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Reply to Pradeep J Nathan and Ben J Harrison

Reply: Pronounced Cognitive Deficits Following an Intravenous L-Tryptophan Challenge in First-degree Relatives of Bipolar Patients Compared to Healthy Controls

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Sir

Thank you very much for your interest in our paper 'Pronounced cognitive deficits following an intravenous L-Tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls' (Sobczak *et al*, 2003b). We appreciate a critical review of the interpretation of our research findings. Next, we will give our response on the three major points of the letter.

GENERAL METHODOLOGICAL CONSIDERATION

If in an experiment, independent variable *X* is manipulated leading to a change in dependent variable *Y*, experimental methodology only permits conclusions attributing the change in *Y* to varying levels of *X*. Suppose that the manipulation of *X* induces changes in *Y*, but these are known to be actually accomplished by *Z*, in such a way that it would have been more appropriate to manipulate *Z* rather than *X*? One should attempt to either manipulate/control *Z* or measure its known indicator(s). According to experimental methodology, one may only speculate but not conclude about the potential mediating influence of *Z*. Obviously, *X* refers to tryptophan (Trp) and *Y* to cognitive performance variables. *Z* refers primarily to serotonin (5-HT), as the fact that manipulating Trp primarily brings about changes in 5-HT would be beyond dispute (but see point 1). The aim of this experiment and a series of other experiments was to study the role of serotonergic vulnerability, expressed as responsivity to serotonergic manipulations in healthy first-degree relatives of bipolar patients (FH). We deliberately did not aim at assessing dopaminergic effects as we assumed that the role of dopamine in bipolar disorders is almost beyond dispute. Therefore, it is only of methodological interest if we should have considered dopaminergic factors in the present experiment. Obviously, an experiment similar to the present, in which dopaminergic turnover would be varied, is also of great interest. Perhaps the best approach would be when the two are combined.

However, several other downstream effects of manipulating Trp should be considered as well. 5-HT is known to have inhibitory influences on noradrenaline, acetylcholine, and dopamine turnover (Robbins, 1997). In particular, inhibitions of acetylcholine transmission brought about by increased 5-HT turnover might explain the large effects of Trp on delayed recall memory (Little *et al*, 1995). On the basis of that, speculation about other potentially mediating neurotransmitters to explain the effect of Trp on memory would point much more into the direction of acetylcholine than dopamine.

POINT 1: SPECIFICITY OF L-TRP CHALLENGE

The specificity of Trp as a serotonergic challenge has been debated several times. Indeed, it has been suggested that increasing Trp in the blood lowers tyrosine and phenylalanine uptake due to competition for the amino-acid transporter (van Praag *et al*, 1987). Dopamine lowers prolactin release, and the increase in prolactin after Trp is suggested to be due to a decrease in this inhibiting activity of dopamine. Prolactin is only an indirect parameter of brain dopaminergic activity. It has been shown that 5-HT also has direct prolactin-stimulating effects via the $5\text{-HT}_{2a/2c}$ receptors (Di Renzo *et al*, 1989, van de Kar *et al*, 1989). The only way to investigate the central effects on these neurotransmitters of Trp infusion is to assess 5-hydroxyindoleacetic acid (5-HIAA) and homovanillic acid (HVA) in

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the cerebrospinal fluid. These are invasive methods and, to our knowledge, such a study has never been described. Another indirect method is measurement of HVA in blood. For each HVA measurement, 1 ml blood is needed. As the amount of blood taken from subjects is limited and other blood parameters (ie hormones, amino acids) are also important to assess, we chose not to include HVA measurements.

Large neutral amino acids (LNAAs; leucine, valine, isoleucine, Trp, tyrosine, phenylalanine) compete for transporter uptake at the level of the blood–brain barrier. The ratios of Trp/LNAAs, phenylalanine/LNAAs, and tyrosine/LNAAs might give another indication of the availability of serotonergic, respectively dopaminergic, precursors in the brain. In the present study, the ratio of Trp/LNAAs increased significantly to a value of 2.3 from a baseline value of 0.13. This is an increase of 1669%. The ratio tyrosine/LNAAs decreased after Trp loading, but this was not significant ($F(1,42) = 0.017$, NS). The ratio phenylalanine/LNAAs was significantly lower after Trp ($F(1,42) = 88.98$, $P < 0.05$). The ratio decreased from 0.11 at baseline to 0.0013. This is a decrease of 103%. As the increase in the ratio of Trp is much higher than the decrease in the ratio of phenylalanine, and the ratio of tyrosine did not change at all, we suggest that the effects of the Trp loading are more likely attributable to central serotonergic effects than to dopaminergic effects.

5-HT acts as a neuromodulator, and important functional interactions between brain 5-HT and dopaminergic systems are known. 5-HT inhibits dopaminergic activity in the mesolimbic system but stimulates this activity in nigrostriatal structures (Manji and Potter, 1997). Thus, we cannot fully exclude that other neurotransmitters are involved in the cognitive effects of Trp and 5-HT.

Despite this we suggest that the cognitive effects of Trp challenge are primary serotonergic mediated because the detrimental effects of Trp on attention and planning in healthy FH have also been found after acute tryptophan depletion (ATD) (Sobczak *et al*, 2002c). Thus, it seems that both an increase and decrease in Trp impair the same cognitive domains that are regulated by the frontal lobe (Sobczak *et al*, 2003b). The specificity of ATD has been pointed out by Klaassen *et al* (1999). They found a decrease in mood and impaired memory performance after ATD but not after lysine depletion. This supports the hypothesis that ATD affects brain 5-HT functioning and not brain protein metabolism in general.

POINT 2: COGNITIVE DEFICITS ON BASELINE ARE MEDIATED BY DOPAMINE

The second suggestion made by Nathan and Harrison (2003) is that the baseline cognitive deficits in FH may be a result of dysregulation of dopaminergic activity in mesocortical regions.

It must be emphasized that the FH subjects in this study were free of any clinical psychiatric symptoms. Thus, cognitive impairments were not related to altered mood states (Sobczak *et al*, 2003b). Therefore, if there was a dopaminergic dysfunction in these subjects at baseline, it would only have affected cognition and not mood.

Independent of Trp, cognitive performance was more impaired in relatives of type I bipolar patients (FH I) compared to relatives of type II patients (FH II). This suboptimal baseline performance in FH I resulted in more pronounced cognitive deficits after Trp (ie on planning). These findings agree with an association of serotonergic vulnerability and cognitive impairments in FH I subjects. FH II subjects were characterized by mood changes after ATD and Trp challenge (Sobczak *et al*, 2002a, b). Taking these findings together, we speculate that FH II subjects share more symptoms of primary affective disorders in which 5-HT plays a prominent role, whereas FH I subjects show characteristics of primary psychotic disorders in which cognitive deficits persist and functional deficits in other neurotransmitters like noradrenaline, acetylcholine and dopamine, or even structural brain abnormalities, may also be involved.

POINT 3: FUTURE STUDY ON THE ROLE OF 5-HT AND DOPAMINE

Indeed the search for biological markers of bipolar disorders is just in its infancy. The present findings must first be replicated in a larger research population including relatives of type I and type II bipolar patients. Of interest is whether there is a biological distinction between type I and type II patients and their relatives. The vulnerability to serotonergic, dopaminergic, and noradrenergic dysfunction should be investigated using ATD, specific serotonergic challenge procedures, and a phenylalanine/tyrosine depletion test. Abnormalities in cholesterol and fatty acids have also been associated with altered brain neurotransmitter activity and psychopathology (Sobczak *et al*, 2003a; Hibbeln and Salem, 1995; Swartz, 1990). Thus, research into the interaction of neurotransmitters, cholesterol, and fatty acids in humans and their association with psychopathology is still a challenge.

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