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Cigarette Smoking and Tryptophan Hydroxylase 2 mRNA in the Dorsal Raphe Nucleus in Suicides

Helene Bach,

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, and
Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY, USA

Victoria Arango,

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, and
Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY, USA

Suham A. Kassir,

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New
York, NY, USA

Andrew J. Dwork,

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, and
Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY, USA

J. John Mann, and

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, and
Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY, USA

Mark D. Underwood

Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, and
Department of Psychiatry, Columbia College of Physicians and Surgeons, New York, NY, USA

Abstract

Cigarette smoking is associated with suicide and mood disorders and stimulates serotonin release. Tryptophan hydroxylase (TPH2) synthesizes serotonin and is over-expressed in suicides. We determined whether smoking is associated with TPH2 mRNA in suicides and controls. TPH2 mRNA was measured postmortem in the dorsal raphe nucleus (DRN) of controls (N=26, 17 nonsmokers and nine smokers) and suicides (N=23, 5 nonsmokers and 18 smokers). Psychiatric history was obtained by psychological autopsy. TPH2 mRNA was greater in suicide nonsmokers than suicide smokers, control smokers and control nonsmokers ($p=0.006$). There was more TPH2 mRNA throughout the DRN. Smoking interferes with the TPH2 mRNA increase observed in suicide nonsmokers. The absence of altered TPH2 expression in non-suicide smokers suggests no pharmacological effect of smoking.

Correspondence concerning this article should be addressed to Mark D. Underwood, PhD, Division of Molecular Imaging and Neuropathology, Department of Psychiatry, Columbia Psychiatry, New York State Psychiatric Institute, 1051 Riverside Drive, Box 42, New York, NY 10032. mu20@columbia.edu.

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Keywords

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INTRODUCTION

Cigarette smoking is more prevalent in psychiatric illnesses, including schizophrenia, major depressive disorder (MDD), and suicidal behavior compared with the general population (Hughes, 2008). Eighty percent of men with MDD and 65% of women with MDD smoke regularly, much higher than the 21% rate in the general population (Glassman et al., 1990). The increased smoking rate in psychiatric illnesses may be because smoking is associated with improved cognition, relief of stress, and improved mood (Aubin, Rollema, Svensson, & Winterer, 2012). Conversely, smoking cessation is sometimes associated with the onset of MDD or depressive symptoms and also with increased suicide behavior in remitted patients (Hughes, 2008).

Nicotine is considered to be the major active ingredient in tobacco exerting its central nervous system effects through nicotinic cholinergic receptors (nAChRs). Nicotinic receptor activation stimulates neurotransmitter release including dopamine, serotonin (5-hydroxytryptophan, 5-HT) and norepinephrine (NE) (Shearman, Rossi, Seršen, & Lajtha, 2005; Singer et al., 2004), and this effect is more pronounced in psychiatric patients compared to smokers in the general population (Herman & Sofuoglu, 2010). In animals, acute administration of nicotine results in 5-HT release in several brain regions including the cerebral cortex, nucleus accumbens and in the ventral tegmental area (VTA) (Schiffer et al., 2001; Singer et al., 2004; Summers & Giacobini, 1995). Long term nicotine administration decreases 5-HT (Balfour & Ridley, 2000). Low doses of nicotine administered directly into the dorsal raphe nucleus (DRN) have anxiolytic effects in a social interaction test and this effect is blocked by co-administration of a 5-HT_{1A} receptor antagonist (Cheeta, Irvine, Kenny, & File, 2001). In *postmortem* human studies, one group reported less 5-HT and 5-hydroxyindoleacetic acid (5-HIAA), the 5-HT metabolite, in hippocampus and median raphe nucleus (MRN) of smokers compared to nonsmokers (Benwell, Balfour, & Anderson, 1990). Others (Klimek et al., 2001), found lower tyrosine hydroxylase immunoreactivity and less binding of adrenergic receptors in the locus coeruleus in smokers compared to nonsmokers. Hence, smoking has potential direct and indirect effects on neurotransmitters and brain regions involved normally and pathologically in mood regulation.

Serotonin producing neurons that innervate the forebrain are located predominantly in the brainstem DRN and MRN. 5-HT is synthesized by the neuron specific rate-limiting enzyme tryptophan hydroxylase (TPH2) (Walther et al., 2003). TPH2 is a stress-sensitive enzyme and is increased in rodents in response to stressful behavioral paradigms (Chamas, Serova, & Sabban, 1999; Chamas et al., 2004). In humans, there is *more* TPH2 mRNA in the DRN and MRN of suicides compared to non-psychiatric controls (Bach-Mizrachi et al., 2006, 2008) and more TPH2 protein in depressed suicides is reported by some (Boldrini, Underwood, Mann, & Arango, 2005; Underwood et al., 1999) but not all (Bonkale, Murdock, Janosky, & Austin, 2004) investigators. To date, no study has examined whether smoking contributes to

TPH2 changes associated with suicide. In the present study, we sought to determine whether smoking history is associated with elevated TPH2 mRNA and whether this association is different in suicides compared to normal controls.

METHODS AND MATERIALS

Subjects

Brain samples were obtained from the Division of Molecular Imaging of Neural Disorders in the New York State Psychiatric Institute. Cases used in this study were collected in the United States and the Republic of Macedonia. All procedures for collection of brain tissue and for the administration of the psychological autopsy were approved by the applicable Institutional Review Boards.

This study includes 26 non-psychiatric controls (17 nonsmokers and 9 smokers) and 23 suicides (5 nonsmokers and 18 smokers). Psychiatric diagnosis was determined by The Structured Clinical Interview for DSM (SCID)-I and a validated psychological autopsy method interviewing next-of-kin as described elsewhere (Kelly & Mann, 1996). Lifetime history of smoking, details of medical and psychiatric illness and treatment, family history, and a developmental history were also obtained from interviews with next-of-kin (Kelly & Mann, 1996). We quantified the amount of smoking as: nonsmoker ($n=22$); light smoker (< 10 cigarettes or half a pack per day, $n=3$), moderate smoker (approximately 20 cigarettes or one pack per day, $n=13$) or heavy smoker (> 40 cigarettes or two packs or more per day, $n=11$). Case and control demographics are provided in Tables 1 and 2.

Brainstems

Brains were collected at autopsy as described previously (Arango et al., 2001). All subjects were free of gross neuropathology and had negative toxicology screens for psychoactive and neurotoxic drugs, except for nicotine. Transverse sections were cut in a cryostat at 20 μ m thickness, mounted on gelatin-subbed glass slides and stored at -80°C until assayed. pH was measured in cerebellar tissue to assess tissue integrity and quality (Harrison et al., 1995). Tissue sections for *in situ* hybridization were collected every millimeter throughout the rostrocaudal extent of the DRN, corresponding to 16–20 sections per case. Sets of adjacent sections from each case, stained for Nissl substance were used to help identify boundaries of the raphe nuclei for the analysis of TPH2 mRNA in *in situ* hybridization autoradiograms.

In situ Hybridization Histochemistry

In situ hybridization was performed on tissue sections spanning the rostrocaudal extent of the DRN. Riboprobes specific for human TPH2 were designed and experiments performed as previously described (Bach-Mizrachi et al., 2006). Briefly, sections were fixed in paraformal-dehyde in phosphate buffered saline (PBS, pH 7.4) for 15 minutes, rinsed in PBS, and acetylated in 0.25% acetic anhydride in 0.1M triethanolamine for 10 minutes. Then sections were dehydrated through increasing concentrations of ethanol and delipidated in chloroform. Sections were incubated in the hybridization solution (50% formamide, 10mM EDTA, 20mM Pipes, 0.75M NaCl, 10% dextran sulfate, 5X Denhardts, 250 μ l/ml tRNA and denatured radiolabeled probe at 3 ng concentration, 2×10^6 counts per 100 μ l)

overnight at 55°C. The sections were then washed in a series of high stringency washes to reduce background, dried and exposed to autoradiography film (Biomax MR, Kodak) with ¹⁴C standard slides (ARC-146, 146A, American Radiolabeled Chemicals, Inc.) for 3 days. Films were developed according to the manufacturer's instructions.

Imaging and Densitometry of *In situ* Hybridization Autoradiograms

Methods for semi-quantification of the *in situ* hybridization autoradiograms have been previously described (Bach-Mizrachi et al., 2006) and are only summarized. Autoradiograms of each section were digitized using an image analysis system (MCID Elite; Imaging Research Inc., St. Catherine's, Ontario, Canada). DRN measurements were made using a circle of fixed area (80mm²). The target area within the circle was user-defined by setting a threshold for pixels that were above background levels. Films that had saturated signal (i.e., pixel values the highest calibration value) were re-exposed for shorter time periods. The measurements taken included the area of the target within the circle (proportional area), the calibrated optical density of label within the circle and the concentration of label within the target. An index of the amount of TPH2 mRNA was calculated as the mean concentration of TPH2 multiplied by the sum of the target cross-sectional areas. Measurements of film background and non-raphe tissue in the section with the same fixed area circle were taken and constituted measures of the amount of non-specific labeling. The DRN is a complex nucleus and is subdivided into subnuclei. Therefore, changes in TPH2 mRNA expression in the DRN were analyzed across the anteroposterior axis and also for individual subnuclei. No single anatomical reference point is visible in all sections or in all cases processed for TPH2 mRNA by *in situ* hybridization. Therefore, the most anterior section of DRN containing the dorsal (DRd), ventrolateral (DRvl), ventral (DRv) and interfascicular (DRif) subnuclei was used as a relative reference starting point. In addition, DRN subnuclei were measured individually with delineations of the subnuclei guided by Nissl stained adjacent sections or sections immunostained for TPH2 protein in cases where they were available. The subnuclei of the DRN defined and sampled included: DRd, DRv, DRvl, DRif and caudal (DRc) subdivisions.

Optical density measurements were calibrated using ¹⁴C standard slides (American Radiolabeled Chemicals, Inc., ARC-146 and ARC-146A) that were co-exposed with the tissue sections. Tissue radioactivity equivalents were adjusted for incorporation of radioactivity into the batch of riboprobe that was labeled for each experiment as a correction for the specific activity of the labeled probe. A threshold level above background was set for each film.

Statistical Analysis

Statistical tests were performed using SPSS (Version 17.0). A chi-square test was used to determine incidence of smoking and psychiatric medication use in the sample. Analysis of Variance (ANOVA) was used to determine differences in TPH2 mRNA expression; the fixed variables were group (control smoker, control nonsmoker, suicide smoker, suicide nonsmoker) and covariates were age, postmortem interval (PMI) or brain pH. To examine TPH2 mRNA expression differences in the different groups and in different DRN subnuclei, Tukey's *post hoc* tests were carried out with Bonferroni correction applied for multiple

comparisons. Regression analysis determined whether there were any correlations between TPH2 expression and age, PMI or amount of smoking.

RESULTS

Incidence of Smoking

Suicides had a higher incidence of smoking compared to non-psychiatric controls (controls: 31% vs. suicides: 75%; Chi-squared=10.15, $p=0.001$). Suicide smokers had more heavy smokers compared to control smokers (29% of control smokers vs. 47% of suicide smokers were heavy smokers, Chi-squared=13.72, $p=0.003$). These rates are consistent with smoking incidence reported in live populations (Lasser et al., 2000; Lineberry, Allen, Nash, & Galardy, 2009).

TPH2 mRNA

TPH2 mRNA localization corresponded to the DRN, MRN and the region corresponding to the B9 cell group of serotonergic neurons (Figure 1). Since we previously found more TPH2 mRNA in suicides than normal controls (Bach-Mizrachi et al., 2006), we examined the effects of cigarette smoking in psychiatric cases and non-psychiatric controls separately. There was no difference in the amount of TPH2 mRNA between control smokers and control nonsmokers ($p>0.05$). Consistent with our previous findings of more TPH2 in suicides, the suicide nonsmokers had more TPH2 mRNA than control smokers or nonsmokers ($p=0.001$). Suicide nonsmokers had approximately two-fold more TPH2 mRNA expression in the DRN than suicide smokers (Figure 2; $p=0.024$ with Bonferroni correction). Neither age, sex, nor PMI explained the difference in TPH2 mRNA between suicide smokers and suicide nonsmokers. Higher TPH2 mRNA in suicide nonsmokers was independent of comorbid psychiatric diagnosis. Suicide smokers had more cases reporting prescription or treatment with psychiatric medications either lifetime or in the prior 3 months before death ($n=9$) than suicide nonsmokers ($n=5$), but the difference in DRN TPH2 mRNA between those groups was not statistically significant ($t=-1.043$, $p=0.318$). There were three suicides that neither smoked nor had psychiatric medication.

TPH2 mRNA in DRN Subnuclei

TPH2 mRNA expression was assessed in the DRN subnuclei of suicide and nonsuicide smokers and nonsmokers. A representative autoradiograph delineating the sampled subnuclei is shown in Figure 3A. In both smokers and nonsmokers, the amount of TPH2 expression was greatest in the DRd, followed by the DRvl>DRc>DRv>DRif. The largest subnucleus of the DRN is the DRd.

There was more TPH2 mRNA (optical density $\times$ area) in suicide nonsmokers than suicide smokers in all subnuclei (Figure 3B, right), but the difference was only statistically significant in the DRvl (250% of suicide nonsmoker, $p=0.025$). The other differences were: DRd (300%, $p=0.25$), DRv (200%, $p=0.10$), DRif (150%, $p=0.20$) and DRc (250%, $p=0.27$). Suicide nonsmokers had more TPH2 in the DRd (ANOVA, $t=-2.43$, $p=0.03$) than both control smokers and control nonsmokers but the difference did not remain statistically significance following correction for multiple comparisons. There was no difference in the

density of TPH2 mRNA in any of the individual subnuclei between control smokers and control nonsmokers ($p>0.05$). This was also true for the index of the total amount of TPH2 mRNA in each subnucleus (Figure 3B, left). TPH2 expression in the subnuclei of controls (smokers and nonsmokers) was not different from suicide smokers (ANOVA, $p>0.05$).

DISCUSSION

We find cigarette smoking is more frequent and heavier in suicides compared with non-psychiatric controls dying from other causes. This is consistent with reports that smoking is associated with suicide behavior (Angst & Clayton, 1998; Hughes, 2008; Malone et al., 2003). Our findings also suggest that cigarette smoking in suicides does not contribute to the elevation in TPH2 that we previously reported in depressed suicides (Bach-Mizrachi et al., 2006). Rather, smoking was associated with less TPH2 mRNA expression compared to nonsmoking suicides or non-psychiatric controls.

We confirmed and expanded our previous finding of elevated TPH2 in suicides, now in a total of 49 cases, only 5 of which were included in our previous study (Bach-Mizrachi et al., 2006). Not only did we replicate that finding in a larger cohort, we extended that observation, finding that the increase in TPH2 mRNA in the DRN of suicides was largely restricted to suicides that did not smoke.

The elevation in TPH2 mRNA in suicides who did not smoke was evident throughout the DRN but only reached statistical significance in a single subnucleus suggesting that the increase in TPH2 mRNA in suicides is widespread throughout the DRN. We also observed greater area of expression of TPH2 mRNA in suicide nonsmokers compared to smokers and this is consistent with what we previously reported for TPH2 mRNA and TPH protein in the DRN of suicides (Bach-Mizrachi et al., 2006; Underwood et al., 1999). In contrast, the distribution of TPH2 mRNA in suicide smokers was similar to non-psychiatric controls. We hypothesize that smoking interferes with the upregulation of TPH2 in the DRN of suicides and thereby diminishes or normalizes the serotonin-synthetic capacity of serotonin neurons as evidenced by the smaller area and lower amount of TPH2 expression in suicides who smoked. A reduced upregulatory response could result in reduced synthesis of 5-HT in smokers and might explain why smoking is a risk factor for suicide. Alternatively, a lower amount of TPH2 expression in suicides could be a predisposition to cigarette smoking addiction.

Cigarette smoking is more prevalent among psychiatric patients than in the general population. This may be explained by the antidepressant and mood stabilizing effects that cigarette smoking is reported to have (George, Sacco, Vessicchio, Weinberger, & Shytle, 2008; Markou & Kenny, 2002; Semba, Matak, Yamada, Nankai, & Toru, 1998). Stress is reported to increase TPH2 expression (Chamas et al., 1999, 2004; Gardner et al., 2009), raising the possibility that TPH2 is increased in nonsmoking suicides as a stress response. Smoking does not appear to be sufficient to reduce suicide since there are more suicides that smoked and or were prescribed medications or treated than suicides that did not smoke and were not treated. It is therefore unlikely that smoking reduced the efficacy of any antidepressant treatment. It is unfortunate that we did not have a group of depressed non-

suicide smokers and a group of depressed non-suicide nonsmokers to be able to better separate the effects of smoking from major depression independent of possible effects attributable to suicide. We simply do not have a sufficient number of such cases to study. Interestingly, suicide smokers had a comparable amount of TPH2 mRNA to controls, while TPH2 mRNA in nonsmoking suicides was increased, raising the possibility that increased TPH2 could contribute to the suicidality. Such a TPH2 increase in suicide, if confirmed, would be contrary to the serotonin deficit hypothesis of suicide etiology.

We previously reported that depressed patients who smoked and made suicide attempts have lower cerebrospinal fluid 5-HIAA and a blunted prolactin response to fenfluramine challenge that correlated with higher aggression scores and higher lethality suicide attempts (Malone et al., 2003). These findings are consistent with two potential explanatory models: 1. chronic smoking results in cortical 5-HT depletion in patients and favors recurrent depression, aggressive traits and more lethal suicidal behavior; or 2. genetic and nongenetic risk factors for suicidal behavior favor a predisposition to addiction to cigarette smoking through the common feature of low serotonergic function in key brain regions. Both models, in conjunction with our data showing no change in TPH2 expression with smoking in non-psychiatric controls suggest a synergistic or additive mechanism by which smoking contributes to depletion of serotonin via indirect modulation of TPH2, but only in depressed individuals at risk for suicide.

Modulation of TPH2 mRNA expression in smokers may occur through genetic variation in the TPH2 promoter. To date, one polymorphism has been found in the TPH2 gene promoter (G-703-T) that has been associated with increased addiction to nicotine (Reuter et al., 2007) and increased suicidality in a depressed population (Yoon & Kim, 2009). This promoter polymorphism is within a haplotype that has been shown to influence TPH2 mRNA expression (Scheuch et al., 2007). However, the T-703-G SNP is rare and other studies have not been able to replicate its association with suicide (Stefulj et al., 2011). It is not known whether smoking affects the expression of this TPH2 polymorphism. Regardless, it is a limitation in the present study that the genotype of the cases is not known.

The present study has other limitations. While we have evidence suggesting that smoking blunts the TPH2 increase in suicide, postmortem studies cannot determine the mechanism by which smoking interferes with the upregulation in TPH2 in suicide. Separating cause from effect cannot be achieved in postmortem studies. Second, although the total number of cases is quite large for a postmortem brain study, the number of cases in individual groups is small and uneven and limits statistical power to detect or explain differences. Replication studies should be done to confirm and extend our findings.

We hypothesize that the blunted amount of TPH2 in suicide smokers is due to an effect of nicotine. However, our study cannot differentiate between the specific pharmacologic actions of nicotine from those of the other combustible cytotoxic compounds in cigarette smoke, or even to some other behavioral or mood stabilizing effect of cigarette smoking. Several of the compounds in cigarette smoke are thought to contribute to neuronal injury or death, decreases in cortical thickness and oxidative damage. However, nicotine has not been shown to cause this type of cell damage (Durazzo, Meyerhoff, & Nixon, 2010), but it has

been shown to improve aspects of cognition, mood and cortisol stress response (Gilbertson, Frye, & Nixon, 2011) and is therefore likely involved in the TPH2 dysregulation that we report here.

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References

- Angst J, Clayton PJ. Personality, smoking and suicide: A prospective study. *Journal of Affective Disorders*. 1998; 51(1):55–62. DOI: 10.1016/s0165-0327(98)00156-6 [PubMed: 9879803]
- Arango V, Underwood MD, Boldrini M, Tamir H, Kassir SA, Hsiung SC, ... Mann JJ. Serotonin 1A receptors, serotonin transporter binding and serotonin transporter mRNA expression in the brainstem of depressed suicide victims. *Neuropsychopharmacology*. 2001; 25(6):892–903. DOI: 10.1016/s0893-133x(01)00310-4 [PubMed: 11750182]
- Aubin HJ, Rolfe H, Svensson TH, Winterer G. Smoking, quitting, and psychiatric disease: A review. *Neuroscience and Behavioral Reviews*. 2012; 36:271–284. DOI: 10.1016/j.neubiorev.2011.06.007
- Bach-Mizrachi H, Underwood MD, Kassir SA, Bakalian MJ, Sibille E, Tamir H, ... Arango V. Neuronal tryptophan hydroxylase mRNA expression in the human dorsal and median raphe nuclei: Major depression and suicide. *Neuropsychopharmacology*. 2006; 31(4):814–824. DOI: 10.1038/sj.npp.1300897 [PubMed: 16192985]
- Bach-Mizrachi H, Underwood MD, Tin A, Ellis SP, Mann JJ, Arango V. Elevated expression of tryptophan hydroxylase-2 mRNA at the neuronal level in the dorsal and median raphe nuclei of depressed suicides. *Molecular Psychiatry*. 2008; 13(5):507–513. DOI: 10.1038/sj.mp.4002143 [PubMed: 18180753]
- Balfour DJ, Ridley DL. The effects of nicotine on neural pathways implicated in depression: A factor in nicotine addiction? *Pharmacology, Biochemistry and Behavior*. 2000; 66(1):79–85. DOI: 10.1016/s0091-3057(00)00205-7
- Benwell MEM, Balfour DJK, Anderson JM. Smoking-associated changes in the serotonergic systems of discrete regions of human brain. *Psychopharmacology*. 1990; 102:68–72. DOI: 10.1007/bf02245746 [PubMed: 1697418]
- Boldrini M, Underwood MD, Mann JJ, Arango V. More tryptophan hydroxylase in the brainstem dorsal raphe nucleus in depressed suicides. *Brain Research*. 2005; 1041(1):19–28. DOI: 10.1016/j.brainres.2005.01.083 [PubMed: 15804496]
- Bonkale WL, Murdock S, Janosky JE, Austin MC. Normal levels of tryptophan hydroxylase immunoreactivity in the dorsal raphe of depressed suicide victims. *Journal of Neurochemistry*. 2004; 88(4):958–964. [PubMed: 14756817]
- Chamas F, Serova L, Sabban EL. Tryptophan hydroxylase mRNA levels are elevated by repeated immobilization stress in rat raphe nuclei but not in pineal gland. *Neuroscience Letters*. 1999; 267(3):157–160. DOI: 10.1016/s0304-3940(99)00340-7 [PubMed: 10381000]
- Chamas FM, Underwood MD, Arango V, Serova L, Kassir SA, Mann JJ, Sabban EL. Immobilization stress elevates tryptophan hydroxylase mRNA and protein in the rat raphe nuclei. *Biological Psychiatry*. 2004; 55(3):278–283. DOI: 10.1016/s0006-3223(03)00788-1 [PubMed: 14744469]
- Cheeta S, Irvine EE, Kenny PJ, File SE. The dorsal raphe nucleus is a crucial structure mediating nicotine's anxiolytic effects and the development of tolerance and withdrawal responses. *Psychopharmacology (Berlin)*. 2001; 155(1):78–85. DOI: 10.1007/s002130100681 [PubMed: 11374339]

- Durazzo TC, Meyerhoff DJ, Nixon SJ. Chronic cigarette smoking: implications for neurocognition and brain neurobiology. *International Journal of Environmental Research and Public Health*. 2010; 7(10):3760–3791. DOI: 10.3390/ijerph7103760 [PubMed: 21139859]
- Gardner KL, Hale MW, Oldfield S, Lightman SL, Plotsky PM, Lowry CA. Adverse experience during early life and adulthood interact to elevate tph2 mRNA expression in serotonergic neurons within the dorsal raphe nucleus. *Neuroscience*. 2009; 163(4):991–1001. DOI: 10.1016/j.neuroscience.2009.07.055 [PubMed: 19647049]
- George TP, Sacco KA, Vessicchio JC, Weinberger AH, Shytle RD. Nicotinic antagonist augmentation of selective serotonin reuptake inhibitor-refractory major depressive disorder: A preliminary study. *Journal of Clinical Psychopharmacology*. 2008; 28(3):340–344. DOI: 10.1097/jcp.0b013e318172b49e [PubMed: 18480694]
- Gilbertson R, Frye RF, Nixon SJ. Nicotine as a factor in stress responsiveness among detoxified alcoholics. *Alcohol and Alcoholism*. 2011; 46(1):39–51. DOI: 10.1093/alcalc/agq070 [PubMed: 21045074]
- Glassman AH, Helzer JE, Covey LS, Cottler PB, Stetner F, Tipp JE, Johnson J. Smoking, smoking cessation, and major depression. *Journal of the American Medical Association*. 1990; 264(12):1546–1549. DOI: 10.1001/jama.1990.03450120058029 [PubMed: 2395194]
- Harrison PJ, Heath PR, Eastwood SL, Burnet PWJ, McDonald B, Pearson RCA. The relative importance of premortem acidosis and postmortem interval for human brain gene expression studies: Selective mRNA vulnerability and comparison with their encoded proteins. *Neuroscience Letters*. 1995; 200:151–154. DOI: 10.1016/0304-3940(95)12102-a [PubMed: 9064599]
- Herman AI, Sofuoglu M. Cognitive effects of nicotine: genetic moderators. *Addiction Biology*. 2010; 15(3):250–265. DOI: 10.1111/j.1369-1600.2010.00213.x [PubMed: 20456288]
- Hughes JR. Smoking and suicide: a brief overview. *Drug and Alcohol Dependence*. 2008; 98(3):169–178. DOI: 10.1016/j.drugalcdep.2008.06.003 [PubMed: 18676099]
- Kelly TM, Mann JJ. Validity of DSM-III-R diagnosis by psychological autopsy: A comparison with clinician ante-mortem diagnosis. *Acta Psychiatrica Scandinavica*. 1996; 94(5):337–343. DOI: 10.1111/j.1600-0447.1996.tb09869.x [PubMed: 9124080]
- Klimek V, Zhu MY, Dilley G, Konick L, Overholser JC, Meltzer HY, ... Ordway GA. Effects of long-term cigarette smoking on the human locus coeruleus. *Archives of General Psychiatry*. 2001; 58(9):821–827. DOI: 10.1001/archpsyc.58.9.821 [PubMed: 11545664]
- Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: A population-based prevalence study. *Journal of the American Medical Association*. 2000; 284(20):2606–2610. DOI: 10.1001/jama.284.20.2606 [PubMed: 11086367]
- Lineberry TW, Allen JD, Nash J, Galardy CW. Population-based prevalence of smoking in psychiatric inpatients: A focus on acute suicide risk and major diagnostic groups. *Comprehensive Psychiatry*. 2009; 50(6):526–532. DOI: 10.1016/j.comppsy.2009.01.004 [PubMed: 19840590]
- Malone KM, Waternaux C, Haas GL, Cooper TB, Li S, Mann JJ. Cigarette smoking, suicidal behavior, and serotonin function in major psychiatric disorders. *American Journal of Psychiatry*. 2003; 160(4):773–779. DOI: 10.1176/appi.ajp.160.4.773 [PubMed: 12668368]
- Markou A, Kenny PJ. Neuroadaptations to chronic exposure to drugs of abuse: relevance to depressive symptomatology seen across psychiatric diagnostic categories. *Neurotoxicity Research*. 2002; 4(4):297–313. DOI: 10.1080/10298420290023963 [PubMed: 12829420]
- Reuter M, Hennig J, Amelang M, Montag C, Korkut T, Hueweler A, Stürmer T. The role of the TPH1 and TPH2 genes for nicotine dependence: A genetic association study in two different age cohorts. *Neuropsychobiology*. 2007; 56(1):47–54. DOI: 10.1159/000110728 [PubMed: 17986837]
- Scheuch K, Lautenschlager M, Grohmann M, Stahlberg SE, Kirchheiner J, Zill P, ... Priller J. Characterization of a functional promoter polymorphism of the human tryptophan hydroxylase 2 gene in serotonergic raphe neurons. *Biological Psychiatry*. 2007; 62(11):1288–1294. DOI: 10.1016/j.biopsych.2007.01.015 [PubMed: 17568567]
- Schiffer WK, Gerasimov MR, Marsteller DA, Geiger J, Barnett C, Alexoff DL, Dewey SL. Topiramate selectively attenuates nicotine-induced increases in monoamine release. *Synapse*. 2001; 42(3):196–198. DOI: 10.1002/syn.10000 [PubMed: 11746717]

- Semba J, Mataka C, Yamada S, Nankai M, Toru M. Antidepressantlike effects of chronic nicotine on learned helplessness paradigm in rats. *Biological Psychiatry*. 1998; 43(5):389–391. DOI: 10.1016/s0006-3223(97)00477-0 [PubMed: 9513755]
- Shearman E, Rossi S, Seršen H, Lajtha HA. Locally administered low nicotine-induced neurotransmitter changes in areas of cognitive function. *Neurochemical Research*. 2005; 30(8): 1055–1066. DOI: 10.1007/s11064-005-7132-9 [PubMed: 16258855]
- Singer S, Rossi S, Verzosa S, Hashim A, Lonow R, Cooper T, ... Lajtha A. Nicotine-induced changes in neurotransmitter levels in brain areas associated with cognitive function. *Neurochemical Research*. 2004; 29(9):1779–1792. DOI: 10.1023/b:nere.0000035814.45494.15 [PubMed: 15453274]
- Stefulj J, Mokrovic G, Hranilovic D, Bordukalo-Niksic T, Bakula M, Kubat M, Jernej B. Functional promoter polymorphism of the neuronal isoform of tryptophan hydroxylase (Tph2) in suicide. *Psychiatry Research*. 2011; 186(2–3):446–447. DOI: 10.1016/j.psychres.2010.08.034 [PubMed: 20869124]
- Summers KL, Giacobini E. Effects of local and repeated systemic administration of (–)nicotine on extracellular levels of acetylcholine, norepinephrine, dopamine, and serotonin in rat cortex. *Neurochemical Research*. 1995; 20(6):753–759. DOI: 10.1007/bf01705545 [PubMed: 7566373]
- Underwood MD, Khaibulina AA, Ellis SP, Moran A, Rice PM, Mann JJ, Arango V. Morphometry of the dorsal raphe nucleus serotonergic neurons in suicide victims. *Biological Psychiatry*. 1999; 46(4):473–483. DOI: 10.1016/s0006-3223(99)00043-8 [PubMed: 10459396]
- Walther DJ, Peter JU, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M. Synthesis of serotonin by a second tryptophan hydroxylase isoform. *Science*. 2003; 299(5603):76.doi: 10.1126/science.1078197 [PubMed: 12511643]
- Yoon HK, Kim YK. TPH2-703G/T SNP may have important effect on susceptibility to suicidal behavior in major depression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2009; 33(3):403–409. DOI: 10.1016/j.pnpbp.2008.12.013 [PubMed: 19162119]

FIGURE 1. Autoradiograms of TPH2 mRNA expression in representative sections of the DRN from a nonsmoker (top) and smoker (bottom) at rostral, mid-level and caudal neuroanatomical levels. Scale bar is equal to 4 mm. Note darker expression of TPH2 in the DRN of the nonsmoker.

FIGURE 2.

Line plots of the amount of TPH2 mRNA (density $\times$ area) along the rostrocaudal axis of the DRN in controls (left) comparing nonsmokers (open circles) and smokers (closed circles). Line plots of suicide nonsmokers (open circles) and suicide smokers (closed circles) are shown on the right. Note that suicides that did not smoke have more TPH2 mRNA than suicide smokers and control nonsmokers and smokers.

FIGURE 3.

(A) Schematic of DRN subnuclei and the MRN. The sampled subnuclei are delineated in black and include the dorsal (DRd), ventral (DRv), ventrolateral (DRvl), interfascicular (DRif) and caudal (DRc). (B) Bar graphs of TPH2 expression in DRN subnuclei in control nonsmokers and smokers (top) and suicide nonsmokers and smokers (bottom). Nonsmokers are represented in grey and smokers are represented in black. One asterisk represents p values < 0.05 comparing suicide nonsmokers to suicide smokers. Two asterisks represent a p value < 0.05 when comparing suicide nonsmokers with controls. Note that suicide nonsmokers have more TPH2 mRNA in all DRN subnuclei except the interfascicular.

TABLE 1

Case Demographics for Nonsmokers

	Subject	Age	Sex	Race	PMI	Brain pH	Manner of death	Brain toxicology	Axis I
1	Control	85	Male	White	7	6.22	CV Disease	Clear	NONE
2	Control	53	Male	White	18.5	NA	CV Disease	Clear	NONE
3	Control	66	Male	White	19	6.10	CV Disease	Clear	NONE
4	Control	51	Male	White	9	6.35	CV Disease	Lidocaine	NONE
5	Control	30	Female	Black	8	6.73	CV Disease	Clear	NONE
6	Control	39	Male	White	14	6.79	CV Disease	Clear	NONE
7	Control	35	Female	White	26	6.73	MVA - Driver	Clear	NONE
8	Control	53	Male	Black	9	NA	Industrial Accident	Clear	NONE
9	Control	37	Male	Black	15	6.75	CV Disease	Clear	NONE
10	Control	43	Male	Black	21	6.76	CV Disease	Clear	NONE
11	Control	44	Male	Asian	21	6.86	Electrocution	Clear	NONE
12	Control	18	Male	Hispanic	16	6.80	MVA - Pedestrian	Clear	NONE
13	Control	19	Male	Black	13	6.84	CV Disease	Clear	NONE
14	Control	43	Male	Black	19	6.49	CV Disease	Clear	NONE
15	Control	24	Male	Hispanic	12	NA	Unknown	Clear	NONE
16	Control	34	Male	White	12	6.38	CV Disease	Lidocaine	NONE
17	Control	56	Male	White	14	6.41	CV	Caffeine	NONE
18	Suicide	28	Female	White	19	6.32	Fall From Height	Clear	Bul/Anor, MDD
19	Suicide	40	Male	White	20	6.77	Hanging	Clear	MDD
20	Suicide	62	Male	White	21	6.37	Drowning	Clear	MDD
21	Suicide	90	Male	White	25	6.76	Hanging	Clear	DELU
22	Suicide	83	Male	White	22	6.60	Gunshot	Clear	MDD

TABLE 2

Case Demographics for Smokers

	Suicide	Age	Sex	Tobacco	Race	PMI	Brain pH	Manner of death	Toxicology	Axis I
1	Control	18	Male	Moderate	Hispanic	11	7.07	Fall From Height-Accident	Clear	NONE
2	Control	35	Male	Moderate	White	10	6.64	MVA	Caffeine	NONE
3	Control	49	Female	Heavy	White	20	5.68	Pneumonia	Theophyllin, Caffeine	NONE
4	Control	31	Male	Moderate	White	10	6.24	MVA	ANX, LIDO, ACE, Caffeine	NONE
5	Control	45	Male	Moderate	White	7	6.30	MVA	Caffeine	NONE
6	Control	69	Male	Moderate	White	8	5.81	Homicide (gunshot)	NA	NONE
7	Control	22	Male	Moderate	White	21	6.68	Homicide (stabbing)	Caffeine	NONE
8	Control	46	Female	Heavy	White	9	6.60	Myocardial Infarction	Caffeine, Nicotine/Cotinine	NONE
9	Control	51	Female	Light	White	7	6.70	Aortic Rupture	Clear	NONE
10	Suicide	63	Male	Heavy	Hispanic	17	6.74	Hanging	Clear	NONE
11	Suicide	59	Male	Moderate	Hispanic	24.5	6.77	Hanging	Clear	MDD
12	Suicide	56	Male	Light	Hispanic	21	6.56	Hanging	Clear	MDD
13	Suicide	31	Male	Heavy	White	10	6.00	Poisoning	ANX	SCZ
14	Suicide	46	Male	Heavy	White	6	6.75	Jump from the building	Clear	SCZD
15	Suicide	64	Male	Heavy	White	10	6.20	Acid ingestion	ANX	MDD
16	Suicide	44	Female	Heavy	White	7	5.70	Acid ingestion	ANX	SZAF
17	Suicide	61	Female	Light	White	24	6.26	Drowning	Clear	MDD
18	Suicide	17	Male	Moderate	White	24	6.42	Benzodiazepine ingestion	ANX, ACE, BARB	MDD
19	Suicide	32	Female	Heavy	White	8	6.71	Hanging	NA	ADJ
20	Suicide	20	Female	Moderate	White	9	6.70	Jump from Height	Nicotine/Cotinine	SCZ
21	Suicide	26	Male	Moderate	White	22	6.77	Hanging	Clear	GAM
22	Suicide	42	Male	Moderate	White	7	6.55	Hanging	Clear	MDD
23	Suicide	51	Female	Moderate	White	12	6.00	Acid ingestion	Caffeine	MDD
24	Suicide	30	Male	Heavy	White	11	6.60	Hanging	Caffeine	MDD
25	Suicide	50	Female	Heavy	White	6	6.70	Jumping in front of a train	ANX, Caffeine	SZAF
26	Suicide	33	Male	Heavy	White	6	6.70	Hanging	Clear	ADJ
27	Suicide	59	Male	Moderate Smoker	White	6	6.10	Hanging	ANX, SER	MDD

Note. Legend and abbreviations: ACE: acetaminophen; ADJ: adjustment disorder; Age in years; ANX: anxiolytic; BARB: barbiturate; Bul/Anor: bulimia/anorexia; CV: cardiovascular; DELU: delusional disorder; GAM: gambling; LIDO: lidocaine; MDD: major depressive disorder; MVA: motor vehicle accident; NA: not available; PMI: postmortem interval in hours; SCZ: schizophrenia; SCZD: paranoid schizophrenia; SZAF: schizoaffective; SER: sertraline.