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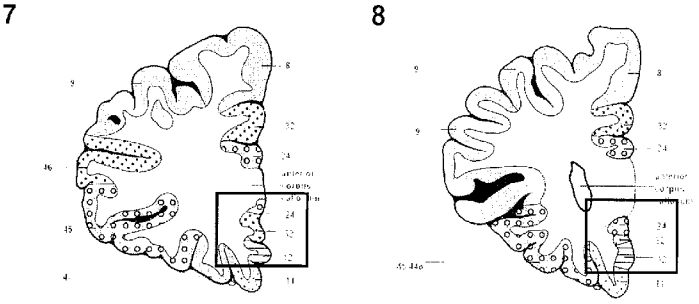


Figure 1: Coronal sections through the adult human forebrain at the anterior limit of the corpus callosum. Boxed areas (in black) mark out the subgenual anterior cingulate cortex.

(57) Abstract: Differential expression of nucleic acids in the brains of subjects suffering from late-onset depression has been demonstrated. The invention provides methods useful in the determination of late-onset depression. Also provided by the present invention is a screening method for the identification of compounds for treatment, prevention or diagnosis of late-onset depression.

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GABA BIOMARKERS FOR DEPRESSION

TECHNICAL FIELD OF THE INVENTION

Biomarkers have been identified that are either upregulated or downregulated in brain tissue samples from subjects suffering from late-onset depression in comparison to brain tissue samples from non-depressed subjects. A screening method is provided by the present invention to identify compounds useful in the treatment, prevention, and diagnosis of late-onset depression. The present invention also provides methods that are useful in the treatment, prevention and diagnosis of late-onset depression.

DESCRIPTION OF RELATED ART

Depression affects 15% of the USA population at some point during their lives, and 100 million people are affected on any given day. The age of onset is fairly evenly spread and can come on suddenly in days or build over years. Over half of people who experience major depression have only one episode. However, with each successive episode, there is a 15% risk that the next episode will be a manic one, changing diagnosis to Bipolar Disorder. Ultimately, approximately 15-20% of those with major depression become chronically depressed and around 15% of patients with major depression may commit suicide; and men commit suicide twice as often as women.

At the current time, there is a limited understanding of the neurobiology involved in depression but it is becoming increasingly evident that this disease is multifaceted and may involve a myriad of elements that act either synergistically or independently to result in mood changes. Depression is a complex disorder and is not dominated by a single pathology that can be used as a marker for the purposes of treatment, diagnosis and screening. A number of neurotransmitter systems are involved, and several targets have been extensively studied, and have resulted in a range of treatment options. Treatments that are currently available include monoamine oxidase inhibitors (MAOIs), tricyclic

antidepressants (TCAs), specific serotonin reuptake inhibitors (SSRIs), noradrenergic reuptake inhibitors (NRIs), serotonin noradrenergic reuptake inhibitors (SNRIs) and noradrenaline dopamine reuptake inhibitors (NDRIs). However, current anti-depressive drugs are unsatisfactory as they have many side effects, and have varying efficacy depending on the patient history and exact condition to be treated.

The gamma-aminobutyric acid (GABA) receptors are a class of receptors that respond to the neurotransmitter GABA, the chief inhibitory neurotransmitter in the vertebrate central nervous system. GABA receptors are reviewed by Bormann (2000 Trends Pharm. Sci.; 1(1): 16-19). There are three classes of GABA receptors: $GABA_A$, $GABA_B$, and $GABA_C$. $GABA_A$ and $GABA_C$ receptors are ionotropic receptors (also known as ligand-gated ion channels), whereas $GABA_B$ receptors are metabotropic receptors (also known as G protein-coupled receptors). The $GABA_A$ receptor directly gates a Cl^- ionophore and has modulatory binding sites for benzodiazepines, barbiturates, neurosteroids and ethanol. $GABA_C$ receptors are Cl^- pores that are insensitive to the drugs bicuculline and baclofen. By contrast, $GABA_B$ receptors couple to Ca^{2+} and K^+ channels via G proteins and second messenger systems; they are activated by the agonist baclofen and are resistant to drugs that modulate $GABA_A$ receptors.

GABA receptors have been linked to mood disorders, including depression.

Emrich et al (Archiv Psychiatrie Nervenkrankheiten 1980; 229: 1–16) demonstrated efficacy of valproate in the treatment of manic depressive patients. They proposed that valproate, through the enhancement of GABA brain concentration, might compensate for a potential GABAergic deficiency. After Emrich's hypothesis, several animal and human studies evaluated the potential role of GABAergic abnormalities in the pathophysiology of depression (Krystal et al 2002 Mol. Psychiatry; 2: S71-S80; Merali et al 2004 J. Neurosci.; 24(6): 1478-1485; Bipolar Disorders, Basic Mechanisms and Therapeutic Implication. Marcel Dekker: New York, 2000, pp. 143–165). As reviewed by Brambilla et al (2003

Molecular Psychiatry; 8: 721-737), animal and clinical studies have suggested that a deficit in GABAergic activity may be involved in the pathophysiology of depression.

US 2005/0209181 discloses the expression profiles of human post-mortem brains from patients diagnosed with schizophrenia and suggests that the markers identified may also be useful in targeting depression. Amongst other targets, this patent publication suggests a link with depression for various subunits of the $GABA_A$ receptor, i.e. alpha 1, alpha 2, alpha 4, alpha 5, alpha 6, beta 1, gamma 2, delta.

In WO 2008/020435, altered expression was found in animal models of depression for various subunits of the $GABA_A$ receptor, i.e. alpha 1, alpha 2, alpha 3, alpha 4, alpha 5, alpha 6, beta 1, beta 2, delta, rho 3, theta. Altered expression was also found for the $GABA_B$ receptor.

In WO 02/20492 tetrahydroindazole derivatives are taught as being useful in the diagnosis and treatment of, amongst other diseases, depression. WO 02/20492 teaches that tetrahydroindazole derivatives act as agonists at alpha 2 or alpha 3 and as such are useful in the treatment of depressive or bipolar disorders.

However, these reported abnormalities may not be specific to depression, as GABAergic alterations have also been suggested in related pathologies, such as anxiety disorder (Kosel *et al* 2004 Neurophsychopharmacol; 29: 347-50) schizophrenia (Int J Neuropsychopharmacol 2002; 5: 159–179) and panic disorder (Biol Psychiatry 2000; 47: 96–106). Furthermore, in a study by Kugaya *et al* (2003 Biol Psychiatry; 54: 792-9) looking at the binding of the $GABA_A$ binding [^{123}I]iomazenil in depressed patients, no difference was noted in depressed subjects in comparison to non-depressed subjects.

The neurobiological basis of late-onset depression remains largely unexplored, hampering the development of effective treatments. In the elderly, depression is the second most common psychiatric disorder after dementia, affecting

approximately 3% of the over 65's, with a further 12% suffering milder yet still debilitating depression (Beekman et al *British Journal of Psychiatry* 174, 307-311 (1999)). Approximately one third of patients do not respond to initial anti-depressant therapy, while those who do respond remain at very high risk of relapse, chronicity and dementia (Cole et al *American Journal of Psychiatry* 156, 1182-1189 (1999)). Consequently late-onset depression is associated with considerable costs in terms of morbidity and mortality as well as health and social care burden. It has been suggested that the clinical management of late-onset depression should be more tailored to its specific pathophysiological profile as compared with depression in younger subjects (Thomas et al *American Journal of Psychiatry* 157, 1682-1684 (2000); Thomas et al *British Journal of Psychiatry* 181, 129-134 (2002)).

There is therefore a need for clinical strategies having particular application in the treatment and diagnosis of late-onset depression.

SUMMARY OF THE INVENTION

Differential expression of genes related to the GABA system has presently been demonstrated in the brains of subjects suffering from late-onset depression as compared to non-depressed subjects. A screening method is provided for the identification of compounds useful in the treatment, prevention or diagnosis of late-onset depression. Also provided are methods useful in the treatment, prevention and diagnosis of late-onset depression. The present invention has the advantage that it provides biomarkers that are specifically related to the pathophysiology of late-onset depression.

DETAILED DESCRIPTION OF THE INVENTION

IN VIVO IMAGING METHOD

The present invention provides an *in vivo* imaging method for use in the determination of whether a subject has or is predisposed to late-onset depression,

said method comprising the steps of:

(i) administering an *in vivo* imaging agent to said subject, wherein said *in vivo* imaging agent comprises a compound that selectively associates with a polynucleotide or polypeptide, said polynucleotide or polypeptide being encoded by a GABAergic receptor gene, and wherein said compound is labelled with an *in vivo* imaging moiety;

(ii) allowing said *in vivo* imaging agent to selectively associate with said polynucleotide and/or said polypeptide expressed in a tissue of said subject;

(iii) detecting by an *in vivo* imaging method signals emitted by said *in vivo* imaging moiety; and,

(iv) generating an image representative of the location and/or amount of said signals.

The term "*in vivo* imaging" as used herein refers to non-invasive techniques that produce images of all or part of the internal aspect of a subject following administration of an *in vivo* imaging agent.

The "subject" of the invention is preferably a mammal, most preferably an intact mammalian body *in vivo*. In an especially preferred embodiment, the subject is a human, and in particular a human suspected to have or to be predisposed to late-onset depression. The term "predisposed to" refers to a subject's susceptibility to develop a disease state based purely on genetic factors; in common parlance "nature" as opposed to "nurture".

"Late-onset depression" refers to major depressive disorder which first emerges in people aged 60 and over. The term "major depressive disorder" refers to a mood disorder involving any of the following symptoms: persistent sad, anxious, or "empty" mood; feelings of hopelessness or pessimism; feelings of guilt, worthlessness, or helplessness; loss of interest or pleasure in hobbies and activities

that were once enjoyed, including sex, decreased energy, fatigue, being "slowed down", difficulty concentrating, remembering, or making decisions, insomnia, early-morning awakening, or oversleeping, appetite and/or weight loss or overeating and weight gain, thoughts of death or suicide or suicide attempts, restlessness or irritability, or persistent physical symptoms that do not respond to treatment, such as headaches, digestive disorders, and chronic pain.

"Administering" the *in vivo* imaging agent is preferably carried out parenterally, and most preferably intravenously. The intravenous route represents the most efficient way to deliver the *in vivo* imaging agent throughout the body of said subject. The *in vivo* imaging agent of the invention is preferably administered as a pharmaceutical composition which comprises the *in vivo* imaging agent along with a biocompatible carrier. The "biocompatible carrier" is a fluid, especially a liquid, in which the *in vivo* imaging agent is suspended or dissolved, such that the composition is physiologically tolerable, i.e. can be administered to the mammalian body without toxicity or undue discomfort. The biocompatible carrier medium is suitably an injectable carrier liquid such as sterile, pyrogen-free water for injection; an aqueous solution such as saline (which may advantageously be balanced so that the final product for injection is either isotonic or not hypotonic); an aqueous solution of one or more tonicity-adjusting substances (e.g. salts of plasma cations with biocompatible counterions), sugars (e.g. glucose or sucrose), sugar alcohols (e.g. sorbitol or mannitol), glycols (e.g. glycerol), or other non-ionic polyol materials (e.g. polyethyleneglycols, propylene glycols and the like). The biocompatible carrier medium may also comprise biocompatible organic solvents such as ethanol. Such organic solvents are useful to solubilise more lipophilic compounds or formulations. Preferably the biocompatible carrier medium is pyrogen-free water for injection, isotonic saline or an aqueous ethanol solution. The pH of the biocompatible carrier medium for intravenous injection is suitably in the range 4.0 to 10.5.

The "compound" comprised in the *in vivo* imaging agent may be a biomolecule, a small molecule, an aptamer, an antisense mRNA a small interference RNA, or an

antibody. The term "biomolecule" includes molecules such as, e.g., lipids, nucleotides, polynucleotides, amino acids, peptides, polypeptides, proteins, carbohydrates and inorganic molecules. The term "small molecule" refers to an organic compound having a molecular weight of between 100 and 1000 Daltons. The term "antibody" refers to a protein produced by cells of the immune system or to a fragment thereof that binds to an antigen. The term "antisense mRNA" refers an RNA molecule complementary to the strand normally processed into mRNA and translated, or complementary to a region thereof. The term "aptamer" refers to an artificial nucleic acid binder (see, e.g., Ellington and Szostak (1990) Nature 346:818-822). The term "small interference RNA" refers to a double-stranded RNA inducing sequence-specific posttranscriptional gene silencing (see, e.g., Elbashir et al. (2001) Genes Dev. 15:188-200). Preferably, the compound comprised in the *in vivo* imaging agent is a small molecule or a biomolecule, most preferably a small molecule. Particular features of an *in vivo* imaging agent suitable for imaging brain tissue are discussed in more detail below.

The term "selectively associates" refers to binding of the *in vivo* imaging agent to the target of interest, i.e. the polynucleotide or polypeptide encoded by a GABAergic receptor gene in preference to other tissues in order to facilitate discrimination of target tissue from non-target tissue by the method of the invention. Binding of the *in vivo* imaging agent to the target of interest may be determined using binding assays such as those described below in relation to the screening method of the invention.

The term "polynucleotide" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. A particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof e.g., degenerate codon substitutions, alleles, orthologs, single-nucleotide polymorphisms, and complementary sequences as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer et al., Nucleic Acid Res. 19:5081 (1991); Ohtsuka et al., J. Biol. Chem. 260:2605-2608

(1985); Rossolini et al., Mol. Cell. Probes 8:91-98 (1994)). The term "nucleic acid" may be used to refer to a gene, complementary deoxyribonucleic acid (cDNA), and messenger ribonucleic acid (mRNA) encoded by a gene.

The term "polypeptide" refers to a polymer of amino acid residues. The term applies to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers. As used herein, the term encompasses amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds. The term "amino acid" refers to naturally occurring and synthetic amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, e.g., hydroxyproline, y-carboxyglutamate, and O-phosphoserine. Amino acid analogs refers to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an alpha-carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, e.g., homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (e.g., norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acids may be referred to herein by either the commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

Where a polynucleotide is "encoded by" a gene, this means that the gene comprises the information required to (i) obtain complementary strands of deoxyribonucleic acid (DNA) by replication, or (ii) obtain mRNA by transcription. cDNA reverse transcribed from mRNA is also encompassed. Where a polypeptide is "encoded by" a gene, this means that the gene comprises the information required to (i) obtain mRNA by transcription, and (ii) obtain said polypeptide from said mRNA by translation. The terms "replication",

"transcription" and "translation" take their accepted meaning in the field of the invention. That is, replication is the process by which DNA is copied into DNA, transcription is the process by which DNA is copied into mRNA, and translation is when the information in mRNA is used as a template for the synthesis of proteins.

A "GABAergic receptor" is a receptor that binds gamma-aminobutyric acid (GABA). A "GABAergic receptor gene" is therefore a gene encoding part of a GAGAergic receptor. Preferably, said GABAergic receptor gene is a gene encoding: $GABA_A$ receptor subunit α_1 (GABRA1); $GABA_A$ receptor subunit α_4 (GABRA4); $GABA_A$ receptor subunit α_5 (GABRA5); $GABA_A$ receptor subunit β_3 (GABRB3); $GABA_A$ receptor subunit γ_1 (GABRG1), $GABA_A$ receptor subunit γ_2 (GABRG2); $GABA_A$ receptor subunit ρ_1 (GABRR1); $GABA_A$ receptor subunit δ (GABRD); $GABA_B$ receptor 2 (GABBR2); or, glycine receptor β (GLRB).

The term "gene" means a segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons). With only a few exceptions, every cell of the body contains a full set of chromosomes and identical genes. Only a fraction of these genes are turned on, however, and it is the subset that is "expressed" that confers unique properties to each cell type. "Gene expression" is the term used to describe the transcription of the information contained within the DNA, the repository of genetic information, into messenger RNA (mRNA) molecules that are then translated into the proteins that perform most of the critical functions of cells. The kinds and amounts of mRNA produced by a cell are a reflection of which genes are expressed, which in turn provides insights into how the cell responds to its changing needs. Gene expression is a highly complex and tightly regulated process that allows a cell to respond dynamically both to environmental stimuli and to its own changing needs. This mechanism acts as both an on/off switch to control which genes are expressed in a cell as well as a volume control that increases or decreases the level of expression of particular genes as necessary.

The "*in vivo* imaging moiety" is any material that is detectable external to said subject's body following its administration to said subject. The presence of the *in vivo* imaging moiety provides a measure indicative of the amount of polypeptide or polynucleotide in the tissue being imaged. The term "labelled with an *in vivo* imaging moiety" means that the *in vivo* imaging moiety can be a constitutive part of the compound, or may be a separate entity conjugated to the compound. Where the *in vivo* imaging moiety is conjugated to the compound, an optional linker moiety links the vector and the *in vivo* imaging moiety together.

Following the administering step and preceding the detecting step, the *in vivo* imaging agent is allowed to selectively associate with said polynucleotide and/or said polypeptide. For example, when the subject is an intact mammal, the *in vivo* imaging agent will dynamically move through the mammal's body, coming into contact with various tissues therein. Once the *in vivo* imaging agent comes into contact with a tissue expressing said polynucleotide and/or said polypeptide, a specific interaction takes place such that clearance of the *in vivo* imaging agent from said tissue takes longer than from other tissues, thereby enabling an image representative of specifically associated *in vivo* imaging agent to be generated.

The term "tissue" is used to describe a collection of cells associated together to perform a particular biological function. The fundamental types of tissues in subjects of the present invention are epithelial, nerve, connective, muscle, and vascular tissues. A preferred tissue in the context of the *in vivo* imaging method of the present invention is brain tissue.

The step of "detecting" the level of the *in vivo* imaging agent selectively associated with said polynucleotide or a polypeptide in said subject is enabled by the presence of said *in vivo* imaging moiety. A suitable *in vivo* imaging technique is one that can detect signals emitted by said *in vivo* imaging moiety and generate data which is indicative of the location and/or amount of *in vivo* imaging moiety present in said tissue of said subject. For example, SPECT can be used as the detection technique where the *in vivo* imaging moiety emits gamma rays.

Preferred brain tissues in the context of the *in vivo* imaging method of the present invention are located in the anterior cingulate and in the nuclear accumbens. Both of these regions of the brain are implicated in the clinical symptoms of depression and are demonstrated herein to be associated with altered expression of GABAergic genes in late-onset depression. Where the brain tissue being imaged is in the anterior cingulate, a preferred GABAergic receptor gene is a gene encoding GABBR2, GABRG1, or GABRR1, most preferably a gene encoding GABBR2 or GABRR1. Where the brain tissue being imaged is in the nucleus accumbens, a preferred GABAergic receptor gene is a gene encoding GABRA4, GABRB3, or GABRG2, most preferably a gene encoding GABRB3.

Particular requirements apply for an *in vivo* imaging agent to be suitable for imaging brain tissue. In the brain, endothelial cells are packed together more tightly than in the rest of the body by means of "tight junctions", which are multifunctional complexes that form a seal between adjacent epithelial cells, preventing the passage of most dissolved molecules from one side of the epithelial sheet to the other. This so-called blood-brain barrier (BBB) blocks the movement of all molecules except those that cross cell membranes by means of lipid solubility (such as oxygen, carbon dioxide, ethanol, and steroid hormones) and those that are allowed in by specific transport systems (such as sugars and some amino acids). Substances with a molecular weight higher than 500 Daltons generally cannot cross the BBB by passive diffusion, while smaller molecules often can. In addition to tight junctions acting to prevent transport in between endothelial cells, there are two mechanisms to prevent passive diffusion. Glial cells surrounding capillaries in the brain pose a secondary hindrance to hydrophilic molecules, and the low concentration of interstitial proteins in the brain also prevents access by hydrophilic molecules.

Lipid solubility is commonly assessed by measuring the octanol-water partition coefficient (P), typically expressed as a $\log_{10}$ value, referred to herein as "logP". The octanol-water partition coefficient represents the distribution of a substance

between an organic and aqueous phase. The logP provides a simple way of determining the lipophilicity or hydrophilicity of a compound.

The ratio is defined as;

Partition = [compound present in octanol]/[compound present in water]

This equation can be expressed as:

Log Partition = $\log_{10}$ [compound present in octanol]/[compound present in water]

In simple terms the greater the positive number of the logP calculation the greater the lipophilicity of the compound. Calculation of the logP is typically determined for potential new pharmaceutical compounds as it provides an insight into how the compound will be compartmentalised within the body following administration.

The logP of an *in vivo* imaging agent suitable for use in the present invention is in the range 1.0-4.5, preferably in the range 1.0-3.5, and most preferably in the range 2.0-3.5. An estimated logP value (AlogP98) can be obtained prior to evaluation *in vitro* and *in vivo*, e.g. using DS MedChem Explorer software (Accelerys). In addition to being advantageous for CNS penetration, lipophilicity in this range permits rapid clearance for *in vivo* imaging, which is particularly important when the radioactive halogen is a relatively short-lived radioisotope, such as ^{18}F.

The BBB penetration properties of a particular *in vivo* imaging agent may be estimated *in silico* by comparison with literature *in vivo* brain penetration data using Accelerys DS MedChem Explorer software. The "logBbR" is the $\log_{10}$ of [brain concentration]/[blood concentration]. The logBbR for *in vivo* imaging agents used in the method of the present invention is suitably in the range 0.0-1.0, preferably in the range 0.3 to 1.0, most preferably in the range 0.5-0.7.

A preferred *in vivo* imaging moiety for use in the *in vivo* imaging method of the invention is chosen from:

(i) a radioactive metal ion;

(ii) a paramagnetic metal ion;

(iii) a gamma-emitting radioactive halogen;

(iv) a positron-emitting radioactive non-metal; and,

(v) a hyperpolarised NMR-active nucleus.

When the *in vivo* imaging moiety is a radioactive metal ion, i.e. a radiometal, suitable radiometals can be either positron emitters such as ^{64}Cu, ^{48}V, ^{52}Fe, ^{55}Co, ^{94m}Tc or ^{68}Ga; or γ-emitters such as ^{99m}Tc, ^{111}In, ^{113m}In, or ^{67}Ga. Preferred radiometals are ^{99m}Tc, ^{64}Cu, ^{68}Ga and ^{111}In. Most preferred radiometals are γ-emitters, especially ^{99m}Tc.

When the *in vivo* imaging moiety is a paramagnetic metal ion, suitable such metal ions include: Gd(III), Mn(II), Cu(II), Cr(III), Fe(III), Co(II), Er(II), Ni(II), Eu(III) or Dy(III). Preferred paramagnetic metal ions are Gd(III), Mn(II) and Fe(III), with Gd(III) being especially preferred.

When the *in vivo* imaging moiety is a gamma-emitting radioactive halogen, the radiohalogen is suitably chosen from ^{123}I, ^{131}I or ^{77}Br. ^{125}I, while suitable for use as a detectable label in the *in vitro* screening method described herein, is not suitable for use as an *in vivo* imaging moiety. A preferred gamma-emitting radioactive halogen is ^{123}I.

When the *in vivo* imaging moiety is a positron-emitting radioactive non-metal, suitable such positron emitters include: ^{11}C, ^{13}N, ^{15}O, ^{17}F, ^{18}F, ^{75}Br, ^{76}Br or ^{124}I. Preferred positron-emitting radioactive non-metals are ^{11}C, ^{13}N, ^{18}F and ^{124}I, especially ^{11}C and ^{18}F, most especially ^{18}F.

When the *in vivo* imaging moiety is a hyperpolarised NMR-active nucleus, such NMR-active nuclei have a non-zero nuclear spin, and include ^{13}C, ^{15}N, ^{19}F, ^{29}Si and ^{31}P. Of these, ^{13}C is preferred. By the term "hyperpolarised" is meant enhancement of the degree of polarisation of the NMR-active nucleus over its equilibrium polarisation. The natural abundance of ^{13}C (relative to ^{12}C) is about

1%, and suitable ^{13}C-labelled compounds are suitably enriched to an abundance of at least 5%, preferably at least 50%, most preferably at least 90% before being hyperpolarised. At least one carbon atom of the *in vivo* imaging agent of the invention is suitably enriched with ^{13}C, which is subsequently hyperpolarised.

Preferred *in vivo* imaging moieties for the present invention are those which can be detected externally in a non-invasive manner following administration *in vivo*, such as by means of SPECT, PET and MRI. Most preferred *in vivo* imaging moieties for *in vivo* imaging are radioactive, especially radioactive metal ions, gamma-emitting radioactive halogens and positron-emitting radioactive non-metals, particularly those suitable for imaging using SPECT or PET.

Preferred *in vivo* imaging agents of the invention do not undergo facile metabolism *in vivo*, and hence most preferably exhibit a half-life *in vivo* of 60 to 240 minutes in humans. The *in vivo* imaging agent is preferably excreted *via* the kidney (i.e. exhibits urinary excretion). The *in vivo* imaging agent preferably exhibits a signal-to-background ratio at diseased foci of at least 1.5, most preferably at least 5, with at least 10 being especially preferred. Where the *in vivo* imaging agent comprises a radioisotope, clearance of one half of the peak level of *in vivo* imaging agent which is either non-specifically bound or free *in vivo*, preferably occurs over a time period less than or equal to the radioactive decay half-life of the radioisotope of the *in vivo* imaging moiety.

Furthermore, the molecular weight of the *in vivo* imaging agent is suitably up to 5000 Daltons. Preferably, the molecular weight is in the range 100 to 3000 Daltons, most preferably 200 to 1000 Daltons. Furthermore, and as mentioned above, for an *in vivo* imaging agent to be suitable for imaging brain tissue, it is desirable for the *in vivo* imaging agent to have a molecular weight of less than 500 Daltons. An especially preferred molecular weight for the *in vivo* imaging agent is therefore in the range 200-500 Daltons.

Where the *in vivo* imaging agent comprises a polypeptide and an *in vivo* imaging moiety, the *in vivo* imaging moiety is conjugated *via* either the polypeptide's N- or C-terminus, or *via* any of the amino acid side chains. Preferably, the *in vivo*

imaging moiety is conjugated to the polypeptide *via* either the N- or C-terminus, optionally *via* a linker such as a polyethylene glycol linker.

Alternatively, functional group of the *in vivo* imaging agent may comprise the *in vivo* imaging moiety. When a functional group comprises an *in vivo* imaging moiety, this means that the *in vivo* imaging moiety forms part of the chemical structure of the *in vivo* imaging agent. For example, the *in vivo* imaging moiety may be a radioactive isotope present at a level significantly above the natural abundance level of said isotope. Such elevated or enriched levels of isotope are suitably at least 5 times, preferably at least 10 times, most preferably at least 20 times; and ideally either at least 50 times the natural abundance level of the isotope in question, or present at a level where the level of enrichment of the isotope in question is 90 to 100%. Examples of such functional groups include iodophenyl groups with elevated levels of ^{123}I, CH_3 groups with elevated levels of ^{11}C, and fluoroalkyl groups with elevated levels of ^{18}F, such that the imaging moiety is the isotopically labelled ^{11}C or ^{18}F atom within the chemical structure.

A compound that selectively associates with a polynucleotide or a polypeptide encoded by a GABAergic receptor gene may be identified and obtained using the screening method of the invention, which is described in more detail below. Where said screening method is a binding assay, it is desirable that the compound binds to the target of interest with nanomolar potency, i.e. having a dissociation constant (K_d) of between 0.01-100nM, preferably between 0.01-10nM and most preferably between 0.01-1.0nM. Labelling of such a compound to provide an *in vivo* imaging agent may conveniently be carried out by reaction of a precursor compound with a suitable source of the desired *in vivo* imaging moiety. A "precursor compound" comprises an unlabelled derivative of the imaging agent, designed so that chemical reaction with a convenient chemical form of the *in vivo* imaging moiety occurs site-specifically; can be conducted in the minimum number of steps (ideally a single step); and without the need for significant purification (ideally no further purification), to give the desired *in vivo* imaging agent. Such precursor compounds are synthetic and can conveniently

be obtained in good chemical purity. The precursor compound may optionally comprise a protecting group for certain functional groups of the precursor compound.

By the term "protecting group" is meant a group which inhibits or suppresses undesirable chemical reactions, but which is designed to be sufficiently reactive that it may be cleaved from the functional group in question under mild enough conditions that do not modify the rest of the molecule. After deprotection, the desired *in vivo* imaging agent is obtained. Protecting groups are well known to those skilled in the art and are suitably chosen from, for amine groups: Boc (where Boc is *tert*-butyloxycarbonyl), Fmoc (where Fmoc is fluorenylmethoxycarbonyl), trifluoroacetyl, allyloxycarbonyl, Dde [i.e. 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] or Npys (i.e. 3-nitro-2-pyridine sulfenyl); and for carboxyl groups: methyl ester, *tert*-butyl ester or benzyl ester. For hydroxyl groups, suitable protecting groups are: methyl, ethyl or *tert*-butyl; alkoxymethyl or alkoxyethyl; benzyl; acetyl; benzoyl; trityl (Trt) or trialkylsilyl such as tetrabutyldimethylsilyl. For thiol groups, suitable protecting groups are: trityl and 4-methoxybenzyl. The use of further protecting groups are described in 'Protective Groups in Organic Synthesis', Theorodora W. Greene and Peter G. M. Wuts, (Third Edition, John Wiley & Sons, 1999).

When the *in vivo* imaging moiety is a metal ion, such as ^{99m}Tc for SPECT or Gd(III) for MRI, the *in vivo* imaging agent preferably comprises a metal complex of the radioactive metal ion with a synthetic ligand. By the term "metal complex" is meant a coordination complex of the metal ion with one or more ligands. It is strongly preferred that the metal complex is "resistant to transchelation", i.e. does not readily undergo ligand exchange with other potentially competing ligands for the metal coordination sites. Potentially competing ligands include other excipients in the preparation *in vitro* (e.g. radioprotectants or antimicrobial preservatives used in the preparation), or endogenous compounds *in vivo* (e.g. glutathione, transferrin or plasma proteins). The term "synthetic" has its conventional meaning, i.e. man-made as opposed to being isolated from natural

sources e.g. from the mammalian body. Such compounds have the advantage that their manufacture and impurity profile can be fully controlled.

Suitable ligands for use in the present invention which form metal complexes resistant to transchelation include: chelating agents, where 2-6, preferably 2-4, metal donor atoms are arranged such that 5- or 6-membered chelate rings result (by having a non-coordinating backbone of either carbon atoms or non-coordinating heteroatoms linking the metal donor atoms); or monodentate ligands which comprise donor atoms which bind strongly to the metal ion, such as isonitriles, phosphines or diazenides. Examples of donor atom types which bind well to metals as part of chelating agents are: amines, thiols, amides, oximes, and phosphines. Phosphines form such strong metal complexes that even monodentate or bidentate phosphines form suitable metal complexes. The linear geometry of isonitriles and diazenides is such that they do not lend themselves readily to incorporation into chelating agents, and are hence typically used as monodentate ligands. Examples of suitable isonitriles include simple alkyl isonitriles such as *tert*-butylisonitrile, and ether-substituted isonitriles such as MIBI (i.e. 1-isocyano-2-methoxy-2-methylpropane). Examples of suitable phosphines include Tetrofosmin, and monodentate phosphines such as *tris*(3-methoxypropyl)phosphine. Examples of suitable diazenides include the HYNIC series of ligands i.e. hydrazine-substituted pyridines or nicotinamides.

The above described ligands are particularly suitable for complexing technetium e.g. ^{94m}Tc or ^{99m}Tc, and are described more fully by Jurisson *et al* [Chem.Rev., 99, 2205-2218 (1999)]. The ligands are also useful for other metals, such as copper (^{64}Cu or ^{67}Cu), vanadium (e.g. ^{48}V), iron (e.g. ^{52}Fe), or cobalt (e.g. ^{55}Co).

Other suitable ligands are described in Sandoz WO 91/01144, which includes ligands which are particularly suitable for indium, yttrium and gadolinium, especially macrocyclic aminocarboxylate and aminophosphonic acid ligands. Ligands which form non-ionic (i.e. neutral) metal complexes of gadolinium are known and are described in US 4885363. Particularly preferred for gadolinium

are chelates including DTPA, ethylene diamine tetraacetic acid (EDTA), triethylene tetraamine hexaacetic acid (TTHA), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), 10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) and derivatives of these.

Where the *in vivo* imaging moiety is radiohalogen, preferred precursor compounds are those which comprise a derivative which either undergoes electrophilic or nucleophilic radiohalogenation or undergoes condensation with a labelled aldehyde or ketone. Examples of the first category are:

(a) organometallic derivatives such as a trialkylstannane (e.g. trimethylstannyl or tributylstannyl), or a trialkylsilane (e.g. trimethylsilyl) or an organoboron compound (e.g. boronate esters or organotrifluoroborates);

(b) a non-radioactive alkyl bromide for halogen exchange or alkyl tosylate, mesylate or triflate for nucleophilic iodination;

(c) aromatic rings activated towards nucleophilic iodination (e.g. aryl iodonium salt aryl diazonium, aryl trialkylammonium salts or nitroaryl derivatives).

The precursor preferably comprises: a non-radioactive halogen atom such as an aryl iodide or bromide (to permit radiohalogen exchange); an organometallic precursor compound (e.g. trialkyltin, trialkylsilyl or organoboron compound); or an organic precursor such as triazenes or a good leaving group for nucleophilic substitution such as an iodonium salt. Preferably for radioiodination, the precursor comprises an organometallic precursor compound, most preferably trialkyltin.

Precursors and methods of introducing radioiodine into organic molecules are described by Bolton [J.Lab.Comp.Radiopharm., 45, 485-528 (2002)]. Suitable boronate ester organoboron compounds and their preparation are described by Kabalka *et al* [Nucl.Med.Biol., 29, 841-843 (2002) and 30, 369-373(2003)]. Suitable organotrifluoroborates and their preparation are described by Kabalka *et al* [Nucl.Med.Biol., 31, 935-938 (2004)].

Radiofluorination may be carried out *via* direct labelling using the reaction of ^{18}F-fluoride with a suitable chemical group in the precursor having a good leaving group, such as an alkyl bromide, alkyl mesylate or alkyl tosylate. ^{18}F can also be introduced by alkylation of N-haloacetyl groups with a $^{18}F(CH_2)_3OH$ reactant, to give $-NH(CO)CH_2O(CH_2)_3{}^{18}F$ derivatives. For aryl systems, ^{18}F-fluoride nucleophilic displacement from an aryl diazonium salt, aryl nitro compound or an aryl quaternary ammonium salt are suitable routes to aryl-^{18}F derivatives.

A ^{18}F-labelled *in vivo* imaging agent may be obtained by formation of ^{18}F fluorodialkylamines and subsequent amide formation when the ^{18}F fluorodialkylamine is reacted with a precursor containing, e.g. chlorine, $P(O)Ph_3$ or an activated ester. Further approaches for radiofluorination, particularly suitable for radiofluorination of peptides, are described in WO 03/080544, which uses thiol coupling, and in WO 04/080492, which makes use of aminoxy coupling. Further details of synthetic routes to ^{18}F-labelled derivatives are described by Bolton, J.Lab.Comp.Radiopharm., 45, 485-528 (2002).

The *in vivo* imaging agent of the method of the invention may be easily obtained by means of a kit. Such kits comprise a suitable precursor compound, preferably in sterile non-pyrogenic form, so that reaction with a sterile source of an *in vivo* imaging moiety gives the desired *in vivo* imaging agent with the minimum number of manipulations. Such considerations are particularly important in the case of radioactive *in vivo* imaging agents, in particular where the radioisotope has a relatively short half-life, for ease of handling and hence reduced radiation dose for the radiopharmacist.

The reaction medium for reconstitution of such kits is preferably a biocompatible carrier, as defined previously herein, such that a pharmaceutical composition comprising said *in vivo* imaging agent is obtained.

In the *in vivo* imaging method of the invention, the detecting step is followed by a step of generating an image representative of the signals emitted by the *in vivo* imaging moiety. This generating step of the method of the invention is carried out

by a computer which applies a reconstruction algorithm to the acquired signal data to yield a dataset. This dataset is then manipulated to generate images showing areas of interest within the subject. These images provide information that is useful in a method for the diagnosis of late-onset depression.

METHOD OF DIAGNOSIS

In another aspect, the present invention provides an *in vivo* imaging agent as defined above in relation to the *in vivo* imaging method for use in a method for the diagnosis of late-onset depression.

Furthermore, the present invention provides a method for the diagnosis of late-onset depression comprising:

(a) the *in vivo* imaging method as defined above; and,

(b) comparing the image generated in step (a) with an *in vivo* image representative of the pattern of uptake of said *in vivo* imaging agent when said *in vivo* imaging method is carried out in non-depressed subjects.

The suitable and preferred embodiments of the tissue and subject of the method of diagnosis are as defined for the method of *in vivo* imaging above.

Variation of levels of a polypeptide or polynucleotide described herein from the image representative of a non-depressed subject (either up or down) indicates that the subject has late-onset depression or is at risk of developing at least some aspects of late-onset depression.

The image representative of uptake of said *in vivo* imaging agent when said *in vivo* imaging method is carried out in non-depressed subjects is obtained by carrying out the *in vivo* imaging method as defined above on a suitably-matched cohort of non-depressed subjects, and producing an image which represents an average of all the images obtained.

METHOD FOR TREATMENT

Compounds that modulate the activity of a GABAergic receptor can be administered to a subject for the treatment of late-onset depression. The present invention therefore provides a method for treatment of a subject suffering from late-onset depression, said method comprising administration of a pharmaceutical composition, said pharmaceutical composition comprising:

(a) a pharmaceutically effective amount of a compound that modulates the activity of a GABAergic receptor by selectively associating with a polynucleotide or polypeptide, said polynucleotide or polypeptide being encoded by a GABAergic receptor gene, said GABAergic receptor gene being a preferred GABAergic receptor gene as defined above in relation to the *in vivo* imaging method of the invention; and,

(b) a biocompatible carrier.

The biocompatible carrier is broadly as defined earlier in the specification. The particular biocompatible carrier selected is determined in part by the particular pharmaceutical composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions (see, e.g. *Remington 's Pharmaceutical Sciences*, 17th ed. 1985)). Formulations suitable for administration include aqueous and non-aqueous solutions, isotonic sterile solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

Administration for treatment is by any of the routes normally used for introducing a pharmaceutical compound into contact with the tissue to be treated and is well known to those of skill in the art. Although more than one route can be used to administer a particular composition, a particular route can often provide a more

immediate and more effective reaction than another route. In the practice of this invention, the pharmaceutical composition can be administered, for example, orally, nasally, topically, intravenously, intraperitoneally, or intrathecally. The pharmaceutical composition can be presented in unit-dose or multi-dose sealed containers, such as ampoules and vials. Solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. The pharmaceutical composition can also be administered as part of a prepared food or drug. A "pharmaceutically effective amount" of a compound is a dose sufficient to affect a beneficial response in the subject over time. The optimal dose level for any patient will depend on a variety of factors including the efficacy of the specific modulator employed, the age, body weight, physical activity, and diet of the patient, on a possible combination with other drugs, and on the severity of the mental disorder. The size of the dose also will be determined by the existence, nature, and extent of any adverse side effects that accompany the administration of a particular pharmaceutical composition to a particular subject.

In determining the effective amount of the pharmaceutical composition to be administered, a physician may evaluate circulating plasma levels of the pharmaceutical composition, pharmaceutical composition toxicity, and the production of anti-pharmaceutical composition antibodies. In general, the dose equivalent of a compound is from about 1 ng/kg to 10 mg/kg for a typical subject. For administration, the pharmaceutical composition can be administered at a rate determined by the LD-50 of the compound, and the side effects of the compound at various concentrations, as applied to the mass and overall health of the subject.

The term "modulates the activity of a GABAergic receptor" means that the compound has an effect on said GABAergic receptor that acts to bring the activity of said receptor closer to that seen in non-depressed subjects.

Furthermore, the *in vivo* imaging agent as defined above in relation to the *in vivo*

imaging method of the invention can be applied for use in a method to decide whether to implement the method for treatment as defined above, said method to decide comprising:

(a) the *in vivo* imaging method as defined herein; and,

(b) evaluating the image generated by the *in vivo* imaging method of step (a) to decide whether to implement said method for treatment.

SCREENING METHOD

In another aspect, the present invention provides a screening method to identify a compound that selectively associates with a polynucleotide or a polypeptide, said method comprising:

(i) contacting said compound with a polypeptide or a polynucleotide, said polynucleotide or polypeptide being encoded by a GABAergic receptor gene; and,

(ii) determining the effect of said compound upon said polypeptide or said polynucleotide;

wherein said GABAergic receptor gene is a gene as defined above for the *in vivo* imaging method of the invention, preferably a gene encoding a GABAergic receptor selected from: $GABA_A$ receptor subunit α_1 (GABRA1); $GABA_A$ receptor subunit α_4 (GABRA4); $GABA_A$ receptor subunit α_5 (GABRA5); $GABA_A$ receptor subunit β_3 (GABRB3); $GABA_A$ receptor subunit γ_1 (GABRG1), $GABA_A$ receptor subunit γ_2 (GABRG2); $GABA_A$ receptor subunit ρ_1 (GABRR1); $GABA_A$ receptor subunit δ (GABRD); $GABA_B$ receptor 2 (GABBR2); or, glycine receptor β (GLRB).

A "compound" useful in the treatment, prevention or diagnosis of late-onset depression may be a biomolecule, a small molecule, an aptamer, an antisense mRNA a small interference RNA, or an antibody. The terms "biomolecule", "small molecule", "antibody", "antisense mRNA", "aptamer", " small interference RNA" take

the meanings provided earlier in the specification.

In its broadest sense, the step of "contacting" said compound with a polypeptide or a polynucleotide means bringing said compound and said polypeptide or polynucleotide into physical contact with each other. This may be accomplished either *in vitro* or *in vivo*, as described in further detail below.

The "effect of the compound" is any specific interaction between the compound and the polynucleotide or the polypeptide. Such specific interaction encompasses specific binding of the compound with the polynucleotide or the polypeptide, and includes any modulation of the level of expression or activity of the polynucleotide or polypeptide induced by the compound.

The step of "determining" the effect of the compound can be carried out by methods well-known in the art. An example of such a well-known screening method is one where the effect determined in the determining step is binding of said compound to said polypeptide or polynucleotide. Such binding assays preferably involve contacting an isolated polypeptide or polynucleotide described herein with one or more compounds and allowing sufficient time for the polypeptide or polynucleotide and compound to form a binding complex. The term "isolated" means separated from other cell components, and may also include synthetic polynucleotides and polypeptides. Any binding complexes formed can be detected using any of a number of established analytical techniques. Protein binding assays include, but are not limited to, methods that measure co-precipitation, co-migration on non-denaturing SDS-polyacrylamide gels, and co-migration on Western blots (see, e.g. Bennet and Yamamura, (1985) "Neurotransmitter, Hormone or Drug Receptor Binding Methods" in *Neurotransmitter Receptor Binding* (Yamamura, H. I., et al., eds., pp. 61-89). The protein utilized in such assays can be naturally expressed, cloned or synthesized. Binding assays are also useful, e.g., for identifying endogenous proteins that interact with a polypeptide. For example, antibodies, receptors or other molecules that bind a polypeptide can be identified in binding assays. In

many cases, at least one of the reactants in the binding assay comprises a detectable label. The term "reactants" in this context including the compound, the polypeptide, the polynucleotide, or any antibodies used to specifically detect them. The "detectable label" can be any material having a detectable physical or chemical property. The presence of detectable label therefore provides a measure indicative of the amount of bound reactant. Depending on the particular detectable label used, a suitable detection technique is used to measure the amount of selectively-bound detectable label. Detectable labels suitable for use in the screening method of the invention include those detectable *in vitro* by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means including:

(i) magnetic beads (e.g., Dynabeads^{TM});

(ii) fluorescent dyes (e. g., fluorescein isothiocyanate, Texas red, rhodamine, and the like);

(iii) radiolabels (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, or ^{32}P);

(iv) enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA); and,

(v) calorimetric labels such as colloidal gold or coloured glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads.

Means of detecting these detectable labels are well known to those of skill in the art. Thus, for example, where the detectable label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the detectable label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence may be detected visually, by means of photographic film, by the use of electronic detectors such as charge-coupled devices (CCDs) or photomultipliers and the like. Similarly,

enzymatic detectable labels may be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product.

The screening method of the present invention may also preferably be carried out *in vitro* wherein said polypeptide or polynucleotide is expressed in a cell and the cell is contacted with the compound. Such methods generally involve conducting cell-based assays in which compounds are contacted with one or more cells expressing a polypeptide or polynucleotide described herein, and then detecting an increase or decrease in expression of transcript, translation product, or catalytic product. The expression level of a polynucleotide described herein in a cell can be determined by measuring the mRNA expressed in a cell with a compound that specifically hybridizes with a transcript (or complementary nucleic acid) of said polynucleotide. Measurement can be conducted by lysing the cells and conducting Northern blots or without lysing the cells using *in situ* hybridization techniques.

A polypeptide can be detected using immunological methods in which a cell lysate is probed with compounds that are antibodies which specifically bind to said polypeptide.

Catalytic activity of polypeptides can be determined by measuring the production of enzymatic products or by measuring the consumption of substrates. Activity refers to either the rate of catalysis or the ability to the polypeptide to bind (K_m) the substrate or release the catalytic product (K_d).

Analysis of the activity of polypeptides can be performed according to general biochemical analyses. Such assays include cell-based assays as well as *in vitro* assays involving purified or partially purified polypeptides or crude cell lysates. The assays generally involve providing a known quantity of substrate and quantifying product as a function of time.

The screening method can also be carried out wherein said contacting step comprises administration of said compound to an animal model of late-onset

depression. The animal models utilized generally are mammals of any kind. Specific examples of preferred animals include, but are not limited to, primates, mice, and rats. In one embodiment, rat models of depression (both chronic and acute), in which the rats are subjected to stress, are used for screening. In one embodiment, invertebrate models such as Drosophila models can be used, screening for modulators of Drosophila orthologs of the human genes disclosed herein. In another embodiment, transgenic animal technology including gene knockout technology, for example as a result of homologous recombination with an appropriate gene targeting vector, or gene overexpression, will result in the absence, decreased or increased expression of a polynucleotide or polypeptide. Transgenic animals generated by such methods find use as animal models of mental disorders and are useful in screening for modulators of mental disorders.

Knockout cells and transgenic mice can be made by insertion of a marker gene or other heterologous gene into an endogenous gene site in the mouse genome *via* homologous recombination. Such mice can also be made by substituting an endogenous polynucleotide with a mutated version of the polynucleotide, or by mutating an endogenous polynucleotide, e.g., by exposure to carcinogens.

In a preferred embodiment, the screening method as described in any of the above embodiments concerns contacting said compound with said polypeptide and determining the effect of said compound on said polypeptide.

METHODS USED IN THE PRESENT INVENTION

Studies are described herein that investigate the expression patterns of genes that are differentially expressed specifically in brain tissue of subjects with late-onset depression. The large spectrum of symptoms associated with depression reflects the complex genetic basis and complex gene expression patterns in these subjects. Furthermore, brain pathways or circuits as well as subcellular pathways are important for understanding the development and diagnosis of mental disorders. The selected brain regions evaluated (anterior cingulate (AC) and nucleus accumbens (NA)) are implicated in the clinical symptoms of depression.

Cytoarchitectual changes in brain regions, expression of key neurotransmitters or related molecules in brain regions, and subcellular pathways in brain regions all contribute to the development of depression.

The data on which the present invention is based was obtained by microarray expression analysis. The arrays used in this kind of analysis are called "expression chips". The immobilized DNA is cDNA reverse transcribed from the mRNA of known genes, and once again, at least in some experiments, the control and sample DNA hybridized to the chip is cDNA reverse transcribed from the mRNA of normal and diseased tissue, respectively. If a gene is overexpressed in a certain disease state, then more sample cDNA, as compared to control cDNA, will hybridize to the spot representing that expressed gene. In turn, the spot will fluoresce red with greater intensity than it will fluoresce green. Once researchers have characterized the expression patterns of various genes involved in many diseases, cDNA derived from diseased tissue from any individual can be hybridized to determine whether the expression pattern of the gene from the individual matches the expression pattern of a known disease. If this is the case, treatment appropriate for that disease can be initiated.

A useful review of microarray methodology can be found in Nature Genetics January 1999 supplement. Of most relevance for the present invention is the article by Botwell on pages 25-32, which describes how to obtain expression data using microarray technology. An overview of the technology is now provided.

DNA "microarrays" are small, solid supports onto which the sequences from thousands of different genes are immobilized, or attached, at fixed locations. The supports themselves are usually glass microscope slides, but can also be silicon chips or nylon membranes. The DNA is printed, spotted, or actually synthesized directly onto the support. It is important that the gene sequences in a microarray are attached to their support in an orderly or fixed way, because the location of each spot in the array is used to identify a particular gene sequence. The spots themselves can be DNA, cDNA, or oligonucleotides. Microarrays can be prepared

by the researcher or sourced commercially, depending on issues of e.g. cost, timing, and human resource available. Genechip® arrays are commercially available arrays that are manufactured using technology that combines photolithography and combinatorial chemistry (www.affymetrix.com). Up to 1.3 million different oligonucleotide probes are synthesized on each array. Each oligonucleotide is located in a specific area on the array called a probe cell. Each probe cell contains hundreds of thousands to millions of copies of a given oligonucleotide.

To obtain cDNA from mRNA, reverse transcription is used, a process in which a DNA polymerase enzyme, known as a reverse transcriptase, transcribes single-stranded ribonucleic acid (RNA) into double-stranded DNA. Typically, a poly-T (thymidine) primer is used as the primer in this reaction as mRNA has a poly-A (adenosine) tail.

The quality of the input mRNA is crucial in order to obtain cDNA suitable for microarray analysis. RNA integrity in post-mortem sampling can be influenced by pre-mortem and post-mortem events, as well as by interrelations between expression level and confounding factors such as donor age of death, pre-mortem hypoxia, agonal events and duration of agonal stage, brain pH, post-mortem interval before sampling, and RNA integrity (Stan et al 2006; Brain Research; 1123(1): 1-11). It is therefore important to screen RNA samples to select those that will be suitable for microarray analysis. A simple pH measurement can be done on the sample as an indication of agonal state, prior to evaluation of the quality of the RNA. Thereafter, various methods can be used to measure RNA quality (Copois et al 2007 J Biotechnol; 127(4): 549-59), one of which is the RNA integrity number (RIN, Agilent Technologies). Using electrophoretic separation on microfabricated chips, RNA samples are separated and subsequently detected via laser induced fluorescence detection. The bioanalyzer software generates an electropherogram and gel-like image and displays results such as sample concentration and the so-called ribosomal ratio (the 18S to 28S ribosomal band ratio). Standardized interpretation of the RNA integrity data is carried out using the RIN software algorithm, which allows for the classification of riboeukaryotic total RNA, based on a

numbering system from 1 to 10, with 1 being the most degraded profile and 10 being the most intact.

DNA microarray technology facilitates the identification and classification of DNA sequence information and the assignment of functions to these new genes. A microarray works by exploiting the ability of a given mRNA molecule to bind specifically to, or hybridize to, the DNA template from which it originated. The term "hybridize" refers to the process of combining, or annealing, complementary single-stranded nucleic acids into a single double-stranded molecule. By using an array containing many DNA samples it is possible to determine, in a single experiment, the expression levels of hundreds or thousands of genes within a cell by measuring the amount of mRNA bound to each site on the array. With the aid of a computer, the amount of mRNA bound to the spots on the microarray is precisely measured, generating a profile of gene expression in the cell.

An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5-10° C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength pH. The Tm is the temperature (under defined ionic strength, pH, and nucleic concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at Tm, 50% of the probes are occupied at equilibrium). "Stringent" conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other 10 salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60° C for long probes (e.g., greater than 50 nucleotides).

Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at

least two times background, optionally 10 times background hybridization. Exemplary stringent hybridization conditions can be as following: 50% formamide, 5X SSC, and 1% SDS, incubating at 42°C, or 5X SSC, 1% SDS, incubating at 65°C, with wash in 0.2X SSC, and 0.1% SDS at 65°C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes.

Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides that they encode are substantially identical. This occurs, for example, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. In such cases, the nucleic acids typically hybridize under moderately stringent hybridization conditions. Exemplary "moderately stringent hybridization conditions" include a hybridization in a buffer of 40% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 1X SSC at 45°C. Such washes can be performed for 5, 15, 30, 60, 120, or more minutes. A positive hybridization is at least twice background. Those of ordinary skill will readily recognize that alternative hybridization and wash conditions can be utilized to provide conditions of similar stringency.

After the hybridization step is complete, the microarray is placed in a "reader" or "scanner" that consists of some lasers, a special microscope, and a camera. Examples include the Packard BioChip Imager, Molecular Dynamics Avalanche and Genetic Microsystems GMS 418 Array Scanner. The fluorescent tags are excited by the laser, and the microscope and camera work together to create a digital image of the array. A typical microarray experiment generates thousands of data points, which means that sophisticated techniques for storing and processing data are required. The tools that are used may comprise software to perform image analysis of data from readers, databases to store and link information, and software that links data from individual clones to web databases, such as GenBank. The GenBank sequence database is an open access, annotated collection of all publicly available nucleotide sequences and their protein translations (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide). This database is produced at National Center for Biotechnology Information (NCBI) as part of the

International Nucleotide Sequence Database Collaboration, or INSDC.

Microarrays were presently used to analyse the mRNA expression profile of samples taken from the post-mortem brains of subjects having late-onset depression. The anterior cingulate and nuclear accumbens were selected for analysis as these areas of the brain are known to be associated with the pathophysiology of depression. Altered expression of the genes related to the GABAergic system (Tables 2 and 4 in the Examples below) has been shown at the mRNA level in selected brain regions of patients diagnosed with depression in comparison with normal individuals. The specific protocols used to obtain the data on which the present invention is based are now described in detail.

Brief Description of the Examples

Example 1 describes the methods employed in carrying out transcriptomics analysis on anterior cingulate samples from depressed and non-depressed subjects.

Example 2 describes the methods employed in carrying out transcriptomics analysis on nuclear accumbens samples from depressed and non-depressed subjects.

Examples

Example 1: Anterior Cingulate Transcriptomics

Example 1(i) Sample Selection

Frozen brain tissue (stored at -80^{0}C) was obtained from the frontal cortex of 10 subjects with late-onset depression, and matched for age, sex, post mortem interval and agonal state with 10 psychiatrically healthy control subjects. All subjects died suddenly, with a mean duration of agonal state of about 7 hours (Li et al *Hum Mol Genet* 13, 609-616 (2004)). These numbers were selected as being sufficient to detect group differences at conventional significance levels on

the microarray of >±2SD, or using DIGE based proteomic analysis. Depressed subjects had all had DSM-IV major depression and case note review confirmed this and they had no other mental illness. Case notes for controls were screened to ensure they had not had any psychiatric disorder. All subjects received a postmortem and neuropathological examination and none had changes consistent with dementia.

The tissue was screened for its suitability for use by assessing pH as a marker of agonal state. Samples were obtained from storage at -80°C, subdissected, thawed and a 10% homogenate (e.g. 1 gram tissue plus 9ml water) prepared by homogenising for 10 seconds at full speed with a UltraTurrax homogeniser. Sample pH was determined using a silver chloride electrode calibrated with aqueous standards.

Example 1(ii) Microarray Analysis

Following evaluation, a restricted set of samples was available for analysis (see Table 1 below). From this sample set, subgenual anterior cingulate cortex (see Figure 1) was selected and microdissected at -20°C. These samples were stored frozen at -80°C and subsequently processed for RNA analysis. For preparation of RNA, samples were thawed and a 10% homogenate prepared by homogenising for 10 seconds at full speed with a UltraTurrax homogeniser. Sample pH was determined and RNA was extracted using guanidine based extraction (TriZOL) and post isolation purification on columns (RNEasy, Qiagen) to remove low molecular weight RNA. RNA quality was determined on the basis of clear 18/26S ribosomal bands using an Agilent Bioanalyzer.

Table 1: Anterior Cingulate RNA Samples Extracted for Microarray Analysis

Sample Number	RIN*	Microarray
AC1	6.5	Yes
AC2	6.2	Yes
AC3	6.7	Yes
AC4	5.5	No
AC5	4.6	No

AC6	6.3	Yes
AC7	4.1	No
AC8	6.2	Yes
AC9	4.8	No
AC10	5.0	Yes
AC11	6.5	Yes
AC12	4.5	No
AC13	7.9	Yes
AC14	6.9	Yes
AC15	7.2	Yes
AC16	7.3	Yes
AC17	5.6	Yes
AC18	7.3	Yes
AC19	5.3	Yes
AC20	6.7	Yes
AC21	5.9	Yes
AC22	6.5	Yes
AC23	7.5	Yes
AC24	6.8	Yes
AC25	7.4	Yes
AC26	6.4	Yes
AC27	6.2	Yes
AC28	5.8	Yes
AC29	2.6	No
AC30	3.1	No
AC31	6.8	Yes
AC32	6.8	Yes

*RIN = RNA Integrity Number

Samples were subjected to a primary analysis using an Agilent 2100 bioanalyser to provide the RNA integrity number (RIN) to estimate the integrity of total RNA samples. The Agilent 2100 software automatically assigned an integrity number to a eukaryote total RNA sample based on the electrophoretic trace of the sample to indicate the presence or absence of degradation products. On the basis of this primary analysis (see Table 1) samples were taken through two rounds of amplification before placing on Affymetrix Plus 2.0 microarrays.

The initial data screen indicated that 4 cases (samples AC17, AC19, and AC21) were possibly not suitable for further analysis due to low 3'/5' ratios and below average % present calls. Statistical analysis was undertaken to determine if any

samples were outliers. Using a Spearmann Rank Correlation approach, three samples provided gene chip results that were outside the main grouping and therefore potential confounders (samples AC17, AC19 and AC21) along with one case which may also be outside the main group (sample AC26). Removal of the outlying samples demonstrated that samples AC17, AC19 and AC21 are possible outliers but that sample AC26 may potentially be retained.

Using both the total dataset and the dataset with samples AC17, AC19 and AC21 filtered out, statistical analysis was undertaken using a stringent and less stringent approach with a maximum of 664 significantly changed genes being identified. Table 2 lists GABAergic receptor genes that were found to be significantly altered in late-onset depression.

Table 2: GABAergic genes transcribed in anterior cingulated samples significantly different in subjects with late-onset depression compared with non-depressed subjects

Fold Change	Gene Name	Map	Description	DEP t-test P-value
1.8	GABRA1	5q34-q35	gamma-aminobutyric acid (GABA) A receptor, alpha 1	0.1204
2.1	GABRA5	15q11.2-q12	gamma-aminobutyric acid (GABA) A receptor, alpha 5	0.0
1.6	GABRB3	15q11.2-q12	gamma-aminobutyric acid (GABA) A receptor, beta 3	0.1029
0.54	GABRG1	4p12	gamma-aminobutyric acid (GABA) A receptor, gamma 1	0.082
1.6	GABRG2	5q31.1-q33.1	gamma-aminobutyric acid (GABA) A receptor, gamma 2	0.7756
2.44	GABRR1	6q14-q21|6q13-q16.3	gamma-aminobutyric acid (GABA) A receptor, rho 1	0.094
2.74	GABBR2	9q22.1-q22.3	gamma-aminobutyric acid (GABA) B receptor, 2	0.005
1.7	GLRB	4q31.3	glycine receptor, beta	0.0062

Example 2: Nuclear Accumbens Transcriptomics

Example 2(i) Sample Selection

Post mortem brain tissue from individuals with late onset depression and individuals without a known neuropsychiatric history has been screened for its suitability for use by assessing pH as a marker of agonal state. Samples from frontal cortex (BA 45) were obtained from storage at -80°C, subdissected, thawed and a 10% homogenate (1 gram tissue plus 9ml water) prepared by homogenising for 10 seconds at full speed with a UltraTurrax homogeniser.

Sample pH was determined using a silver chloride electrode calibrated with aqueous standards.

Example 2(ii) Microarray Analysis

Following sample selection, a restricted set of samples was available for analysis (see Table 3, below). From this sample set, nucleus accumbens (see Figure 2) was selected and microdissected at -20°C. These samples were stored frozen at -80°C and subsequently processed for RNA isolation and microarray analysis.

Table 3: Nucleus Accumbens RNA Samples Extracted for Microarray Analysis

Sample Number	RIN*	Microarray
NA1	8.5	Yes
NA2	N/A	No
NA3	6.6	Yes
NA4	6.1	Yes
NA5	8.7	Yes
NA6	8.3	Yes
NA7	7.1	Yes
NA8	8.1	Yes
NA9	7.4	Yes
NA10	N/A	No
NA11	6.9	Yes
NA12	7.8	Yes
NA13	7.9	Yes
NA14	7.3	Yes
NA15	N/A	Yes
NA16	7.6	Yes
NA17	7.7	Yes
NA18	N/A	No
NA19	6.7	Yes
NA20	8.0	Yes
NA21	6.9	Yes
NA22**	7.0	Yes
NA23	6.1	Yes
NA24	7.6	Yes
NA25	5.9	No
NA26	7.8	Yes
NA27	7.3	Yes
NA28	8.2	Yes

NA29	7.4	Yes
NA30	6.5	Yes
NA31	6.2	Yes

*RIN = RNA Integrity Number

** Putamen sample used for comparative purposes for previous anterior cingulate experiment

Samples were subjected to a primary analysis using an Agilent 2100 bioanalyser and on the basis of this (see Table 3) samples NA2, NA10, NA18 and NA25 were not processed for further analysis. Other samples were taken through two rounds of amplification before placing on Affymetrix Plus 2.0 microarrays.

From the results of cluster analysis, samples NA20 and NA26 have noticeably different expression patterns to the other samples. These samples also group together in the results of principal component analysis (PCA) analysis. Samples NA20 and NA26 were therefore removed, and the data was re-analysed by hierarchical clustering and PCA.

Using both the dataset with samples NA20 and NA26 filtered out, statistical analysis was undertaken using a stringent and less stringent approach with 121 genes significantly differently expressed in depressed subjects compared with non-depressed subjects. Table 4 lists GABAergic receptor genes that were found to be most significantly altered in late-onset depression.

Table 4: GABAergic genes transcribed in nucleus accumbens samples significantly different in subjects with late-onset depression compared with non-depressed subjects

Fold Change	Gene Name	Map	Description	DEP t-test P-value
1.9	GABRA1	5q34-q35	gamma-aminobutyric acid (GABA) A receptor, alpha 1	0.0003
0.70	GABRA4	4p12	gamma-aminobutyric acid (GABA) A receptor, alpha 4	0.106
2.0	GABRA5	15q11.2-q12	gamma-aminobutyric acid (GABA) A receptor, alpha	0.0066

			5	
1.6	GABRB3	15q11.2-q12	gamma-aminobutyric acid (GABA) A receptor, beta 3	0.014
1.6	GABRG2	5q31.1-q33.1	gamma-aminobutyric acid (GABA) A receptor, gamma 2	0.0462
2.3	GABRD	1p|1p36.3	gamma-aminobutyric acid (GABA) A receptor, delta	0.0011
1.8	GABBR2	9q22.1-q22.3	gamma-aminobutyric acid (GABA) B receptor, 2	0
1.7	GLRB	4q31.3	glycine receptor, beta	0.0001

Claims

1) An *in vivo* imaging method for use in the determination of whether a subject has or is predisposed to late-onset depression, said method comprising the steps of:

   (i) administering an *in vivo* imaging agent to said subject, wherein said *in vivo* imaging agent comprises a compound that selectively associates with a polynucleotide or polypeptide, said polynucleotide or polypeptide being encoded by a GABAergic receptor gene, and wherein said compound is labelled with an *in vivo* imaging moiety;

   (ii) allowing said *in vivo* imaging agent to selectively associate with said polynucleotide and/or said polypeptide expressed in a tissue of said subject;

   (iii) detecting by an *in vivo* imaging method signals emitted by said *in vivo* imaging moiety; and,

   (iv) generating an image representative of the location and/or amount of said signals.

2) The *in vivo* imaging method as defined in Claim 1 wherein said GABAergic receptor gene is a gene encoding: $GABA_A$ receptor subunit α_1 (GABRA1); $GABA_A$ receptor subunit α_4 (GABRA4); $GABA_A$ receptor subunit α_5 (GABRA5); $GABA_A$ receptor subunit β_3 (GABRB3); $GABA_A$ receptor subunit γ_1 (GABRG1), $GABA_A$ receptor subunit γ_2 (GABRG2); $GABA_A$ receptor subunit ρ_1 (GABRR1); $GABA_A$ receptor subunit δ (GABRD); $GABA_B$ receptor 2 (GABBR2); or, glycine receptor β (GLRB).

3) The *in vivo* imaging method as defined in either Claim 1 or Claim 2 wherein said subject is an intact mammalian body *in vivo*.

4) The *in vivo* imaging method as defined in any one of Claims 1-3 wherein said tissue is brain tissue.

5) The *in vivo* imaging method as defined in Claim 4 wherein said brain tissue is in the anterior cingulate.

6) The *in vivo* imaging method as defined in Claim 5 wherein said GABAergic receptor gene is a gene encoding GABBR2, GABRG1, or GABRR1.

7) The *in vivo* imaging agent as defined in Claim 6 wherein said GABAergic receptor gene is a gene encoding GABBR2 or GABRR1.

8) The *in vivo* imaging method as defined in Claim 4 wherein said brain tissue is in the nucleus accumbens.

9) The *in vivo* imaging method as defined in Claim 8 wherein said GABAergic receptor gene is a gene encoding GABRA4, GABRB3, or GABRG2.

10) The *in vivo* imaging method as defined in Claim 9 wherein said GABAergic receptor gene is a gene encoding GABRB3.

11) The *in vivo* imaging method as defined in any one of Claims 1-10 wherein said *in vivo* imaging moiety is chosen from:

(i) a radioactive metal ion;

(ii) a paramagnetic metal ion;

(iii) a gamma-emitting radioactive halogen;

(iv) a positron-emitting radioactive non-metal; and,

(v) a hyperpolarised NMR-active nucleus.

12) The *in vivo* imaging method as defined in Claim 11 wherein said *in vivo* imaging moiety is one of (i), (iii) or (iv).

13) A method for the diagnosis of late-onset depression comprising:

(a) the *in vivo* imaging method as defined in any one of Claims 1-12; and,

(b) comparing the image generated in step (a) with an *in vivo* image representative of the pattern of uptake of said *in vivo* imaging agent when said *in vivo* imaging method is carried out in non-depressed subjects.

14)A method for treatment of a subject suffering from late-onset depression, said method comprising administration of a pharmaceutical composition, said pharmaceutical composition comprising:

(a) a pharmaceutically effective amount of a compound that modulates the activity of a GABAergic receptor by selectively associating with a polynucleotide or polypeptide, said polynucleotide or polypeptide being encoded by a GABAergic receptor gene, said GABAergic receptor gene GABAergic as defined in the method of Claim 2; and,

(b) a biocompatible carrier.

15)An *in vivo* imaging agent as defined in the method of any one of Claims 1, 2, 11 or 12 for use in the method of diagnosis as defined in Claim 13.

16)An *in vivo* imaging agent as defined in the method of any one of Claims 1, 2, 11 or 12 for use in a method to decide whether to implement the method for treatment as defined in Claim 14, said method to decide comprising:

(a) the *in vivo* imaging method as defined in any one of Claims 1-12; and,

(b) evaluating the image generated by the *in vivo* imaging method of step (a) to decide whether to implement said method for treatment.

17)A screening method to identify a compound that selectively associates with a polynucleotide or a polypeptide, said method comprising:

(i) contacting said compound with a polypeptide or a polynucleotide, said polynucleotide or polypeptide as defined in the method of any one of Claims 1, 2, 6, 7, 9 or 10; and,

(ii) determining the effect of said compound upon said polypeptide or said polynucleotide.

18) The screening method as defined in Claim 17 wherein said effect is binding of said compound to said polypeptide or said polynucleotide.

19) The screening method as defined in either Claim 17 or Claim 18 wherein said polypeptide or polynucleotide is isolated.

20) The screening method as defined in Claim 17 or Claim 18 wherein said polypeptide or polynucleotide is expressed in a cell *in vitro*.

21) The screening method as defined in either Claims 17 or Claim 18 wherein said contacting step comprises administration of said compound to an animal model of late-onset depression, and wherein said polypeptide or said polynucleotide is expressed in a tissue of said animal model.

22) The screening method as defined in Claim 21 wherein said animal model of late-onset depression is a primate, mouse or rat model of late-onset depression.

23) The screening method as defined in any one of Claims 17-22 wherein said compound is contacted with said polypeptide.

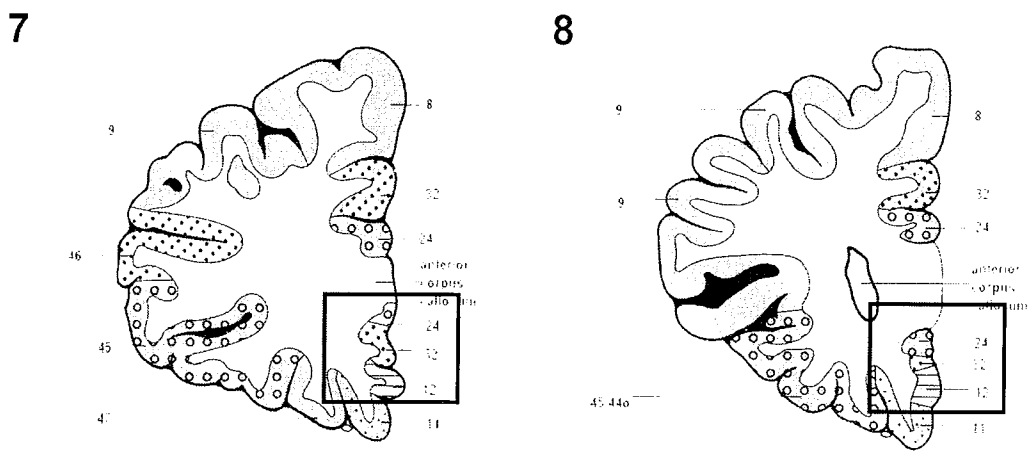


Figure 1: Coronal sections through the adult human forebrain at the anterior limit of the corpus callosum. Boxed areas (in black) mark out the subgenual anterior cingulate cortex.

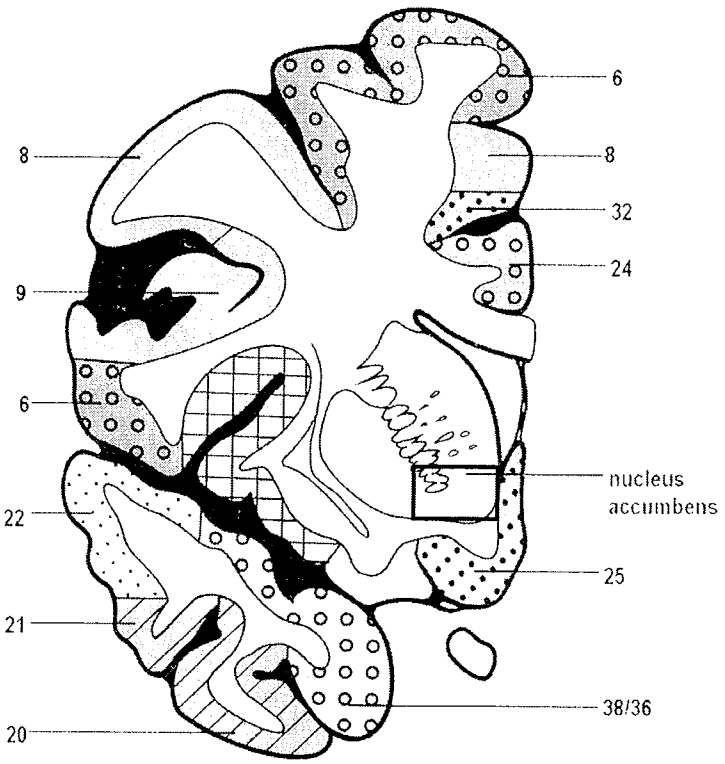


Figure 2: Schematic coronal section through the adult human forebrain at the anterior limit of the striatum. Boxed area (in black) marks out the nucleus accumbens.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/055944

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	KUGAYA AKIRA ET AL: "Cerebral benzodiazepine receptors in depressed patients measured with [123I]iomazenil SPECT." BIOLOGICAL PSYCHIATRY 15 OCT 2003, vol. 54, no. 8, 15 October 2003 (2003-10-15), pages 792-799, XP002541945 ISSN: 0006-3223 cited in the application the whole document ----- -/--	1-14

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 August 2009	18/09/2009

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Quirin, Katharina

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/055944

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOEPPING ET AL: "Synthesis of fluorine substituted pyrazolopyrimidines as potential leads for the development of PET-imaging agents for the GABAA receptors" BIOORGANIC & MEDICINAL CHEMISTRY, ELSEVIER SCIENCE LTD, GB, vol. 16, no. 3, 26 November 2007 (2007-11-26), pages 1184-1194, XP022453091 ISSN: 0968-0896 the whole document	1-14,17, 18,20,23
X	DERRY JASON M C ET AL: "Identification of a residue in the gamma-aminobutyric acid type A receptor alpha subunit that differentially affects diazepam-sensitive and -insensitive benzodiazepine site binding." JOURNAL OF NEUROCHEMISTRY MAR 2004, vol. 88, no. 6, March 2004 (2004-03), pages 1431-1438, XP002541946 ISSN: 0022-3042 the whole document page 1433	17,18, 20,23
T	SMITH DONALD F ET AL: "Molecular tools for assessing human depression by positron emission tomography." EUROPEAN NEUROPSYCHOPHARMACOLOGY : THE JOURNAL OF THE EUROPEAN COLLEGE OF NEUROPSYCHOPHARMACOLOGY SEP 2009, vol. 19, no. 9, September 2009 (2009-09), pages 611-628, XP002541440 ISSN: 1873-7862 the whole document	1-14, 17-23
A	PIMLOTT SALLY L: "Radiotracer development in psychiatry." NUCLEAR MEDICINE COMMUNICATIONS MAR 2005, vol. 26, no. 3, March 2005 (2005-03), pages 183-188, XP009121587 ISSN: 0143-3636 the whole document	1-14, 17-23
A	BRAMBILLA P ET AL: "GABAergic dysfunction in mood disorders." MOLECULAR PSYCHIATRY AUG 2003, vol. 8, no. 8, August 2003 (2003-08), pages 721-737 , 715, XP002541947 ISSN: 1359-4184 cited in the application the whole document	1-14, 17-23

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SANACORA G ET AL: "IMPAIRMENT OF GABAERGIC TRANSMISSION IN DEPRESSION: NEW INSIGHTS FROM NEUROIMAGING STUDIES" CRITICAL REVIEWS IN NEUROBIOLOGY, CRC PRESS, BOCA RATON, FL, US, vol. 14, no. 2, 1 January 2000 (2000-01-01), pages 23-45, XP001029967 ISSN: 0892-0915 the whole document	1-14, 17-23
A	HASLER GREGOR ET AL: "Reduced prefrontal glutamate/glutamine and gamma-aminobutyric acid levels in major depression determined using proton magnetic resonance spectroscopy." ARCHIVES OF GENERAL PSYCHIATRY FEB 2007, vol. 64, no. 2, February 2007 (2007-02), pages 193-200, XP002541441 ISSN: 0003-990X the whole document	1-14, 17-23
A	SMITH DANIEL J ET AL: "The use of single photon emission computed tomography in depressive disorders" NUCLEAR MEDICINE COMMUNICATIONS, LIPPINCOTT WILLIAMS AND WILKINS, XX, vol. 26, no. 3, 1 March 2005 (2005-03-01), pages 197-203, XP009121389 ISSN: 0143-3636 the whole document	1-14, 17-23
A	VAISHNAVI SANDEEP ET AL: "Neuroimaging in late-life depression" INTERNATIONAL REVIEW OF PSYCHIATRY, CARFAX, ABINGDON, GB, vol. 18, no. 5, 1 October 2006 (2006-10-01), pages 443-451, XP009121384 ISSN: 0954-0261 the whole document	1-14, 17-23
A	SANACORA GERARD ET AL: "GABAergic contributions to the pathophysiology of depression and the mechanism of antidepressant action." CNS & NEUROLOGICAL DISORDERS DRUG TARGETS APR 2007, vol. 6, no. 2, April 2007 (2007-04), pages 127-140, XP009121630 ISSN: 1871-5273 the whole document	1-14, 17-23

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/055944

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GARERI P ET AL: "Conventional and new antidepressant drugs in the elderly." PROGRESS IN NEUROBIOLOGY JUL 2000, vol. 61, no. 4, July 2000 (2000-07), pages 353-396, XP002541949 ISSN: 0301-0082 the whole document page 382 page 386 - page 387	1-14, 17-23

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 15,16

Present claims 15 and 16 relate to compounds which have a given desired property or effect, namely that :
- they specifically associate with a GABAergic receptor or a nucleic acid encoding a GABAergic receptor in the brain of a living organism, and
- that their binding pattern enables diagnosis or treatment of late-onset depression.

However, the description does not provide sufficient disclosure in the sense of Article 5 PCT because it discloses no such compounds. The subject-matter of claims 15 and 16 covers limitless and untried downstream developments in relation to yet to be demonstrated molecular activities. The claims amount to no more than an invitation to set up further research programs for which no guidance is forthcoming and, therefore, it is an undue burden to put the claimed subject-matter into practice, i.e. to structurally identify the relevant compounds and show a potential effect in diagnosis and therapy.

Moreover, the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained, contrary to Article 6 PCT. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2)PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2009/055944

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 15,16
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.