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EDITORIAL REVIEW

'It's not over when it's over': persistent neurobiological abnormalities in recovered depressed patients

Z. Bhagwagar[1] and P. J. Cowen[2]*

[1] *Department of Psychiatry, Yale University, Newhaven, USA*
[2] *University Department of Psychiatry, Warneford Hospital, Oxford, UK*

The tendency of major depression to recur is a leading problem in clinical management and is responsible for much of the illness burden. Until recently, biological studies of depression have focused on the mechanisms involved in acute illness but there are now many data to suggest that neurobiological abnormalities persist when depressed patients are clinically recovered and withdrawn from medication. These abnormalities encompass a number of neurochemical and neuropsychological mechanisms that could be relevant to recurrence, including changes in the availability of serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes, decreases in cortical γ-aminobutyric acid (GABA), increases in cortisol secretion and negative biases in the processing of emotional information. Studies of groups at high risk of depression before illness onset will help to clarify which biological abnormalities precede the development of depression and which are the product of recurrent illness. Ultimately this work should lead to a better understanding of the neurobiology of vulnerability to depression and more innovative approaches to primary and secondary prevention.

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Introduction

One of the more remarkable features of clinical depression is its tendency to remit, either spontaneously or during the course of treatment. For William Styron, the 'men and women who have recovered from the disease … bear witness to what is probably its only saving grace: it is conquerable' (Styron, 2004). However, the tendency to recovery is accompanied by an equally great tendency to recurrence; for example, at least 80% of recovered depressed patients will experience further major depression, and over a 25-year follow-up, patients will suffer, on average, about five further episodes (Mueller *et al*. 1999; Angst, 2000). The long-term outcome of those hospitalized for depression is particularly poor (Lee & Murray, 1988).

These epidemiological data suggest that even when depressed patients are clinically recovered, they possess risk factors that place them at increased liability of future episodes. Several clinical predictors of depressive recurrence have been identified, including the number of previous episodes, age of illness onset and persisting subsyndromal symptomatology (Angst, 2000). It also appears that with increasing number of depressive episodes, the role of life events in triggering illness in individuals decreases; this has been suggested to represent a kind of 'kindling' of susceptibility (Kendler *et al*. 2001). The neurobiological basis of vulnerability to recurrent depression has been less studied but there is growing evidence that recovered depressed patients manifest several abnormalities in mood-related biological mechanisms that could be relevant to increased risk of relapse.

Traditionally, biological studies of conditions such as depression have focused on abnormalities associated with the acute episode. A difficulty with this approach is that any abnormalities detected could represent epiphenomena of the abnormal clinical state or its treatment rather than mechanisms relevant to pathophysiology. There is therefore a good argument for developing a complementary strategy in which biological studies are carried out in people with a history of recurrent depression who are currently recovered and medication free. Neurobiological abnormalities identified in these circumstances could be markers of trait vulnerability to recurrent illness and arguably valuable as illness endophenotypes (Hasler *et al*. 2004; Flint & Munafò, 2007). Whatever the merits of the latter proposition, a better understanding of trait neurobiological abnormalities might lead to improved ways of identifying those at high risk of

* Address for correspondence: Professor P. J. Cowen, Neurosciences Building, Warneford Hospital, Oxford OX3 7JX, UK.
(Email: phil.cowen@psych.ox.ac.uk)

recurrence and to novel methods of prevention. In this review we outline current developments in this area, focusing specifically on studies where recovered patients have been free of medication. We concentrate on unmedicated recovered subjects because, not surprisingly, antidepressant drugs can influence many of the neurobiological mechanisms relevant to vulnerability. It should also be noted that in most cases it is unclear whether neurobiological abnormalities might have been present before the development of illness or might instead be a consequence of the illness and its treatment (the so-called 'scar' effect).

Monoamine neurotransmission

Serotonin (5-hydroxytryptamine, 5-HT)

Acutely depressed patients show changes in many aspects of 5-HT neurotransmission (Cowen, 2005). The efficacy of 5-HT-potentiating agents such as selective serotonin re-uptake inhibitors (SSRIs) in the treatment of depression suggests that low 5-HT activity might be associated with the state of depression itself; however, changes in 5-HT mechanisms are also present in recovered unmedicated depressed patients. For example, both acutely depressed (Drevets *et al*. 1999; Sargent *et al*. 2000) and recovered depressed (Bhagwagar *et al*. 2004*a*) patients demonstrate rather generalized decreases in brain 5-HT_{1A} receptor availability measured by positron emission tomography (PET).

This finding is of interest because the phenotype of the 5-HT_{1A} receptor knockout mouse is characterized by increased anxiety (Ramboz *et al*. 1998). Perhaps, therefore, low 5-HT_{1A} receptor availability in depressed patients could be a developmental phenomenon exposing affected individuals to an increased risk of emotional disorders. Indeed, a recent multimodal imaging study by Fisher *et al*. (2006) in healthy subjects demonstrated that increased reactivity of the amygdala to negative facial expressions correlated with lower 5-HT_{1A} receptor binding in the raphe nuclei. Thus, low 5-HT_{1A} receptor availability could predispose to emotional disorders by facilitating increased processing of negative emotional stimuli in limbic regions (Vuillemier, 2005).

We have also found that recovered depressed patients have increased cortical 5-HT_{2A} receptor binding measured by PET in conjunction with [^{11}C]MDL 100907 (Bhagwagar *et al*. 2006). Because the status of 5-HT_{2A} receptor binding in acute depression is controversial (Meyer *et al*. 2003; Mintun *et al*. 2004), this finding requires replication. However, 5-HT_{1A} and 5-HT_{2A} receptors are often co-localized on cortical neurones, where they appear to have opposing physiological roles (Piquet & Galvan, 1994; Martin-Ruiz *et al*. 2001). Thus, our data would be consistent with a persistent dysregulation of the ascending 5-HT input to cortex and limbic regions, presumably including the circuitry involved in the representation and regulation of emotion.

One of the most striking 5-HT-linked abnormalities in unmedicated recovered depressed patients is the liability to re-experience depressive symptomatology when undergoing acute tryptophan depletion (ATD), a dietary manipulation that decreases brain 5-HT function by limiting the availability of tryptophan for brain 5-HT synthesis (Smith *et al*. 1997; Ruhé *et al*. 2007). This effect is not seen in people who have no history of depression and no personal risk factors (Ruhé *et al*. 2007). In addition, while ATD may produce some subjective lowering of mood on visual analogue scales in people with a strong family history of depression, this does not reach the level of clinical symptomatology (Ruhé *et al*. 2007). This indicates that depressive reactions to ATD are probably associated with a personal history of major depression. As a result of ATD, the brain apparently acquires an organizational state in which negative emotions and their associations are much more readily accessed.

The unusual psychological effects of ATD in recovered depressed patients could be attributable to underlying deficits in 5-HT neurotransmission, such that the effect of ATD to impair 5-HT function in recovered depressives is greater than in non-depressed controls. This would imply that a sufficient lowering of 5-HT neurotransmission would cause depression even in non-vulnerable individuals. Perhaps a more likely explanation might be the presence of persistent abnormalities in the cortical and limbic neural circuitry with which 5-HT pathways interact. PET imaging studies suggest that ATD-induced depressive relapse is associated with altered activity in orbitofrontal cortex, dorsolateral prefrontal cortex, anterior cingulate cortex and thalamus (Smith *et al*. 1999; Neumeister *et al*. 2004); the integrated activity of these brain regions is known to underpin the processing of emotional information (Phillips *et al*. 2003). It is possible that underlying deficits in this circuitry in recovered depressed patients are 'unmasked' by ATD. The fact that the effects of ATD appear to be present only in people who have experienced depression suggests that depression itself can cause neurobiological changes that increase psychological vulnerability to low 5-HT states.

Could the striking effects of ATD in recovered depressed patients be a consequence of previous antidepressant treatment, particularly with SSRIs? It is difficult to exclude this possibility completely, but in our own ATD study of recovered depressed patients

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we also saw symptomatic relapse in patients who had not been treated with antidepressants (Smith *et al*. 1997), suggesting that this is not the explanation.

Catecholamines

Treatment of recovered depressed patients with the catecholamine synthesis inhibitor α-methyl-*para*-tyrosine (AMPT) also produces acute depressive relapse (Berman *et al*. 1999). However, dietary depletion of tyrosine, the amino acid precursor of noradrenaline and dopamine, does not (McTavish *et al*. 2005). While AMPT diminishes both noradrenaline and dopamine synthesis, the effects of tyrosine depletion appear limited to dopamine activity, perhaps because of the greater utilization of tyrosine by dopaminergic neurones (McTavish *et al*. 1996). This suggests that the effect of AMPT to produce depressive relapse is caused by lowered noradrenaline activity. Importantly, a PET study of AMPT in recovered depressed patients implicated similar brain circuitry to that involved in ATD-induced depressive relapse, that is, orbitofrontal cortex, dorsolateral prefrontal cortex and thalamus (Bremner *et al*. 2003). Thus, if this circuitry is indeed potentially dysfunctional in recovered depressed patients, it appears to be susceptible to the effects of impaired noradrenaline neurotransmission as well as lowered 5-HT function.

Gamma-aminobutyric acid (GABA)

As noted earlier, ascending 5-HT pathways make synaptic connection with both GABA interneurones and glutamatergic pyramidal neurones in both cortical and limbic regions (Taylor *et al*. 2003). Studies using proton magnetic resonance spectroscopy (MRS) have shown lowered GABA levels in occipital cortex in acutely depressed patients, particularly those with a melancholic syndrome (Sanacora *et al*. 2004). Both SSRI treatment (Sanacora *et al*. 2002) and electroconvulsive therapy (ECT) (Sanacora *et al*. 2003) increase GABA levels in depressed patients; however, this effect is not seen with cognitive behaviour therapy despite clinical improvement (Sanacora *et al*. 2006).

The latter finding suggests that the effect of SSRIs and ECT to increase GABA levels in depressed patients might be an effect of treatment rather than a consequence of clinical recovery. Consistent with this we found lowered GABA levels in occipital cortex in recovered, unmedicated depressed patients (Bhagwagar *et al*. 2007), which suggests that diminished GABA availability might be a trait maker of vulnerability to depression. It is unclear whether this abnormality is a consequence of recurrent depression or might also be present in high-risk groups; however, the non-invasive nature of MRS should make it feasible to study those at high risk of depression before the onset of clinical illness. It is possible that changes in GABA levels might be caused by the glial cell deficits that have been reported in neuropathological studies of patients with recurrent mood disorders (Harrison, 2002; Sanacora *et al*. 2004).

Cortisol hypersecretion and sleep

Increased secretion of cortisol is a common accompaniment of major depression and is usually regarded as a state marker of illness (Holsboer, 2000). However, some aspects of hypothalamo–pituitary–adrenal (HPA) axis function may remain abnormal in remitted patients, particularly those at high risk of recurrence (Zobel *et al*. 2001). We have used the waking increase in salivary cortisol as a measure of HPA axis activity (Pruessner *et al*. 1997). Our studies have shown that waking salivary cortisol is increased both in acute depression (Bhagwagar *et al*. 2005) and in recovered depressed patients (Bhagwagar *et al*. 2003). Furthermore, we have found a similar increase in waking salivary cortisol in young people who have not been depressed themselves but who have a depressed parent (Mannie *et al*. 2007). The increase in cortisol secretion in the latter group could not be explained by symptomatic status, childhood adversity or recent life events.

Taken together, the waking salivary cortisol data show that recovered depressed patients continue to demonstrate abnormal HPA axis activity. Moreover, judging from the increase in cortisol secretion in children of depressed parents, this particular HPA axis abnormality probably precedes the onset of clinical illness. Persistently increased cortisol secretion could represent a risk factor for subsequent depression; for example, a recent study reported that increased cortisol levels at age 13 independently predicted depressive symptomatology 3 years later (Halligan *et al*. 2007). These findings support studies from other high-risk groups of adolescents and adults showing that elevated cortisol secretion is a risk factor for subsequent major depression (Goodyer *et al*. 2000; Harris *et al*. 2000).

Increased cortisol secretion is often suggested to be a risk factor for neuronal atrophy and has been linked to decreased hippocampal volume in patients with recurrent depression (Campbell & MacQueen, 2004). Although the role of cortisol in this effect is not yet clearly established, there does seem to be an association between recurrent depression and hippocampal atrophy (Campbell & MacQueen, 2006). This could be another example of the way in which depression itself may result in neurobiological changes

that complicate the course of the illness. However, it has not yet been demonstrated clearly that hippocampal volume is decreased in fully recovered depressed patients (Campbell & MacQueen, 2006). It is, however, certainly possible that persistently increased cortisol secretion could underlie some of the medical complications associated with chronic depression, including cardiovascular disease and obesity (Sherwood *et al*. 2004).

There is also a considerable body of work on the HPA axis in depression using the dexamethasone–corticotrophin releasing hormone (DEX/CRH) test. Acutely depressed patients show increased cortisol release in the DEX/CRH test, which is attenuated following antidepressant treatment and clinical improvement (see Holsboer, 2000). However, remitted patients who continue to demonstrate increased cortisol release are at higher risk of subsequent relapse (Zobel *et al*. 2001). This raises the possibility that abnormal responses to the DEX/CRH test might represent a marker of vulnerability to depression. The 'Munich vulnerability study in affective disorders' involves a longitudinal investigation of individuals at high familial risk of depression and initially it was reported that cortisol responses to DEX/CRH were abnormally increased in this group (Holsboer *et al*. 1995); however, current findings indicate that the DEX/CRH test is not abnormal in people who develop depression prior to the onset of illness (Ising *et al*. 2005). These latter observations are difficult to reconcile at present.

The Munich study has also investigated changes in the sleep polysomnogram (electroencephalogram, EEG) in patients at risk of depression. Patients with acute depression have fairly reliable abnormalities in rapid eye movement (REM) sleep, including shortened latency to REM sleep and increased density of REM sleep (increased frequency of eye movements during REM sleep periods) (see Hasler *et al*. 2004). Previous studies have suggested that recovered depressed patients continue to manifest a short latency to REM sleep and that this abnormality co-segregates in first-degree relatives (Giles *et al*. 1993, 1998). The Munich study did not find abnormal REM latency in people at increased familial risk of depression, but both high-risk individuals and those who eventually developed depression had increased REM density (Modell *et al*. 2005). Thus, the regulation of REM sleep remains a possible endophenotypic marker of vulnerability to depression (Hasler *et al*. 2004).

Discussion

Recovered depressed patients who are off medication and essentially asymptomatic continue to manifest abnormalities in many of the neurobiological mechanisms that are impaired in acute depression. This means that it is not yet clear what neurobiological changes might be responsible for the acute experience of depression itself. The best current candidates in this respect probably remain the monoamine neurotransmitters because lowering of 5-HT and noradrenaline function can cause acute depression in people with a previous history of illness (see above). However, depletion studies in healthy subjects indicate that lowered monoamine function is not sufficient to cause depression and the variable response of depressed patients to monoamine potentiating drugs suggests that diminished monoamine function is probably not a necessary cause of depression either.

Current data suggest that recovered depressed patients have abnormalities in aspects of 5-HT neurotransmission and in the regulation of GABA activity. Ascending 5-HT pathways make important synaptic connection with GABA interneurones in cortical and limbic regions and GABA neurones in turn influence the activity of 5-HT cell bodies (Taylor *et al*. 2003). What might be the neuropsychological consequences of dysfunction in these neuronal networks?

GABA and 5-HT neurones are among the neuronal elements involved in the processing of emotional information. In the amygdala, for example, lowered GABA and 5-HT function would be expected to lead to increased excitability (Stutzman & LeDoux, 1999) and there is evidence from functional magnetic resonance imaging (fMRI) studies that the amygdala is hyperresponsive to negative facial expressions both in acute depression (Sheline *et al*. 2001; Fu *et al*. 2004) and in recovered unmedicated depressed patients (Neumeister *et al*. 2006). Increased activity of the amygdala might be relevant to vulnerability to depression because it could facilitate automatic processing of negative emotional stimuli (Vuillemier, 2005). Consistent with this, we found that recovered depressed patients showed increased ability to detect fearful facial expressions and that this increased sensitivity was attenuated by a single intravenous dose of the SSRI citalopram (Bhagwagar *et al*. 2004*b*). This is consistent with the effect of intravenous citalopram to lower amygdala reactivity to negative emotional stimuli in healthy controls (Del-Ben *et al*. 2005). Attenuating the sensitivity of the amygdala to negative stimuli is a plausible mechanism of action for SSRIs in the prevention of depression in those at risk (Drevets, 2003) and, as noted above, persistent overactivity of the neural responses of the amygdala to negative stimuli might also be characteristic of recovered depressed patients (Neumeister *et al*. 2006).

Taken together, the data suggest that many of the neurobiological abnormalities found in recovered

depressed patients are associated with changes in the processing of emotional information and that in people at risk of recurrent depression the brain is in a state in which negative information is preferentially processed. Much appraisal of emotional information, for example that carried out by the amygdala, occurs at a non-conscious (automatic) level (Vuillemier, 2005). Thus, it is possible that excessive negative biases in emotional appraisal could be present in patients who do not subjectively experience depressive symptomatology. However, because of selective attention to negative stimuli, this processing style would be likely to put people at risk of more intense and prolonged negative emotions, particularly in adverse circumstances. This could lead in turn to the emergence and maintenance of depressed mood (Johnson-Laird *et al*. 2006).

In this respect it is worth noting that recovered depressed patients undergoing ATD can briefly re-experience the totality of the depressive syndrome including, for example, physical symptoms as well as associated negative cognitions about the self and the world (Smith *et al*. 1997). This suggests that, in patients who have suffered several episodes of depression, relatively modest psychological or biological triggers can be sufficient to recruit many depressive phenomena into conscious awareness. Recurrent depression may therefore lead to the formation of an associative network in which sadness and anxiety can readily bring to mind depressive memories, beliefs and experiences.

This formulation gives importance to the neurobiological effects of the depressive experience itself in increasing vulnerability to future episodes of illness. From this viewpoint it will be important to establish which of the biological abnormalities we have described are present before the onset of illness and which apparently occur after the first few depressive episodes. It may be that, in the future, more therapeutic attention needs to be given to the biological consequences of acute depressive episodes, perhaps through the development of treatments with neuroprotective effects or those that might regulate the formation and recall of emotional memories (Lee *et al*. 2006).

Another issue for future studies is how far any of the abnormalities we have described here might be useful predictors of future prognosis. This work is difficult because it requires prospective follow-up studies. However, there is preliminary evidence that susceptibility to the mood-lowering effects of ATD may be of prognostic significance in patients with recurrent depression (Ruhé *et al*. 2007). In addition, it appears that elevated cortisol secretion may have a role in predicting depression in high-risk individuals (Goodyer *et al*. 2000; Harris *et al*. 2000). Neurobiological markers, such as elevated cortisol, could help to guide early intervention studies in young people before the onset of recurrent illness. While the use of antidepressants in this group would not be appropriate, it is possible that, at this stage, other forms of intervention, for example psychological or nutritional therapies, might have a preventative role. This kind of approach might enable us eventually to decrease the long-term personal and societal burden of depression, which continues to grow despite the availability of reasonably effective symptomatic treatments.

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