Commentary

Don't panic. A guide to tryptophan depletion with disorder-specific anxiety provocation

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SD Hood[1], CJ Bell[2], SV Argyropoulos[3] and DJ Nutt[3]

Abstract

The 2002 paper "Does 5-HT restrain panic? A tryptophan depletion study in panic disorder patients recovered on paroxetine" by Bell and colleagues – reprinted in this issue of the Journal – reports on a study undertaken in the halcyon days of David Nutt's Psychopharmacology Unit at the University of Bristol, England. In this invited commentary authors of the original work discuss the impact of this paper on the field of acute tryptophan depletion research (especially in the field of clinical anxiety disorders) and the development of disorder-specific anxiogenic provocations over the past decade.

Keywords

Psychopharmacology, anxiety, tryptophan depletion, serotonin

Our paper "Does 5-HT restrain panic? A tryptophan depletion study in panic disorder patients recovered on paroxetine." (Bell et al., 2002) – reprinted in this issue of the Journal – reports on a study undertaken in the halcyon days of David Nutt's Psychopharmacology Unit at the University of Bristol, England. The turn of the century was a formative period for the junior authors of this research, undertaken in a milieu that was rich in intra- and interdisciplinary outputs and camaraderie despite the undistinguished physical locale near waste bins in the campus basement.

It was a time of much promise for psychopharmacology research, significant pharmaceutical company sponsorship of clinical research, cascading launches of new medicines to treat depressive and anxiety disorders – much of which, a decade and a half later, has failed to deliver. Looking now at the withdrawal of much of pharma from neuroscience R&D, the failure of medicines such as the neuropeptides to reach the market (Christmas et al., 2008), the relative stasis in anxiety clinical guidelines over this period (Hood, 2015), the tightening of competitive research budgets and the preoccupation of government health authorities with overtly translational research to the exclusion of basic sciences research, one can be forgiven for the concluding that this "golden age" of clinical psychopharmacology research is over!

Our paper explored the role of synaptic 5-HT in the effects of selective serotonin reuptake inhibitors (SSRIs) to restrain panic in patients with panic disorder. To do this we employed the acute tryptophan depletion (ATD) method to deplete synaptic 5-HT (Delgado et al., 1989). This methodology was developed for the study of 5-HT in the actions of antidepressants in depression treatment where negative mood changes were found following ATD in patients recovered on SSRIs but not on noradrenaline-targeting drugs. In our study we used this methodology in patients with panic disorder who had also responded to treatment with an SSRI. An important difference in the design, however, was that we employed a disorder-specific provocation challenge in addition to the ATD to provoke an anxiety response. We measured spontaneous and provoked changes in anxiety in a within-subject, repeated-measures design, with participants blindly receiving a tryptophan-containing "control" mixture on a separate testing occasion.

The provocation we chose to use was flumazenil, as our group had shown it to provoke panic in an earlier paper (Nutt et al., 1990). The control condition was normal saline infusion near the time of maximal ATD effect. The primary study finding was that flumazenil produced a panic attack (defined by changes in the panic inventory) in seven out of 14 patients when tryptophan depleted and one out of 14 on the control day ($p<0.02$). It was a lesser proportion than we found in untreated panic disorder patients where almost all panicked – eight of ten (Nutt et al., 1990). We suggested that this was supportive of the theory proposed by Deakin and Graeff, who suggested that 5-HT has a restraining effect on panic symptoms in patients who had responded to treatment with an SSRI (Deakin and Graeff, 1991). That some treated patients did not panic after ATD and flumazenil challenge may reflect a failure to adequately deplete 5-HT *intracerebrally*, as plasma measures showed they had the same levels of peripheral ATD depletion. Lack of response of some patients to ATD in depression had been reported (Booij et al., 2002), so an alternative explanation is that these subjects are less affected by changes to brain 5-HT. Another explanation may be that some people have moved into a stage of recovery that means that they no longer require an increase in 5-HT in the synapse to

[1]School of Psychiatry & Clinical Neurosciences (M521), University of Western Australia, Perth, WA, Australia
[2]Mental Health Clinical Research Unit, Department of Psychological Medicine, University of Otago, Christchurch, New Zealand
[3]Centre for Neuropsychopharmacology, Division of Brain Sciences, Imperial College London, London, UK

Corresponding author:
SD Hood, Associate Dean (Community & Engagement), Head (School of Psychiatry & Clinical Neurosciences), Faculty of Medicine, Dentistry & Health Sciences, The University of Western Australia, 35 Stirling Highway, Crawley, Perth WA 6009, Australia.
Email: sean.hood@uwa.edu.au

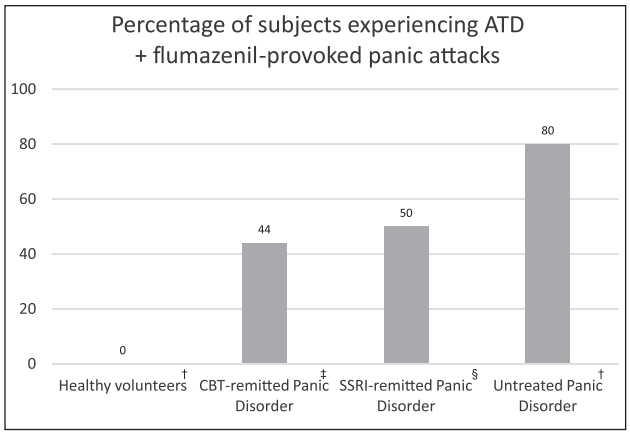

Figure 1. Increasing susceptibility to panic symptoms in response to challenge.
† Nutt et al. 1990; ‡ Bell et al. 2011; § Bell et al. 2002.

restrain panic. Similar findings have also been reported in ATD in depressed patients recovered on SSRIs, where the most profound effects are seen early in recovery from the episode, with the proportion of patients sensitive to ATD decreasing the longer they have stayed well.

Conceived at the same time as our study, but completed and then published a decade later, was a sister protocol that applied the same provocation schedule to patients who had responded to cognitive behavioural therapy (CBT) (Bell et al., 2011). This study reported that flumazenil produced a panic attack in four out of nine patients when tryptophan depleted and two out of nine on the control day ($p<0.06$), that is, a non-significant trend. There was a significant interaction between ATD and flumazenil and some (but not all) self-report measures of anxiety (visual analogue scale total scores and the Spielberger State Anxiety Inventory (Spielberger et al., 1983)). These findings suggest ATD increases some measures of anxiety in response to flumazenil in patients who had responded to treatment with CBT, which also has similarities with the findings of Smith and Cowen in depression where recovered patients – some of whom had only been treated with CBT – also relapsed under ATD (Smith et al., 1997).

Taken together the results from these treated panic disorder studies could be interpreted as suggesting a role for serotonergic processes in both treatment modalities. The other, and we now feel more likely, explanation of the anxiogenic effects of ATD in some treated panic disorder patients is that these subjects have a vulnerability to changes to 5-HT function and that ATD uncovers or activates a neural circuit, as has previously been suggested to explain the effects of ATD in treated depression (Booij et al., 2002; Neumeister et al., 2006). ATD would therefore, in reducing 5-HT function, result in a more activated anxiety network, and this may explain the results in both the SSRI and CBT responder studies.

Figure 1 below summarises the responses to flumazenil and ATD in the three studies in patients with panic disorder (the untreated (Nutt et al., 1990), the SSRI responders (Bell et al., 2002) and CBT responders (Bell et al., 2011)). This suggests (i) that the panicogenic response to flumazenil can be at least partially blocked by effective treatment (either by CBT or SSRI); and (ii) that ATD increases the vulnerability to panic.

This research project, the first in a series of ATD studies in anxiety partially supported by the Wellcome Trust, established some of the standards of our clinical trial provocations to follow and in retrospect was the springboard for a significant body of related research that continues to the current day.

Disorder-specific provocations were designed for our subsequent ATD studies. In our study examining patients with social anxiety disorder who had responded to SSRIs three behavioural challenges were used – an autobiographical script relating to an intensely embarrassing personal experience, a verbal performance task in which the patient was instructed to give an impromptu oral presentation to three observers on a topic related to their professional activities or general interests, and a neutral (non-anxiogenic subject matter) script as a behavioural control (Argyropoulos et al., 2004). In the post-traumatic stress disorder (PTSD) study an autobiographic audio narrative of the traumatic event was used to provoke memories of the traumatic event (Corchs et al., 2009). In the obsessive–compulsive disorder (OCD) study an exposure paradigm targeted to the individual's feared situation was the provocation (Hood, 2010), and in generalised anxiety disorder (GAD) a 12–20 minute inhalation of 7.5% CO_2 was used (Hood et al., 2010). ATD studies in other groups were also subsequently conducted by our group, including in patients with irritable bowel syndrome (IBS) who demonstrated intermediate levels of anxiety and depressive symptoms after ATD (Shufflebotham et al., 2006), and normal volunteers subjected to serotonergic manipulation (Hood et al., 2006).

Pooled analyses of our data supported a separation between TD-responsive anxiety disorders (panic/social anxiety/PTSD) versus TD-non-responsive disorders (OCD and GAD) in a manner that parallels other developments in psychiatric nomenclature, for example differentiating fear and worry disorders (Corchs et al., 2015). These pooled data are in concordance with previous theories and their predictions (Deakin and Graeff, 1991). According to these theories, fear disorders would be related to aversive contingencies in which the organism needs to move away from a threat and in which 5-HT acutely modulates sensitivity to fear-related stimuli. Once undermined by ATD, a relapse occurs. In contrast to the fear disorders, these theories also predict that anxiety disorders would be related to aversive contingencies in which the organism has to approach the threat.

ATD studies in other groups were also conducted by our group, including patients with IBS who demonstrated levels of anxiety and depressive symptoms intermediate between that of normal controls and the SSRI-remitted patient groups described above (Shufflebotham et al., 2006). Another study by our group examining ATD in normal volunteers subjected to a single-breath 35% CO_2 inhalation (Hood et al., 2006) demonstrated that ATD did not augment the psychological response to a 35% CO_2 stressor in healthy controls. Furthermore, there appeared to be no additive effect of ATD on endocrine or cardiovascular responses to this challenge.

There were other less expected, but nevertheless scientifically important sequelae of this research. Firstly, Jayne Bailey subsequently led the establishment of the 7.5% CO_2 model of GAD, prompted in large part by our desire to find a suitable disorder-specific provocation for TD-GAD (Bailey et al., 2011). We reported a pressor effect of about 9 mm Hg systolic in this paper, with cardiovascular measures initially included largely as a safety monitoring item that we have reported on elsewhere (Hood

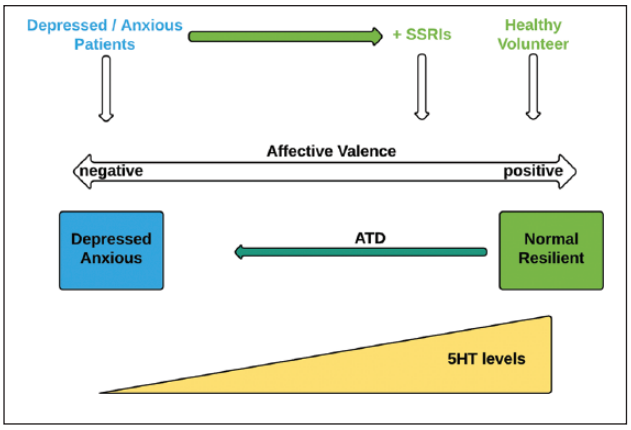

Figure 2. What does 5HT do in the brain?

et al., 2009b). Presciently, we have noted a similar pressor response in the TD-responsive anxiety disorders (Corchs et al., 2015) that corresponds to observations of serotonin-autonomic-panic disturbances in hypertensive patients investigated over the past decade by some of us (Davies et al., 2006, 2008).

Taken together, the idea of 5-HT acting to promote emotional resilience developed as in Figure 2. This incorporates the findings from both our anxiety and others' depression studies using ATD. Psychological analyses of depression and anxiety disorders agree that each disorder is associated with a psychological trait of negative affect, the tendency to see the world as a more hostile and threatening place than other people without these disorders do. Anxiety and depression differ in that in depression there is a deficit of the ability to experience positive affect (loss of hedonic ability) that is preserved in anxiety disorders. We have suggested elsewhere (Nutt et al., 2007) that the hedonic axis is driven by the activity of dopamine and noradrenaline neurotransmitters and the affective valence one by 5-HT.

According to this theory SSRIs (and other antidepressants that enhance 5-HT function) move patients from negative to neutral or even positive valence, and they become similar to normal people. This improvement in outlook is accompanied by an increase in resilience to stress, something well demonstrated in animals. However, when 5-HT levels are reduced by ATD the underpinning state of negative affective valence returns – that is, the patient relapses.

We are aware of and anticipate related research in the future. Although the study reviewed here and subsequent ATD experiments by our group used the standard ATD mixture as detailed by Young et al. (Hood et al., 2005; Young et al., 1985), colleagues have also used a gelatine-based mixture (Lieben et al., 2004) in patients with panic disorder with discrepant findings (Colasanti et al., 2011) – possibly reflecting differences in the formulations (Sobczak and Schruers, 2014) and prompting a call for standardisation (Badawy and Dougherty, 2015). Nevertheless, other ATD formulations – such as the simplified Moja-De preparation (Sanchez et al., 2015) – are becoming popular, and we await the emergence of a preference formulation for clinical psychopharmacology.

Next, flumazenil was used as an IV bolus panicogen delivered within 10 minutes in our 2002 paper; however, our group and others have been investigating the utility of slow infusions delivered over days as a *therapy* to treat benzodiazepine dependence (Hood et al., 2009a; Hulse et al., 2013). It is intriguing that altering the rate of delivery has such a marked difference on symptom outcome, providing insights into the mechanisms underlying the effect of a medicine that is considered in routine medical care as a benzodiazepine antagonist (Hood et al., 2014).

The importance of nutrition and dietary factors in psychiatric illnesses is emphasised in recent literature and popular media (Lopresti et al., 2012; Sarris et al., 2015), but the pioneering work of the early tryptophan depletion studies in establishing the conventional serotonin-deficit hypothesis of depression and related disorders is often forgotten (Zepf et al., 2015). Despite the limitations of researching small sample sizes using the tryptophan depletion method with disorder-specific provocations, this remains an area of ongoing research activity. Readers interested in this methodology are encouraged to contact us, or related organisations active in this field such as the International Society for Tryptophan Research (ISTRY, 2016).

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