

Antisense Inhibition of 5-Hydroxytryptamine_{2A} Receptor Induces an Antidepressant-Like Effect in Mice

ETIENNE SIBILLE, ZOLTAN SARNYAI, DANIEL BENJAMIN, JUDIT GAL, HARRIET BAKER, and MIKLOS TOTH

Department of Pharmacology, Cornell University Medical College, New York, New York 10021 (E.S., J.G., M.T.), Laboratories of Neuroendocrinology and Biology of Addictive Diseases, Rockefeller University, New York, New York 10021 (Z.S.), Division of Neuropharmacology, Center of Alcohol Studies, Rutgers University, Piscataway, New Jersey 08855 (D.B.), and Cornell University Medical College, Burke Medical Research Institute, White Plains, New York 10605 (J.G., H.B.)

Received May 27, 1997; Accepted September 6, 1997

SUMMARY

Treatment with different antidepressants is invariably accompanied by the down-regulation of the 5-hydroxytryptamine_{2A} (5-HT_{2A}) receptor. To determine whether receptor down-regulation is an essential part of antidepressant action, we manipulated levels of the 5-HT_{2A} receptor by using a nonpharmacological approach. Here, we report that down-regulation of the 5-HT_{2A} receptor by intracerebroventricular injection of antisense oligonucleotides resulted in an antidepressant-like effect in mice. Animals with 5-HT_{2A} receptor deficiency showed less immobility in the Porsolt's forced swim test, a well established animal model that is used to identify drugs with an antidepressant effect. The overall locomotor activity of the receptor-deficient animals was not altered, demonstrating the specificity of

the behavioral change in the Porsolt's forced swim test. Reduced immobility in this test was accompanied by a greater c-Fos response in piriform cortex. Because 5-HT_{2A} receptors have been localized on γ -aminobutyric acid interneurons, the inhibitory activity of these neurons may be impaired at low receptor levels, leading to a greater c-Fos response in the piriform cortex and increased mobility in the Porsolt's forced swim test. These experiments demonstrate that down-regulation of the 5-HT_{2A} receptor alone is sufficient to achieve an antidepressant-like effect in mice and suggest that receptor down-regulation may be an essential part of the antidepressant drug action.

A variety of different compounds have been found to have antidepressant activity. Although the pharmacological actions of these antidepressants are prompt, the clinical effects require weeks or even months to become manifest (see review in Ref. 1). This delayed response suggests that the development of antidepressant effect requires a plastic change in brain initiated by the drug treatment. A representative change, induced by long term antidepressant treatment, is the modulation of the 5-HT_{2A} receptor. Virtually all antidepressants down-regulate the level of 5-HT_{2A} receptor, and this down-regulation is temporally correlated with the onset of clinical efficacy (2–5). Antidepressants that down-regulate the 5-HT_{2A} receptor include tricyclics, SSRIs, monoamine oxidase inhibitors, and atypical antidepressants such as mianserin (3). Tricyclic antidepressants block both norepinephrine and 5-HT uptake; SSRIs inhibit 5-HT transport; and monoamine oxidase inhibitors prevent the inactivation of monoamines. These drugs can be considered indirect agonists because they increase the availability of monoamines,

especially 5-HT, in synaptic cleft. Increased levels of 5-HT in turn may initiate receptor down-regulation (6, 7). It is paradoxical that mianserin, a 5-HT_{2A/2C} receptor antagonist with an antidepressant effect also elicits receptor down-regulation (2) because chronic treatment with antagonists generally induces disuse supersensitivity, a state characterized by an increase in receptor density (8). Nevertheless, the extent of down-regulation with mianserin is comparable to that achieved by tricyclics and SSRIs (2, 9).

Because treatment with different antidepressants modulates the level of 5-HT_{2A} receptor, it is intriguing to hypothesize that receptor down-regulation is involved in or even required for the development of antidepressant effect. If receptor down-regulation is indeed an essential part of the antidepressant drug action, drugs with selectivity to the 5-HT_{2A} receptor could be used to relieve certain symptoms of depression. On the other hand, if receptor down-regulation is not directly linked to the therapeutic effect, drugs that avoid receptor effect may be more selective in the treatment of depression. To determine whether receptor down-regulation is an essential part of antidepressant drug action, the 5-HT_{2A}

This work was supported by a grant from the National Alliance for Research on Schizophrenia and Depression.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; GABA, γ -aminobutyric acid; SSRI, selective serotonin reuptake inhibitor; AS, antisense; MS, mismatched; aCSF, artificial cerebrospinal fluid; LSD, lysergic acid diethylamide; FST, Porsolt's forced swim test; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; PTZ, pentylenetetrazol; PCR, polymerase chain reaction.

receptor was down-regulated directly and specifically by intracerebroventricular injection of a receptor-specific AS oligonucleotide. In contrast to the pharmacological approach, which blocks receptor function by antagonists, the AS approach provides down-regulation of the 5-HT_{2A} receptor number that is more analogous to the effect of chronic antidepressant treatment. Moreover, selectivity of AS oligonucleotides is greater than that of most receptor antagonists. AS oligonucleotides can specifically recognize receptor mRNA, facilitate its degradation, or interfere with its translation, resulting in a reduced receptor level (10). Here, we report that AS-induced down-regulation of the 5-HT_{2A} receptor results in an antidepressant-like effect in mice. This result suggests that down-regulation of 5-HT_{2A} receptor alone may have some therapeutic benefit in the treatment of mood disorders.

Materials and Methods

Oligonucleotide treatment. Balb/C mice (6–8 weeks old) were injected alternatively into the left and right lateral ventricles every 12 hr for 4 days with 10 μ g of either AS (5'-AGACACTTCTGT-TATAGA-3') or MS (5'-AGTCACTGCTGTTATGGA-3') oligonucleotide in 5 μ l of aCSF. The oligonucleotides corresponded to the 5'-translated region of the 5-HT_{2A} receptor. The MS oligonucleotide differed in three positions from the AS oligonucleotide. A control group of mice was injected with 5 μ l of aCSF. All behavioral tests were performed on the fifth day.

For localization of the oligonucleotide, animals (three mice) were injected with a biotin-labeled AS oligonucleotide five times over 2.5 days. Control animals were injected with either aCSF (two mice), unlabeled oligonucleotide (two mice) or 0.2 mM biotin in aCSF (two mice). Twelve hours after the last oligonucleotide injection, animals were anesthetized with pentobarbital sodium (Nembutal Sodium, 150 mg/kg; Abbot Laboratories, King-of-Prussia, PA) and perfused intracardially with 4% paraformaldehyde. Brain sections (40 μ m) were obtained on a freezing microtome, treated with 0.5% H₂O₂ to remove endogenous peroxidase activity, and incubated with the ABC complex of the Vector Elite Kit (1:100) (Vector Laboratories, Burlingame, CA) as well as with substrate, according to the manufacturer's instructions.

Autoradiography. 5-HT_{2A} receptor binding was carried out on brain sections using ¹²⁵I-labeled LSD (50 pM), according to a published procedure (11). 5-HT_{2A}-nonspecific binding (\approx 30% of total) was determined in the presence of 200 nM spiperone. Spiperone blocks both the 5-HT_{2A} and dopamine D₂ receptors. The D₂ receptor component of the total binding was 19% in cortical layers and 26% in striatum, as determined by competitive displacement on parallel sections with increasing concentrations of haloperidol (IC₅₀ = 0.4 nM). The 5-HT_{2A} receptor-specific component of binding was calculated from the total binding by subtracting nonspecific and D₂ receptor-specific binding. Sections were exposed overnight to Hyperfilm (Amersham, Arlington Heights, IL), and computed densitometry was performed with the NIH Image program. Quantification was based on a series of ¹²⁵I-autoradiographic internal standards (Amersham).

5-HT_{2C} receptor binding was measured in the choroid plexus, a region abundant in these receptors, by using ¹²⁵I-labeled LSD in the presence of spiperone. Because 200 nM spiperone displaced 5-HT_{2A} receptor binding (IC₅₀ = 8 nM) as well as D₂ receptor binding, the remaining signal was solely due to 5-HT_{2C} receptor binding. Nonspecific binding was measured in the presence of 2 μ M mianserin. Mianserin displaces 5-HT_{2A}, 5-HT_{2C}, and D₂ receptor binding. Sections were exposed for 8 days.

c-Fos immunohistochemistry. The assay was performed as described previously (12). Two hours after FST, animals were anesthetized with pentobarbital sodium (150 mg/kg) and perfused intra-

cardially with 4% paraformaldehyde. Free floating sections (40 μ m) were incubated with a c-Fos antiserum (Fos and related antigens, 1:8000 dilution; Cambridge Research Biochemicals, Northwich, UK). The antigen was visualized with the ABC Vector Elite Kit. c-Fos immunoreactive nuclei were counted on parallel slides with a bright-field microscope at 10 \times magnification. c-Fos-positive nuclei were counted for the whole frontal piriform cortex, without correction, in three successive sections per mouse brain. The numbers of animals involved in this test were four for aCSF, five for MS, and five for AS.

Quantitative RT-PCR. RT-PCR was performed essentially as described previously (13). Briefly, total RNA was prepared from frontal cortex by using TRIZOL Reagent (GIBCO BRL, Gaithersburg, MD). Then, 9 μ g of total RNA was incubated with 1 unit of RNase-free DNase I (GIBCO BRL) in the presence of 20 units of RNasin ribonuclease inhibitor (Promega, Madison, WI) to remove any remaining genomic DNA. The DNA-free RNA was reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (GIBCO BRL) using a primer corresponding to bases -381 to -399 in the 5'-untranslated region of the 5-HT_{2A} receptor (5'-AAACAG-CATGAGATCCAA-3'). Reverse-transcribed cDNA, corresponding to 62, 31, and 15.5 ng of total RNA, was used for PCR amplification. Primers complementary to bases -381 to -399 (see sequence above) and -781 to -761 (5'-CTCAAAGAGAGGGGATTCCACA-3') yielded a 400-bp product. During PCR, [³²P]dATP was incorporated into the product to allow for quantification in a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Behavioral studies. Headshakes were registered during a 10-min period at 30 min after a 2.5 mg/kg intraperitoneal dose of DOI. In FST, mice were forced to swim in a 8-in wide water-filled cylinder, essentially as described by Porsolt *et al.* (14). In this test, immobility of the mice is measured in blocks of 2 min for a total of 6 min. All animals were naive and submitted only once to each test. The open field apparatus consisted of a 15- \times 21-inch black box divided into six (2- \times 3-inch) even-sized rectangles. The number of crosses in open field was recorded for 10 min. The elevated plus maze was performed according to standard procedures (15) using a cross-maze with 12- \times 2-inch arms. The number of entries into and time spent in the open arms as well as the total number of entries were recorded in a 10-min test. Care of all mice in this work was in accordance with institutional guidelines.

Seizure susceptibility measured by PTZ test. Mice were injected with 85 mg/kg PTZ intraperitoneally, and seizure events were videotaped during an observation period of 20 min. Mice were scored for the sequence of four seizure behaviors produced by the drug (i.e., myoclonic jerks, clonic convulsions, tonic phase, and death). A human-guided computer-assisted scoring system was used to evaluate seizures (16). The timing of the seizure behaviors, as well as their duration after the PTZ injection, was recorded. Myoclonus was defined as a single movement of the mouse that involved a downward motion of the head, combined with a single jerk of the body, and a brief upward extension of the tail. Clonus usually was the second seizure behavior to occur chronologically after PTZ injection; it was defined as rapidly repetitive jerks of the mouse that involved the entire body such that the mouse would fall to the side. The tonic stage of the seizure, when reached, was defined as a slow hindlimb extension. In these mice, the tonic stage was invariably followed by death.

Statistical analysis. Performance in behavioral studies, absorbance of autoradiographs, and c-Fos positive nuclei were compared between groups using a one-way analysis of variance followed by Scheffé's *post hoc* test. Statistical difference was determined at a level of $p < 0.05$. For autoradiography and c-Fos quantification, multiple successive sections were quantified in each sector sampled, and the average for this sector was calculated in each mouse. The results of the PTZ test were calculated by nonparametric analysis of variance followed by the χ^2 test.

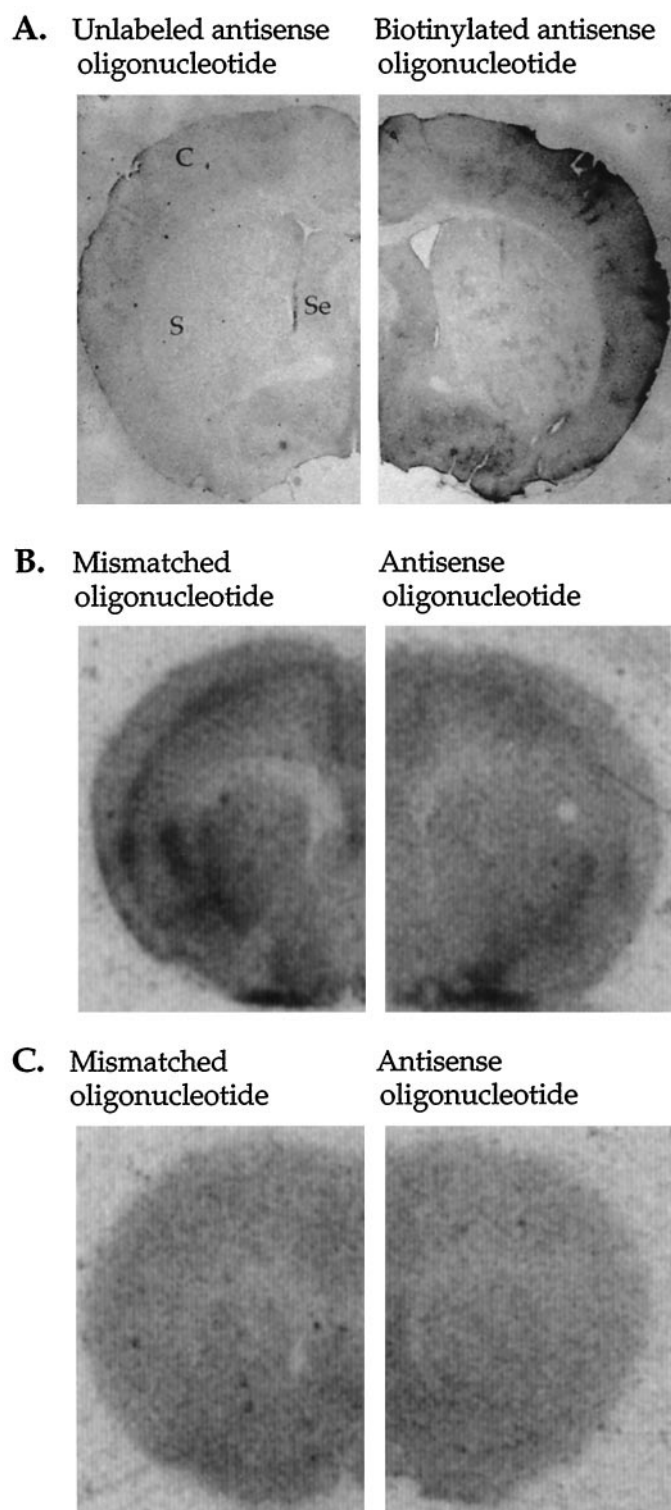


Fig. 1. Intracerebroventricular injection of oligonucleotides into mouse brain. A, Biotin-labeled AS oligonucleotide administered alternatively to the left and right lateral ventricles (*right*) is taken up preferentially by the cortex (C). The oligonucleotide was visualized by a peroxidase enzymatic reaction. Subcortical regions such as striatum (S) and septum (Se) accumulated less oligonucleotide compared with the cortex. Thalamus and hypothalamus are not visible at the anatomic level displayed but also contained low levels of the administered oligonucleotide. No specific staining was found in brains of animals injected with unlabeled oligonucleotide (*left*). B, AS oligonucleotide injection down-regulated cortical 5-HT_{2A} receptors (*right*), as measured by receptor autoradiography using ¹²⁵I-labeled LSD. Results of the measurements on regional

Results

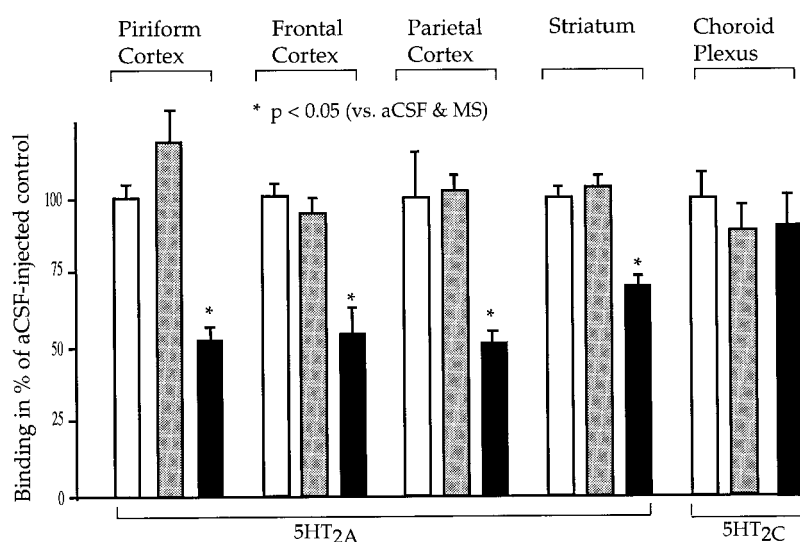
AS oligonucleotide treatment selectively down-regulates 5-HT_{2A} receptor levels and attenuates receptor function. In the brain, 5-HT_{2A} receptors are found mostly in the cortical layers and striatum. Because the most dramatic antidepressant-induced receptor down-regulation occurs in the cortex (3), we tested whether oligonucleotides injected into the lateral ventricles reached the cortical layers. A biotin-labeled oligonucleotide was repeatedly injected (intracerebroventricular injections every 12 hr for 2 days), and its localization was visualized by peroxidase enzymatic staining (Fig. 1A). A predominantly cortical distribution was observed, presumably due to the transport of the oligonucleotide to the subarachnoid space that overlies the entire cortex and provides a large surface for uptake. Subcortical regions such as striatum or thalamus accumulated fewer oligonucleotides. Intermediate levels were found along the midline, in the septum, and in the hypothalamus. The highest levels of oligonucleotides were found in the choroid plexus, presumably because it was in a direct contact with the oligonucleotides injected into the lateral ventricles. Mice injected with unlabeled oligonucleotide (Fig. 1A), aCSF, or biotin alone (data not shown) did not show any detectable staining.

Repeated intracerebroventricular injection of a 18-mer AS oligodeoxynucleotide, corresponding to the 5'-translated region of the 5-HT_{2A} receptor (10 μg in 5 μl of aCSF every 12 hr for 4 days), resulted in 47%, 46%, and 48% decreases in specific binding in frontal, parietal, and piriform cortices, as measured by autoradiography with ¹²⁵I-labeled LSD (Figs. 1B and 2A). Because the central nervous system has a low nuclease activity, unmodified phosphodiester oligonucleotides were used, avoiding the toxicity inherent to the more stable phosphorothioate oligonucleotides. Injection of neither MS oligonucleotides (three mismatched bases in the AS sequence) nor aCSF altered receptor binding. In the striatum, the down-regulation by the AS oligonucleotide was only 30%, which is in agreement with the lower level of oligonucleotide accumulation in this region (Figs. 1A and 2A). Other regions, such as thalamus, hypothalamus, and hippocampus, showed a low level of specific binding, and the effect of AS oligonucleotide in these regions could not be reproducibly measured.

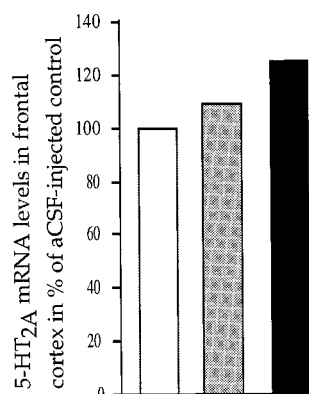
To evaluate the specificity of the AS oligonucleotide for the 5-HT_{2A} receptor, we tested its effect on the 5-HT_{2C} receptor. The 5-HT_{2A} and 5-HT_{2C} receptors are closely related; their ligand binding properties, coupling, and amino acid sequence are similar (17). However, their nucleotide sequences are not entirely identical, and the AS oligonucleotide was selected so it would not interact with the 5-HT_{2C} receptor sequence. 5-HT_{2C} receptor binding was measured in the choroid plexus, a region abundant in these receptors. Because the choroid plexus contained the highest level of oligonucleotide, it was an ideal brain region in which to assess whether the 5-HT_{2A} receptor-specific oligonucleotide can alter 5-HT_{2C} receptor binding. As Fig. 2A shows, no attenuation of the 5-HT_{2C} receptor was measured after repeated oligonucleotide injection; thus, the effect of AS oligonucleotide was selective for the 5-HT_{2A} receptor.

5-HT_{2A} receptor concentration are displayed in Fig. 2. C, Nonspecific binding in the presence of 200 nM spiperone.

A.



B.



C.

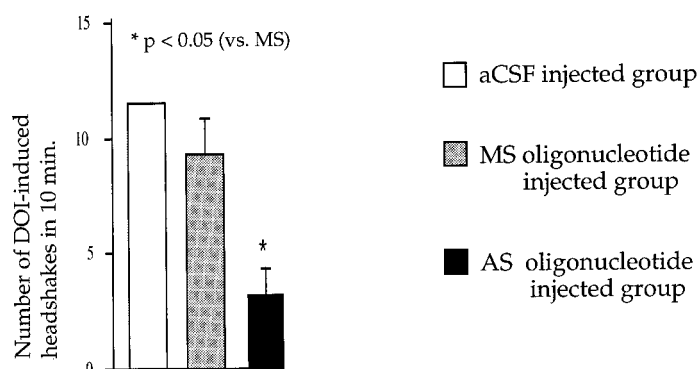


Fig. 2. Receptor down-regulation and functional receptor deficit in AS oligonucleotide-injected animals. A, Receptor down-regulation in cortical regions of AS oligonucleotide-injected animals measured by receptor autoradiography. Striatum showed a less profound but statistically significant receptor down-regulation. The level of the closely related 5-HT_{2C} receptor was not significantly altered, demonstrating the selectivity of the down-regulation for the 5-HT_{2A} receptor. Values (mean \pm standard error) are shown for aCSF (five animals), MS (six animals), and AS (six animals) oligonucleotide-injected animals. B, The 5-HT_{2A} receptor mRNA level was not affected by AS oligonucleotide injection in cortex as measured by reverse transcription PCR from total RNA derived from frontal cortex. Values are mean of four RNA samples isolated from two animals in each group. C, Down-regulation of the 5-HT_{2A} receptor resulted in a functional receptor deficit. The number of DOI-induced headshakes, mediated by 5-HT_{2A} receptors, was significantly decreased in AS oligonucleotide-injected animals compared with MS oligonucleotide- and aCSF-injected mice. Values (mean \pm standard error) are shown for aCSF (two animals), MS (six animals), and AS (four animals) oligonucleotide-injected animals.

Interestingly, attenuation of receptor binding by AS oligonucleotide injection in the frontal cortex was not accompanied by the down-regulation of 5-HT_{2A} receptor mRNA (Fig. 2B), suggesting a post-transcriptional/translational mechanism for the AS oligonucleotide action. To determine whether the receptor down-regulation was accompanied by a functional attenuation, we measured the number of DOI-induced headshakes (18). Headshakes induced by DOI are thought to originate in the brainstem and be facilitated by diencephalic structures (19), but alterations in 5-HT_{2A} receptor density in frontal cortex can also modify the headshake response (20, 21). Headshakes induced by DOI were significantly decreased in AS oligonucleotide-injected animals compared

with control mice (Fig. 2C). Although the decreased headshake response cannot be attributed to a specific pool of receptors, it nevertheless demonstrated a functional 5-HT_{2A} receptor deficit in the brain of AS oligonucleotide-injected mice.

Taken together, these results demonstrated that repeated intracerebroventricular injection of AS oligonucleotide can selectively decrease 5-HT_{2A} receptor binding, mostly in the cortex, leading to the attenuation of receptor function.

Receptor down-regulation increases mobility in FST. FST is an animal model of depression that is used routinely for preclinical testing of antidepressants (14). FST is sensitive to tricyclic antidepressants, monoamine oxidase

inhibitors, and atypical antidepressants. FST is also sensitive to SSRIs in both mice and rats (22); however, some SSRIs are less active and sometimes even inactive in rats (23). The test is based on the observation that when forced to swim in a restricted space from which there is no escape, mice will gradually cease attempts to escape and become immobile. It was suggested that immobility reflects a state of despair that can be reduced by a variety of drugs and treatments that are therapeutically effective in depression (22).

As Fig. 3A shows, immobility of AS oligonucleotide-injected animals was reduced significantly compared with control animals (aCSF and MS oligonucleotide-injected animals). The test was validated by using the atypical antidepressant mianserin at the highest nonsedative dose of 3 mg/kg. As expected, mianserin had an anti-immobility effect in FST comparable to that induced by AS oligonucleotide injection (Fig. 3B). However, mianserin can block both the 5-HT_{2A} and 5-HT_{2C} receptors. On the other hand, the selective 5-HT_{2A}

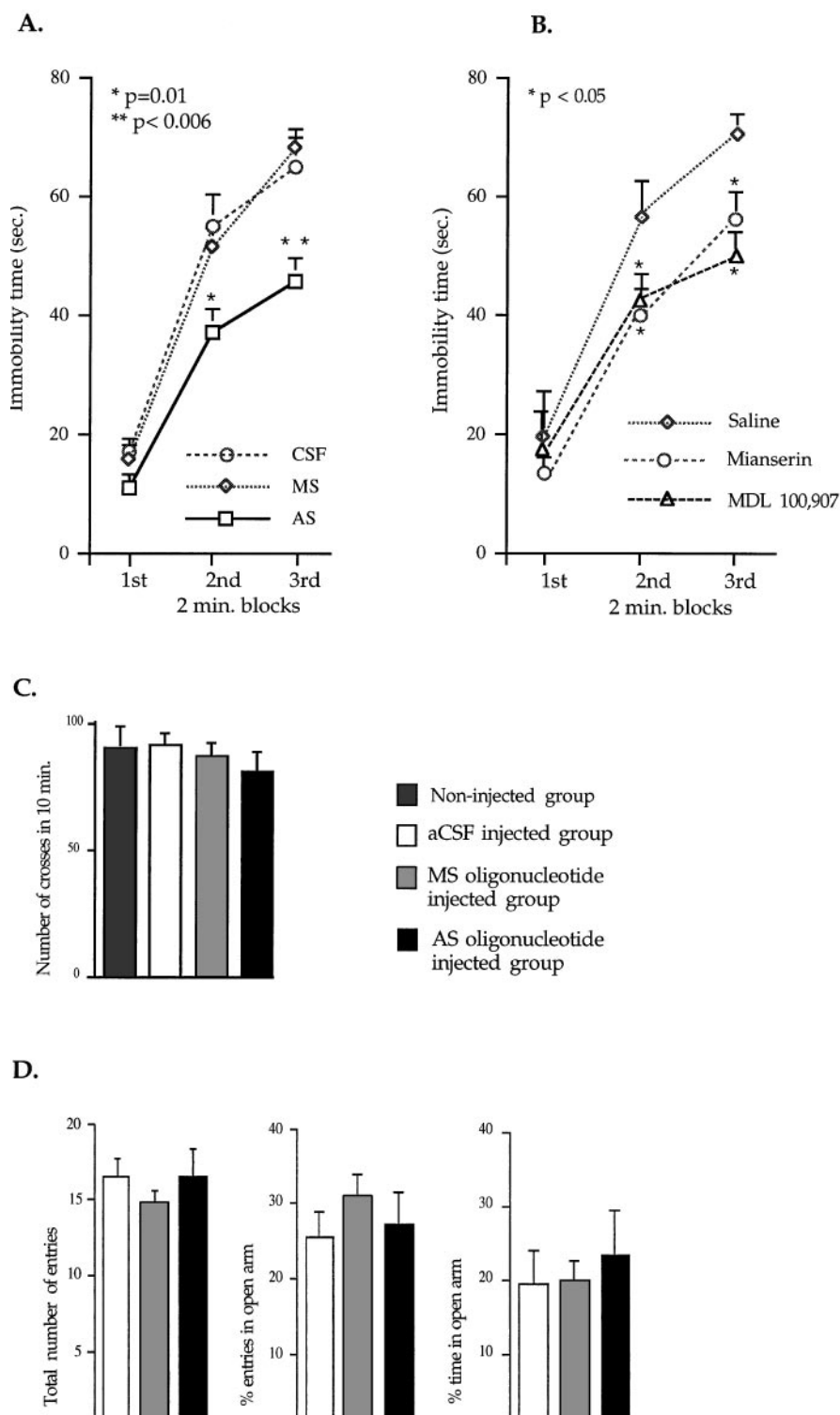


Fig. 3. Behavioral characterization of mice with a deficit in cortical 5-HT_{2A} receptor. A, FST. Animals exposed to swim stress show an intense escape-directed behavior in the first block of the test. aCSF and MS oligonucleotide-injected animals subsequently display a gradually diminishing mobility throughout the second and third blocks of the test. AS oligonucleotide-injected animals showed less immobility. Values (mean \pm standard error) are shown for aCSF (13 animals), MS (20 animals), and AS (22 animals) oligonucleotide-injected animals. B, Mianserin (3 mg/kg; 10 animals) and MDL 100907 (0.15 mg/kg; 7 animals) reproduce the AS oligonucleotide-induced behavioral effect in FST. Control animals were injected with saline (10 animals). C, Locomotor activity of AS oligonucleotide-injected animals is not altered in the open field (noninjected, 8 animals; aCSF, 8 animals; MS, 8 animals; AS, 9 animals). D, Elevated maze. AS-treated animals did not differ from control groups in percentage of time and entries in the open arms as well as in total number of entries in all arms of the maze (aCSF, 14 animals; MS, 17 animals; AS, 19 animals).

receptor antagonist MDL 100,907 (24) was also effective in FST, demonstrating that blockade of the 5-HT_{2A} receptor can lead to an anti-immobility effect (Fig. 3B).

The anti-immobility effect in AS oligonucleotide-injected animals was not due to increased basal locomotion because activity of these animals in open field was similar to that of control mice (Fig. 3C). In addition, receptor down-regulation had no detectable effect in the elevated maze (Fig. 3D). We conclude that a reduction in 5-HT_{2A} receptor level is sufficient to increase mobility in FST.

Increased c-Fos response of AS oligonucleotide-injected animals in the piriform cortex. FST induces the expression of the immediate-early gene *c-fos*, indicating an extensive neuronal activation during swimming (25). Using immunohistochemistry directed toward the c-Fos protein and related antigens, we observed no difference between AS and MS oligonucleotide-injected animals in subcortical nuclei (lateral septal nucleus, bed nucleus of the stria terminalis, and hypothalamic and thalamic paraventricular nuclei; data not shown). However, a greater increase in immunoreactivity was found in the piriform cortex of AS oligonucleotide-injected animals compared with control animals [$150 \pm 11.7\%$ of control (mean \pm standard error), $p < 0.005$] (Fig. 4). The FST-induced c-Fos response was not significantly increased in other cortical regions of AS oligonucleotide-injected animals compared with control (MS- and aCSF-injected) mice (data not shown).

The increased c-Fos response in AS oligonucleotide-injected animals after the forced swim raised the possibility of a general hyperexcitability as a consequence of down-regulation of the 5-HT_{2A} receptor. The overall excitability of the brain of AS oligonucleotide-injected mice was measured by using the seizure-inducing agent PTZ. PTZ seems to decrease

the potency of GABA-mediated inhibition in brain (26) and, depending on the dosage, can produce myoclonic jerks, clonic convulsions, tonic seizures, and death in animals. The 85 mg/kg dose of PTZ used in this experiment was equally potent to produce myoclonic jerks and clonic convulsions in both AS and MS oligonucleotide-injected animals (Table 1). However, there was a tendency for more lethality among AS oligonucleotide-injected mice, albeit the difference between AS oligonucleotide-injected and control animals did not reach statistical significance. These results argue for no increased excitability in AS oligonucleotide-injected animals, at least not in brain regions involved in initiating PTZ seizures, such as the reticular formation and the anterior thalamus (27, 28). These data also suggest that increased neuronal activation in AS oligonucleotide-injected animals is restricted to certain regions that may correspond to areas rich in 5-HT_{2A} receptor.

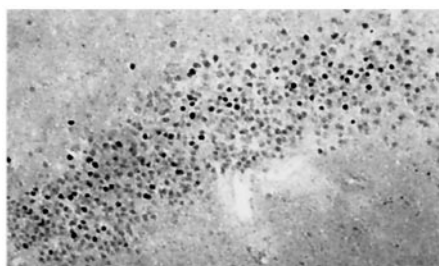
Discussion

The down-regulation of the 5-HT_{2A} receptor by virtually all antidepressants raised the possibility of receptor involvement in drug action. However, antidepressants down-regulate the 5-HT_{2A} receptor by different mechanisms, and it has been difficult to determine whether the receptor down-regulating and therapeutic effect of antidepressants are linked. For example, fluoxetine, a 5-HT transporter blocker and effective antidepressant probably has no direct action on the receptor itself; rather, it is the elevated level of 5-HT that may down-regulate the receptor. In contrast, mianserin, a 5-HT_{2A/2C} receptor antagonist and atypical antidepressant presumably acts on the receptor itself (29).

To study whether down-regulation of the receptor leads to an antidepressant-like effect, the level of 5-HT_{2A} receptor was reduced by injecting AS oligonucleotides into the brain of mice. The antisense approach provided down-regulation of the 5-HT_{2A} receptor that was analogous to the reduction in receptor number induced by chronic antidepressant treatment. Even the extent of the down-regulation of the receptor in the frontal cortex by AS oligonucleotide injection ($\approx 47\%$, Figs. 1 and 2) was similar to that achieved by antidepressant treatment (up to 50%) (3, 30). The effect of AS oligonucleotide injection was selective for the 5-HT_{2A} receptor because no attenuation of the 5-HT_{2C} receptor was detectable (Fig. 2A). Receptor down-regulation was accompanied by a functional attenuation because DOI-induced headshakes were significantly decreased in AS oligonucleotide-injected animals (Fig. 2C).

As Fig. 3A demonstrates, down-regulating the level and attenuating the function of the 5-HT_{2A} receptor by intracerebroventricular injection of a receptor-specific AS oligonucleotide resulted in a significant change in the behavioral response of mice. Receptor down-regulation inhibited immobility in FST, which is an indication of an antidepressant effect. The extent of anti-immobility effect in receptor-deficient animals was comparable to that induced by the atypical antidepressant mianserin (Fig. 3B). Clinically active antidepressants have an anti-immobility effect in FST without altering locomotor activity in open field. Likewise, no locomotor activation was seen in AS oligonucleotide-injected animals (Fig. 3C). Taken together, experiments involving AS oligonucleotide injections demonstrate that down-regulation of the

Antisense oligonucleotide



Mismatched oligonucleotide

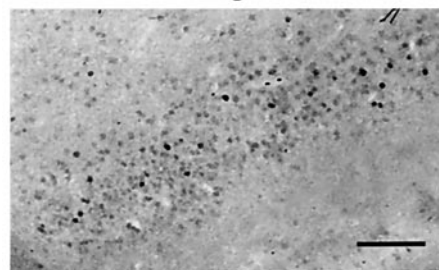


Fig. 4. Increased c-Fos response after FST in the piriform cortex of a mouse with a deficit in 5-HT_{2A} receptors (*top*, AS oligonucleotide-injected animal) compared with a control (*bottom*, MS oligonucleotide-injected animal). Bar, 40 μ m. Number of c-Fos-positive nuclei/0.01 mm² were 66 ± 5.6 for AS, 44.3 ± 3.4 for MS, and 48 ± 5.0 for aCSF.

TABLE 1

Susceptibility of AS oligonucleotide-injected and control animals to PTZ-induced seizures

	aCSF (n = 8)	MS (n = 8)	AS (n = 8)
Animals displaying jerks (n)	7	8	7
Mean number of jerks in 20 min	50 ± 10	57 ± 2	47.4 ± 9.9
Time spent in jerks in 20 min (sec)	28.9 ± 10.8	39 ± 11	34.7 ± 15
Animals displaying clonus (n)	7	8	7
Mean number of clonus in 20 min	1.4 ± 0.3	2 ± 0.5	1.4 ± 0.9
Time spent in clonus in 20 min (sec)	15.3 ± 5.6	15.3 ± 4	13.4 ± 5.7
Animals displaying tonic seizures (n)	1	2	4 ^{a,b}
Animals that died (n)	1	2	4 ^{a,b}

^a $p = 0.10$ versus aCSF; ^b $p = 0.3$ versus MS, χ^2 test.

receptor alone is sufficient to achieve an antidepressant-like effect in mice.

How does down-regulation of 5-HT_{2A} receptor inhibit immobility in FST? Although it is attractive to interpret immobility as a sign of despair and compare it with depression, there is no evidence to support this notion. Rather, behavior of mice in FST is more similar to a typical response to stress. Initially, stress activates a number of effector systems within the central nervous system that promote arousal and vigilance. However, when stress becomes chronic, the acute behavioral stress responses are gradually diminished, presumably because excessive stress reactions would be counterproductive (exhaustion to swim). The stress nature of FST is supported by the activation of the hypothalamic-pituitary-adrenal axis and the induction of the early-immediate gene *c-fos* after swimming (25, 31). Both AS and MS oligonucleotide-injected animals showed an ≈ 4 -fold increase in plasma corticosterone levels 10 min after the initiation of FST (data not shown). Also, the temporal and spatial patterns of *c-fos* activation were very similar to that induced by stressful stimuli such as restrain (Ref. 25; see also Fig. 4). Therefore, the gradually developing immobility in FST can represent a containment process (32) rather than a despair. Whether the behavior in FST is a stress response or more of a despair-like reaction, the data presented here unequivocally demonstrate that it can be significantly altered by the 5-HT_{2A} receptor.

The anti-immobility effect of receptor down-regulation may be explained by the specific localization of the receptors in the central nervous system. 5-HT is present at the terminal area of fine 5-HT immunoreactive axons in the cortex that arise from the dorsal raphe nucleus (33). In these areas, 5-HT_{2A} receptors are frequently found on interneurons. Based on morphology and electrophysiological properties, 5-HT_{2A} receptor-bearing interneurons are likely GABAergic cells (34–36). 5-HT_{2A} receptor-bearing interneurons form a dense band on layer III in the piriform cortex (34) and could provide an inhibitory cortical input. Low 5-HT_{2A} receptor levels in AS oligonucleotide-injected animals may lead to increased pyramidal activity due to less activation of the inhibitory GABAergic interneurons during FST. In turn, the increased neuronal activity could result in an anti-immobility effect by augmenting neuronal pathways that mediate the behavioral stress responses such as the escape-directed behavior.

Although GABAergic interneurons expressing 5-HT_{2A} receptors are also localized in the frontal/parietal cortex, the overall number of these cells represents a relatively small portion of the total cell number in these regions (35). Moreover, the 5-HT_{2A} receptor is also expressed in pyramidal cells

in the frontal/parietal cortex, which could counteract the effect on GABAergic function by activating pyramidal cells directly. Therefore, AS down-regulation of the receptor in neocortex could have less impact on the overall neuronal activity, and this situation would render it undetectable by *c-Fos* immunostaining. The localized nature of increased neuronal activity is also supported by the lack of overall brain hyperexcitability shown in the PTZ test (Table 1). Although not revealed by our immunostaining experiments, a 5-HT_{2A} receptor-mediated tonic inhibition may exist in the medial prefrontal cortex. Schmidt *et al.* (24) showed that blocking 5-HT_{2A} receptors by the selective antagonist MDL 100,907 results in an increase in dopamine efflux in rats. These results raise the possibility that the behavioral effects of the AS oligonucleotide injection may also be due to an increased dopamine release during forced swim. Taken together, the data presented here support the hypothesis that down-regulation of 5-HT_{2A} receptors disinhibits piriform cortex and perhaps other receptor-rich cortical areas in mice that could lead to a state of increased psychomotor activity, visible as anti-immobility effect in FST.

The antidepressant-like effect induced by AS oligonucleotide injection in mice is consistent with the beneficial effect of pharmacological blockade of the 5-HT_{2A} receptor in dysthymic disorders. Studies with ritanserin, a 5-HT_{2A/2C} receptor antagonist, showed that a group of patients with anxiety syndrome felt less tired and more energetic after treatment (37). This observation prompted further studies that showed a benefit of ritanserin in patients with dysthymic disorder characterized by anergy, lack of motivation, and depressive mood (37). It is tempting to speculate that block of 5-HT_{2A} receptors would increase psychomotor activity that could counterbalance the psychomotor retardation present in these patients. However, antidepressant action is certainly more complex than producing a state of psychomotor activation. Nevertheless, psychomotor activation could be an important part of antidepressant drug action. Indeed, sympathomimetic stimulants, such as amphetamine and amphetamine surrogates, are occasionally used as antidepressants.

Another disease characterized by psychomotor retardation is schizophrenia, which may also respond to the manipulation of the 5-HT_{2A} receptor (38). Indeed, the 5-HT_{2A/2C} receptor antagonist ritanserin and the 5-HT_{2A}/D2 receptor antagonist clozapine showed a benefit in schizophrenia, in particular by ameliorating negative symptoms (39, 40).

Taken together, we propose that down-regulation of the 5-HT_{2A} receptor contributes to the beneficial effect of antidepressants by producing a state of increased psychomotor activity. Drugs with selectivity to the 5-HT_{2A} receptor could be used to relieve certain symptoms of depression. Recently,

MDL 100,907, a selective 5-HT_{2A} receptor antagonist, has been developed (40). MDL 100,907, is currently in clinical trial for schizophrenia, but it would be interesting to test this compound in depression, too.

Acknowledgments

MDL 100,907 was a gift from Hoechst Marion Roussel Research Institute, Hoechst Marion Roussel, Inc. (Cincinnati, OH).

References

- Loo, H., and T. Brochier. Long-term treatment with antidepressive drugs. *Ann. Med. Psychol. (Paris)* **153**:190–197 (1995).
- Blackshear, M. A., and E. Sanders-Bush. Serotonin receptor sensitivity after acute and chronic treatment with mianserin. *J. Pharmacol. Exp. Ther.* **221**:303–308 (1982).
- Peroutka, S. J., and S. H. Snyder. Long-term antidepressant treatment decreases spiroperidol-labeled serotonin receptor binding. *Science (Washington D. C.)* **210**:88–90 (1980).
- Peroutka, S. J., and S. H. Snyder. Regulation of serotonin₂ (5-HT₂) receptors labeled with [³H]spiroperidol by chronic treatment with the antidepressant amitriptyline. *J. Pharmacol. Exp. Ther.* **215**:582–587 (1980).
- Goodwin, G. M., A. R. Green, and P. Johnson. 5-HT₂ receptor characteristics in frontal cortex and 5-HT₂ receptor-mediated head-twitch behaviour following antidepressant treatment to mice. *Br. J. Pharmacol.* **83**:235–242 (1984).
- Hadcock, J. R., H. Y. Wang, and C. C. Malbon. Agonist-induced destabilization of β -adrenergic receptor mRNA: attenuation of glucocorticoid-induced up-regulation of β -adrenergic receptors. *J. Biol. Chem.* **264**:19928–19933 (1989).
- Collins, S., M. G. Caron, and R. J. Lefkowitz. From ligand binding to gene expression: new insights into the regulation of G-protein-coupled receptors. *Trends Biochem. Sci.* **17**:37–39 (1992).
- Samanin, R., T. Mennini, A. Ferraris, C. Bendotti, and F. Borsini. Hyper- and hyposensitivity of central serotonin receptors: [³H]serotonin binding and functional studies in the rat. *Brain Res.* **189**:449–457 (1980).
- Roth, B. L., and R. D. Ciaranello. Chronic mianserin treatment decreases 5-HT₂ receptor binding without altering 5-HT₂ receptor mRNA levels. *Eur. J. Pharmacol.* **207**:169–172 (1991).
- Wahlstedt, C. Antisense oligonucleotide strategies in neuropharmacology. *Trends Pharmacol. Sci.* **15**:42–46 (1994).
- Pazos, A., A. Probst, and J. M. Palacios. Serotonin receptors in the human brain. IV. Autoradiographic mapping of serotonin-2 receptors. *Neuroscience* **21**:123–139 (1987).
- Weiser, M., H. Baker, T. C. Wessel, and T. H. Joh. Differential spatial and temporal gene expression in response to axotomy and deafferentation following transection of the medial forebrain bundle. *J. Neurosci.* **13**:3472–3484 (1993).
- Toth, M., J. Grimsby, G. Buzsaki, and G. P. Donovan. Epileptic seizures caused by inactivation of a novel gene, jerky, related to centromere binding protein-B in transgenic mice. *Nat. Genet.* **11**:71–75 (1995).
- Porsolt, R. D., A. Bertin, and M. Jalfre. Behavioral despair in mice: a primary screening test for antidepressants. *Arch. Int. Pharmacodyn. Ther.* **229**:327–336 (1977).
- Pellow, S., P. Chopin, S. E. File, and M. Briley. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods* **14**:149–167 (1985).
- Donovan, G. P., C. Harden, J. Gal, L. Ho, E. Sibille, R. Trifiletti, L. J. Gudas, and M. Toth. Sensitivity to Jerky gene dosage underlies epileptic seizures in mice. *J. Neurosci.* **17**:4562–4569 (1997).
- Julius, D. Molecular biology of serotonin receptors. *Annu. Rev. Neurosci.* **14**:335–360 (1991).
- Pranzatelli, M. R. Evidence for involvement of 5-HT₂ and 5-HT_{1C} receptors in the behavioral effects of the 5-HT agonist 1-(2,5-dimethoxy-4-iodophenyl aminopropane)-2 (DOI). *Neurosci. Lett.* **115**:74–80 (1990).
- Bedard, P., and C. J. Pycock. 'Wet-dog' shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology* **16**:663–670 (1977).
- Biegón, A., and M. Israeli. Quantitative autoradiographic analysis of the effects of electroconvulsive shock on serotonin-2 receptors in male and female rats. *J. Neurochem.* **48**:1386–1391 (1987).
- Moorman, J. M., D. G. Grahame-Smith, S. E. Smith, and R. A. Leslie. Chronic electroconvulsive shock enhances 5-HT₂ receptor-mediated head shakes but not brain C-fos induction. *Neuropharmacology* **35**:303–313 (1996).
- Porsolt, R. D., A. Bertin, N. Blavet, M. Deniel, and M. Jalfre. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur. J. Pharmacol.* **57**:201–210 (1979).
- Borsini, F. Role of the serotonergic system in the forced swimming test. *Neurosci. Biobehav. Rev.* **19**:377–395 (1995).
- Schmidt, C. J., C. K. Sullivan, and G. M. Fadayel. Blockade of striatal 5-hydroxytryptamine₂ receptors reduces the increase in extracellular concentrations of dopamine produced by the amphetamine analogue 3,4-methylenedioxymethamphetamine. *J. Neurochem.* **62**:1382–1389 (1994).
- Cullinan, W. E., J. P. Herman, D. F. Battaglia, H. Akil, and S. J. Watson. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience* **64**:477–505 (1995).
- Wilson, W. A., and A. V. Escueta. Common synaptic effects of pentylenetetrazol and penicillin. *Brain Res.* **72**:168–171 (1974).
- Velasco, F., M. Velasco, F. Estrada-Villanueva, and J. P. Machado. Specific and nonspecific multiple unit activities during the onset of pentylenetetrazol seizures. I. Intact animals. *Epilepsia* **16**:207–214 (1975).
- Mirski, M. A., and J. A. Ferrendelli. Anterior thalamic mediation of generalized pentylenetetrazol seizures. *Brain Res.* **399**:212–223 (1986).
- Toth, M., and T. Sherk. Antagonist-mediated down-regulation of 5-hydroxytryptamine type 2 receptor gene expression: modulation of transcription. *Mol. Pharmacol.* **45**:1095–1100 (1994).
- Blackshear, M. A., L. L. Martin, and E. Sanders-Bush. Adaptive changes in the 5-HT₂ binding site after chronic administration of agonists and antagonists. *Neuropharmacology* **25**:1267–1271 (1986).
- Young, E. A., S. Akana, and M. F. Dallman. Decreased sensitivity to glucocorticoid fast feedback in chronically stressed rats. *Neuroendocrinology* **51**:536–542 (1990).
- West, A. P. Neurobehavioral studies of forced swimming: the role of learning and memory in the forced swim test. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **14**:863–877 (1990).
- Blue, M. E., K. A. Yagaloff, L. A. Mamounas, P. R. Hartig, and M. E. Molliver. Correspondence between 5-HT₂ receptors and serotonergic axons in rat neocortex. *Brain Res.* **453**:315–328 (1988).
- Sheldon, P. W., and G. K. Aghajanian. Serotonin (5-HT) induces IPSPs in pyramidal layer cells of rat piriform cortex: evidence for the involvement of a 5-HT₂-activated interneuron. *Brain Res.* **506**:62–69 (1990).
- Morilak, D. A., S. J. Garlow, and R. D. Ciaranello. Immunocytochemical localization and description of neurons expressing serotonin₂ receptors in the rat brain. *Neuroscience* **54**:701–717 (1993).
- Marek, G. J., and G. K. Aghajanian. Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100,907, a highly selective 5-HT_{2A} receptor antagonist. *Eur. J. Pharmacol.* **259**:137–141 (1994).
- Reyntjens, A., Y. G. Gelders, M. L. J. A. Hoppenbrouwers, and G. Vanden Busche. Thymosthenic effects of ritanserin (R55667), a centrally acting serotonin-S₂ receptor blocker. *Drug Dev. Res.* **8**:205–211 (1986).
- Busatto, G. F., and R. W. Kerwin. Perspectives on the role of serotonergic mechanisms in the pharmacology of schizophrenia. *J. Psychopharmacol.* **11**:3–12 (1997).
- Leyen, J. E., P. M. Janssen, A. Schotte, W. H. Luyten, and A. A. Megens. Interaction of antipsychotic drugs with neurotransmitter receptor sites in vitro and in vivo in relation to pharmacological and clinical effects: role of 5HT₂ receptors. *Psychopharmacology (Berl)* **112** (Suppl. 1):S40–S54 (1993).
- Kehne, J. H., B. M. Baron, A. A. Carr, S. F. Chaney, J. Elands, D. J. Feldman, R. A. Frank, P. L. van Giersbergen, T. C. McCloskey, M. P. Johnson, D. R. McCarty, M. Poirot, Y. Senyah, B. W. Siegel, and C. Widmaier. Preclinical characterization of the potential of the putative atypical antipsychotic MDL 100,907 as a potent 5-HT_{2A} antagonist with a favorable central nervous system safety profile. *J. Pharmacol. Exp. Ther.* **277**:968–981 (1996).

Send reprint requests to: Dr. Miklos Toth, Department of Pharmacology, LC 519, Cornell University Medical College, 1300 York Avenue, New York, NY 10021. E-mail: mtoth@mail.med.cornell.edu