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REVIEW

The $GABA_B$ receptor as a target for antidepressant drug action

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Preclinical and clinical data suggest that a modification in $GABA_B$ receptor expression and function may contribute to the symptoms of major depression and the response to antidepressants. This includes laboratory animal experiments demonstrating that antidepressants modify brain $GABA_B$ receptor expression and function and that $GABA_B$ receptor antagonists display antidepressant potential in animal models of this condition. Clinical and post-mortem studies reveal changes in GABAergic transmission associated with depression as well as depression-related changes in $GABA_B$ subunit expression that are localized to the cortical depression network. Detailed in this review are the preclinical and clinical data implicating a role for the $GABA_B$ receptor system in mediating symptoms of this disorder and its possible involvement in the response to antidepressants. Particular emphasis is placed on clinical and post-mortem studies, including previously unpublished work demonstrating regionally-selective modifications in $GABA_B$ receptor subunit expression in brain samples obtained from depressed subjects. Together with the earlier preclinical studies, these new data point to a role for the $GABA_B$ system in major depression and support the antidepressant potential of $GABA_B$ receptor antagonists.

Abbreviations

BDNF, brain-derived neurotophic factor; GABA, γ-aminobutyric acid; $GABA_{B1a}$, $GABA_{B1b}$, $GABA_{B2}$, γ-aminobutyric $acid_B$ receptor subunits; PMI, postmortem interval; RGS, regulators of G-protein signaling; RIN, RNA integrity number

Introduction

It has been over five decades since the discovery of antidepressants (Kuhn, 1958). During that time, neuronal pathways and neurochemical systems that appear critical for mediating the symptoms of this disorder have been identified (Rajkowska *et al.*, 1999; Nestler *et al.*, 2001; Krystal *et al.*, 2002; Mayberg, 2003; Seminowicz *et al.*, 2004). These discoveries were due, in part, to the insights gained on the mechanisms of action of these drugs. Even with this progress, however, little has changed with regard to the types of agents employed to treat this condition, with this class still dominated by drugs that directly interact with monoamine systems (Kelsey and Nemeroff, 1998). This is not due to a lack of effort, as there remains a need for novel treatments (Enna and Williams, 2009). While newer antidepressants are generally safer than older agents, little progress has been made in decreasing the percentage of non-responders and in speeding the onset of action. Inasmuch as the first antidepressants were discovered empirically in the clinic, and there is no consistently identifiable neuropathology associated with this condition, the difficulties associated with discovering more efficacious antidepressants suggests that fundamental information is still lacking concerning the underlying neurobiological abnormalities responsible for this disorder. Indeed, it is possible that major depression is not a single entity unto itself, but rather a constellation of symptoms that only manifest in association with other

psychiatric conditions. This could explain why no single mechanistic approach, such as enhancement of monoaminergic transmission, would be effective in all, or even the majority, of depressed patients because of the variable nature of the underlying cause.

Numerous attempts have been made to identify and develop antidepressants that target neurotransmitter systems other than those directly associated with the monoamines. Included have been antagonists for neurokinin-1 receptors (Herpfer and Lieb, 2005; Alvaro and Di Fabio, 2007), melanin-concentrating hormone-1 receptors (Shimazaki *et al.*, 2006), corticotrophin-releasing factor-1 receptors (Valdez, 2009) and metabotropic glutamate receptors (Lesage and Steckler, 2010). While there are substantial preclinical data supporting the antidepressant potential of these agents, clinical studies have not as yet demonstrated their superiority over conventional therapies. This lack of success is likely due to several factors. One is that the animal models typically employed for screening antidepressant candidates were developed on the basis of their response to the clinically effective monoaminergic drugs. Although many of these models appear to have face validity, construct validity remains questionable given the lack of understanding of the disease process, as does the predictive validity for candidates that do not directly activate monoaminergic transmission. Moreover, as the response to many clinically effective psychotherapeutics involves interactions with multiple sites, it is possible that the most effective antidepressant may be one that targets several transmitter systems. As designing this type of drug is difficult given the number of possible target combinations, such an agent is more likely to be discovered with a pharmacometric approach (Enna and Williams, 2009).

The chances of successfully developing novel antidepressants are likely to be enhanced if there is direct evidence from preclinical, clinical or post-mortem studies directly linking the intended target with major depression in humans. The $GABA_B$ receptor is a candidate that fulfills this criterion (Table 1). Thus, since its discovery in the early 1980s (Bowery, 2010), animal studies have indicated that the $GABA_B$ receptor system is modified by chronic administration of antidepressants (Enna and Bowery, 2004). These findings, in turn, led to clinical studies aimed at identifying the consequences of stimulating this receptor system in depressed patients. Moreover, post-mortem brain studies demonstrate alterations in GABAergic neurons and possible changes in brain GABA synthesis in depressed subjects (Cryan and Slattery, 2010). Most recently, post-mortem analyses has indicated changes in $GABA_B$ receptor subunit gene expression in discrete areas of the brain within the proposed cortical depression network (Klempan *et al.*, 2009; Sequeira *et al.*, 2009).

Outlined in this review are the preclinical and clinical data implicating a role for the $GABA_B$ receptor system in mediating symptoms of major depression, and its possible involvement in the response to antidepressants. Particular emphasis is placed on clinical and post-mortem studies, with a detailed description of previously unpublished work demonstrating regionally selective modifications in $GABA_B$ receptor subunit expression in brain samples obtained from depressed subjects. Together with the earlier clinical and preclinical work (Table 1), these findings point to a role for the $GABA_B$ system in major depression and support the antidepressant potential for $GABA_B$ receptor antagonists.

$GABA_B$ receptors

Two pharmacologically and molecularly distinct GABA receptors have been identified, $GABA_A$ and $GABA_B$ (Enna, 2007). Whereas the $GABA_A$ site is a pentameric, ligand-gated ion channel allosterically modulated by benzodiazepines and other anxiolytic and hypnotic agents, the $GABA_B$ receptor, a G protein-coupled heterodimer, is the site of action for baclofen, a muscle relaxant (Bowery, 2010). A class III metabotropic site, the $GABA_B$ receptor is composed of two 7-transmembrane spanning proteins (Kubo and Tateyama, 2005; Binet *et al.*, 2006). While there are multiple subunit isoforms in various animal species, $GABA_{B1a,}$ $GABA_{B1b}$ and $GABA_{B2}$ predominate, with coupling of either of the $GABA_{B1}$ subtypes with $GABA_{B2}$ essential for insertion into the plasma membrane and receptor function (Kaupmann *et al.*, 1998; Chronwall *et al.*, 2001; Enna and Bowery, 2010). Although $GABA_B$ receptors are widely distributed throughout the neuroaxis, subunit expression differs among brain regions (Bischoff *et al.*, 1999; Towers *et al.*, 2001; Vigot *et al.*, 2006; Farb *et al.*, 2007), suggesting the possible existence of regionally and molecularly distinct $GABA_B$ receptor subtypes.

The activation of $GABA_B$ receptors causes neuronal hyperpolarization by decreasing Ca^{2+} and increasing K^+ membrane efflux, the latter through direct coupling to Kir3 K^+ channels (Enna, 2001; Ladera *et al.*, 2008; Fernandez-Alacid *et al.*, 2009; Pinard *et al.*, 2010). Located both pre- and post-synaptically, $GABA_B$ receptors influence cAMP production through coupling to G_i and G_o. The stimulation of this site can either inhibit or enhance formation of this second messenger depending upon whether there is a simultaneous activation of a G_s-coupled site in the same neuronal

Table 1

Chronological listing of selected reports on the relationship between $GABA_B$ receptors and major depression

Authors	Date	Type of report	Journal	Findings
Pilc and Lloyd	1984	Preclinical	*Life Sci*	Chronic antidepressant administration increases $GABA_B$ receptors binding in the rat brain
Lloyd *et al.*	1985	Preclinical	*J Pharmacol Exp Ther*	$GABA_B$ receptor number in brain is increased by electroshock or antidepressant administration in the rat
Suzdak and Gianutsos	1986	Preclinical	*Eur J Pharmacol*	Repeated antidepressant or $GABA_B$ agonist administration modifies $GABA_B$ receptor binding and function in rat brain
Gray and Green	1987	Preclinical	*Br J Pharmacol*	Antidepressants or electroconvulsive shock increases $GABA_B$ receptor function in mouse brain
Cross *et al.*	1988	Clinical	*Psychiatry Res*	$GABA_B$ receptor binding profiles similar between suicide and control
Martin *et al.*	1989	Preclinical	*Neuropsychobiology*	Antidepressant drugs reverse decrements in $GABA_B$ receptor expression in a rat model of major depression
Marchesi *et al.*	1991	Clinical	*Psychoneuroendocrinology*	$GABA_B$ regulation of growth hormone in major depression
Post *et al.*	1991	Clinical	*Int Clin Psychopharmacol*	Baclofen exacerbates symptoms of major depression
Arranz *et al.*	1992	Clinical	*Neuropsychobiology*	$GABA_B$ receptor binding profiles in suicide and control subjects
O'Flynn and Dinan	1993	Clinical	*Am J Psychiatry*	$GABA_B$ regulation of growth hormone release in major depression
Pratt and Bowery	1993	Preclinical	*Br J Pharmacol*	Repeated administration of $GABA_B$ antagonist or desipramine up-regulates $GABA_B$ receptor binding in rat brain
Petty	1995	Review	*J Affect Disord*	A GABAergic hypothesis of depression
Nakagawa *et al.*	1996	Preclinical	*Brain Res*	Baclofen displays antidepressant activity in a rat model of depression
Davis *et al.*	1997	Clinical	*Psychoneuroendocrinology*	Lack of growth hormone response to baclofen in patients with major depression
Nakagawa *et al.*	1999	Preclinical	*Eur J Pharmacol*	$GABA_B$ antagonist reduces helplessness in a rat model of major depression
Sanacora *et al.*	2000	Clinical	*Crit Rev Neurobiol*	Neuroimaging and GABAergic function in major depression
Krystal *et al.*	2002	Clinical	*Mol Psychiatry*	Magnetic resonance spectroscopy indicates reduced cortical GABA levels in depression
Sands *et al.*	2003b	Preclinical	*Life Sci*	$GABA_B$ receptor subunit gene expression in rat brain is differentially modified in models of major depression and schizophrenia
Froestl *et al.*	2004	Preclinical	*Biochem Pharmacol*	$GABA_B$ antagonist SGS742 displays antidepressant activity in animal models of depression
Mombereau *et al.*	2004	Preclinical	*Neuropsychopharmacology*	$GABA_{B1}$ subunit deletion mutant mice displays antidepressant phenotype in the forced-swim test
Sands *et al.*	2004	Preclinical	*Biochem Pharmacol*	Repeated administration of antidepressants modifies $GABA_B$ receptor function in rat hippocampus
Enna and Bowery	2004	Review	*Biochem Pharmacol*	Review of physiological and pharmacological manipulations that alter $GABA_B$ receptor expression and function
Fatemi *et al.*	2005	Clinical	*Schizophr Res*	GAD expression is altered in mood disorders and schizophrenia
Mombereau *et al.*	2005	Preclinical	*Neuroreport*	$GABA_{B2}$ receptor subunit deletion mutant mice displays antidepressant phenotype in forced-swim test
Slattery *et al.*	2005	Preclinical	*J Pharmacol Exp Ther*	$GABA_B$ receptor antagonists display antidepressant activity in rodent models of major depression
McCarson *et al.*	2005	Preclinical	*Biochem Pharmacol*	Effect of antidepressants on $GABA_B$ receptor expression in rat spinal cord is state-dependent

Table 1

Continued

Authors	Date	Type of report	Journal	Findings
Frieling and Bleich	2006	Review	*Eur Arch Psychiatry Clin Neurosci*	Tranylcypromine and $GABA_B$ receptor function
McCarson *et al.*	2006	Preclinical	*Brain Res*	Antidepressant administration or repeated stress alters $GABA_B$ receptor expression and function in rat spinal cord
Nowak *et al.*	2006	Preclinical	*Br J Pharmacol*	$GABA_B$ receptor antagonists display antidepressant activity in rodent models of major depression
Vigot *et al.*	2006	Preclinical	*Neuron*	$GABA_B$ receptor subunits regulate receptor location and function in mouse hippocampal neurons
Bielau *et al.*	2007	Clinical	*Ann NY Acad Sci*	GAD staining reveals altered GABAergic neuron terminal density in post-mortem brain samples from depressed patients
Rajkowska *et al.*	2007	Clinical	*Neuropsychopharmacology*	Reduced GABAergic neuron number in prefrontal cortex in major depression
Cornelisse *et al.*	2007	Preclinical	*J Neurophysiol*	Selective serotonin reuptake inhibitor treatment reduces $GABA_B$ receptor function in rat brain
Frankowska *et al.*	2007	Preclinical	*Pharmacol Rep*	Baclofen displays antidepressant activity in animal models of major depression
Klempan *et al.*	2009	Clinical	*Mol Psychiatry*	$GABA_A$ and $GABA_B$ receptor expressions are altered in the prefrontal cortex of suicides
Maciag *et al.*	2009	Clinical	*Biol Psychiatry*	Reductions in cortical GABAergic neurons in major depression
Sequeira *et al.*	2009	Clinical	*PLoS One*	Gene expression linkage analysis implicates $GABA_B$ mechanisms in major depression
Levinson *et al.*	2010	Clinical	*Biol Psychiatry*	$GABA_B$-mediated cortical silence is prolonged in major depression
Cryan and Slattery	2010	Review	*Advances in Pharmacology*	Overview of research implicating a role for $GABA_B$ receptors in major depression

compartment (Karbon and Enna, 1985). Thus, the response to $GABA_B$ receptor stimulation or inhibition may vary as a function of the pre-existing state of the affected cell. Given their widespread distribution, and myriad of effects on second messenger production and ion channel activity, it is not surprising that laboratory animal and human studies indicate that alterations in the $GABA_B$ receptor system contribute to the symptoms of a host of clinical conditions, including seizures, cognitive deficits, depression, anxiety, spasticity, drug abuse, schizophrenia, pain and gastro-oesophageal reflux disease (Enna, 2001; Enna and Bowery, 2004; Froestl, 2010).

Because of these findings, efforts have been expended to develop orthosteric $GABA_B$ receptor agonists and antagonists, and allosteric modulators (Froestl, 2010). Included are agonists such as arbaclofen placarbil (Gerson *et al.*, 2010; Lal *et al.*, 2009), a baclofen prodrug, and lesogabaran (Bredenoord, 2009), and orthosteric antagonists such as CGP36742, CGP54626 and SCH50911 (Froestl, 2010). Positive allosteric modulators, including CGP7930 and GS39783, have been designed to more selectively activate subsets of the $GABA_B$ receptor system and thereby minimize the side effects encountered with orthosteric agonists (Cryan *et al.*, 2004; Mannoury la Cour *et al.*, 2008).

While evidence suggests the possibility of pharmacologically distinct $GABA_B$ receptors (Bonanno and Raiteri, 1993a,b; Cunningham and Enna, 1996), their existence has been a matter of dispute (Waldmeier *et al.*, 1994). Indeed, the discovery that heterodimerization is required for receptor function, and the fact that the number of $GABA_B$ receptor subunits is limited, argue against variability among orthosteric binding sites, which are exclusively located on the $GABA_{B1}$ subunit. The identification of pharmacologically distinguishable sites is important therapeutically as generalized activation or inhibition of $GABA_B$ receptors would be anticipated to be accompanied by numerous side effects, as is the

case with baclofen, an orthosteric $GABA_B$ receptor agonist. It is encouraging therefore that in recent years the evidence supporting pharmacologically distinct $GABA_B$ receptors has grown (Pinard *et al.*, 2010). Data are accumulating to suggest that the differential relative affinities and responses reported for $GABA_B$ receptor agonists and antagonists could be due to differences in the expression or function of regulators of G-protein signaling (RGS) proteins, which can influence $GABA_B$ receptor and K^+ channel responsiveness (Mutneja *et al.*, 2005). Moreover, four sequence-related cytosolic proteins have been discovered that bind as tetramers to the C-terminal domain of the $GABA_{B2}$ subunit, influencing the pharmacology and kinetics of the receptor response (Pinard *et al.*, 2010; Schwenk *et al.*, 2010).

Thus, the functional responsiveness of $GABA_B$ receptors is dependent upon the production of $GABA_{B1}$ and $GABA_{B2}$ subunits, the coupling of the latter to G proteins, the phosphorylation state of the receptor, and the scaffolding provided by RGS and cytosolic proteins. Taken together, these observations, along with earlier studies, provide evidence for possible pharmacological heterogeneity among $GABA_B$ receptors. Such findings are crucial for customizing compounds to selectively interact with subsets of $GABA_B$ receptors in developing new therapies for the treatment of CNS disorders, including major depression.

$GABA_B$ receptors and depression

It has been speculated for some time that $GABA_B$ receptors are modified in depression and in response to antidepressant therapies (Enna and Bowery, 2004) (Table 1). While there have been conflicting findings (Cryan and Slattery, 2010), the preclinical studies generally indicate that chronic, but not acute, administration of antidepressants or electroconvulsive shock increases the number and function of $GABA_B$ receptors in rodent brain (Pilc and Lloyd, 1984; Lloyd *et al.*, 1985; Suzdak and Gianutsos, 1986; Gray and Green, 1987; Martin *et al.*, 1989; Pratt and Bowery, 1993; Frieling and Bleich, 2006; Cornelisse *et al.*, 2007), and that brain $GABA_B$ receptor number is decreased in rat models of depression (Martin *et al.*, 1989). Notably, antidepressant-induced increases in $GABA_B$ receptor binding occur in only certain rat brain regions (Pratt and Bowery, 1993).

Although these manipulations generally increase $GABA_B$ receptor sensitivity, changes in the magnitude and direction of $GABA_B$ subunit expression vary with the central nervous system area examined. Thus, it appears that regionally selective changes in the production, assembly and processing of brain $GABA_B$ subunits may be important homeostatic mechanisms for controlling CNS activity. For example, antidepressant-induced increases in $GABA_B$ receptor binding occur in only certain areas of the rat brain frontal cortex, including laminas I and VI. In contrast, chronic administration of these agents causes no changes in $GABA_B$ binding in laminas II, III and V (Pratt and Bowery, 1993). This regional specificity may explain why some have been unable to detect such changes and suggests this receptor modification is not due to a direct interaction of the drug with $GABA_B$ receptors, but rather is secondary to an effect on some other, most likely monoaminergic, system (Slattery *et al.*, 2005).

With the cloning of the $GABA_B$ receptor subunit genes (Kaupmann *et al.*, 1997; 1998; Jones *et al.*, 1998; White *et al.*, 1998; Chronwall *et al.*, 2001), it became possible to examine whether the changes noted in $GABA_B$ receptor binding and function are due to modifications in the transcription or translation of these proteins (McCarson and Enna, 1999; Sands *et al.*, 2003a,b; 2004; McCarson *et al.*, 2005; 2006). Studies indicate that chronic administration of antidepressants, stress or pain differentially modifies $GABA_B$ receptor subunit gene expression and receptor responsiveness in rat spinal cord and hippocampus, and alters $GABA_B$ receptor responsiveness in these subjects. The chronic administration of classical antidepressants generally increases $GABA_B$ receptor function and $GABA_{B1a}$ gene expression in rat hippocampus and dorsal horn of the spinal cord, while having a variable effect on the expression of the $GABA_{B2}$ subunit gene. These data suggest that antidepressants cause an up-regulation in $GABA_B$ receptor expression and function by decreasing GABAergic tone, supporting the notion that depression is characterized by an overabundance of brain GABAergic activity (Sands *et al.*, 2004).

The antidepressant-induced increase in $GABA_B$ receptor function is evidenced by an enhancement in baclofen-stimulated cAMP production in brain and spinal cord tissue obtained from animals that are chronically administered any one of a number of such agents (Sands *et al.*, 2003a; 2004; McCarson *et al.*, 2006) Likewise, repeated administration of amitryptyline or electroconvulsive shock enhances baclofen-induced inhibition of K^+-stimulated serotonin release from mouse frontal cortex (Gray and Green, 1987). Both of these findings are consistent with other data indicating these drugs increase $GABA_B$ receptor number in this brain area.

An enhancement of receptor number and responsiveness could suggest that antidepressants either correct a depression-related underactive system or decrease GABAergic tone, leading to a supersentive receptor state. While there has been a

report suggesting that baclofen displays antidepressant activity in some animal models of depression (Frankowska *et al.*, 2007), the overwhelming weight of evidence suggests that a decrease in $GABA_B$ receptor activity is more typically associated with an antidepressant response. Thus, $GABA_B$ receptor antagonists display antidepressant properties in most, but not all (Mombereau *et al.*, 2004), animal models of this condition (Nakagawa *et al.*, 1999; Froestl *et al.*, 2004; Mombereau *et al.*, 2005; Nowak *et al.*, 2006), and $GABA_B$ receptor stimulation exacerbates learned helplessness in rats (Nakagawa *et al.*, 1996), a behaviour interpreted as a model for human depression. Also, mice lacking functional $GABA_B$ receptors behave as though they were receiving an antidepressant, suggesting regionally selective enhancements in brain GABAergic function in depression (Mombereau *et al.*, 2004). Interpretation of these findings is limited, however, by the possibility that this behavioural response is due to secondary adaptive changes in these genetically modified animals rather than being a faithful representation of a phenotype that results solely from a selective decline in $GABA_B$ receptor activity.

It has been reported that $GABA_B$ receptor positive allosteric modulators display weak anxiolytic activity in some (Cryan *et al.*, 2004; Jacobson and Cryan, 2008), but not all (Jacobson and Cryan, 2008; Paterson and Hanania, 2010), animal models of anxiety. Moreover, anxiety-like behaviour has been noted in $GABA_B$ receptor-deficient mice (Mombereau *et al.*, 2005).These findings suggest that $GABA_B$ receptor blockade might exacerbate or precipitate an anxiety disorder in susceptible subjects, such as individuals with major depression. However, the clinical importance of this finding remains questionable given the inconsistency of the anxiolytic response to $GABA_B$ positive allosteric modulators in animal models, and the lack of any reported anxiogenic effect of an orthosteric $GABA_B$ receptor antagonist following administration to humans (Froestl *et al.*, 2004).

Other preclinical data supporting the hypothesis that $GABA_B$ receptor blockade may alleviate depression include the finding that $GABA_B$ receptor antagonists increase gene expression and protein levels of nerve growth factor and brain-derived neurotophic factor (BDNF) in various regions of the rat brain (Heese *et al.*, 2000; Enna *et al.*, 2006). The relevance of this discovery to depression is based on reports that various classes of antidepressants, as well as electroconvulsive shock, increase the expression of BDNF in the rat hippocampus, and that BDNF displays antidepressant-like activity when placed directly into this brain region (Duman and Monteggia, 2006). In addition, hippocampal levels of BDNF are decreased in a mouse model of depression (Tsankova *et al.*, 2006). It has been proposed that this effect of antidepressants on BDNF expression induces hippocampal neurogenesis, which is thought to be an important factor in alleviating depression (Miller *et al.*, 2008). However, as noted by Tanti and Belzung (2010), BDNF polymorphisms are associated with a number of conditions, the effect of antidepressants on hippocampal neurogenesis is species-dependent and this response is not observed with all agents that display antidepressant activity in other models. Thus, the predictive value of enhanced BDNF production in assessing antidepressant potential remains uncertain.

Nonetheless, the results from nearly 30 years of research suggest that antidepressants cause an up-regulation of $GABA_B$ receptor number and function secondary to a decrease in GABAergic activity that may result from prolonged activation of monoaminergic systems (Sands *et al.*, 2004). Serotonergic transmission appears to be particularly important in this regard in that $GABA_B$ receptor antagonists no longer display antidepressant properties following administration of para-chlorophenalanine, an inhibitor of tryptophan hydroxylase (Slattery *et al.*, 2005). It has also been reported that antidepressants decrease the function of presynaptic serotonin-3 receptors on GABA neurons, resulting in a decrease in GABA release (Nakagawa and Ishima, 2003). Such findings have led to speculation that depression is characterized by an enhanced $GABA_B$ tone, perhaps as a result of a decrease in serotonergic activity, and that the response to antidepressants is dependent upon a reduction in $GABA_B$ receptor stimulation which, in turn, leads to a supersensitive $GABA_B$ receptor system.

Human studies on the $GABA_B$ system and neuropsychiatric disorders

A number of clinical and post-mortem studies support a causal relationship between the GABAergic system and depression (Cryan and Slattery, 2010) (Table 1). Thus, GABA levels in the cerebral cortex, plasma and CSF are lower than normal in depressed patients, as is the number of GABA neurons in layer II of the orbitofrontal cortex (Petty, 1995; Rajkowska *et al.*, 1999; Sanacora *et al.*, 2000; Krystal *et al.*, 2002). A $GABA_B$ receptor involvement is suggested by the findings of some (Marchesi *et al.*, 1991; O'Flynn and Dinan, 1993; Lucey *et al.*, 1994), but not others (Davis *et al.*, 1997), that the growth hormone response to baclofen is blunted in depressed individuals as compared with controls, suggesting altered $GABA_B$ receptor responsiveness in these patients. Also, an efficacy study with baclofen indicates that this $GABA_B$ receptor agonist worsens

symptoms of depression (Post *et al.*, 1991). Although the sample size is too small for drawing firm conclusions from this study, these data are interesting in light of the subsequent preclinical work suggesting that depression may be associated with an overstimulation of the $GABA_B$ system.

Post-mortem studies reveal that those diagnosed with an affective disorder display a decreased expression of cerebellar glutamic acid decarboxylases (GAD) (Fatemi *et al.*, 2005), the enzymes responsible for the synthesis of GABA, and differences in GAD immunohistochemistry in various regions of the cerebral cortex and the hippocampus as compared with controls (Bielau *et al.*, 2007). There have also been reports of differences between depressed and control subjects in the size and density of cerebral cortical GABA neurons (Rajkowska *et al.*, 2007; Maciag *et al.*, 2009). While such studies are important for establishing a GABAergic dysfunction in depression, they do not directly address whether, and to what extent, these changes influence, or are related to, the $GABA_B$ receptor system.

This issue was addressed directly by a study showing that the cortical silent period, a measure of cortical inhibition thought to be a reflection of $GABA_B$ receptor function, is prolonged in depressed individuals (Levinson *et al.*, 2010). As baclofen administration lengthens the cortical silent period in normal subjects, the finding with depressed patients supports the preclinical work suggesting that this disorder is characterized by an enhancement in $GABA_B$ receptor activity. In contrast, however, earlier binding studies on post-mortem tissue (Cross *et al.*, 1988; Arranz *et al.*, 1992) found no differences in $GABA_B$ receptor number or affinity between controls and suicide subjects in frontal and temporal cortices and hippocampal samples. However, the interpretation of these results is compromised by the fact that these studies were conducted using relatively gross brain regions, which may dilute any changes that occur in highly discreet brain areas, and that ligand binding alone reveals nothing about the functional state of the receptor.

Efforts have been made to determine whether modifications in the expression of $GABA_B$ receptor subunits are associated with neuropsychiatric illness. Inasmuch as both $GABA_{B1}$ and $GABA_{B2}$ must be present to form a functional receptor, a change in the production of either could signal an alteration in the responsiveness of this system. As detailed previously, laboratory animal studies indicate that antidepressant administration, as well as chronic pain and stress, alters $GABA_B$ subunit expression and receptor function in rat brain and spinal cord (Sands *et al.*, 2003a; 2004; McCarson *et al.*, 2006), demonstrating the utility of analysing subunit expression as an indicator of receptor modifications. As both $GABA_B$ receptor subunits are found throughout the human brain (Billinton *et al.*, 2000; Berthele *et al.*, 2001; Waldvogel *et al.*, 2004), it is likely that alterations in their expression could result in CNS disturbances, the nature of which would depend on the specific brain region involved. Indeed, reports indicate regionally selective changes in $GABA_B$ subunit expression in association with schizophrenia (Mizukami *et al.*, 2002), temporal lobe epilepsy (Furtinger *et al.*, 2003; Princivalle *et al.*, 2003) and autism (Fatemi *et al.*, 2009). Microarray studies of post-mortem brain tissue obtained from depressed and non-depressed suicide victims indicate modifications in the expression of genes responsible for the production of both $GABA_A$ and $GABA_B$ receptors in various regions of the prefrontal cortex and in selected subcortical areas (Klempan *et al.*, 2009; Sequeira *et al.*, 2009). The $GABA_{B2}$ subunit expression appeared to be particularly affected, being elevated in the depressed suicide group relative to non-depressed individuals (Sequeira *et al.*, 2009). A full appreciation of the significance of these findings awaits replication of this work and a determination as to whether these alterations in gene expression are indicative of a change in subunit protein. Besides hinting at an association between changes in the production of $GABA_B$ receptor subunits and depression, these studies confirm the importance of examining this issue in well-defined regions, as the gene alterations are not global, but rather circumscribed to rather discreet brain areas.

Human $GABA_B$ receptor subunit expression in depression

To confirm that depression is associated with selective, regionally defined changes in human brain $GABA_B$ receptor subunits, a preliminary study was undertaken with post-mortem brain samples obtained from depressed individuals and control subjects. The primary hypothesis was that regionally selective alterations in brain $GABA_B$ receptor subunit gene expression are a characteristic of this disorder. The brain areas examined were the hippocampus, subgenual cingulate and orbitofrontal cortex, regions implicated in the pathophysiology of depression (Sheline, 1996; Sheline *et al.*, 1999; Mayberg, 2003; Seminowicz *et al.*, 2004; Pittenger and Duman, 2008; Hajszan *et al.*, 2009; Koolschijn *et al.*, 2009; Yucel *et al.*, 2009; Price and Drevets, 2010). Particular emphasis was placed on subsections of the hippocampus as this is a region where the balance between excitatory and inhibitory inputs appears to be particularly critical. Examining the molecular contribution of $GABA_B$ alterations

within the extended cortical depression system complements a focus on the reward regions in depression. The entire extended network includes the subgenual cingulate, the dorsolateral prefrontal cortex, orbitofrontal cortex, pregenual anterior cingulate and frontal pole, as well as the amygdala, hippocampus and insula. The regions of greatest importance to major depression are prefrontal cortex, anterior cingulate, amygdala, anterior thalamus and regions within the hippocampus. Inasmuch as these brain areas are richly innervated by monoaminergic and GABAergic neurons, they are most likely to display changes in $GABA_B$ receptor subunit expression if, as indicated by the preclinical studies, there is a functional interplay between these transmitter systems in depression and the response to antidepressants.

Because major depression is a neuroanatomically complex condition, it is critical to examine in discrete brain regions the possible molecular and neurochemical changes associated with this condition to reduce the possibility of overlooking a meaningful alteration because of a diluting effect of adjacent tissue. Changes in the volume, function and interaction among cortical-limbic brain areas are particularly evident in depression (Sheline, 1996; Sheline *et al.*, 1999; Mayberg, 2003; Seminowicz *et al.*, 2004; Pittenger and Duman, 2008). Within the hippocampus, alterations in the morphology of the dentate gyrus (DG), CA1 and CA3 regions occur in association with stress, depression and antidepressant therapy (Malberg *et al.*, 2000; Pittenger and Duman, 2008; Hajszan *et al.*, 2009). Likewise, depression-related volume changes occur in the subgenual cingulate cortex (Yucel *et al.*, 2009) and the orbital frontal cortex (Koolschijn *et al.*, 2009). Thus, these brain regions, along with cerebellar cortex, an area not believe to contribute to the symptoms of this disorder, were selected for studying possible changes in $GABA_B$ subunit expression in depression.

Human brain tissue from depression and control cases was obtained from the Dallas Brain Collection (Stan *et al.*, 2006). The tissue was collected only after acquiring consent from the next of kin along with permission to review medical records and to conduct a telephone interview with a primary caregiver. All clinical information on each case was evaluated by at least three research psychiatrists and diagnoses were made using DSM-IV criteria. Blood screens for drugs of abuse, alcohol and prescription medications were conducted on each subject. Cases were excluded when there was a known history of neurological disorders or of an axis I psychiatric condition other than major depression.

The human post-mortem material was obtained from 12 cases diagnosed with major depressive disorder and 12 control subjects. The two groups were matched as closely as possible for age, brain pH, post-mortem interval (PMI), and RNA integrity number (RIN), an indicator of human post-mortem tissue RNA quality (Stan *et al.*, 2006) (Table 2). While half the members of the depression group were suicides, only two subjects in this total cohort had detectible blood levels of medication at the time of death (Table 2). The groups were analysed in a paired design. The hippocampus, anterior cingulate and orbitofrontal cortices were selected for study because of the *in vivo* imaging data suggesting their involvement in the clinical manifestations of depression and the response to antidepressant treatments. The tissue samples were dissected from the anterior cingulate (BA24) and orbitofrontal cortex (BA11), as well as hippocampal subfields and cerebellum. Other than for the hippocampus, the samples were frozen immediately in a mixture of dry ice and isopentane (1:1, v : v), pulverized on dry ice and stored at –80°C until analysed.

The entire hippocampus was removed from the fresh brain, embedded longitudinally into a mold with Histomer polymer (Histotech, Frederiksberg, Denmark). Tissue blocks were then taken at 5 mm intervals and frozen immediately in a mixture of dry ice and isopentane (1:1, v : v). Blocks from the mid-level of the hippocampus were used for the study. Four samples, each 300 μm thick, were cryostat sectioned at –20°C, then stored at –80°C. Nissl staining of 14 μm sections adjacent to the samples was used to determine orientation.

In each of the 300 μm sections, the parahippocampal gyrus was first dissected away from the hippocampus proper, then a series of cuts was made to isolate the CA3, CA1, subiculum and DG (Figure 1).

The $GABA_B$ receptor subunit expression assays were performed blind to diagnosis (depressed or control) using paired samples from the two groups of subjects. Sample pairing was performed by someone not involved with the biochemical assay to ensure that tissues tested on any given day included an equal number of samples from the same brain regions of depressed and control individuals.

Modified versions of human $GABA_{B1a}$ and $GABA_{B2}$ expression vectors provided by Dr Klemens Kaupmann (Novartis, Basel, Switzerland) were used for probe synthesis. The probe sequences were bases 276-613 of human $GABA_{B1a}$ cDNA (Accession Number AJ225028) and bases 2746-3188 of human $GABA_{B2}$ cDNA (Accession Number BC035071.2). Human β-actin mRNA expression was quantified with probes generated using a pGEM-T vector containing bases 374-1093 (Accession Number NM_001101) of the human β-actin sequence (Nandan and Reiner, 1997).

Table 2

Characteristics of depressed and control group subjects

Controls							Depression							
Case	Age (years)	PMI (hours)	RIN	Gender	Race	Cause of death	Case	Age (years)	PMI (hours)	RIN	Gender	Race	Meds	Cause of death
C1	48	20	6.8	M	C	HT CVD	D1	46	11	8.3	F	C	yes	HT CVD
C2	60	11	9.3	M	AA	HT CVD	D2	54	6	8.9	M	C	no	AS CVD
C3	20	21	8.2	M	C	Blunt force injury	D3	35	9	7.4	M	C	no	suicide
C4	31	16	8.1	M	C	HT CVD	D4	33	18	8.5	M	C	no	suicide
C5	60	20	8.5	M	C	Myocardial infarction	D5	61	20	8.6	M	C	no	suicide
C6	43	15	6.1	M	C	HT CVD	D6	50	23	8.8	M	C	no	suicide
C7	65	11	8	F	C	Aortic dissection	D7	57	16	6.6	F	C	no	suicide
C8	83	13	8.5	F	C	Sharp force injury	D8	59	10	9	F	C	no	HT CVD
C9	34	23	6.2	M	C	HT CVD	D9	40	18	6.9	M	C	no	suicide
C10	48	15	9.5	M	C	HT CVD	D10	41	13	8	F	AA	yes	Diabetic ketoacidosis
C11	63	14	7.7	M	C	AS CVD	D11	65	14	7.6	M	C	no	HT CVD
C12	19	20	9	M	C	Gunshot wound to chest	D12	26	19	8.1	F	C	no	Accidental drowning
av	47.83	16.58	7.99	2F/10M	11C/1AA		av	47.25	14.75	8.06	5F/7M	11C/1AA		
SD	19.45	4.08	1.12				SD	12.39	5.10	0.79				

PMI, post-mortem interval; RIN, RNA integrity number; meds, antidepressant medications at time of death; HT CVD, hypertensive cardiovascular disease; AS CVD, atherosclerotic cardiovascular disease; F, female; M, male; C, Caucasian; AA, African American; av, average; SD, standard deviation.

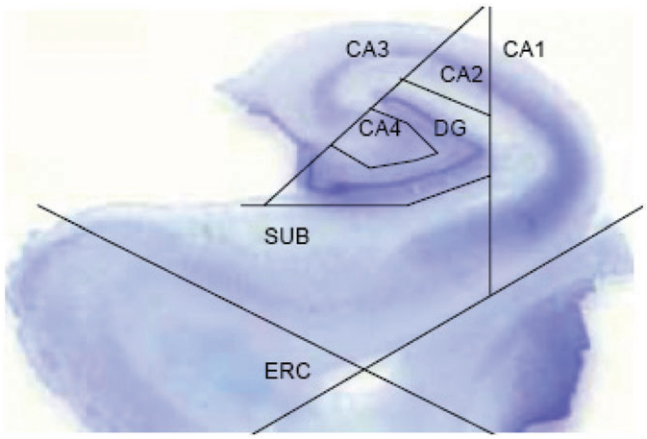

Figure 1

Representation of the coordinates used to dissect the dentate gyrus (DG), CA1 and CA3 regions of the hippocampus.

Total RNA was isolated from tissue samples using a rapid-guanidinium method (Chomczynski and Sacchi, 1987) and assayed separately for $GABA_{B1a}$, $GABA_{B2}$ and β-actin mRNAs using solution hybridization–nuclease protection assays (McCarson and Krause, 1994).

The primary analyses were designed to test whether regionally selective alterations occur in $GABA_B$ receptor subunit expression in the hippocampus, anterior cingulate and orbitofrontal cortex in cases of depression compared with controls. Secondary analyses were conducted to explore the influence of age and gender on receptor subunit expression. An independent samples *t*-test was employed for comparing these results given the relatively large sample size, the continuity and normal distribution of the gene expression values, the convergence of measures of central tendency, and the similar variance in each data set. Spearman rank correlations were used to assess possible correlations between mRNA levels with RIN and PMI. Unpaired *t*-tests were conducted to verify that the two diagnostic groups were matched on demographic variables, age, RIN and PMI. In all analyses, differences were considered statistically significant with $P \leq 0.05$.

Both $GABA_{B1a}$ and $GABA_{B2}$ receptor subunit gene expression were detectable in all brain regions examined (Table 3). Of the hippocampal subfields studied, only the DG displayed a significant difference between depressed and control groups. The expression of both subunit genes in the DG differed significantly between the depressed subjects and controls, with a 30% decrease in $GABA_{B1a}$ ($t = 2.18$, df_{19}, $P = 0.04$) and a 50% increase in $GABA_{B2}$ gene expression ($t = 2.21$, df_{20}, $P = 0.04$) in this brain region (Table 3). The latter result confirms an earlier finding from a microarray study indicating that $GABA_{B2}$ subunit expression is elevated in brain tissue from depressed suicide subjects as compared with non-depressed individuals (Sequeira *et al.*, 2009). Differential modifications in the expression of $GABA_{B1}$ and $GABA_{B2}$ subunit gene expression have been found in rat brain (Sands *et al.*, 2003b) and spinal cord (McCarson *et al.*, 2006) tissue. It is unknown whether these changes are occurring in the same or different cellular elements. Regardless, an increase or decrease in gene expression of either one or both subunits in a single cell is likely to cause, and reflect, an alteration in $GABA_B$ receptor function. The results also indicated a significant negative correlation between $GABA_{B1a}$ subunit gene expression and age ($R = –0.43$, $P = 0.04$) in the CA3 region of the hippocampus. Covarying for age did not alter the absence of a group difference in $GABA_{B1a}$ expression in CA3. Comparisons between groups revealed a significant decrease in $GABA_{B1a}$ subunit expression in the CA3 subfield in depressed male subjects compared with control males ($t = 2.55$, df_{11}, $P = 0.03$; Figure 2).

No significant differences were noted between depressed and control groups in $GABA_{B1a}$ or $GABA_{B2}$ subunit gene expression in the orbital frontal and anterior cingulate cortices or cerebellum (Table 3). The $GABA_{B2}$ receptor subunit expression in the orbital frontal cortex was, however, nearly twice as high in male depressed subjects as in male controls ($t = 2.3$, $P = 0.04$; Figure 3), and there was a strong trend towards a significant increase in the expression of the $GABA_{B2}$ subunit ($P < 0.06$) in this brain region of depressed subjects compared with controls (Cohen's d effect size = 0.83) (Table 3). While there were no significant correlations between age, RIN or PMI and $GABA_{B2}$ receptor subunit gene expression for these three brain regions, and for $GABA_{B1a}$ expression in the orbital frontal cortex, a correlation was noted between $GABA_{B1a}$ expression and PMI ($R = 0.41$, $P = 0.05$) and RIN ($R = 0.46$, $P = 0.02$) for the anterior cingulate cortex and cerebellum respectively.

Because major depression has been associated with changes in the volume of some brain regions (Rajkowska, 2000; Rajkowska *et al.*, 2007; Maciag *et al.*, 2009), it is possible that measurement of beta-actin gene expression may not be an appropriate reference for control as the quantity of this marker could differ between the two groups as a result of cell loss. However, analysis of the beta-actin gene revealed no significant differences in expression in the brain areas examined in the depressed and control groups (data not shown). This finding indicates that despite any loss of volume or decrease in

Table 3

$GABA_{B1a}$ and $GABA_{B2}$ subunit gene expressions in various brain regions of depressed and control subjects

Brain region	$GABA_{B1a}$		$GABA_{B2}$	
	Depressed	Control	Depressed	Control
CA1	17 ± 4	23 ± 3	Not measured	
CA3	86 ± 18	83 ± 8	Not measured	
Dentate gyrus	25 ± 2*	37 ± 5	17 ± 2*	11 ± 1
Subgenual cingulate cortex	14 ± 2	16 ± 2	13 ± 2	17 ± 3
Orbital frontal cortex	9 ± 2	7 ± 1	64 ± 13	36 ± 6
Cerebellum	4 ± 1	4 ± 1	19 ± 6	23 ± 5

Values are the mean pg subunit specific mRNA/ng β-actin ± SEM.
*$P < 0.05$ compared with corresponding control, two-tailed Student's *t*-test.

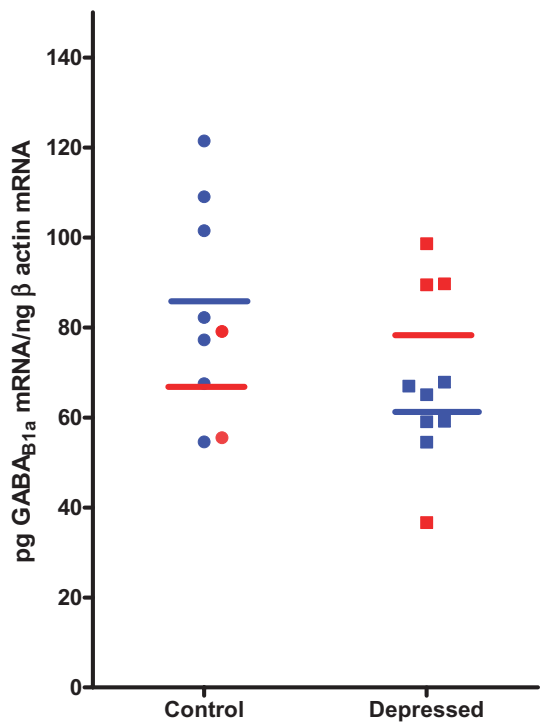

Figure 2

$GABA_{B1a}$ subunit gene expression in the CA3 region of the hippocampus of depressed (n = 6) and control (n = 7) male (blue) and depressed (n = 4) and control (n = 2) female (red) subjects. Horizontal lines indicate the means for each group. The level of significance for the difference between means for the male subjects is P = 0.03, as determined by an independent samples *t*-test.

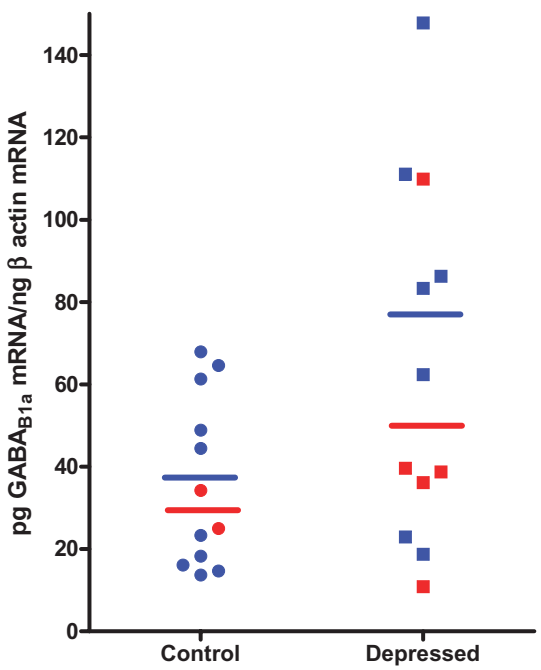

Figure 3

$GABA_{B2}$ subunit gene expression in the orbital frontal cortex of depressed (n = 7) and control (n = 9) male (blue) and depressed (n = 5) and control (n = 2) female (red) subjects. Horizontal lines indicate the means for each group. The level of significance for the difference between means for the male subjects is P = 0.04, as determined by an independent samples *t*-test.

cell number, the expression of this marker relative to total RNA levels remains unchanged in depression. Accordingly, beta-actin appears to be an appropriate control gene for normalizing the levels of $GABA_B$ receptor subunit gene expression under these circumstances.

These results suggest a decrease in $GABA_{B1a}$ and an increase in $GABA_{B2}$ subunit expression in the DG of depressed individuals as compared with controls. Because dysfunction in this brain region has been previously linked with depression, this change in $GABA_B$ receptor subunit expression could be associated with the illness itself. Because of the size of the tissue samples, it was not possible to analyse $GABA_{B2}$ subunit expression in the CA1 and CA3 regions of the hippocampus, leaving open the possibility of a depression-related modification in the expression of

this $GABA_B$ receptor subunit in these areas. Nonetheless, the fact that no significant changes in $GABA_{B1a}$ subunit gene expression were noted in the CA1 or CA3 regions of the hippocampus, or in $GABA_{B1a}$ and $GABA_{B2}$ expression in the subgenual cingulate cortex, two areas also thought to be involved in the depression brain circuit, nor in the cerebellum, an area outside this circuit (Pittenger and Duman, 2008), suggests that the changes observed in the DG are selective and not a reflection of a generalized abnormality in $GABA_B$ subunit gene expression as a result of the disorder, drug treatment or death.

The finding of an apparent decrease in $GABA_{B1a}$ subunit expression in the CA3 region of male subjects, and a doubling of the $GABA_2$ subunit expression in the orbital frontal cortex of these individuals as compared with male controls, suggests that the $GABA_B$ system may be modified in these depression circuit brain areas as well (Figures 2 and 3). Inasmuch as the $GABA_{B1}$ subunit gene expression data for the CA3 and orbital frontal cortex nearly attained statistical significance when comparing all samples (Table 3), it seems probable these areas are affected, with the lack of statistical significance for the present findings possibly being due to the influence of variation because of the sample size. Given the small number of female subjects, it is impossible to determine whether these changes are related to gender (Figures 2 and 3). Nevertheless, the modifications noted in the DG alone demonstrate that the $GABA_B$ receptor system is altered in a critical brain region associated with major depressive illness.

It is also noteworthy that a negative correlation was found between $GABA_{B1a}$ subunit gene expression in the CA3 region and age, while no significant correlations were detected between $GABA_B$ receptor subunit gene expression and drug history or postmortem delay. It could be speculated that this age-related decline in $GABA_{B1a}$ expression in the CA3 may contribute to the increase in susceptibility to depression in the elderly.

From these data, it is impossible to know whether the changes in $GABA_B$ receptor subunit expression lead to a change in the production of subunit protein or receptor function. However, numerous studies have indicated that alterations in $GABA_B$ subunit expression are usually accompanied by a change in receptor sensitivity (Sands *et al.*, 2003a; 2004; McCarson *et al.*, 2005; 2006; Merlo *et al.*, 2007). As the production of $GABA_{B2}$ subunits appears to be the rate-limiting step in the formation of functional $GABA_B$ receptors (Thuault *et al.*, 2004), these results, like previous work in laboratory animals, suggest that the system is up-regulated in depressed subjects in the DG, and possibly the CA3 region of the hippocampus and in the orbital frontal cortex. As a change in $GABA_B$ receptor activity alters the expression of brain-derived and glial cell line-derived neurotrophic factors (Heese *et al.*, 2000; Enna *et al.*, 2006; Fiorentino *et al.*, 2009), it is possible that alterations in $GABA_B$ receptor expression and function influences hippocampal neurogenesis, which may be a component of the clinical response to antidepressants (Pittenger and Duman, 2008).

As these data were obtained from a predominantly antidepressant-free cohort of cases (Table 2), the results suggest that the changes in $GABA_B$ receptor subunit expression may be part of the molecular phenotype of this psychiatric condition. It is, however, possible that they are long-lasting responses to prior antidepressant treatment. This is a critical issue as it has been reported that the $GABA_B$ receptor response to antidepressants is state-dependent (McCarson *et al.*, 2005), making it impossible to predict the effect of these drugs on human brain $GABA_B$ receptors without knowing whether this system is modified by the condition itself. As discussed previously, an increase in $GABA_B$ function could be a response to a persistent antidepressant-induced reduction in GABAergic tone, or it might be an evidence of a disease-related supersensitive $GABA_B$ receptor system. The latter possibility is consistent with the discovery that $GABA_B$ receptor antagonists display an antidepressant profile in animal models of this condition (Nakagawa *et al.*, 1999; Froestl *et al.*, 2004; Slattery *et al.*, 2005; Nowak *et al.*, 2006).

The findings that baclofen, a $GABA_B$ receptor agonist, worsens the symptoms of depression (Post *et al.*, 1991) and, like depression, prolongs the cortical silent period in humans (Levinson *et al.*, 2010), and that mice lacking functional $GABA_B$ receptors behave as though they have been administered an antidepressant, all support the notion that an overactive GABA system contributes to the symptoms of this disorder. They also argue strongly against the idea that the antidepressant response to $GABA_B$ receptor antagonists might be due to an enhancement in GABA release secondary to the blockade of $GABA_B$ autoreceptors.

These data confirm and extend previous studies (Klempan *et al.*, 2009; Sequeira *et al.*, 2009) indicating a direct relationship between a modification in $GABA_B$ receptor subunit gene expression and major depression, with receptor subunit changes being most evident in the DG. Given the proposed relationship between the DG and affective illness (Malberg *et al.*, 2000), these findings suggest a direct link between modifications in human brain $GABA_B$ receptor subunit gene expression and depression, and provide insights into the molecular mecha-

nisms that may be responsible, at least in part, for some of the neurochemical and behavioural changes associated with this condition. These discoveries support the notion that GABAergic medications, in particular $GABA_B$ receptor antagonists, may represent a novel approach for the treatment of this disorder.

While it has been some time since the development of orally active $GABA_B$ receptor antagonists (Froestl *et al.*, 1995), only one of these phosphinic acid GABA analogues has been examined clinically (Froestl *et al.*, 2004). In this study, SGS742 progressed through Phase II clinical trials as a potential treatment for cognitive deficits. Although no serious side effects were noted at the doses tested, and some benefits were reported for patients diagnosed with mild cognitive impairment, clinical trials were halted because the efficacy was insufficient to warrant commercial development. Given the difficulties associated with demonstrating clinical antidepressant activity, and the low affinity of SG742 for the $GABA_B$ receptor site, no effort has yet been made to test the hypothesis that $GABA_B$ receptor antagonists are antidepressants. Proof of principle must await the development of more potent, orally active and pharmacokinetically appropriate members of this class.

To fully exploit these preliminary findings on receptor subunit expression in post mortem brain-tissue, future work should focus on determining the functional correlate of these changes and on whether these alterations are drug induced or part of the pathophysiological process. Ultimately, the relationship between $GABA_B$ receptors and depression can only be conclusively tested by a thorough clinical assessment of the antidepressant properties of $GABA_B$ receptor antagonists.

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Conflict of interest

The authors declare no competing financial interests in relation to the work described in this report.

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