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REVIEW

# Serotonin$_{1A}$ receptors in mood disorders: a combined genetic and genomic approach

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**The serotonin$_{1A}$ (5-HT$_{1A}$) receptor has been under intense investigation, mostly due to its putative role in both the etiology and therapeutic treatments of depression and anxiety-related behaviors. However, the exact contribution of this receptor to normal brain physiology and disease processes remains poorly understood, due to a complex expression pattern and multiple functions. Recent development in genetic and genomic approaches allow not only for more refined functional dissection, but also for probing large gene databases for unknown gene product interactions. Here, we describe an experimental approach that is based on a combination of regional and temporal genetic manipulations of the 5-HT$_{1A}$ receptor with large-scale gene expression profiling to attempt to untangle the distinct roles for this receptor in particular brain regions, as well as to identify molecular partners that mediate its function. In turn, new leads for understanding mechanisms of anxiety, depression and their pharmacological treatments may be generated. © 2001 Lippincott Williams & Wilkins.**

**Keywords: serotonin, anxiety, depression, antidepressant, genetic, genomic, knock-out, microarray, mouse**

## INTRODUCTION

Pharmacological, biochemical and neuroanatomical studies are beginning to establish that depressive disorders are brain diseases with unique histopathological and molecular features. In particular, the serotonin system appears to be involved in the etiology of several neuropsychiatric disorders, including anxiety and depression. Initially, the classical 'monoamine hypothesis' stated that a monoamine deficiency might underlie depression. The fact that most antidepressant drugs, including tricyclics (TCA) and selective serotonin reuptake inhibitors (SSRIs), increase the synaptic availability of serotonin in a time course that parallels the improvement of the disease symptoms further supported a role for increased serotonergic signaling in the therapeutic treatment of depression. However, despite these pharmacological breakthroughs, the development of new and better treatments for depression remains a major area of research for both academia and pharmaceutical companies. The main factor supporting this research effort resides in the fact that all available drug regimens still require 2–4 weeks of chronic treatment before the full therapeutic effects are experienced by patients. Furthermore, as clinical diagnostics become more refined, an increasing number of patients become exposed to antidepressant treatments for a variety of related disorders. For many of these patients with less severe symptoms, the latest generations of antidepressant drugs have levels of side-effects that are unacceptable, resulting in decreased compliance to treatment. Finally, a significant portion of depressed patients are still refractory to most current drug regimens.

Pharmacological agents that target components of the serotonin system have been successful at treating some of the symptoms of these disorders, possibly by regulating the processes that appear to have been dysregulated during the course of the disease. Several lines of evidence point towards the serotonin$_{1A}$ (5-HT$_{1A}$) receptor as a putative key player in the etiology and therapeutic treatment of depression and anxiety disorders. However, the multiplicity of additional receptor subtypes, and of their interaction with other neurotransmitters systems, as well as the impact of environment and genetic

factors, have limited a more refined cellular or molecular understanding of the actual mechanisms that are involved in the disease process. Specific agonist and antagonist compounds were developed for the 5-$HT_{1A}$ receptor and have been beneficial at unraveling some of the contribution of this receptor subtype in mood control (De Vry, 1995; Cowen, 2000). More recently, the application of transgenic technologies confirmed the critical role of the 5-$HT_{1A}$ receptor in the modulation of anxiety-related behaviors in rodent systems (Heisler *et al*., 1998; Parks *et al*., 1998; Ramboz *et al*., 1998). However, the complexity of the expression pattern and functions of this receptor subtype has required the development and application of more sophisticated experimental approaches to investigate fully its function in the modulation of the neural networks that may be involved in neuropsychiatric disorders. We present a combination of genetic and genomic approaches here, to attempt to answer key questions, such as: (1) Which brain regions or neural circuits mediate the role of the 5-$HT_{1A}$ receptor in neuropsychiatric disorders? (2) Does development play a role in the adult function of this receptor? and (3) What are the genes or gene products that interact with 5-$HT_{1A}$ receptors to bring about a disease phenotype or therapeutic improvements?

## SEROTONIN, 5-$HT_{1A}$ RECEPTOR AND MOOD DISORDERS

Serotonin is a biogenic amine with a widespread distribution in the central nervous system (CNS) which makes it an essential modulatory neurotransmitter of most brain functions. Extensive documentation supports the role of serotonin in feeding, sleep and thermoregulation, as well as in mood regulation, stress response, aggression, memory and learning (Baumgarten and Gothert, 1997). The serotonin system also appears to be involved in the etiology of several neuropsychiatric disorders, including depression and anxiety. In addition to this wide variety of sensory, motor and cortical functions in the CNS, serotonin modulates diverse peripheral functions, such as enteric reflexes, platelet aggregation, smooth muscle contraction, cardiovascular function and nociception.

The implication of serotonin in such a wide variety of functions is less surprising when one considers the overall structure of the serotonergic system in the brain. The serotonin system is among the most extensively distributed systems in the CNS. In mammals, most serotonergic neurons reside along the midline of the midbrain, within the raphe nuclei (Baumgarten and Grozdanovic, 1997; Jacobs, 1997). The two anterior nuclei, the median raphe and the slightly lateralized dorsal raphe nuclei contain large neurons that send dense parallel projections to the large majority of forebrain structures. The posterior nuclei project to the lower brain stem and the spinal cord. The projection fields of the dorsal and median raphe nuclei have distinct patterns and occasionally overlap. In particular, axons emanating from the dorsal raphe nuclei generally have fine and extensive collateral branches with tiny varicosities that occupy target fields abundantly and, therefore, are well adapted to modulate the function of the innervated cells (Kosofsky and Molliver, 1987; Blue *et al*., 1988). Thick varicose fibers emanate from the median raphe with basket terminals in the cortical upper layer and hippocampus (Kosofsky and Molliver, 1987).

The variety of functions regulated by serotonin is also reflected at the level of the receptors. At least 14 different receptors have been identified and cloned (Hartig, 1997). The fact that all of the receptors are G-protein coupled, with the exception of the 5-$HT_3$ subtype, which is an ion channel, emphasizes the primary modulatory function of serotonin. In contrary to fast-acting neurotransmitters, such as glutamate or $\gamma$-aminobutyric acid (GABA), that activate ion channels directly and induce excitatory or inhibitory postsynaptic potentials, serotonin mainly modulates the function and responsiveness of the target cell through second messenger systems. Effector systems include, among others, adenylate cyclase, phospholipase C and potassium channels. Figure 1 displays a schematic representation of the serotonin system. Excitability and firing of the serotonergic neurons is negatively regulated by 5-$HT_{1A}$ receptors in the raphe, while the release at the terminal field is negatively regulated by presynaptic 5-$HT_{1B}$ receptors. The action of serotonin is terminated by reuptake into serotonergic neurons via the serotonin transporter (5-HTT). Pyramidal cells express a variety of postsynaptic receptors (5-$HT_{1A}$, 5-$HT_{2A}$, 5-$HT_{2C}$, 5-$HT_3$, etc.). Serotonergic neurons have a slow and spontaneous rhythmic activity of 4–5 Hz during the wake cycle, become less regular during non-REM sleep and fall silent during REM sleep (Jacobs and Fornal, 1991). Disruptions or abnormalities in both serotonin levels (or in 5-hydroxyindoleacetic acid (5-HIAA), its main metabolite) and serotonin receptor function have been implicated in neuropsychiatric disorders. Furthermore, pharmacological manipulations of serotonin levels or receptor function can either worsen or alleviate the disease symptoms. This review concentrates on the role of the 5-$HT_{1A}$ receptor in disease.

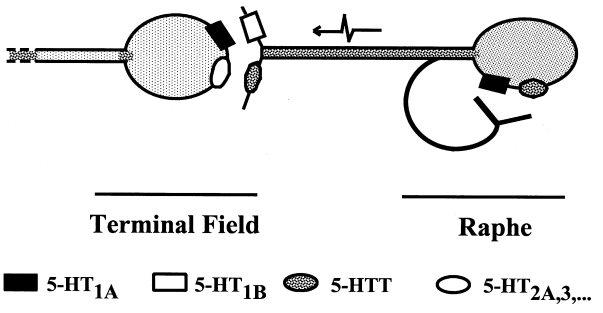


FIGURE 1. Schematic serotonin system. Serotonergic neurons in the raphe nuclei project to most forebrain regions. Excitability and firing of serotonergic neurons are regulated by 5-HT$_{1A}$ receptors (black square) on serotonergic cell bodies (presynaptic autoreceptor), while the release at the terminal is negatively regulated by 5-HT$_{1B}$ presynaptic receptors (white rectangle). The action of serotonin is terminated by reuptake into serotonergic neurons by the serotonin transporter (dotted circle). At the postsynaptic levels, the action of serotonin is mediated by several receptor subtypes (white circle), including the 5-HT$_{1A}$ receptor (black square).

The 5-HT$_{1A}$ receptor is the classical autoreceptor that exerts a feedback inhibition on serotonergic neurons in the raphe nuclei (reviewed by Hamon, 1997). Activation of this receptor by agonists results in a reduction of the firing rate of serotonergic neurons (both *in vivo* and *in vitro*, see Blier *et al*., 1987; Jolas *et al*., 1993) and leads to the suppression of serotonin synthesis and turnover. 5-HT$_{1A}$ receptor activation also reduces serotonin release in diverse projection areas and decreases serotonergic function at a variety of postsynaptic receptors. Therefore, this receptor represents a potentially important modulatory site for the overall regulation of the serotonin system. 5-HT$_{1A}$ receptors are also directed to the somatodendritic compartment of postsynaptic neurons in the cortex, hippocampus, hypothalamus, septum and amygdala. Similarly, activation of postsynaptic receptors is generally believed to induce a membrane hyperpolarization and a consequent decrease in the firing rate of the postsynaptic cell (Andrade, 1992).

Activation of the 5-HT$_{1A}$ receptor by agonists results in an anxiolytic effect (De Vry, 1995). Correlations were found between the time and dose dependency of the anxiolytic effect, the inhibition of serotonergic firing in the dorsal raphe nuclei, and the inhibition of serotonin release after systemic administration of 5-HT$_{1A}$ receptor agonists (Jolas *et al*., 1995). The 5-HT$_{1A}$ receptor partial agonists, buspirone and a series of congeners, also produce this neurochemical effect and are clinically effective at treating symptoms of anxiety (Coplan *et al*., 1995). Buspirone is the only non-benzodiazepine drug that is used in the clinic exclusively to relieve anxiety disorders and has shown efficacy in major depressive disorder and post-traumatic stress disorder (Apter and Allen, 1999). Based on these findings, it has been proposed that 5-HT$_{1A}$ receptor agonists stimulate presynaptic receptors, which inhibit serotonin release and consequently reduce serotonin signaling at a multitude of diverse target receptors. These may include 5-HT$_{2A}$, 5-HT$_{2C}$ and 5-HT$_3$ receptors, since selective antagonists acting on these receptors have also been shown to be anxiolytic in some behavioral tests (Griebel, 1995).

5-HT$_{1A}$ receptor antagonists appear to accelerate and enhance the antidepressant activity of the SSRIs, such as fluoxetine/Prozac. SSRIs increase the availability of serotonin in the synaptic cleft by blocking the uptake of this monoamine. However, a 2- to 4-week delay is observed before the onset of therapeutic action of this class of drugs (reviewed by Blier and de Montigny, 1994; Loo and Brochier, 1995). Blocking the inhibitory feedback by 5-HT$_{1A}$ receptor antagonism may augment the ability of SSRIs to increase extracellular serotonin levels by bypassing the period of time that is necessary to downregulate presynaptic autoreceptors upon prolonged SSRI medication (Artigas *et al*., 1996; Trillat *et al*., 1998). This observation suggests that 5-HT$_{1A}$ autoreceptor desensitization is an integral part of the antidepressant effect (Blier and de Montigny, 1994).

In parallel to presynaptic desensitization, postsynaptic hippocampal 5-HT$_{1A}$ receptor activation may be involved in the antidepressant effect of multiple treatments. Repeated administration (14–21 days) of the tricyclic antidepressant imipramine, the SSRI paroxetine, the monoamine oxidase inhibitor befloxatone A, the adrenergic $\alpha 2$ blocker mirtazapine, or the 5-HT$_{1A}$ partial agonist gepirone, or seven consecutive days of electroconvulsive shocks, all induced a tonic activation of postsynaptic hippocampal 5-HT$_{1A}$ receptors in rats (Haddjeri *et al*., 1998). The fact that gepirone, a 5-HT$_{1A}$ partial agonist, also induced a tonic postsynaptic activation is consistent with earlier reports of pre- but not postsynaptic desensitization of 5-HT$_{1A}$ receptors upon prolonged agonist exposure at doses that have antidepressant activity (Blier and de Montigny, 1987). Differences in reserve between pre- and postsynaptic receptor pools were suggested to be responsible for this phenomenon. These findings may explain the fact that buspirone, another 5-HT$_{1A}$ receptor partial agonist, has long-term antidepressant activity on its own (Apter and Allen, 1999). The presence of an effective partial agonist in a context of presynaptic desensitization suggests that the antidepressant effect of

buspirone may be postsynaptically mediated (Haddjeri *et al*., 1998).

Evidence from clinical studies also points towards a correlation between altered 5-$HT_{1A}$ receptor physiology and depressive disorders. For instance, 5-$HT_{1A}$ agonist challenges induce a release in adrenocorticotropic hormone and peripheral cortisol. These hormonal responses have consistently been shown to be decreased in unmedicated subjects with depression, suggesting a central deficit in 5-$HT_{1A}$ function (reviewed by Cowen, 2000). Binding measurements have yielded conflicting results, with studies showing no differences, decrease in raphe, cortex and hippocampus, or tendencies towards increase in raphe (reviewed by Drevets *et al*., 2000). However, recent *in vivo* imaging, combined with careful clinical sampling, have confirmed a widespread reduction of 5-$HT_{1A}$ receptors in major depression (Sargent *et al*., 2000) and bipolar disorders (Drevets *et al*., 2000).

## GENETIC MANIPULATIONS OF THE 5-$HT_{1A}$ RECEPTOR: FUNCTIONAL INSIGHTS INTO MOOD REGULATION

To study the role of the 5-$HT_{1A}$ receptor in the regulation of mood, and in particular to test the hypothesis of decreased 5-$HT_{1A}$ receptor function and anxiety or depression, a genetic model of receptor hypofunction was created. Three independent research groups have produced lines of mice with genetically altered 5-$HT_{1A}$ receptor function and have all reported an identical phenotype in the mutant mice (Heisler *et al*., 1998; Parks *et al*., 1998; Ramboz *et al*., 1998). 5-$HT_{1A}$ receptor knockout (KO) mice displayed increased anxiety-like behaviors in several conflict-based behavioral tests (reviewed by Gross *et al*., 2000; Olivier *et al*., 2001), confirming the critical role of the 5-$HT_{1A}$ receptor in the modulation of mood in general and anxiety in particular. In view of the pharmacological data from both animal models and the 5-$HT_{1A}$-mediated therapeutic relief of anxiety symptoms in human patients, the increased anxiety phenotype of 5-$HT_{1A}$ KO mice was altogether not surprising, but rather a confirmation of a large body of previous research studies. However, the constitutive nature of the genetic deletion in both pre- and postsynaptic brain regions raised several questions with regards to the interpretation of the resulting phenotype.

### 5-$HT_{1A}$ KO phenotype: a pre- or postsynaptic effect?

Due to the dual localization and functions of the 5-$HT_{1A}$ receptor, the observed phenotype could result from either a functional deficit in presynaptic serotonergic neurons, or to a lack of 5-$HT_{1A}$ receptor-mediated signaling in postsynaptic brain regions. A presynaptic deficit may manifest as a deregulated or increased serotonergic tone with altered kinetics of signaling at postsynaptic sites. Microdialysis experiments revealed normal extracellular 5-HT levels and release in striatum of KO mice (He *et al*., 2001). Likewise, total 5-HT tissue contents were identical between KO and wild-type (WT) mice in all brain regions measured, while 5-HT metabolites were increased in the raphe nuclei of KO mice (Ase *et al*., 2000). These observations suggest the presence of increased 5-HT turnover at presynaptic sites but normal 5-HT availability and release at postsynaptic sites. Such a discrepancy may reflect the fact that terminal 5-$HT_{1B}$ autoreceptors are more abundant in target fields than in raphe nuclei, and may therefore better compensate for 5-HT release in target regions (Ramboz *et al*., 1998) than in raphe nuclei. Alternatively, other neurotransmitter systems may have compensated to maintain proper release at serotonergic terminals. Taken together, these data suggest that the increased anxiety phenotype of KO mice is more likely to result from the absence of 5-$HT_{1A}$ receptor-mediated signaling in postsynaptic brain regions.

### 5-$HT_{1A}$ KO phenotype: an adaptive consequence or the lack of maturation of proper neural networks?

5-$HT_{1A}$ receptor KO mice do not fully mimic the effects of pharmacological interventions. For instance, WAY 100635, a 5-$HT_{1A}$ receptor specific antagonist, is not anxiogenic in conflict tests such as the elevated plus-maze in mice, while 5-$HT_{1A}$ KO mice displayed increased anxiety-like behavior in this test. This observation suggests that genetic deletion does not equate with pharmacological blockade of the receptor, and that the observed phenotype may represent the expression of compensatory events that are several steps removed from the original genetic manipulation. Accordingly, the absence of the receptor during development could lead to either a lack of proper maturation of specific neural networks (deficit phenotype) or to adaptations in other systems (compensated phenotype) that can confound the interpretation of the subsequent phenotype.

In order to answer these questions, new genetic targeting technologies are being developed that allow the control of the level of expression of a particular gene in a temporal and/or regional fashion. These new approaches should circumvent some of the complications that are inherent to the constitutive deletion of a gene in germ lines (Lucas and

Hen, 1995; Stark *et al*., 1998). Figure 2 displays the strategy that is being employed in our laboratory to achieve temporal and regional manipulations of the 5-HT$_{1A}$ receptor. Traditionally in KO studies, the function of a gene is disrupted by removing part of the coding region of the gene and by replacing it by homologous recombination in embryonic stem (ES) cells with a neomycin-resistant DNA cassette. In the present design, a DNA sequence (STOP cassette) and a minimal operator sequence (tetO) for the bacterial tetracycline system were introduced immediately downstream of the transcription initiation site of the gene. This STOP cassette efficiently shuts down all transcriptional activity, resulting in a null mutant for the 5-HT$_{1A}$ receptor. The tetO operator serves as a docking site for the tetracycline transactivator (tTA) transcription factor, which efficiently drives the transcription of the 5-HT$_{1A}$ receptor (Bujard, 1999). The expression of the tTA protein is driven, in a separate line of transgenic mice, to several forebrain regions, including the hippocampus, cortex, amygdala and striatum, under the control of the $\alpha$-$Ca^{2+}$-calmodulin kinase II (CamKII) promoter (Ghavami *et al*., 1997). When cross-bred with 5-HT$_{1A}$ STOP receptor knock-in mice, double transgenic mice express the 5-HT$_{1A}$ receptor under the control of the tTA protein in the brain regions described above. The receptor is only reawakened in postsynaptic sites, as tTA is not expressed in the raphe nuclei. Furthermore, the binding of tTA to its promoter, tetO, can be prevented by tetracycline antibiotic drugs. When provided in the food or in the drinking water, doxycycline (dox) binds efficiently to tTA, prevents binding to tetO and shuts off all transcriptional activity at the 5-HT$_{1A}$ receptor locus. Taken together, the tTA system provides an inducible transcriptional system that allows the induction or repression, in a temporal manner, of the expression of a particular gene in brain regions that are determined by the promoter of the transgenic tTA.

As shown in Figure 3, the 5-HT$_{1A}$ receptor can now be induced only in postsynaptic brain regions, as demonstrated by the absence of 5-HT$_{1A}$ receptor binding in raphe nuclei. Furthermore, the expression of the receptor can be induced or repressed by doxycycline either during development or only in the adult animal, thus allowing the separation of developmental contributions of the receptor from its adult role. In addition, the restricted pattern of tTA expression permits us to study the contribution of the receptor in selected brain regions, for instance only in pre- or postsynaptic regions that normally express the 5-HT$_{1A}$ receptor. Additional transgenic lines with promoters driving the expression of tTA in midbrain presynaptic brain regions, or with more restricted forebrain patterns of expression, are currently being developed.

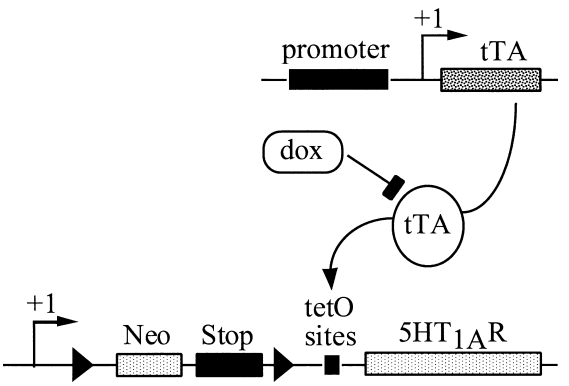


FIGURE 2. Schematic for temporal and regional genetic manipulations of the 5-HT$_{1A}$ receptor. A DNA sequence consisting of a neomycin resistance cassette and a STOP sequence, both flanked by LoxP sites, as well as a minimal tetracycline operator sequence (tetO), are introduced by homologous recombination in embryonic stem cells (knock-in) immediately downstream from the site of initiation of transcription. This DNA cassette efficiently shuts down all transcriptional activity, resulting in a null mutant for the 5-HT$_{1A}$ receptor. The transcriptional activity of the gene can be reawakened by the binding of the tetracycline transactivator (tTA) transcription factor. The tTA protein is produced under the control of a CaMkinase II promoter (Mayford *et al*., 1996). The binding of tTA to its promoter, tetO, is prevented by tetracycline antibiotic drugs (doxycycline, dox). When provided in the food or in the drinking water, doxycycline efficiently binds to tTA, prevents binding to tetO, and shuts off all transcriptional activity at the 5-HT$_{1A}$ receptor locus. Taken together, a temporal modulation of the transcriptional activity of the 5-HT$_{1A}$ receptor is achieved by the inducible tetracycline system, while the regional control of the expression pattern is provided by the specificity of the promoter that drives the expression of tTA.

## GENOMIC APPROACH TO THE CONSEQUENCES OF GENETIC DELETION OF THE 5-HT$_{1A}$ RECEPTOR

As mentioned above, 5-HT$_{1A}$ receptor KO mice do not fully mimic the effects of pharmacological interventions in conflict-based behavioral assays, suggesting the presence of adaptations in the brain of KO mice. Evidence of compensatory mechanisms in other neurotransmitter systems was recently described in 5-HT$_{1A}$ receptor KO mice. After initial observations of reduced behavioral responses to a benzodiazepine challenge in the mutant mice, significant structural and functional adaptations in the GABAergic system were documented in the amygdala of mice lacking the 5-HT$_{1A}$ receptor (Sibille *et al*., 2000). This new and unexpected interaction between these two independent neurotransmitter systems is intriguing since both systems are known

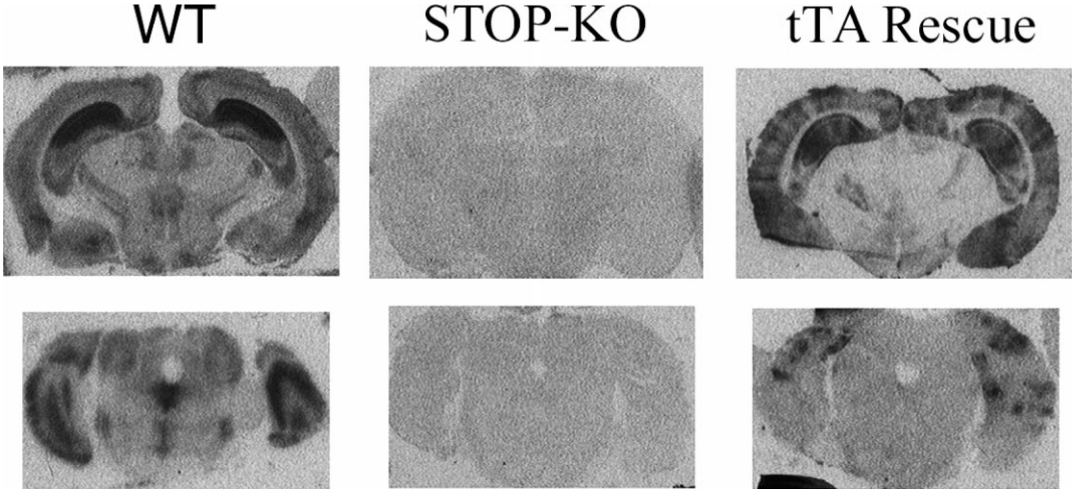


FIGURE 3. Genetic manipulation of the 5-$HT_{1A}$ receptor: $^{125}$I-MPPI autoradiography. $^{125}$I-MPPI autoradiographs depict 5-$HT_{1A}$ receptor binding at two anatomical levels: hippocampus (postsynaptic heteroreceptors) and raphe (presynaptic autoreceptors). WT: normal 5-$HT_{1A}$ receptor levels; STOP-KO: the STOP cassette efficiently blocks 5-$HT_{1A}$ receptor transcriptional activation at both pre- and postsynaptic sites; tTA Rescue: CamKII–tTA rescues receptor expression in hippocampus and cortex, but not in raphe.

to be involved in the modulation of mood, and possibly also in the etiology of several neuropsychiatric disorders (see Olivier *et al*., 2001). This existence of compensatory mechanisms in other neurotransmitters systems in KO mice is not without precedent. For instance, in mice lacking the 5-$HT_{1B}$ receptor, the phenotype of mutant mice does not fully mimic pharmacological blockade or inhibition of the receptor. For instance, 5-$HT_{1B}$ KO mice demonstrated an increased locomotor response to cocaine and a higher tendency to self-administer the drug (Rocha *et al*., 1998), while injection of a 5-$HT_{1B}$ receptor antagonist, GR127935, decreased the locomotor effects of cocaine and had no effects on self-administration (Castanon *et al*., 2000).

In order to characterize putative differences between 5-$HT_{1A}$ receptor KO and wild-type control mice, a large-scale search for markers of altered cellular function was undertaken. Gene expression profiling allows the monitoring of thousands of genes or expressed sequenced tags (ESTs) in a simultaneous and unbiased way. Current methodology for investigating gene expression includes protocols that are based either on unbiased transcript sampling or on knowledge of defined sets of genes and ESTs. RNA transcript sampling includes differential display, serial analysis of gene expression (SAGE) (Velculescu *et al*., 1995) or restriction endonuclease partitioning (GeneCalling [Shimkets *et al*., 1999], TOGA [Sutcliffe *et al*., 2000]). These approaches are sensitive and could, in theory, detect any expressed transcript. However, they require time-consuming cloning, sequencing and confirmation processes. The development of DNA microarrays has significantly enhanced the capacity of screening large sets of genes and ESTs. Arrays allow for the expression of multiple genes, displayed as multiple probes on a fixed matrix, to be investigated simultaneously in a single hybridization reaction. Adaptation of photolithographic masking techniques used in semiconductor manufacture has greatly extended the power of this approach. With the aid of sophisticated computer-based analysis algorithms, it is now possible to analyze up to 400 000 distinct oligonucleotides in a single array (Lockhart *et al*., 1996) (Figure 4). To increase the specificity of hybridization reactions that interrogate the whole transcriptome (set of all RNAs expressed in the biological sample) in a single reaction, the knowledge of sequence information stemming from the effort to sequence whole genomes was used to design specific oligonucleotides for thousands of genes. As few as 200–300 bases of gene or EST sequences are sufficient to design 15–20 non-overlapping, unique 25-mer oligonucleotides, which represent specific probes for this given sequence. The use of probe redundancy to assess the expression level of a specific transcript greatly improves the signal to noise ratio (efficiency of hybridization is averaged over 20 probes), increases the accuracy of RNA quantification (removal of outlier data) and significantly reduces the rate of false positives. Such arrays are made available commercially through Affymetrix, Inc. (Santa Clara, CA, USA).

Recent evidence of altered hippocampal volume in depressed patients has helped re-focus attention on the hippocampus, as a putative critical brain

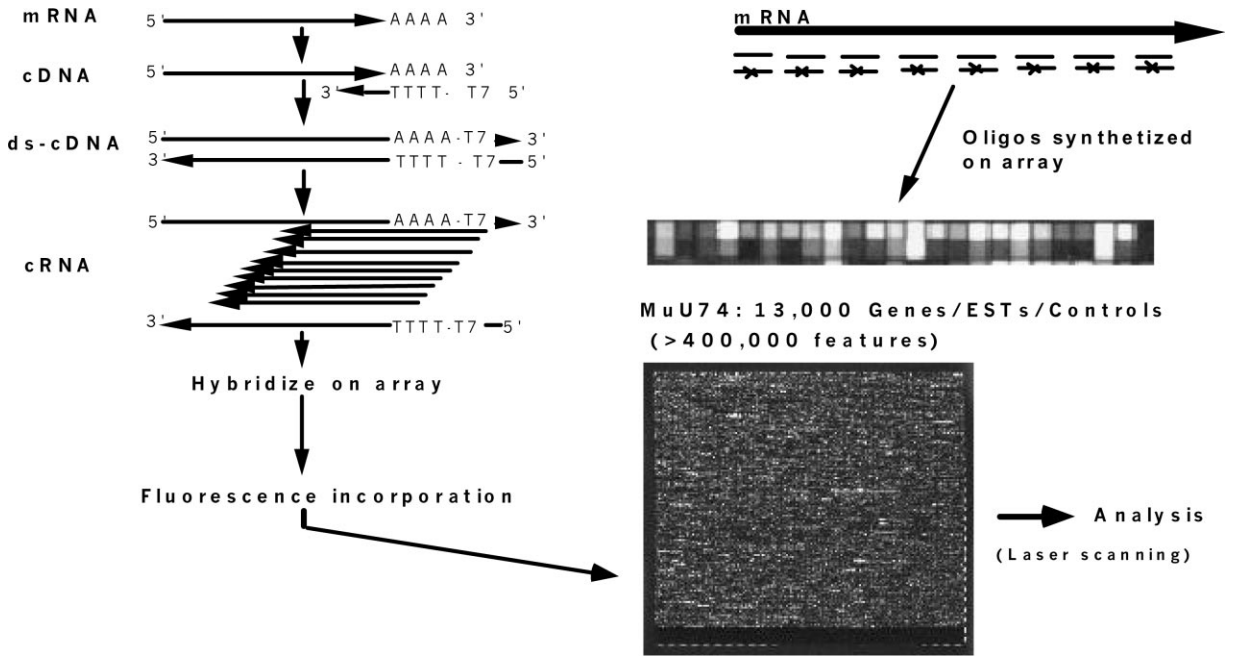


FIGURE 4. Oligonucleotide DNA microarray. On Affymetrix oligonucleotide DNA microarray, each gene is represented by 16–20 specific complementary oligonucleotides (20–25 base pairs long). An equal number of mismatch oligonucleotides controls for the specificity of the signal. All oligonucleotides are synthesized directly on to the glass slide. On Mu11K arrays, 13 000 genes, ESTs and controls are represented by more than 400 000 features on two microarrays. The newest generation of U74 chips condense all 13 000 genes on a single chip. To prepare samples, total or polyA RNA is extracted and converted into ds-cDNA. A copy RNA or cRNA is then transcribed *in vitro* and biotinylated. This cRNA is then hybridized on to the microarrays. Fluorescence is coupled to hybridized probes by avidin and biotin–antibody coupled fluorophore. Finally, a laser scan is obtained for the analysis. Two kinds of analysis are performed. On a single chip analysis, a gene is determined to be present or absent, depending on its pattern of hybridization across all features representing this transcript on the array. An estimate of the expression level for each transcript is provided by the average of the difference in signal intensity between hybridization at perfect match and mismatch oligonucleotides. In a pairwise comparison, the efficiency of hybridization at each oligonucleotide is compared between two samples that were hybridized on two separate arrays. For both types of analyses, quantitative (average differences) and qualitative (present, absent, increased or decreased) calls are the results of decision matrices that take into account several parameters and metrics that describe the hybridization of each transcript.

region in depressive disorders and their therapeutic treatments (Sheline *et al*., 1999; Drevets *et al*., 2000; Rajkowska, 2000). Chronic drug treatment with SSRIs and lithium increase neurogenesis in the dentate gyrus of experimental animals (Chen *et al*., 2000), while lithium can also induce a reversal in decrease of hippocampal volume in human patients (Moore *et al*., 2000). Therefore, in view of the high level of expression of the 5-HT$_{1A}$ receptor in this brain region and its implication in mood disorders, studies of gene expression profiling were undertaken on hippocampal samples obtained from 5-HT$_{1A}$ KO and control mice. It is hypothesized that this receptor may exert a key role in pathways and neural networks that are responsible for establishing normal anxiety levels. It is also hypothesized that normal hippocampal homeostasis and drug-induced plastic changes might be disrupted in 5-HT$_{1A}$ KO mice.

From an experimental point of view, the variability between animals was reduced by pooling total hippocampal RNA from 2–3 mice, which was then used to generate probes for Affymetrix oligonucleotide murine 11K (Mu11K) arrays. On these arrays, approximately 11 000 full-length genes and EST cluster sequences are represented on two separate arrays (Mu11K subA and subB). On average, 43 $\pm$ 4% of the genes or sequences printed on the arrays were detected as present in mouse hippocampal samples. Thus, the expression levels of approximately 5000 genes can be monitored reliably with these types of gene arrays. Based on Affymetrix dual-comparison analysis, more than 3% of these genes or ESTs were detected as increased or decreased between individual samples. Repeated experiments (5 arrays per genotype) helped determine that this large number of genes (2–300) with different expression levels between samples mostly represented biological variability between samples (different groups of mice) and normal fluctuations in experimental procedures (sample preparation, hybridization and arrays). A small group of genes and ESTs, which displayed consistent decreases or increases in expression levels across all sample com-

parisons, were identified for further characterization. Altered transcriptional activities of these genes are currently being confirmed on different technological platforms, including real-time PCR, *in situ* hybridization and Northern blot. Figure 5 displays an *in situ* hybridization on coronal sections of wild-type and KO mice for an EST that showed a consistentdownregulation of expression level in the hippocampus of 5-$HT_{1A}$ KO mice, when compared to wild-type controls. Currently, the nature of this gene is being investigated, as well as its transcriptional activity after drug treatments, such as chronic antidepressant treatment. Furthermore, genes with altered transcriptional activity in 5-$HT_{1A}$ KO mice will be investigated in additional lines of mice with altered anxiety phenotypes that were developed in the laboratory. Such lines include mice with mutations in the serotonin system, i.e. 5-$HT_{1A}$ KO mice with postsynaptic rescue of the receptor and mice lacking the 5-$HT_{1B}$ receptor, or in other neurotransmitter systems, such as the peptide substance-P system. Mice lacking the substance-P receptor, NK1, display decreased anxiety-related behaviors (Santarelli *et al*., 2001). Interestingly, serotonin and central 5-$HT_{1A}$ receptor functions were also shown to be altered in NK1 KO mice, suggesting the possibility of common pathways across the neurotransmitter system for the modulation of mood-related behavior.

In the search for new directions in depression and anxiety research, a correlation between altered expression levels of particular genes and anxiety phenotype across several strains of mutant mice or pharmacological treatments would represent a strong argument for the involvement of these gene products in the modulation of mood.

## SUMMARY

Despite considerable effort over past decades, the mechanisms involved in the etiology of most neuropsychiatric disorders remain unknown. Experimental approaches to study the mechanisms of these disorders have relied on the knowledge obtained from the modes of actions of pharmacological compounds, as well as from markers of abnormal functions in patients. Accordingly, the 5-$HT_{1A}$ receptor has been under intense investigation due to its putative role in both the etiology and treatment of these disorders. Here, we have presented a novel experimental approach, consisting of applications of inducible bacterial systems to modify current transgenic approaches, in combination with large-scale gene expression profiling. By being able to control the level of expression of the 5-$HT_{1A}$ receptor in a temporal and regional manner, it will be possible to discriminate between the putative dif-

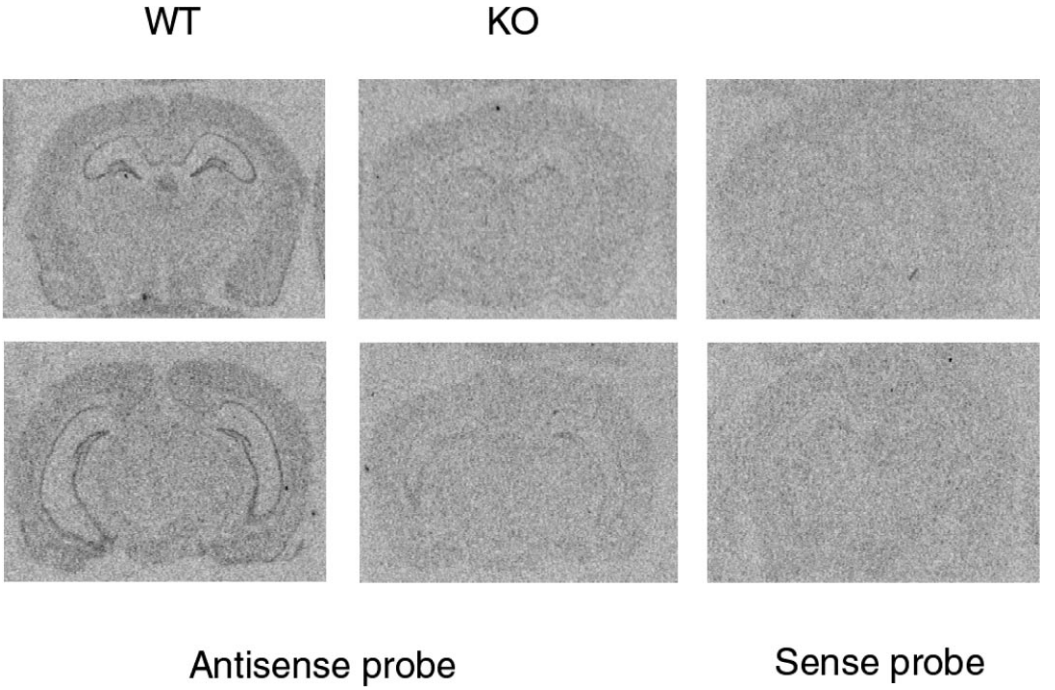


FIGURE 5. Altered gene expression in 5-$HT_{1A}$ KO mice. $^{33}P$ *in situ* hybridization of an unknown EST. Decreased signal intensity is observed throughout the hippocampus and cortical layers. Based on internal autoradiograph standards, KO levels were determined at $29.6 \pm 5.9\%$ of WT levels ($n = 4$ per genotype, repeated sections per animal).

ferent roles of this receptor subtype either during development or in the adult organism, as well as in separate brain regions. This approach represents a considerable advantage over classical pharmacological studies, which, despite the availability of selective agonists and antagonists, have not been able to discriminate clearly between several behavioral and physiological effects of 5-HT$_{1A}$ receptor activation or blockade, especially *in vivo*, in freely behaving animals.

In this postgenomic era, much emphasis is being placed on identifying gene or gene products that interact with, or which are downstream from, candidate genes. By monitoring altered gene expression in combination with temporal and regional manipulations of the 5-HT$_{1A}$ receptor, we expect to identify markers or downstream effectors of the receptor function, expression levels of which correlate, on the one hand, with the phenotype in behavioral screens, and, on the other hand, with the expression of the receptor in a particular brain region at a defined time. It is hypothesized that the nature of these new molecular leads may reveal information as to the actual mechanisms that are either responsible for the direct expression of a therapeutic phenotype, or that may underlie the neural plasticity that occurs during disease progression. The identification of an EST with decreased expression level in the brain of mice lacking the 5-HT$_{1A}$ receptor highlights two important considerations. First, new genes will be found having expression levels that correlate with complex behavioral phenotypes; and, secondly, the current status of knowledge and annotation of the gene databases is still relatively poor, leaving considerable latitude for genetic characterization and unraveling of putative new pathways.

## REFERENCES


Andrade R (1992). Electrophysiology of 5-HT$_{1A}$ receptors in the rat hippocampus and cortex. *Drug Dev Res* **26**:275–286.

Apter JT, Allen LA (1999). Buspirone: future directions. *J Clin Psychopharmacol* **19(1)**:86–93.

Artigas F, Romero L, de Montigny C, Blier P (1996). Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT$_{1A}$ antagonists. *Trends Neurosci* **19(9)**:378–383.

Ase AR, Reader TA, Hen R, Riad M, Descarries L (2000). Altered serotonin and dopamine metabolism in the CNS of serotonin 5-HT(1A) or 5-HT(1B) receptor knockout mice. *J Neurochem* **75(6)**:2415–2426.

Baumgarten HG, Gothert M (1997). *Serotoninergic Neurons and 5-HT Receptors in the CNS*. New York: Springer.

Baumgarten HG, Grozdanovic (1997). Anatomy of central serotoninergic projection systems. In: *Serotoninergic Neurons and 5-HT Receptors in the CNS*. Van de Kar LD (editor). New York: Springer, pp. 41–89.

Blier P, de Montigny C (1987). Modification of 5-HT neuron properties by sustained administration of the 5-HT$_{1A}$ agonist gepirone: electrophysiological studies in the rat brain. *Synapse* **1(5)**:470–480.

Blier P, de Montigny C (1994). Current advances and trends in the treatment of depression. *Trends Pharmacol Sci* **15(7)**:220–226.

Blier P, de Montigny C, Tardif D (1987). Short-term lithium treatment enhances responsiveness of postsynaptic 5-HT$_{1A}$ receptors without altering 5-HT autoreceptor sensitivity: an electrophysiological study in the rat brain. *Synapse* **1(3)**:225–232.

Blue ME, Yagaloff KA, Mamounas LA, Hartig PR, Molliver ME (1988). Correspondence between 5-HT$_2$ receptors and serotonergic axons in rat neocortex. *Brain Res* **453(1–2)**:315–328.

Bujard H (1999). Controlling genes with tetracyclines. *J Gene Med* **1(5)**:372–374.

Castanon N, Scearce-Levie K, Lucas JJ, Rocha B, Hen R (2000). Modulation of the effects of cocaine by 5-HT$_{1B}$ receptors: a comparison of knockouts and antagonists. *Pharmacol Biochem Behav* **67(3)**:559–566.

Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK (2000). Enhancement of hippocampal neurogenesis by lithium. *J Neurochem* **75(4)**:1729–1734.

Coplan J, Wolk S, Klein D (1995). Anxiety and the serotonin$_{1A}$ receptor. In: *Psychopharmacology: The Fourth Generation of Progress*. Bloom F, Kupfer D (editors). New York: Raven Press, pp. 1301–1310.

Cowen PJ (2000). Psychopharmacology of 5-HT(1A) receptors. *Nucl Med Biol* **27(5)**:437–439.

De Vry J (1995). 5-HT$_{1A}$ receptor agonists: recent developments and controversial issues. *Psychopharmacology (Berl.)* **121(1)**:1–26.

Drevets WC, Frank E, Price JC, Kupfer DJ, Greer PJ *et al*. (2000). Serotonin type-1A receptor imaging in depression. *Nucl Med Biol* **27(5)**:499–507.

Ghavami A, Stark KL, Jareb M, Ramboz S, Segu L, Hen R (1999). Differential addressing of the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors in epithelial cells and neurons. *J Cell Sci* **112**:967–976.

Griebel G (1995). 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. *Pharmacol Ther* **65(3)**:319–395.

Gross C, Santarelli L, Brunner D, Zhuang X, Hen R (2000). Altered fear circuits in 5-HT(1A) receptor KO mice. *Biol Psychiatry* **48(12)**:1157–1163.

Haddjeri N, Blier P, de Montigny C (1998). Long-term antidepressant treatments result in a tonic activation of forebrain 5-HT$_{1A}$ receptors. *J Neurosci* **18(23)**:10150–10156.

Hamon M (1997). The main features of the central 5-HT$_{1A}$ receptors. In: *Serotoninergic Neurons and 5-HT Receptors in the CNS*. Van de Kar LD (editor). New York: Springer, pp. 238–268.

Hartig PR (1997). Molecular biology and transductional characteristics of 5-HT receptors. In: *Serotoninergic Neurons and 5-HT Receptors in the CNS*. Van de Kar LD (editor). New York: Springer.

He M, Sibille E, Benjamin D, Toth M, Shippenberg T (2001). Differential effects of 5-HT(1A) receptor deletion upon basal and fluoxetine-evoked 5-HT concentrations as revealed by *in vivo* microdialysis. *Brain Res* **902(1)**:11–17.

Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P *et al*. (1998). Elevated anxiety and antidepressant-like responses in serotonin 5-HT$_{1A}$ receptor mutant mice. *Proc Natl Acad Sci USA* **95(25)**:15049–15054.

Jacobs BL (1997). Physiology and pharmacology of brain sero-

toninergic neurons. In: *Serotoninergic Neurons and 5-HT Receptors in the CNS*. Van de Kar LD (editor). New York: Springer, pp. 91–116.

Jacobs BL, Fornal CA (1991). Activity of brain serotonergic neurons in the behaving animal. *Pharmacol Rev* **43(4)**:563–578.

Jolas T, Haj-Dahmane S, Lanfumey L, Fattaccini CM, Kidd EJ *et al*. (1993). (−)Tertatolol is a potent antagonist at pre- and postsynaptic serotonin 5-$HT_{1A}$ receptors in the rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **347(5)**:453–463.

Jolas T, Schreiber R, Laporte AM, Chastanet M, De Vry J *et al*. (1995). Are postsynaptic 5-$HT_{1A}$ receptors involved in the anxiolytic effects of 5-$HT_{1A}$ receptor agonists and in their inhibitory effects on the firing of serotonergic neurons in the rat? *J Pharmacol Exp Ther* **272(2)**:920–929.

Kosofsky BE, Molliver ME (1987). The serotoninergic innervation of cerebral cortex: different classes of axon terminals arise from dorsal and median raphe nuclei. *Synapse* **1(2)**:153–168.

Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV *et al*. (1996). Expression monitoring by hybridization to high-density oligonucleotide arrays [see comments]. *Nat Biotechnol* **14(13)**:1675–1680.

Loo H, Brochier T (1995). [Long-term treatment with antidepressive drugs]. *Ann Med Psychol (Paris)* **153 (3)**:190–196; discussion 196–197.

Lucas JJ, Hen R (1995). New players in the 5-HT receptor field: genes and knockouts. *Trends Pharmacol Sci* **16(7)**:246–252.

Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD *et al*. (1996). Control of memory formation through regulated expression of a CaMKII transgene. *Science* **274(5293)**:1678–1683.

Moore GJ, Bebchuk JM, Wilds IB, Chen G, Manji HK (2000). Lithium-induced increase in human brain grey matter. *Lancet* **356(9237)**:1241–1242.

Murphy DL, Wichems C, Li Q, Heils A (1999). Molecular manipulations as tools for enhancing our understanding of 5-HT neurotransmission. *Trends Pharmacol Sci* **20(6)**:246–252.

Olivier B, Pattij T, Wood SJ, Oosting R, Sarnyai Z, Toth M (2001). The 5-$HT_{1A}$ receptor knockout mouse and anxiety: is the $GABA_A$–benzodiazepine receptor complex involved? *Behav Pharmacol*, in press.

Parks CL, Robinson PS, Sibille E, Shenk T, Toth M (1998). Increased anxiety of mice lacking the serotonin1A receptor. *Proc Natl Acad Sci USA* **95(18)**:10734–10739.

Rajkowska G (2000). Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol Psychiatry* **48(8)**:766–777.

Ramboz S, Oosting R, Ait Amara D, Kung HF, Blier P *et al*. (1998). Serotonin receptor 1A knockout: An animal model of anxiety-related disorder. *Proc Natl Acad Sci USA* **95**:14476–14481.

Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N *et al*. (1998). Increased vulnerability to cocaine in mice lacking the serotonin-1B receptor. *Nature* **393(6681)**:175–178.

Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P *et al*. (2001). Genetic and pharmacological disruption of neurokinin 1 receptor function decreases anxiety-related behaviors and increases serotonergic function. *Proc Natl Acad Sci USA* **98(4)**: 1912–1917.

Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C *et al*. (2000). Brain serotonin1A receptor binding measured by positron emission tomography with [$^{11}$C]WAY-100635: effects of depression and antidepressant treatment. *Arch Gen Psychiatry* **57(2)**:174–180.

Sheline YI, Sanghavi M, Mintun MA, Gado MH (1999). Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J Neurosci* **19(12)**:5034–5043.

Shimkets RA, Lowe DG, Tai JT, Sehl P, Jin H *et al*. (1999). Gene expression analysis by transcript profiling coupled to a gene database query. *Nat Biotechnol* **17(8)**:798–803.

Sibille E, Pavlides C, Benke D, Toth M (2000). Genetic inactivation of the Serotonin(1A) receptor in mice results in downregulation of major GABA(A) receptor alpha subunits, reduction of GABA(A) receptor binding, and benzodiazepine-resistant anxiety. *J Neurosci* **20(8)**:2758–2765.

Stark KL, Oosting RS, Hen R (1998). Novel strategies to probe the functions of serotonin receptors. *Biol Psychiatry* **44(3)**:163–168.

Sutcliffe JG, Foye PE, Erlander MG, Hilbush BS, Bodzin LJ *et al*. (2000). TOGA: an automated parsing technology for analyzing expression of nearly all genes. *Proc Natl Acad Sci USA* **97(5)**:1976–1981.

Trillat AC, Malagie I, Mathe-Allainmat M, Anmella MC, Jacquot C *et al*. (1998). Synergistic neurochemical and behavioral effects of fluoxetine and 5-$HT_{1A}$ receptor antagonists. *Eur J Pharmacol* **357(2–3)**:179–184.

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995). Serial analysis of gene expression. *Science* **270(5235)**:484–487.

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General: throughout, the first author only of each reference has been cited in the text. I have changed these to include the name of the second author (when there are only 2 authors of a reference) or 'et al.' (when $>2$ authors). Following on from this, some 'a', 'b' references (two refs with the same first author and year) have been changed. Please check that these corrections are OK at proof stage.

## Uncited references

Ghavami et al., 1999; Murphy et al., 1999

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| Fig. 1 | | Caption refers to a‘dotted circle’-please check this (no dotted circle on my copy) |
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| | | Baumgarten and Gothert (1997)-Please check the ref. Should it be‘ In: *Serotoninergic Neurons and 5-HT Receptors in the CNS* Van de Kar LD (editor)... pp.??-??’? Chapter title? (see Baumgarten HG, Grozdanovic (1997)) |
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| | | Heisler LK, Chu HM, Brennan TJ, Danao JA, Bajwa P *et al*-please give six authors before *et al*. |
| | | Jolas T, Haj-Dahmane S, Lanfumey L, Fattaccini CM, Kidd EJ, *et al*-please give six authors before *et al*. |
| | | Jolas T, Schreiber R, Laporte A M, Chastanet M, De Vry J, *et al* (1995)-please give six authors before *et al*. |
| | | Lockhart DJ, Dong H, Byrne MC, Follettie MT, Gallo MV *et al*. (1996)-please give six authors before *et al*. |
| | | Mayford M, Bach ME, Huang YY, Wang L, Hawkins RD *et al*. (1996)-please give six authors before *et al*. |
| | | Murphy D L, Wichems C, Li Q, & Heils A (1999)-not in text. Add to text or delete here. |
| | | Ramboz S, Oosting R, Ait Amara D, Kung HF, Blier P *et al* (1998)-please give six authors before *et al*. |
| | | Rocha BA, Scearce-Levie K, Lucas JJ, Hiroi N, Castanon N *et al*.(1998)-please give six authors before *et al*. |
| | | Santarelli L, Gobbi G, Debs PC, Sibille ET, Blier P *et al*. (2001)-please give six authors before *et al*. |
| | | Sargent PA, Kjaer KH, Bench CJ, Rabiner EA, Messa C *et al* (2000).-please give six authors before *et al*. |
| | | Shimkets RA, Lowe DG, Tai JT, Sehl P, Jin H *et al*. (1999)-please give six authors before *et al*. |
| | | Sutcliffe JG, Foye PE, Erlander MG, Hilbush BS, Bodzin LJ *et al*. (2000).-please give six authors before *et al*. |
| | | Trillat AC, Malagie I, Mathe-Allainmat M, Anmella MC, Jacquot C *et al*. (1998)-please give six authors before *et al*. |