Brain mechanisms associated with depressive relapse and associated cognitive impairment following acute tryptophan depletion

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Background Acute tryptophan depletion lowers brain serotonin synthesis and results in a transient, but striking, clinical relapse in recovered depressed patients.

Aims To identify brain regions which change their activity as an acute depressive relapse evolves and to determine how pathological mood might modulate neural activity during a cognitive task.

Method We used $H_2{}^{15}O$ positron-emission tomography (PET) to study eight recovered depressed men after tryptophan depletion and after a control procedure. During both PET scan sessions, subjects performed a paced verbal fluency task which alternated with a control verbal repetition task.

Results Increasing levels of depression after tryptophan depletion were associated with diminished neural activity in the ventral anterior cingulate, orbitofrontal cortex and caudate nucleus regions. In addition, depressive relapse attenuated cognitive task-related activation in the anterior cingulate cortex.

Conclusions Our data indicate that changes in neural activity in distinct brain regions mediate the clinical phenomena of depression and depression-related cognitive impairment following acute tryptophan depletion. These changes could be associated with the widespread distribution of serotonin neurons in brain pathways associated with the expression of affect and cognitive performance.

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There is extensive evidence that brain serotonin plays an important role in the pathophysiology of major depression and the psychotropic effects of antidepressant drug treatment (Smith & Cowen, 1997). Tryptophan depletion is a dietary technique which involves the administration of an amino acid mixture which lacks tryptophan, the amino acid precursor of serotonin. Studies in both animals and humans have shown that a tryptophan-free amino acid mixture lowers plasma and brain tryptophan levels and decreases brain serotonin synthesis (Young *et al*, 1985; Nishizawa *et al*, 1997). Previous studies have shown that tryptophan depletion can induce acute temporary depressive relapse in euthymic patients maintained on serotonergic antidepressants (Delgado *et al*, 1990) and in drug-free recovered depressed subjects (Smith *et al*, 1997). The aim of the present study was to use tryptophan depletion in conjunction with positron-emission tomography (PET) to identify the brain regions associated with acute depressive relapse and its associated cognitive impairment.

METHOD

Subjects

Eight male subjects (mean age 39.1 years, range 27–64) took part in the study. Males were used in the study because of the difficulties in using neuroimaging techniques involving radioactivity in females of childbearing age. All subjects had a history of at least two episodes of DSM-III-R major depression (American Psychiatric Association, 1987) as assessed by the Structured Clinical Interview for DSM-III-R (SCID; Spitzer *et al*, 1990). All subjects met criteria for full recovery from depression (Frank *et al*, 1991) and had been free of significant symptoms for at least six months. All subjects gave informed consent to the study and were explicitly informed that the procedure might cause a reappearance of some or all of their depressive symptoms. The study had received approval from the local research ethics committees and from the Administration of Radioactive Substances Advisory Committee (ARSAC, UK). Two of the subjects had been medication-free for at least six months, the other six were maintained on antidepressant medication (three on selective serotonin reuptake inhibitors, one on amitriptyline, one on a monoamine oxidase inhibitor (MAOI) and one on a combination of amitriptyline and MAOI). Two subjects on antidepressants were also taking lithium.

Experimental procedure

Subjects received two amino acid mixtures in a double-blind balanced-order design. One mixture was nutritionally balanced and contained tryptophan ('balanced mixture'), the other was identical except that it contained no tryptophan ('TRP-free mixture'). The composition of the mixtures was identical to those previously described (by Young *et al*, 1985). The TRP-free mixture contained L-alanine 5.5 g, L-arginine 4.9 g, L-cysteine 2.7 g, glycine 3.2 g, L-histidine 3.2 g, L-isoleucine 8 g, L-leucine 13.5 g, L-lysine monhydrochloride 11 g, L-methionine 3 g, L-phenylalanine 5.7 g, L-proline 12.2 g, L-serine 6.9 g, L-threonine 6.5 g, L-tyrosine 6.9 g and L-valine 8.9 g. The balanced drink contained the same amino acids plus 2.3 g L-tryptophan. Subjects followed the same protocol as a previous study of recovered depressed patients (Smith *et al*, 1997). They were tested on two days separated by at least a week. Prior to each test day they followed a low protein diet to maximise the effects of subsequent tryptophan depletion. On each test day, the amino acid mixture was given at 09.00 h. Following the mixture, subjects sat quietly in a testing room and were allowed to read neutral material. Subjects were given a low-tryptophan lunch at +3 hours and the PET scan started at +5 hours. Mood was rated objectively using the Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1960), modified for use over a short period of time by removing five items (initial, middle and delayed insomnia, genital symptoms and loss of weight) which could not change during the course of the test (Smith *et al*, 1997). The HAM-D was used because previous studies of tryptophan depletion (Delgado *et al*, 1990) have shown that the main effects are on objective ratings of mood

measured using this scale. The HAM–D test was completed by one of the investigators (K.S., who was blind to test status) at baseline, before and immediately after the PET scan. Partial and full reappearance of symptoms were judged by a modified HAM–D score of greater than 5 or greater than 11 respectively, as previously described (Smith *et al*, 1997). For the purpose of analysis, values of HAM–D were assigned to each PET scan assuming a linear relationship between pre- and post-scan HAM–D ratings. Following the PET scan, subjects were taken home and monitored as clinically indicated.

Biochemical measures

Blood samples for measurement of total and free tryptophan were taken immediately before administration of the mixture and seven hours later. Plasma tryptophan levels were determined by high-performance liquid chromatography with amperometric detection.

Psychological tasks

During scanning, subjects performed either task 'a', a paced word repetition task, in which words were spoken by the experimenter every five seconds and immediately repeated by the subject, or task 'b', a paced orthographic verbal fluency task, in which a letter was repeated every five seconds by the experimenter and subjects generated a word beginning with that letter for each repetition. If the subject could not generate a word, he said "pass" and was given a new letter which was repeated in the same way. A new letter was automatically introduced after ten repeats of a single letter. The tasks were alternated across the six scans in each session, with half the subjects having the order A-B-A-B-A-B and half B-A-B-A-B-A.

Image acquisition

Scans of the distribution of $H_2{}^{15}O$ were obtained using a Siemens/CPS ECAT EXACT HR+ PET scanner operated in high sensitivity 3D mode. Subjects received a total of 350 Mbq of $H_2{}^{15}O$ over 20 seconds through a forearm cannula for each scan, and activity was measured during a 90-second time window. Each subject had two sessions of six scans (i.e. a total of 12 scans). The PET images comprised $2 \times 2 \times 2$ mm voxels with a 6.4 mm transaxial and a 5.7 mm axial resolution (full width at half maximum). The data were analysed with statistical parametric mapping (SPM96, Wellcome Department of Cognitive Neurology, London; http://www.fil.ion.ucl.ac.uk/spm) implemented on Matlab. Structural magnetic resonance images (MRIs) from each subject were co-registered to the PET data following realignment of the PET time series. All the scans were then transformed into a standard stereotactic space (Talairach & Tournoux, 1988; Friston *et al*, 1995*b*). The scans were smoothed using a Gaussian filter set at 12 mm full width at half maximum. The measurements were adjusted to a global mean of 50 ml/dl/min.

Statistical analysis

A blocked (by subject) ANCOVA model was fitted to the data at each voxel, with a condition effect for each psychological task in each of the depleted or balanced sessions, and global cerebral blood flow (CBF) as a confounding covariate. Predetermined contrasts of the condition effects at each voxel were assessed using a *t*-statistic image for each contrast. Levels of total tryptophan and values from the HAM–D rating scale were used to generate covariates of interest for separate SPM96 analyses. Individual values for each scan were assigned by linear interpolation between pre- and post-scanning results. The general methods employed by SPM are described in detail elsewhere (Friston *et al*, 1995*a*,*b*).

RESULTS

Ratings of clinical depression

In agreement with previous studies, tryptophan depletion produced a full or partial reappearance of depressive symptoms in six of the eight subjects according to the HAM–D criteria described above. One subject became partially symptomatic on the day he was given the balanced mixture (although his depression score was greater after tryptophan depletion). Reappearance of symptoms did not correlate with the presence or the type of medication on which the subjects were maintained. HAM–D scores on the tryptophan depletion and placebo days are shown in Fig. 1.

The effect of increasing clinical depression on regional cerebral blood flow

We used a regression analysis to assess the effect of mood change on both the tryptophan-depleted and balanced-mixture days, taking into account the effect of changing tryptophan levels. Increasing depression scores were significantly correlated ($P<0.001$, uncorrected) with decreased activity in the orbitofrontal cortex, subgenual anterior cingulate, left caudate nucleus, left extrastriate cortex and superior parietal cortex bilaterally (Fig. 2). There were no increases in activity associated with increasing HAM–D score. The coordinates and associated *Z*-scores for mood-related decreases in regional cerebral blood flow are given in Table 1.

The effect of psychological task on regional cerebral blood flow

The regional cerebral blood flow pattern produced by the psychological tasks (verbal fluency contrasted with repetition) resulted in activations in the orbitofrontal cortex,

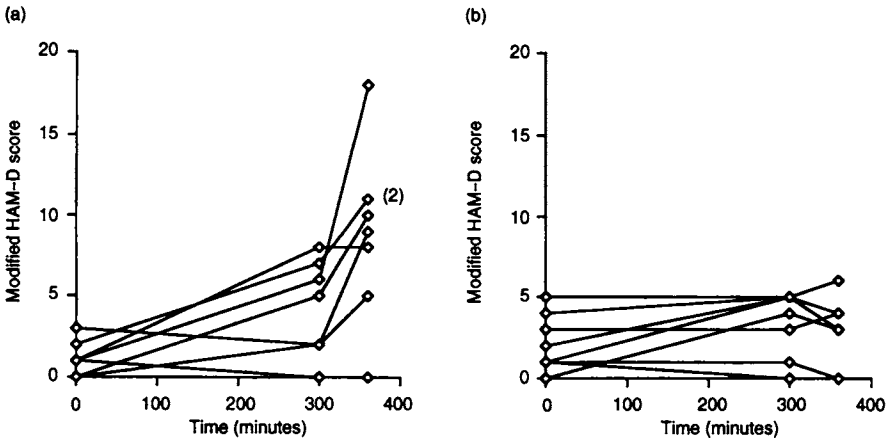

Fig. 1 Changes in mood on the tryptophan depletion (Fig. 1a) and the balanced day (Fig. 1b) (when the control amino acid mixture was given). Mood was assessed using the modified Hamilton Rating Scale for Depression at time 0 (just prior to the administration of the amino acid mixture), at +5 hours (before the positron-emission tomography (PET) scan) and at +6 hours (after the PET scan). (2) denotes a line representing two individuals.

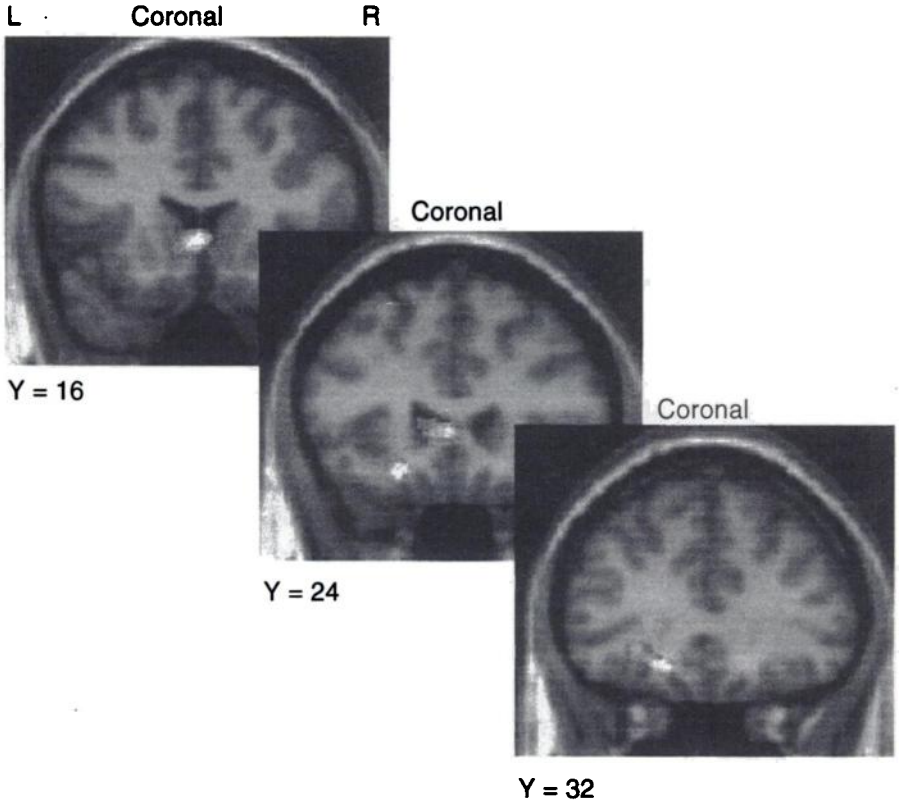

Fig. 2 Brain regions which show a decrease in activity with increasing depression (white areas) when the modified Hamilton Rating Scale for Depression scores were used as the covariate of interest in a statistical parametric mapping analysis. The regions showing significant change in neural activity have been projected onto serial coronal magnetic resonance imaging slices at y=16, 24 and 32 mm (i.e. moving from the back to front of the brain and including the subgenual cingulate, left caudate nucleus and orbitofrontal cortex) (see Table 1 for coordinates).

Table 1 Areas of decreased neural activity associated with increasing severity of depression

Area	Coordinates (x, y, z)	Z-score
Left extrastriate cortex (BA18)	−16, −98, −16	4.96
Left superior parietal cortex (BA1)	−26, −78, 52	4.40
Right superior parietal cortex (BA1)	20, −78, 50	4.21
Left orbitofrontal cortex (BA11)	−22, 26, −14	4.05
Subgenual anterior cingulate cortex (BA24)	0, 16, 2	3.93
Caudate	−30, 10, 16	3.82

Table 2 Areas where increasing depressed mood attenuates neural activation associated with verbal fluency

Area	Coordinates (x, y, z)	Z-score
Superior frontal cortex (BA6)	−8, 14, 66	4.03
Anterior cingulate (BA32)	−2, 42, 28	3.40
Parietal cortex (BA38)	40, 10, −24	3.39
Parietal cortex (BA40)	−42, −52, 50	3.36

middle temporal cortex, dorsal anterior cingulate cortex and superior and middle frontal cortex. Deactivations were seen in the superior temporal cortex bilaterally, right superior frontal cortex and occipital cortex. The critical analysis, however, is the effect of mood change on cognitive-task-associated activations. This interaction, between mood and cognitive task, was analysed using an orthogonalised HAM-D covariate with respect to changing tryptophan levels. We compared the difference in slope between the two regressions of fluency and repetition on corrected HAM–D scores across both scanning sessions, tested at every voxel, to produce a statistical parametric map. No predicted areas of increased activation were revealed in the interaction of increasing depression and verbal fluency. By contrast, a predicted effect was seen in the dorsal anterior cingulate, where there was a significant attenuation of activation ($P<0.001$, uncorrected) (Fig. 3) under the fluency condition with increasing severity of depression.

Plasma tryptophan levels

After ingestion of the TRP-free mixture the mean (s.e.) total plasma tryptophan level fell from 13.66 (0.6) to 2.3 (0.3), a reduction of 83%. On the balanced day, there was no significant change in total tryptophan levels (14.2 (1.1) to 15.3 (1.2)). Similar results were found for free tryptophan levels, with a fall of 87% after tryptophan depletion (data not shown).

DISCUSSION

Regional cerebral blood flow and depressive relapse

Our data highlight the role of the medial prefrontal and orbital cortices in the manifestation of clinical depression. These data extend previous findings based on single time measurements (Goodwin, 1996), where it is difficult to assess the relative importance of state and trait factors. The advantage of the tryptophan depletion paradigm is that it allows subjects to be used as their own controls in a manner which enables changes in neural activity to be explicitly linked to relapse of depressed mood. On this basis, we found that increasing severity of depression was associated with decreased perfusion in the subgenual anterior cingulate, orbitofrontal cortex and caudate nucleus. A previous PET study using tryptophan depletion, but involving the

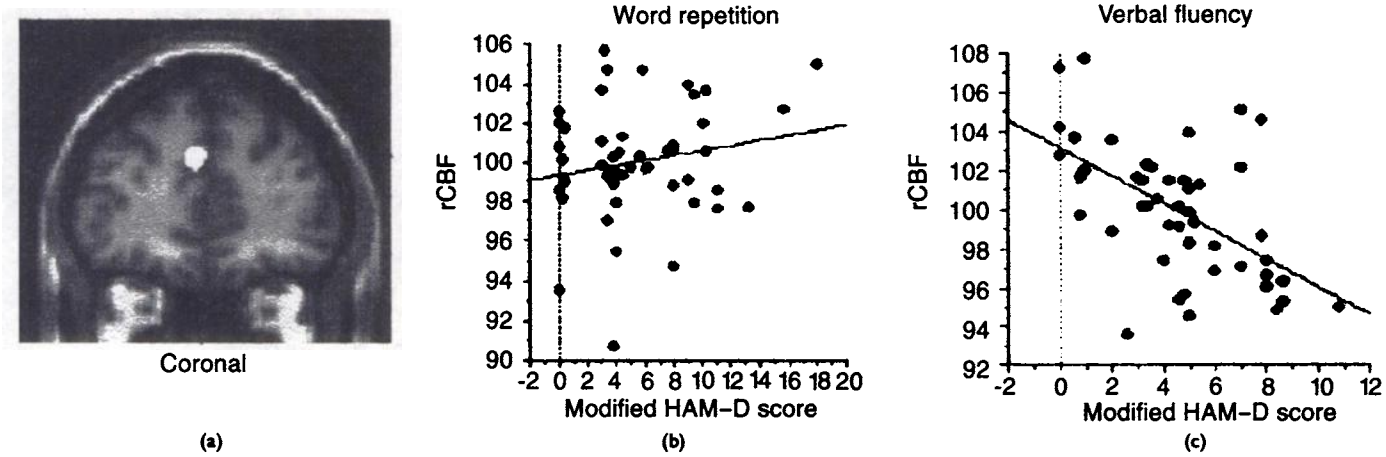

Fig. 3 The region (white area, centre) of the anterior cingulate which shows modulation of its activation by depressed mood when subjects perform a verbal fluency task. Separate regressions for the verbal fluency and word repetition tasks were calculated at every voxel using modified Hamilton Rating Scale for Depression (HAM–D) scores (orthogonalised to exclude variance attributable solely to changing tryptophan levels). Task-related differences in regression slopes were tested in a statistical parametric mapping analysis. Increasing depressed mood resulted in decreased regional cerebral blood flow (rCBF) in the anterior cingulate during fluency but not in the repetition task. (b) and (c) Regression plots of the rCBF values for the maximal voxel in the anterior cingulate ($x=-2$, $y=42$, $z=28$) in the repetition and fluency tasks. Each dot refers to a single scan for that condition in adjusted rCBF values.

deoxyglucose method, reported results consistent with our investigation, including decreased metabolism in the orbitofrontal, cingulate and parietal cortex and the caudate nucleus (Bremner *et al*, 1997). Taken together, these observations imply that the decreased activity in these regions described in previous functional neuroimaging investigations of patients with major depression (Goodwin, 1996) reflect state-related phenomena.

A functional deficit, localised in the subgenual anterior cingulate cortex, has been reported in acutely depressed patients in association with morphometric changes (Drevets *et al*, 1997). Our study, using subjects as their own controls, makes us confident that structural differences between the two scanning sessions cannot provide an exclusive explanation for the observed functional differences in this latter region. Our results, involving on-line changes in clinical state, imply that the functional deficit in this area needs to be explained in terms of state as well as trait.

Brain circuits involved in depression

Patients with anterior cingulate lesions display diminished motivation and a loss of interest (Devinsky *et al*, 1995) – key symptoms of major depression. The anterior cingulate has projections to the orbitofrontal cortex and limbic striatum (Kunishio & Haber, 1994), and this circuit has been hypothesised to function abnormally in depression (Ebert & Ebmeier, 1996). Consistent with this proposal, we observed decreasing activity associated with increasing severity of relapse in the orbitofrontal cortex and caudate nucleus. Animal and human studies indicate that the orbitofrontal cortex plays a central role in affective behaviour (Rolls *et al*, 1994), while changes in activity in the caudate nucleus could underpin psychomotor changes which are prominent in depressive relapse during tryptophan depletion (Smith *et al*, 1997).

Cognitive deficit in depression

Our data also provide information on the brain regions involved in the impairment in cognitive performance seen in depression. Use of an appropriate cognitive task allows a degree of control over subjects' mental state during scanning. More critically, the use of a cognitive task enabled us to address the consequences of acute relapse into depression on the brain regions involved in an aspect of cognitive performance.

Our findings showed that increasing depression attenuated the responsiveness of the dorsal anterior cingulate to a verbal fluency task. Interestingly, a similar finding has been reported in healthy subjects during verbal fluency activation in a state of induced low mood (Baker *et al*, 1997). The paced nature of our tasks ensured that differential task performance cannot explain our findings, although this procedure prevented an assessment of on-line performance data. However, it should be noted that clinical depression is associated with impaired performance on verbal fluency tasks (Trichard *et al*, 1995).

Decreased activity of the dorsal anterior cingulate has been reported in previous studies of depressed patients (Ebert & Ebmeier, 1996), while 'normalisation' of activity in this region accompanies recovery from depression (Goodwin, 1996). Taken together, the findings suggest that decreased neural activity in the dorsal anterior cingulate cortex may underpin some of the reversible cognitive deficits associated with depressive illness.

Serotonin function and depression

Serotonin pathways heavily innervate cortical and subcortical brain regions, including those identified in our study as being linked to increasing depression scores (Cudennec *et al*, 1993). How changes in serotonin function exert their behavioural effects has hitherto been an unanswered question. We suggest that decreasing serotonin function exerts its effects on mood by specifically impairing neural activity in the caudate nucleus, ventral anterior cingulate and orbitofrontal cortex regions which underpin the expression of clinical depressive symptoms. Furthermore, impaired serotonin neurotransmission in the dorsal anterior cingulate cortex may be involved in the expression of the interaction between pathological mood states and cognition.

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REFERENCES

American Psychiatric Association (1987) *Diagnostic and Statistical Manual of Mental Disorders* (3rd edn, revised) (DSM-III-R). Washington, DC: APA.

Baker, S. C., Frith, C. D. & Dolan, R. J. (1997) The interaction between mood and cognitive function studied with PET. *Psychological Medicine*, **27**, 565–578.

Bremner, J. D., Innis, R. B., Salomon, R. M., et al (1997) Positron emission tomography measurement of cerebral metabolic correlates of tryptophan depletion-induced depressive relapse. *Archives of General Psychiatry*, **54**, 364–374.

Cudennec, A., Bonvento, G., Duverger, D., et al (1993) Effects of dorsal raphe nucleus stimulation on cerebral blood flow and flow–metabolism coupling in the conscious rat. *Neuroscience*, **55**, 385–401.

Delgado, P. L., Charney, D. S., Price, L. H., et al (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.

Devinsky, O., Morrell, M. J. & Vogt, B. A. (1995) Contributions of anterior cingulate to behaviour. *Brain*, **118**, 279–306.

Drevets, W. C., Price, J. L., Simpson, J. R., et al (1997) Subgenual prefrontal cortex abnormalities in mood disorders. *Nature*, **386**, 824–827.

Ebert, D. & Ebmeier, K. P. (1996) The role of the cingulate gyrus in depression: from functional anatomy to neurochemistry. *Biological Psychiatry*, **39**, 1044–1050.

Frank, E., Prien, R. F., Jarrett, R. B., et al (1991) Conceptualization and rationale for consensus definitions in major depressive disorder. *Archives of General Psychiatry*, **48**, 851–855.

Friston, K. J., Ashburner, J., Poline, J.-P., et al (1995a) Spatial registration and normalization of images. *Human Brain Mapping*, **3**, 165–189.

—, Holmes, A. P., Worsley, K. J., et al (1995b) Statistical parametric maps in functional imaging: a general linear approach. *Human Brain Mapping*, **2**, 189–210.

Goodwin, G. M. (1996) Functional imaging, affective disorder and dementia. *British Medical Bulletin*, **52**, 495–512.

Hamilton, M. (1960) A rating scale for depression. *Journal of Neurology, Neurosurgery and Psychiatry*, **23**, 56–62.

Kunishio, K. & Haber, S. N. (1994) Primate cingulostriatal projection: limbic striatal versus sensorimotor striatal input. *Journal of Comparative Neurology*, **350**, 337–356.

Nishizawa, S., Benkelfat, C., Young, S. N., et al (1997) Differences between males and females in rates of serotonin synthesis in the human brain. *Proceedings of the National Academy of Sciences*, **94**, 5308–5313.

Rolls, E. T., Hornak, J., Wade, D., et al (1994) Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. *Journal of Neurology, Neurosurgery and Psychiatry*, **57**, 1518–1524.

Smith, K. A. & Cowen, P. J. (1997) Serotonin and depression. In *Depression: Neurobiological, Psychopathological and Therapeutic Advances* (eds A. Honig, M. Ansseau & H. van Praag), pp. 129–146. London: John Wiley & Sons.

—, Fairburn, C. G. & Cowen, P. J. (1997) Release of depression after rapid depletion of tryptophan. *Lancet*, **349**, 915–919.

Spitzer, R. L., Williams, J. B. W., Gibbon, M., et al (1990) *Structured Clinical Interview for DSM-III-R*. Washington, DC: American Psychiatric Press.

Talairach, J. & Tournoux, P. (1988) *Co-Planar Stereotaxic Atlas of the Human Brain*. New York: Threme Medical Publishers.

Trichard, C., Martinot, J. L., Alagille, M., et al (1995) Time course of prefrontal lobe dysfunction in severely depressed in-patients: a longitudinal neuropsychological study. *Psychological Medicine*, **25**, 79–85.

Young, S. N., Smith, S. E., Pihl, R. O., et al (1985) Tryptophan depletion causes a rapid lowering of mood in normal males. *Psychopharmacology*, **87**, 173–177.

CLINICAL IMPLICATIONS

- Clinical depressive symptoms are linked to decreased neural activity in the subgenual anterior cingulate, orbitofrontal cortex and caudate nucleus.
- Distinct brain mechanisms involving the dorsal anterior cingulate cortex may mediate certain aspects of the cognitive impairment associated with depressive relapse.
- Tryptophan depletion may cause depressive relapse by lowering serotonin neurotransmission in key brain circuits involved in mood regulation.

LIMITATIONS

- As with most neuroimaging studies, the results are based on relatively small numbers of subjects.
- Most of the subjects were taking antidepressant medications, which could have interacted with the neurochemical effects of tryptophan depletion.
- Because we studied only men we cannot necessarily extrapolate the findings to women.

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