



Pergamon

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

**ADDICTIVE
BEHAVIORS**

Addictive Behaviors 29 (2004) 665–671

Double-blind trial of the effects of tryptophan depletion on depression and cerebral blood flow in smokers

Michele Pergadia^{a,b,c,*}, Bonnie Spring^{b,c}, Lukasz M. Konopka^c,
Barbara Twardowska^c, Parvez Shirazi^c, John W. Crayton^c

^aWashington University School of Medicine, St. Louis, MO, USA

^bUniversity of Illinois at Chicago, Chicago, IL, USA

^cHines VA Hospital, USA

Abstract

Studies of clinically depressed patients have documented left frontal lobe hypoactivity. Smokers also show an increased prevalence of depression and evidence that nicotine normalizes qEEG indices of left frontal lobe activity. Tryptophan depletion (TD) has been shown to increase negative mood in smokers, particularly those with recurrent depression. Thus, in smokers, we expected that increased depression during TD would be associated with decreased cerebral blood flow, specifically in the left frontal lobe. Hamilton depression scores and relative regional cerebral blood flow (rCBF) were measured with SPECT using ^{99m}Tc-hexamethylpropyleneamineoxime in seven smokers after TD and after a control procedure. Decreased bilateral cerebral blood flow to the inferior frontal (IF) lobe following TD relative to placebo was associated with increased depressed mood ($r = -.653$, $P < .05$). Among smokers, a decrease in brain serotonin is associated with increased depressed mood and with focal bilateral decreases in IF activity. Chronic nicotine exposure appears to be associated with cortical responses suggestive of depressive vulnerability.

© 2004 Elsevier Ltd. All rights reserved.

Keywords: Smoking; Depression; Tryptophan depletion; SPECT; Inferior frontal cortex

1. Introduction

Studies assessing cerebral blood flow via SPECT and PET images show that, prior to treatment, depressed patients exhibit hypoperfusion in the frontal regions, particularly in the

* Corresponding author. Department of Psychiatry, Washington University School of Medicine, 40 N. Kingshighway, Suite One, St. Louis, MO 63108, USA. Tel.: +1-314-286-2270; fax: +1-314-286-2213.

E-mail address: michelep@matlock.wustl.edu (M. Pergadia).

left frontal lobe (Baxter, 1991; Drevets & Raichle, 1992; Martinot et al., 1990). Treatment with antidepressants results in a normalization of frontal blood flow (Martinot et al., 1990). Electrocardinal studies (qEEG) also show a pattern of decreased activity in the left frontal lobe during depression and a rise in activity following stabilization of mood with treatment (Davidson, Ekman, Saron, Senulis, & Friesen, 1990; Henriques & Davidson, 1991). Using qEEG in smokers, Gilbert (1987) found that nicotine increased left frontal lobe activity relative to right, particularly among more introverted smokers. Nicotine withdrawal, in contrast, decreased left frontal activity relative to right, and was associated with sustained vigilance impairments lasting as long as 1 month post-cessation for smokers high in neuroticism (Gilbert et al., 1992). These findings suggest that nicotine may act similarly to antidepressant treatment for smokers by normalizing left frontal lobe activity.

A recent Cochrane review by Shaw, Turner, and Del Mar (2002) evaluated 108 controlled clinical trials examining the antidepressant effect of 5-hydroxytryptophan (5-HTP) or tryptophan compared to placebo. Reviewers found some evidence that 5-HTP and tryptophan may be more effective than placebo in decreasing depressive symptoms. Using tryptophan depletion (TD) as a biological strategy to increase dysphoric mood, Bremner et al. (1997) examined brain metabolism via PET in remitted depressed patients currently medicated with SSRIs. Results showed that TD decreased regional frontal lobe metabolism compared to placebo. In addition, Bremner et al. (1997) found that decreases in frontal lobe activity were correlated with increases in depressed mood. Using PET in eight remitted depressed patients, Smith, Morris, Friston, Cowen, & Dolan (1999) found that TD increased depressed mood compared to PBO, and increased depression was correlated with decreased activity in the left orbitofrontal cortex. On the other hand, Yatham et al. (2001) found TD did not increase depressed mood in participants without a history of psychiatric illness; however, PET images showed bilateral decreases in serotonin receptor (5-HT₂) binding activity in localized brain areas, including frontal regions. These studies provide evidence that TD is a sufficiently powerful dietary challenge to temporarily alter brain activity, particularly in patients with a history of depression.

To date, no study has examined the effect of a TD challenge on dysphoria and localized brain activity in smokers. The primary aim of this study was to determine whether TD, a challenge that functionally depletes plasma tryptophan and presumably, brain serotonin, would alter mood and brain activity in smokers. More specifically, we hypothesized that smokers would exhibit increased depression and decreased left inferior frontal (LIF) blood flow after TD as compared to PBO, and that changes in depression would correlate with blood flow. An exploratory aim was to examine whether effects of TD appeared to be more pronounced for smokers with a history of depression.

2. Method

2.1. Participants

Participants in this study were volunteers who responded to posted advertisements recruiting for smokers with or without a history of depression. Seven smokers participated

in the study [mean age 32.85 (S.D. = 15.68), range 19–64 years]. They were heavy smokers, smoking an average of 18.86 (S.D. = 10.04) cigarettes per day for at least 1 year. All participants underwent a general physical examination and CBC differential testing, and were noted as unremarkable by study physicians. Three participants had a history of at least one episode of *DSM-IV* major depression (American Psychiatric Association, 1994) as assessed by the Structured Clinical Interview for *DSM-IV* (SCID; Spitzer, Williams, & Gibbon, 1994). The four other smokers had never had an episode of major depressive disorder. All participants had been medication-free and lacking significant symptoms for at least 6 months. The study received approval from the local research ethics committee. All enrollees gave informed consent to participate and were explicitly informed that the study procedure might cause a reappearance of dysphoric mood. The study nurse monitored participants' vital signs and general condition throughout testing.

2.2. Procedures

Smokers were tested double-blind in a counterbalanced, randomized design in which they received tryptophan-depleting and a taste-matched placebo mixtures on 2 days separated by a 1-week interval. Mood was assessed at baseline, and again at 3, 5, and 7 h postconsumption. Moreover, at 5 h postconsumption, the time of peak plasma TD, brain activity was assessed via SPECT imaging.

Participants arrived at the laboratory after fasting from 8 p.m. on the evening before testing. All breakfast, lunch, and midday snack foods were provided during test days and were the low-tryptophan diet used by Delgado et al. (1990). The tryptophan-depleted mixture consisted of a combination of 15 amino acids given in a chocolate-based drink. L-methionine, L-cysteine, and L-arginine were encapsulated to mask their unpalatable taste. The remaining 12 amino acids (L-alanine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine, and L-valine) were mixed with 3 oz of tonic water and blended with crushed ice, psyllium, baking soda, chocolate syrup, and peppermint extract. In the placebo condition (PBO), participants ingested capsules containing confectioner's sugar, and drank placebo beverage consisting of the same tonic water mixture. Following the drink, participants sat quietly in a testing room and were allowed to read neutral material and to smoke ad lib. Five hours after drink consumption, participants underwent a negative mood induction during which they listened over headphones to personalized scripts that had been equated across test days for affective intensity. A 25-mCi injection of HMPAO (^{99m}Tc -hexamethylpropyleneamineoxime) was administered intravenously during the mood induction.

The Hamilton Rating Scale for Depression (HAM-D; Hamilton, 1960), modified for recurrent use over a short period of time, was administered at baseline and again 3, 5 and 7 h after the challenge drinks. The modified HAM-D included nine items that Delgado et al. (1991) found to be sensitive to TD: loss of energy, decreased concentration, loss of interest, depressed mood, decreased appetite, loss of pleasure, ruminative thinking, worthlessness/failure, and psychic anxiety. To insure that participants were maintaining their usual level of nicotine intake, carbon monoxide readings were taken at 2-h intervals throughout both test days.

2.3. Image acquisition and processing

Participants were tested in a soundproof room awake and seated with their eyes closed. Technetium-99 m-HMPAO (Amersham International) was prepared and a 25-mCi (740-MBq) dose was administered intravenously. After approximately 30 min, a rotating gamma camera system equipped with ultra high resolution fan beam collimators (Trionix system) scanned each participant's brain. Images were obtained in a 256×128 format with an acquisition time of 50 s per step. Fan beam projection data were converted to parallel data in 128×128 format and the SPECT reconstruction was performed with a Henning filter. Approximately 60 transverse slices were generated per participant.

Individual images were coregistered and resliced to generate the same number of slices per study. This facilitated placement of Regions of Interest (ROIs) template. Images were coregistered using commercially available software (Medx) by Sensor systems.

The Talairach atlas guided the placement of ROIs. Symmetrical hand-drawn ROIs were combined into functional areas by using standard landmarks and classification schemes to define the following: inferior frontal (IF) cortex, superior frontal (SF) cortex, temporal cortex, cingulate cortex, parietal cortex, the thalamus, and the caudate for both the left and right hemispheres. A rCBF was calculated for each ROI using the average activity in the region normalized to the activity level of the whole brain.

2.4. Statistical analysis

A 2×2 mixed model ANOVA was used to test the a priori hypothesis that depression would increase and LIF blood flow would decrease after TD as compared to PBO. Condition (TD vs. PBO) was the within-participants factor and history of depression acted as a between-participants factor. We predicted a main effect of condition, such that TD, compared to placebo would increase depression and decrease LIF blood flow in smokers. We also explored any evidence suggesting an interaction such that the magnitude of the effects would be greater for smokers with a history of depression compared to those without a history of depression. A Pearson correlation was computed to test the strength of the relationship between depression and cerebral blood flow to the LIF region following TD.

To investigate the specificity of TD effects on regional brain activity, mixed model ANOVAs were also used to explore whether TD compared to PBO would also decrease blood flow to other ROIs. The comparative regions included bilateral IF, SF, cingulate, thalamus, caudate, temporal lobes, and the parietal lobes.

3. Results

Results showed that smokers' depressive symptoms were significantly affected by TD, independently of history of depression. Level of depression increased significantly ($t = 3.42$; $P < .01$) from an average Hamilton score of 1.42 (S.D. = 1.72) at baseline to 4.00 (S.D. = 3.16) at peak depletion period (5 h after TD). Depression 5 h after TD ($m = 4.00$; S.D. = 3.16) also

Table 1
Mean regional blood flow after PBO versus TD^a

Brain region	Placebo	Tryptophan depletion	<i>t</i>
Inferior frontal	617.98 (22.88)	601.37 (30.80)	2.03*
Superior frontal	633.75 (41.84)	623.25 (42.31)	1.34
Cingulate	646.03 (18.45)	636.08 (27.54)	1.23
Thalamus	568.89 (41.36)	560.46 (13.00)	1.68
Caudate	566.64 (24.51)	565.54 (7.03)	0.19
Temporal	515.77 (24.12)	501.96 (27.13)	1.24
Parietal	619.70 (21.64)	605.34 (35.31)	1.19

^a Values are given as mean (\pm S.D.). Regional blood flow data are normalized to the whole brain.

* ($t=2.03$, $P<.05$).

tended to be greater than depression 5 h after PBO ($m=2.14$; S.D. = 1.67; $P<.10$). There was no evidence that the mood deterioration in response to TD was greater for smokers with a history of depression than those lacking depressive history.

Bilateral blood flow to the IF lobe as measured by SPECT decreased significantly following TD ($m=601.37$; S.D. = 30.80) as compared to PBO ($m=617.98$; S.D. = 22.88; $t=-2.03$; $P<.05$). Moreover, the degree of decrease in IF cerebral blood flow produced by TD correlated significantly with the degree of increase in depressed mood induced by TD ($r=-.653$, $P<.05$).

The specific decrease in blood flow to the left frontal lobe following TD ($m=599.23$; S.D. = 32.29) as compared to PBO ($m=607.31$; S.D. = 24.50) was no greater than the overall bilateral change. Again, there was no difference between smokers with, versus without, a history of depression.

Cerebral blood flow to regions outside of the IF cortex showed no significant differences between PBO and TD conditions (see Table 1). Nor were there correlations between altered blood flow to these regions following TD and changes in depression (all $P_s>.05$).

4. Discussion

Study results indicate that double-blind TD increased symptoms of depression and decreased blood flow to the prefrontal cortex of smokers. Based on prior neurophysiological studies of TD and depressive symptoms (Smith et al., 1999; Bremner et al., 1997), we had expected to observe only left-sided hypofrontality. Instead, we found that TD produced bilateral decreases in IF activity. Moreover, increased depression in response to TD correlated significantly with decreased IF blood flow. These results were highly specific to the IF cortex, in that no other brain region was significantly affected by TD, nor were changes in other regions correlated with changes in depression.

Whereas prior studies have detected TD-related mood changes chiefly among remitted depressed people who are medicated with SSRIs (Bremner et al., 1997; Smith et al., 1999), we observed significant changes across smokers, including those who lacked a history of depression. There was no evidence in this sample to suggest that either mood or blood flow

changes in response to TD were greater among smokers with a prior episode of depression than those who lacked such a history. Their observed mood changes after tryptophan were comparable and, overall, no extreme fluctuations in mood were noted. It may be that chronic nicotine use alters brain activity bilaterally within the IF cortex in a manner that conveys a vulnerability to depression (Henriques & Davidson, 1991; Mayberg, Lewis, Regenold, & Wagner, 1994; Quattrocki, Baird, & Yurgelun-Todd, 2000). The observation that smokers with or without a history of depression respond to TD with depressive, subjective, and biological signs is consistent with the suggestion that chronic nicotine self-administration augments depressive vulnerability (Balfour & Ridley, 2000; Goodman & Capitan, 2000). That nicotine engenders changes in brain function similar to those associated with depression might help to explain why successful antidepressant pharmacotherapies for nicotine dependence show comparable efficacy regardless of whether or not smokers have a history of depression (Hall et al., 1998; Hayford et al., 1999).

Results require replication in a larger sample, but they highlight the usefulness of TD as a paradigm to unmask both depressive symptoms and cortical markers associated with nicotine dependence. Greater understanding of how nicotine and depression conjointly influence serotonin receptor regulation should enhance the development of treatments for comorbid nicotine dependence and depression.

Acknowledgements

This study was supported by grants from VA Merit Review (BS), American Heart Association (MP), and Amersham Medi + Physics (LMK).

Portions of this work were presented in abstract form at the 2000 annual meeting of the Society of Biological Psychiatry and the 2001 meeting of the Society for Research on Nicotine and Tobacco. We express appreciation to Elizabeth Massey, Francine O'Connor, Pamela Kohlbeck, and Regina Pingitore for their assistance on this project.

References

- American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders*. (4th ed.). Washington, DC: American Psychiatric Association.
- Balfour, D. J. K., & Ridley, D. L. (2000). The effects of nicotine on neural pathways implicated in depression: A factor in nicotine addiction? *Pharmacology, Biochemistry and Behavior*, *66*, 79–85.
- Baxter, L. R. (1991). PET studies of cerebral function in major depression and obsessive-compulsive disorder: the emerging prefrontal cortex consensus. *Annals of Clinical Psychiatry*, *3*, 103–109.
- Bremner, J. D., Innis, R. B., Salomon, R. M., Staib, L. H., Ng, C. K., Miller, H. L., Bronen, R. A., Krystal, J. H., Duncan, J., Rich, D., Price, L. H., Malison, R., Dey, H., Soufer, R., & Charney, D. S. (1997). Positron emission tomography measurement of cerebral metabolic correlates of tryptophan depletion-induced depressive relapse. *Archives of General Psychiatry*, *54*, 364–374.
- Davidson, R. J., Ekman, P., Saron, C., Senulis, J., & Friesen, W. V. (1990). Approach/withdrawal and cerebral asymmetry: Emotional expression and brain physiology. *Journal of Personality and Social Psychology*, *58*, 330–341.

- Delgado, P. L., Charney, D. S., Price, L. H., Aghajanian, G. K., Landis, H., & Heninger, G. R. (1990). Serotonin function and the mechanism of antidepressant action. Reversal of antidepressant-induced remission by rapid depletion of plasma tryptophan. *Archives of General Psychiatry*, *47*, 411–418.
- Delgado, P. L., Price, L. H., Miller, H. L., Salomon, R. M., Licinio, J., Krystal, J. H., Heninger, G. R., & Charney, D. S. (1991). Rapid serotonin depletion as a provocative challenge test for patients with major depression: Relevance to antidepressant action and the neurobiology of depression. *Psychopharmacology Bulletin*, *27*, 321–330.
- Drevets, W. C., & Raichle, M. E. (1992). Neuroanatomical circuits in depression: Implications for treatment mechanism. *Psychopharmacology Bulletin*, *28*, 261–274.
- Gilbert, D. G. (1987). Effects of smoking and nicotine on EEG lateralization as a function of personality. *Personality and Individual Differences*, *8*, 933–941.
- Gilbert, D. G., Meliska, C. J., Welsler, R., Scott, S., Jensen, R. A., & Meliska, J. (1992, March). *Individual differences in the effects of smoking cessation on EEG, mood and vigilance*. Paper presented at the Thirteenth Annual Meeting of the Society of Behavioral Medicine, New York.
- Goodman, E., & Capitman, J. (2000). Depressive symptoms and cigarette smoking among teens. *Pediatrics*, *106*, 748–755.
- Hall, S. M., Reus, V. I., Munoz, R. F., Sees, K. L., Humfleet, G., Hartz, D. T., Frederick, S., & Triffleman, E. (1998). Nortriptyline and cognitive-behavioral therapy in the treatment of cigarette smoking. *Archives of General Psychiatry*, *55*, 683–690.
- Hamilton, M. (1960). A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry*, *23*, 56–62.
- Hayford, K. E., Patten, C. A., Rummans, T. A., Schroeder, D. R., Offord, K. P., Croghan, I. T., Glover, E. D., Sachs, D. P., & Hurt, R. D. (1999). Efficacy of bupropion for smoking cessation in smokers with a former history of major depression or alcoholism. *British Journal of Psychiatry*, *174*, 173–178.
- Henriques, J. B., & Davidson, R. J. (1991). Left frontal hypoactivation in depression. *Journal of Abnormal Psychology*, *100*, 535–545.
- Mayberg, H. S., Lewis, P. J., Regenold, W., & Wagner Jr., H. N. (1994). Paralimbic hypoperfusion in unipolar depression. *The Journal of Nuclear Medicine*, *35*(6), 929–934.
- Martinot, J. L., Hardy, P., Feline, A., Huret, J., Mazoyer, B., Attar-Levy, D., Pappata, S., & Syrota, A. (1990). Left prefrontal glucose hypometabolism in the depressed state: A confirmation. *American Journal of Psychiatry*, *147*, 1313–1317.
- Quattrocki, E., Baird, A., & Yurgelun-Todd, D. (2000). Biological aspects of the link between smoking and depression. *Harvard Review of Psychiatry*, *8*(3), 99–110.
- Shaw, K., Turner, J., & Del Mar, C. (2002). Tryptophan and 5-hydroxytryptophan for depression (Cochrane review). *The cochrane library, issue 2*. Oxford: Updated Software.
- Smith, K. A., Morris, J. S., Friston, K. J., Cowen, P. J., & Dolan, R. J. (1999). Brain mechanisms associated with depressive relapse and associated cognitive impairment following acute tryptophan depletion. *British Journal of Psychiatry*, *174*, 525–529.
- Spitzer, R. L., Williams, J. B. W., & Gibbon, M. (1994). *Structured clinical interview for DSM-IV*. New York: New York State Psychiatric Institute, Biometrics Research Department.
- Yatham, L. N., Liddle, P. F., Shiah, I., Lam, R. W., Adam, M. J., Zis, A. P., & Ruth, T. J. (2001). Effects of rapid tryptophan depletion on brain 5-HT₂ receptors: A PET study. *British Journal of Psychiatry*, *178*, 448–453.