronic immobilization stress (Chamas et al., 1999, 2004), chronic restraint stress (Abumaria et al., 2008) and chronic social stress (Abumaria et al., 2006), However, as discussed in detail above, further studies are required to determine if the increased *tph1* expression reflects stress-induced increases in *tph1* expression in serotonergic neurons or non-neuronal cells.

4. D × E influences on *tph1* and *tph2* expression

Lowry and colleagues have investigated D × E influences on *tph2* expression in rats (Gardner et al., 2009). Briefly, rats were exposed to neonatal handling, maternal separation, or animal facility rearing control conditions during P2–P14. As adults, rats were exposed to either social defeat or a control condition, which consisted of exposure to a novel cage environment. Rats exposed to neonatal handling as pups and social defeat as adults had decreased *tph2* mRNA expression, relative to animal facility reared controls, in the DRD, dorsal raphe nucleus, ventral part (DRV), and DRVL/VLPAG region. When *tph2* expression was considered at specific rostrocaudal levels of specific subdivisions of the DR, it emerged that differences between neonatal handled and animal facility reared control rats, subsequently exposed to defeat, in the DRD and DRV were restricted to the mid-rostrocaudal and caudal parts of these subregions (DRD, −8.25, −8.34, −8.42 mm bregma; DRV, −8.25, −8.42 mm bregma; cf. Fig. 1B and D). Thus, neonatal handling decreased stress-induced *tph2* expression in regions that have been associated with regulation of anxiety states and anxiety-related behavior.

As adults, rats previously exposed to maternal separation during development had increased *tph2* expression following social defeat in specific subdivisions of the DR, measured using *in situ* hybridization histochemistry, relative to animal facility reared control rats exposed to social defeat (Gardner et al., 2009). Defeated rats exposed to maternal separation as pups had increased *tph2* mRNA expression, relative to defeated animal facility reared controls in the DRD, DRV and DRVL/VLPAG. In addition, social defeat-induced *tph2* expression was increased in the DRD, DRV, DRVL/VLPAG and DRI of rats exposed to maternal separation as pups relative to neonatally handled rats (cf. Fig. 1B and F), suggesting polarized responses of maternally separated and neonatally handled rats to a stressful experience during adulthood. When *tph2* expression was considered at specific rostrocaudal levels of specific subdivisions of the DR, maternally separated rats exposed to social defeat had increased *tph2* expression in the rostral DRV relative to animal facility reared controls exposed to social defeat. The rostral DRV receives input from cortical areas including the lateral orbital cortex and subregions of the amygdala including anterior, anterior cortical and basomedial nuclei (Peyron et al., 1998) and therefore may be involved in the regulation of emotional behavior.

Among rats exposed to any given early life experience condition, only rats exposed to maternal separation responded with social defeat-induced increases in *tph2* expression, and this effect was restricted to the DRVL/VLPAG region (cf. Fig. 1E and F). This suggests that maternally separated rats had a vulnerability to stress-induced increases in *tph2* expression. As mentioned previously, *tph2* seems somewhat resilient to stress-induced alterations in expression; however, these data suggest that adverse early life experience results in a unique vulnerability to stress-induced changes (Gardner et al., 2009).

It is currently unknown how *tph2* expression relates to the activity state of serotonergic neurons. For example, if stress-induced TPH2 is preferentially trafficked to the dendrites, this could potentially result in a decrease in serotonergic activity; conversely, if stress-induced TPH2 is preferentially trafficked to the axon terminals, this could potentially result in an increase in serotonergic activity. It will be important, in future studies, to understand the consequences of stress-induced increases in *tph2* expression and serotonergic activity.

5. Conclusions

Evidence suggests that adverse experience during adulthood increases *tph1* mRNA expression in microdissected tissues from the midbrain raphe complex. Further studies are required to determine if the stress-induced increases in *tph1* expression are due to altered expression in neuronal or non-neuronal sources, such as the cerebral vasculature or T cells. A stress-induced increase in *tph1* mRNA expression in T cells would be of interest in its own right, given recent studies demonstrating a role for T cells in regulation of cognitive function (Derecki et al., 2010b; Derecki et al., 2010a). The expression of *tph2* appears to be relatively insensitive to either acute or chronic stress in adulthood. However, some studies have identified stress-induced increases in *tph2* expression in the mid-rostrocaudal and caudal DR, similar to what has been described in depressed suicide patients. Therefore, further studies investigating the effects of stress on *tph2* expression in topographically organized subpopulations of serotonergic neurons are warranted. Finally, studies in rodents suggest that adverse early life experience increases the vulnerability to stress-induced increases in *tph2*, while neonatal handling decreases *tph2* expression, and may increase the resilience to stress-induced increases in *tph2* later in life.

Acknowledgements

The authors gratefully acknowledge Professor Donald M. Kuhn for providing the monospecific polyclonal antibodies for TPH1 and TPH2 used in Fig. 2. This work was supported by Award Number R01MH086539 to CAL and R01MH065702 to AS and CAL from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health.

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