100 boys). Future studies should explore gender-specific effects of functional polymorphic genes involved in the activation of the hypothalamo–pituitary–adrenal axis and the role in stress-related psychopathology.

Conflict of interest

The authors declare no conflict of interest.

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Converging evidence for central 5-HT effects in acute tryptophan depletion

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Acute tryptophan depletion (ATD), a dietary technique for manipulating brain serotonin (5-HT) function, has advanced our understanding of 5-HT mechanisms in the etiology and treatment of depression and other affective disorders.[1] A recent review article in *Molecular Psychiatry* questioned the validity of ATD.[2] Although we agree that ATD's effects on 5-HT activity at the molecular level need further clarification, van Donkelaar *et al.*[2] goes too far in challenging whether ATD exerts its effects through serotonergic mechanisms. There is strong evidence that ATD reduces brain 5-HT and disrupts stimulated 5-HT release,[3,4] and converging translational findings support a central role for brain 5-HT in ATD's effects on cognition and behavior.[5–7]

Van Donkelaar *et al.*[2] does not dispute the fact that ATD reduces 5-HT synthesis and brain 5-HT levels.[4] Their arguments converge on two issues. First, they claim that non-serotonergic mechanisms could explain ATD effects, including altered peripheral metabolic processes, cerebrovascular abnormalities and confounding stress effects. Although we welcome additional research to explore these complex alternative pathways, the most parsimonious explanation of ATD's effects on human cognition and behavior is that ATD influences central 5-HT function. ATD has produced results paralleling those following other 5-HT manipulations in humans and animals. For example, ATD has reliable and highly selective effects on emotional processing in humans, inducing a cognitive bias toward negative stimuli and away from positive stimuli.[5] In line with these findings, a recent translational study comparing the effects of citalopram and neurotoxin-induced global 5-HT depletions in rats showed that reducing 5-HT function increased sensitivity to negative stimuli and reduced sensitivity to positive stimuli, whereas enhancing 5-HT function had the opposite effect.[6] Many other equivalent examples abound.[7] These converging findings have been replicated across different laboratories, and support a primary role for brain 5-HT in mediating the effects of ATD.

Moreover, the argument that stress confounds the interpretation of ATD's effects is untenable. ATD studies are placebo-controlled for precisely this reason, so that the unpleasant aspects of the procedure are matched across placebo and depletion conditions. Although we acknowledge it is difficult to examine the effects of ATD (relative to placebo) under

non-stressful conditions in animals, this argument could equally be applied to most studies in experimental neuroscience, including those cited to support the criticisms of ATD. It is possible that stress could function synergistically with ATD, but not placebo, and contribute to the effects of ATD; however, there is some evidence suggesting that acute stress does not underlie the effects of ATD in humans. ATD can be administered in liquid form, which is highly unpleasant, or in capsule form, which is less unpleasant; these different procedures produced similar effects on fear recognition.[8] We also note that dietary depletion of other amino acids, such as tyrosine and phenylalanine, has effects on cognition, distinct from those of ATD.[9] Furthermore, most of the proposed alternative mechanisms would likely predict broad attentional and executive impairments following ATD, but these have not been reported;[8] rather, ATD produces highly selective effects on emotional processing and episodic memory.[5,8]

Second, van Donkelaar *et al*.[2] claim there is insufficient proof that ATD alters 5-HT release, citing two microdialysis studies that report no effects of ATD on basal 5-HT efflux in the medial prefrontal cortex or raphé nuclei.[2] However, these studies cannot rule out the possibility that ATD reduced basal 5-HT efflux in other regions. As 5-HT projections are highly diffuse, it is premature to draw conclusions about the entire 5-HT system, based on null results in just two brain regions.

More importantly, measuring basal 5-HT efflux is a poor test of whether ATD influences 5-HT neurotransmission. For example, post-weaning social isolation in rats has clear disruptive effects on the 5-HT system, and reduces 5-HT efflux during a task sensitive to 5-HT lesions, but has no effect on basal 5-HT efflux.[10] Therefore, a more appropriate test of whether ATD influences 5-HT release is to show that ATD reduces 5-HT efflux in conditions in which 5-HT release is stimulated. Several studies have confirmed this is the case.[2,3]

Because some of these studies used a serotonin-specific reuptake inhibitor (SSRI) in the dialysate fluid to enhance detection sensitivity, van Donkelaar *et al*.[2] conclude that ATD reduces 5-HT release only after systemic 5-HT reuptake blockade, making the strong claim that 'direct evidence that ATD decreases extracellular 5-HT concentrations is lacking'. However, this argument inappropriately equates the effects of SSRIs in dialysate fluid with those of systemic SSRI treatment. Although systemic SSRIs can reduce 5-HT release via presynaptic 5-HT_{1A} autoreceptors, previous microdialysis studies using SSRIs in the dialysate fluid have demonstrated that selective 5-HT_{1A} antagonists do not increase 5-HT efflux.[11] This suggests that the doses of SSRIs used to enhance microdialysis sensitivity are insufficient to influence 5-HT_{1A} autoreceptor function, being several orders of magnitude lower than systemic SSRI doses demonstrated to reduce 5-HT release.

The effects of ATD at the molecular level are undoubtedly complex, and the extent to which ATD alters 5-HT release in humans may vary across individuals and contexts. Future studies in animals might combine ATD with electrophysiology in the raphé, microdialysis or cyclic voltammetry under conditions known to activate 5-HT neurons, such as footshock regimens. Although existing positron emission tomography studies in humans are suggestive of serotonergic mechanisms in ATD, improved 5-HT tracer ligands are critically needed to reliably measure endogenous 5-HT.[12] Additional techniques for altering 5-HT function in humans, such as selective receptor antagonists, are also needed. Developing these techniques is especially important because the relevance for humans of ATD studies in rats is unclear. These studies will enhance our understanding of not only the effects of ATD but also the role of 5-HT in cognition and behavior. Until then, however, we suggest that the evidence is at least persuasive: ATD reduces 5-HT levels in brain tissue,[4] reduces stimulated 5-HT release[3] and alters human behavior in ways that converge with other 5-HT manipulations across species.[5–7] The most parsimonious explanation for these findings is that ATD alters central 5-HT function.

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