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Role of 5-HT in Stress, Anxiety, and Depression

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GRAEFF, F. G., F. S. GUIMARÃES, T. G. C. S. DE ANDRADE AND J. F. W. DEAKIN. *Role of 5-HT in stress, anxiety, and depression.* PHARMACOL BIOCHEM BEHAV **54**(1) 129–141, 1996.—There are conflicting results on the function of 5-HT in anxiety and depression. To reconcile this evidence, Deakin and Graeff have suggested that the ascending 5-HT pathway that originates in the dorsal raphe nucleus (DRN) and innervates the amygdala and frontal cortex facilitates conditioned fear, while the DRN-periventricular pathway innervating the periventricular and periaqueductal gray matter inhibits inborn fight/flight reactions to impending danger, pain, or asphyxia. To study the role of the DRN 5-HT system in anxiety, we microinjected 8-OH-DPAT into the DRN to inhibit 5-HT release. This treatment impaired inhibitory avoidance (conditioned fear) without affecting one-way escape (unconditioned fear) in the elevated T-maze, a new animal model of anxiety. We also applied three drug treatments that increase 5-HT release from DRN terminals: 1) intra-DRN microinjection of the benzodiazepine inverse agonist FG 4172, 2) intra-DRN microinjection of the excitatory amino acid kainic acid, and 3) intraperitoneal injection of the 5-HT releaser and uptake blocker D-fenfluramine. All treatments enhanced inhibitory avoidance in the T-maze. D-Fenfluramine and intra-DRN kainate also decreased one-way escape. In healthy volunteers, D-fenfluramine and the 5-HT agonist mCPP (mainly 5-HT_{2C}) increased, while the antagonists ritanserin (5-$HT_{2A/2C}$) and SR 46349B (5-HT_{2A}) decreased skin conductance responses to an aversively conditioned stimulus (tone). In addition, D-fenfluramine decreased, whereas ritanserin increased subjective anxiety induced by simulated public speaking, thought to represent unconditioned anxiety. Overall, these results are compatible with the above hypothesis. Deakin and Graeff have suggested that the pathway connecting the median raphe nucleus (MRN) to the dorsal hippocampus promotes resistance to chronic, unavoidable stress. In the present study, we found that 24 h after electrolytic lesion of the rat MRN glandular gastric ulcers occurred, and the immune response to the mitogen concanavalin A was depressed. Seven days after the same lesion, the ulcerogenic effect of restraint was enhanced. Microinjection of 8-OH-DPAT, the nonselective agonist 5-MeO-DMT, or the 5-HT uptake inhibitor zimelidine into the dorsal hippocampus immediately after 2 h of restraint reversed the deficits of open arm exploration in the elevated plus-maze, measured 24 h after restraint. The effect of the two last drugs was antagonized by WAY-100135, a selective 5-HT_{1A} receptor antagonist. These results are compatible with the hypothesis that the MRN–dorsal hippocampus 5-HT system attenuates stress by facilitation of hippocampal 5-HT_{1A}-mediated neurotransmission. Clinical implications of these results are discussed, especially with regard to panic disorder and depression.

5-HT Dorsal raphe nucleus Median raphe nucleus Dorsal hippocampus Elevated T-maze Elevated plus-maze Restraint stress Conditioned skin conductance responses Simulated public speaking Stress Anxiety Depression

ACUTE or chronic stress arouses emotional states such as anger, anxiety, and depression. Although complex emotional states cannot be reduced to imbalances of a single neurotransmitter, a prominent participation of 5-HT in depression and anxiety is generally acknowledged (25,36,56). Nevertheless, there are many controversies about the exact role 5-HT plays in these conditions. For example, in animal models that produce response inhibition, such as conflict tests, drugs, or brain lesions that reduce 5-HT output have a benzodiazepine-like anxiolytic effect (106). Also, microinjection of 5-HT receptor antagonists into the amygdala releases punished behavior, whereas 5-HT receptor activation increases response suppres-

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sion (47,92). These results support the classical view that 5-HT is anxiogenic (53,111). In contrast to conflict tests, however, models in which animals actively escape or avoid aversive brain stimulation point to an anxiolytic role for 5-HT. Thus, systemic administration of the inhibitor of 5-HT synthesis, PCPA, or of 5-HT antagonists facilitates learned escape from electrical stimulation of the dorsal periaqueductal gray (DPAG) of the rat. In contrast, 5-HT reuptake blockers, the synthesis precursor, 5-HTP, and some 5-HT receptor agonists impair the same behavior. Even more clearly, intra-DPAG administration of 5-HT, 5-HT_{1A}, and 5-$HT_{2A/2C}$ receptor agonists, 5-HT uptake blockers, and 5-HT_{1B} autoreceptor blockers all raise the threshold of aversive electrical stimulation of the DPAG following their microinjection into the same brain structure. Therefore, 5-HT seems to inhibit aversion generated in the DPAG, a brain structure related to anxiety [for a detailed review of the evidence see (34–36,39)].

Since their introduction, antidepressant drugs were thought to enhance 5-HT neurotransmission. As a consequence, depression has been associated with lack of 5-HT (20). Because depression and anxiety often occur together in the same patient, it is hard to reconcile the lack of 5-HT hypothesis of depression with the excess of 5-HT hypothesis of anxiety, chiefly because antidepressants also improve many types of pathologic anxiety, including panic, obsessive compulsive disorder, and even generalized anxiety disorder or GAD (82). Although it has been argued that 5-HT uptake inhibitors may reduce 5-HT release via a predominant (indirect) action on autosomic 5-HT_{1A} receptors that reduce 5-HT neuron firing, results from electrophysiological (9) as well as microdialysis (3) studies show that this reduction is likely to occur only at the early phase of drug administration. This correlates with initial aggravation of anxiety (56,82). When clinical improvement occurs, 5-HT neurotransmission is probably enhanced due to desensitization of autosomic and presynaptic autoreceptors (5-HT_{1B} or 5-HT_{1D}) as well as postsynaptic events increasing responses to 5-HT_{1A} receptor stimulation (22). Nevertheless, because most antidepressant drugs downregulate 5-$HT_{2A/2C}$ receptors in the frontal cortex following chronic administration, it has been argued that these receptors could be supersensitive in disorders like panic and obsessive compulsive disorder (56). If this were true, 5-$HT_{2A/2C}$ receptor antagonists should improve these conditions. This hypothesis has been tested by giving ritanserin to panic disorder patients. The results of two independent studies showed either no effect or even an aggravation of panic disorder by ritanserin given chronically, a regimen that in addition to antagonism is expected to (paradoxically) downregulate 5-$HT_{2A/2C}$ receptors [(29,52), Guimarães, Mabaya, and Deakin, unpublished results]. These observations clearly do not support the 5-HT receptor supersensitivity theory, at least in regard to 5-$HT_{2A/2C}$ receptors.

A THEORETICAL MODEL

To reconcile this seemingly contradictory evidence, Deakin and Graeff (23) suggested that different 5-HT pathways and receptor subtypes modulate the neural substrates of depression, panic, and generalized anxiety, respectively (Fig. 1). According to this assumption, the ascending 5-HT pathway that originates in the dorsal raphe nucleus (DRN), runs along the medial forebrain bundle, and innervates the amygdala and frontal cortex facilitates active escape or avoidance behaviors that occur in response to potential or distal threat (8). These

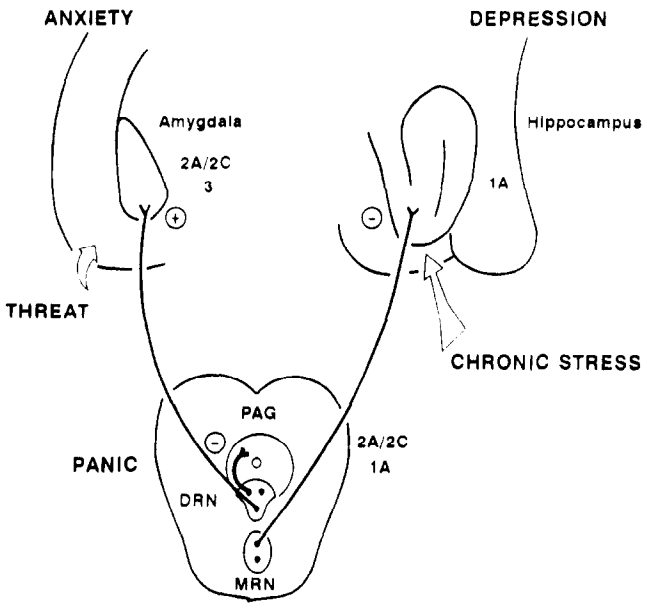

FIG. 1. Schematic representation of the role of 5-HT in the modulation of emotions evoked by acute (threat) or chronic stress. 5-HT neurons in the dorsal raphe nucleus (DRN) are supposed to be activated in threatening situations. Their ascending projections facilitate neurons that evaluate danger in the amygdala and inhibit neurons commanding flight in the dorsal periaqueductal gray (PAG). 5-HT neurons in the median raphe nucleus (MRN) would be activated by persistent and uncontrollable stress. In the hippocampus, 5-HT would promote disconnecting mechanisms that result in more tolerance to stress and, thus, prevent depression.

behavioral strategies rely on learning and, thus, relate to conditioned or anticipatory anxiety and, possibly, GAD. Postsynaptic 5-$HT_{2A/2C}$ and 5-HT_3 receptors are likely to be activated by this pathway. In turn, the DRN-periventricular pathway innervates the periventricular and periaqueductal gray matter. In these regions 5-HT inhibits inborn fight or flight reactions triggered by proximal danger (8), acute pain, or asphyxia that may relate to panic disorder. This function of 5-HT is likely to be mediated by both 5-$HT_{2A/2C}$ and 5-HT_{1A} postsynaptic receptors.

The two pathways discussed above regulate adaptive responses to acute stress. However, there are instances in which aversive stimulation cannot be escaped or avoided, the organism having, thus, to cope with chronic stress. Deakin and Graeff (23) further suggested that the pathway connecting the median raphe nucleus (MRN) to the hippocampus promotes resistance to chronic stress by disconnecting the aversive events from psychobiological processes underlying appetitive and social behaviors, thus allowing the animal or person to lead an almost normal life despite persistent adversity. Depression supervenes when this coping mechanism fails. 5-HT_{1A} receptors are likely to be the main target of the MRN-hippocampal pathway.

The original experimental and clinical evidence supporting these hypotheses has been thoroughly reviewed before (23–25,36). In the following, we summarize the results of recent experiments carried out in laboratory animals as well as in healthy volunteers to test specific predictions derived from the present model.

THE DUAL 5-HT-FEAR HYPOTHESIS

A dual role for 5-HT has been suggested by Deakin and Graeff (23) in the mediation of different types of anxiety. Thus, 5-HT released from nerve terminals from the DRN is supposed to increase learned anxiety at the amygdala, whereas 5-HT released from DRN terminals innervating the DPAG would inhibit unconditioned fear. They argued that a brain system that promotes highly integrated defensive behaviors (in the amygdala) while at the same time inhibiting primitive fight/flight reactions (in the DPAG) would have clear survival value.

Animal Experiments

The elevated T-maze. To test this dual 5-HT-fear hypothesis, a new animal model of anxiety (and memory), named the elevated T-maze, was developed (39,108). The aim was to generate conditioned and unconditioned fear in the same rat. The apparatus is derived from the elevated x or plus-maze (43,91), a widely used animal model of anxiety. The elevated T-maze consists of three arms of equal dimensions, 50 cm above the floor (Fig. 2). One of the arms is enclosed by walls and stands perpendicular to the two open opposed arms. The procedure described below allows measurement of both inhibitory (passive) avoidance of the open arms and one-way escape from one of the open arms in the same animal. The former is supposed to represent learned fear, and the latter, innate fear (78,105). Inhibitory avoidance is assessed by placing the rat at the end of the enclosed arm of the maze and recording the time to withdraw from this arm with the four paws during three consecutive trials. Soon afterwards, the rat is placed at the end of one of the open arms and the time to withdraw from this arm with the four paws, while escaping towards the enclosed arm, is recorded. In validating studies, the BZD anxiolytic diazepam and the 5-HT_{1A} ligand ipsapirone impaired inhibitory avoidance in a dose-dependent way. In contrast, one-way escape behavior remained unchanged (39,108).

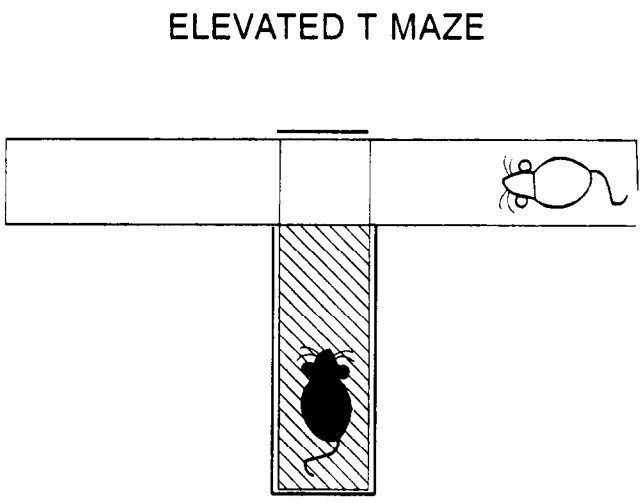

FIG. 2. Schematic representation of the elevated T-maze. An arm enclosed by 40 cm high walls is perpendicular to two open arms. The apparatus is 50 cm above the ground. The rat is placed three times in succession at the end of the enclosed arm for inhibitory (passive) avoidance training. Soon afterwards the same rat is placed at the end of the left open arm to measure one-way escape.

Therefore, two pharmacologically distinct types of fear seem to be generated in the elevated T-maze.

The dual 5-HT-fear hypothesis predicts that inhibition of 5-HT release from DRN terminals will decrease learned anxiety while increasing innate fear. Thus, in the elevated T-maze inhibitory avoidance should be impaired and one-way escape facilitated by drug treatments that inhibit the activity of DRN 5-HT neurons. We tested this prediction by microinjecting the 5-HT_{1A} receptor agonist 8-OH-DPAT into the DRN. Reported results show that this drug markedly decreases the firing rate of 5-HT neurons through stimulation of autosomic receptors (1) and, consequently, reduces 5-HT release from axon terminals (10). The obtained results partially fulfilled the above expectations, because intra-DRN 8-OH-DPAT markedly impaired inhibitory avoidance. Nevertheless, one-way escape remained unchanged (Fig. 3). Interestingly, the anxiolytic drugs diazepam and ipsapirone, given IP, had a similar profile (108). Furthermore, the present results agree with published reports showing that intra-DRN administration of 5-HT_{1A} receptor agonists has anxiolytic effects in animal models that produce response inhibition (46,98).

Conversely, activation of the DRN 5-HT pathways is expected to facilitate inhibitory avoidance, whereas one-way escape is to be impaired. For testing these predictions, we used three ways of increasing 5-HT activity in the territories innervated by the DRN: 1) microinjection of the BZD inverse agonist FG 7142 into the DRN, to counteract tonic GABAergic inhibition of 5-HT neurons (55,102); 2) intra-DRN microinjection of a subtoxic dose of kainic acid, to directly stimulate DRN 5-HT neurons; and 3) systemic administration of D-fenfluramine (75), a drug that seems to release 5-HT selectively from terminals of the DRN (see below).

The results showed that intra-DRN FG 7142 (40 pmol) facilitated inhibitory avoidance, but did not affect one-way escape (37). Kainic acid also enhanced inhibitory avoidance and, in addition, impaired one-way escape (Fig. 4). Intra-DRN administration of the same dose of kainate did not change locomotor activity or rearing in rats placed inside a circular arena (37). Therefore, the increases in both avoidance and escape latencies caused by kainate are unlikely to be due to sedation or motor incapacitation.

Systemically administered D-fenfluramine tended to facilitate inhibitory avoidance, although a clear dose–effect relationship was not obtained. In addition, D-fenfluramine increased one-way escape latencies in a dose-dependent way (Fig. 5).

Thus, with the exception of of the lack of effect of 8-OH-DPAT and of FG 7142 on one-way escape, the changes caused by the drugs tested in the elevated T-maze were predicted by the dual 5-HT-fear hypothesis. The negative results with 8-OH-DPAT and FG 7142 on escape contrasts with the effectiveness of both drugs on avoidance. This may be an indication that the DRN-periventricular 5-HT system has no inhibitory tone, and acts on DPAG neurons controlling defense and aversion in a phasic way. Indeed, this hypothesis has already been suggested on the basis of the absence of behavioral changes following the microinjection of 5-$HT_{2A/2C}$ receptor blockers into the DPAG (35).

In vivo microdialysis. The use of D-fenfluramine in the behavioral test described above, relies on the assumption that this drug releases 5-HT selectively from DRN terminals. In this respect, Mamounas et al. (68) have shown that such nerve endings differ from MRN terminals both morphologically and in the sensitivity to neurotoxic compounds. While DRN termi-

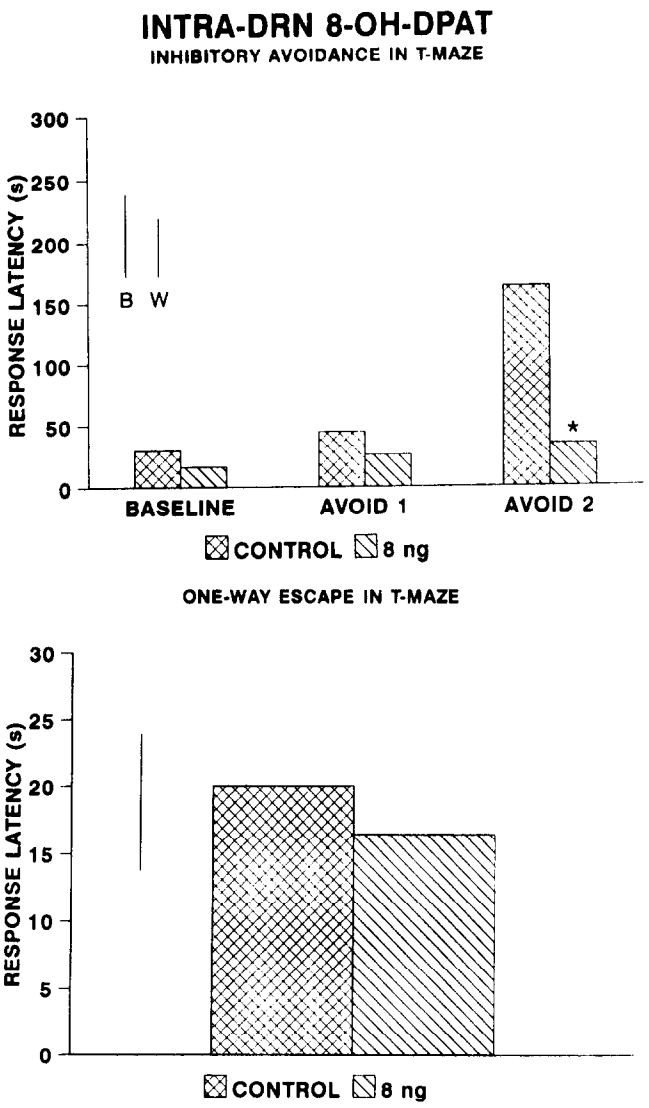

FIG. 3. Effect of intra-DRN 8-OH-DPAT on inhibitory avoidance and one-way escape in the elevated T-maze. Columns represent mean of seven rats. Vertical bars represent 2 SED (B, between group and W, within group comparisons). BASELINE, avoidance 1 (AVOID 1), and avoidance 2 (AVOID 2) latencies were measured at 30-s intervals, beginning immediately after the intracerebral injection of either drug or saline. In the inhibitory avoidance test, two-way ANOVA showed a significant effect of trials, $F(2, 24) = 11.78$, $p < 0.001$, of drug, $F(1, 12) = 7.78$, $p = 0.016$, and a significant drug × trial interaction, $F(2, 24) = 7.58$, $p = 0.003$. The Student's t test showed a significant difference (*) between drug and control at AVOID 2, $t(12) = 2.95$, $p = 0.012$. Therefore, the drug impaired both performance and acquisition of inhibitory avoidance. One-way escape was not significantly affected by the drug, $t(12) = 0.333$, $p = 0.575$. 8-OH-DPAT was dissolved in saline and microinjected (0.2 μl/2 min) into the dorsal raphe nucleus 10 min before the experimental session.

nals are lesioned by substituted amphetamines, among which are PCA and D-fenfluramine itself, MRN terminals are preserved. In addition, Series et al. (96) showed that the acute 5-HT-releasing effect of D-fenfluramine, measured through microdialysis in frontal or parietal cortex of anesthetized rats, was markedly reduced 2 weeks after the animals had been treated by a neurodegenerative regimen of PCA, D-fenfluramine, or another amphetamine derivative, MDMA. These results are consistent with the hypothesis that the acute release of 5-HT evoked by D-fenfluramine occurs via those terminals destroyed by the substituted amphetamines.

To further explore this subject, M. B. Viana conducted microdialysis experiments in collaboration with R. Silveira, at the Instituto de Investigaciones Biologicas Clemente Estable,

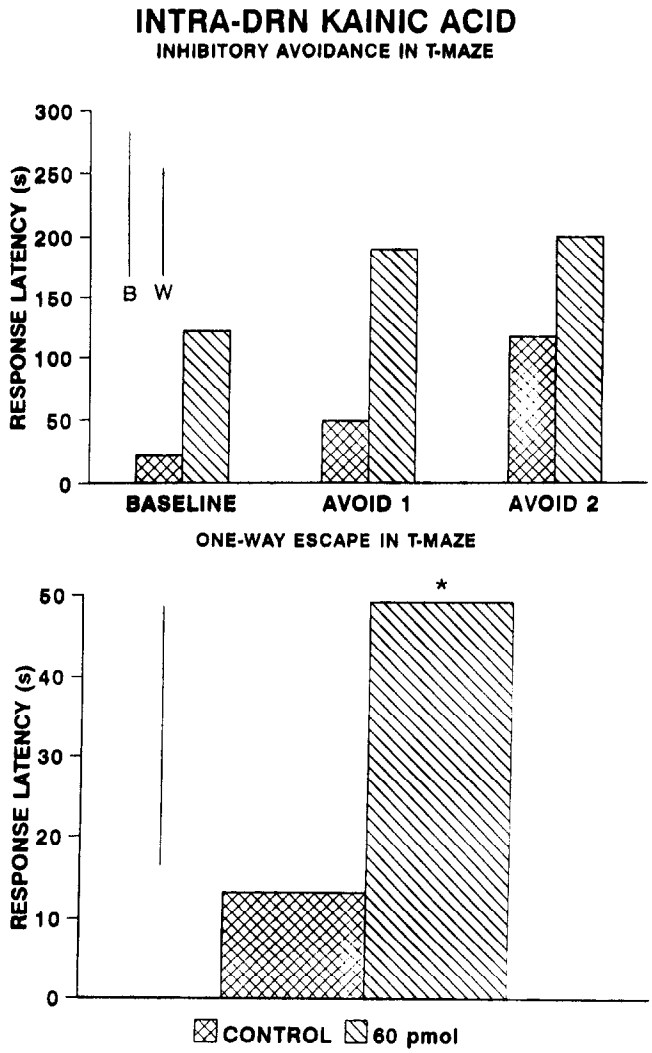

FIG. 4. Effect of intra-DRN kainic acid on inhibitory avoidance and one-way escape in the elevated T-maze. Columns represent mean of 10 rats for the control group and 8 rats for the group treated with kainate. In the inhibitory avoidance test, two-way ANOVA showed a significant effect of trials, $F(2, 32) = 4.14$, $p = 0.025$, and of drug, $F(1, 16) = 10.51$, $p = 0.005$, but a nonsignificant drug × trial interaction, $F(2, 32) = 0.49$, $p = 0.618$. Therefore, the drug enhanced inhibitory avoidance performance without affecting avoidance acquisition. One-way escape was significantly impaired (*) by the drug, $t(16) = 4.92$, $p = 0.041$. Kainic acid (60 pmol) was dissolved in saline and microinjected (0.2 μl/2 min) into the dorsal raphe nucleus immediately before the experimental session. Further specifications are in the legend of Fig. 3.

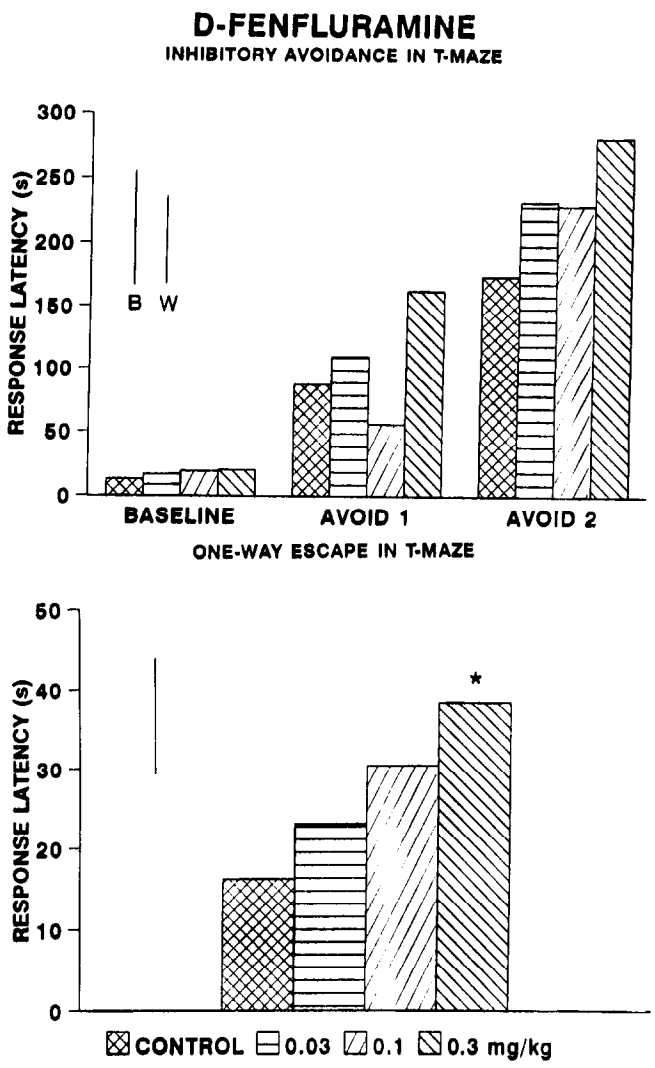

FIG. 5. Effect of D-fenfluranime on inhibitory avoidance and one-way escape in the elevated T-maze. Columns represent mean of 13 rats for the group treated with 0.3 mg/kg D-fenfluramine and of 12 rats for the remaining groups. In the inhibitory avoidance test, two-way ANOVA revealed a significant effect of trials, $F(2, 90) = 85.42$, $p < 0.001$, a nearly significant effect of drug, $F(3, 45) = 2.42$, $p = 0.079$, but a nonsignificant drug × trial interaction, $F(6, 90) = 1.57$, $p = 0.165$. In the escape test, one-way ANOVA showed a significant effect of treatment, $F(3, 45) = 3.39$, $p = 0.026$. $^*p < 0.05$ with respect to control by the Student-Newman-Keuls test. D-fenfluramine was dissolved in saline and injected (1 ml/kg), IP, 25 min before the experimental session. Further specifications are in the legend of Fig. 3.

Montevideo, Uruguay. In this study, regional differences in 5-HT innervation were used to assess the selectivity of D-fenfluramine releasing action. Although many nervous territories receive 5-HT input from both MRN and DRN, there are brain structures that get 5-HT innervation almost exclusively from one of these nuclei. For example, the amygdala is chiefly innervated by the DRN, while the dorsal hippocampus gets 5-HT input mainly from the MRN (4). If D-fenfluramine acts upon DRN 5-HT terminals only, the drug is expected to release 5-HT in the amygdala, but not in the dorsal hippocampus. To verify this prediction, extracelullar 5-HT concentration was measured with in vivo microdialysis in the amygdala and dorsal hippocampus of urethane-anesthetized rats treated with 10 mg/kg, IP, of D-fenfluramine with the same dose used by Series et al. (96). As expected, marked increases in the concentration of 5-HT were obtained in the dialysate from the amygdala, but no change in extracellular amine concentration occurred in the dialysate collected from the dorsal hippocampus (38,107).

Tests in Healthy Volunteers

To test the dual 5-HT-fear hypothesis in human beings, healthy volunteers were submitted to two experimental models believed to induce conditioned and unconditioned anxiety, respectively [for discussion, see (25,27)]. The first model was a conditioned fear test that measures changes in skin conductance occurring in response to a tone associated once with an aversive noise (42). The second model was a simulated public speaking test, consisting of talking in front of a videocamera (73). In addition to physiological measurements, such as arterial blood pressure and heart rate, subjective anxiety was estimated through self-rating scales, the most sensitive of which is an analog scale known as the Visual Analog Mood Scale (VAMS). The following drugs were used: 1) the 5-HT uptake blocker and 5-HT releasing agent D-fenfluramine, discussed above; 2) mCPP, a mixed 5-HT receptor agonist, with preferential affinity for 5-HT_{2C} and 5-HT_{1B} receptors (49); 3) the $5\text{-HT}_{2A/2C}$ receptor antagonist, ritanserin (49); and 4) a selective 5-HT_{2A} receptor antagonist synthesized by Sanofi Recherche (SR 46349B). A double-blind, placebo-controlled procedure was used.

For the simulated public speaking test, the following procedure was used: after a 15-min adaptation period, baseline measurements (B) were taken followed by drug or placebo intake. Prestress measurements (P) were done after a time interval variable as a function of the drugs being studied. Immediately thereafter the subject watched a prerecorded videotape on a video screen with the instructions about the task he/she would have to perform. Subjects were told that they would have 2 min to prepare a 4-min speech about the episodes in their lives that most caused anxiety, and that the speech would be recorded on videotape and later analyzed by a psychologist. Anticipatory anxiety measurements (A) were taken before the subject started speaking in front of the videocamera while viewing his/her own image on the video screen. The speech was interrupted in the middle, and performance anxiety measurements (S) were taken. Poststress measurements (F) were performed 15 min after the end of speech.

For the conditioned fear test, subjects having skin conductance electrodes attached to the medial phalanx of their second and third finger of the left hand were presented through a headphone a sequence of 10 neutral tones (75 dB, rapid onset, 1 s duration) at pseudorandom intervals (mean interval = 60 s). This allows habituation to the tone. The 11th tone was immediately followed by a burst of loud (95 dB) white noise (acquisition). Subsequently, there were ten extinction trials with the same tone (now a CS) being presented again as before.

The predictions were that in the classical aversive conditioning test direct or indirect (D-fenfluramine) 5-HT receptor agonists increase anxiety, while antagonists attenuate anxiety. In the public speaking model (unconditioned anxiety), 5-HT

agonists would restrain anxiety, whereas antagonists would potentiate anxiety.

As expected, oral administration of D-fenfluramine markedly decreased the rise in anxiety caused by speaking in front of a videocamera [(45), Fig. 6]. Also in the predicted direction, the dose of 15 mg of D-fenfluramine tended to enhance conditioned fear (Fig. 7). These results in human beings are in close correspondence with the effects of D-fenfluramine in the rat elevated T-maze described above. In both cases measures that are believed to represent conditioned anxiety tended to be increased by D-fenfluramine, whereas those thought to reflect unconditioned fear were markedly attenuated.

The direct agonist mCPP significantly increased conditioned fear while not affecting public speaking anxiety (18). The former result agrees with evidence reported by others suggesting that the 5-HT_{2C} receptor subtype is important for anxiety [e.g., (59)]. The lack of effect on public speaking anxiety may be due to the fact that mCPP acts on several receptor subtypes that may affect this kind of anxiety in opposite directions.

As expected, both ritanserin and SR 46349B attenuated anxiety in the aversive conditioning test. The dose of 10 mg of ritanserin significantly decreased the amplitude of skin conductance responses after the CS-US association, without affecting habituation to the neutral tone (44). This suggests that ritanserin interferes with the expression of aversively conditioned responses rather than their acquisition. In turn, SR 46349B affected both habituation and extinction phases; it also significantly reduced the number of spontaneous fluctuations. Nevertheless, the two compounds differed in the public speaking test. Ritanserin (10 mg) significantly increased the level of anxiety at the last phase of the experimental session (25). This anxiogenic effect not only was predicted by dual 5-HT-fear hypothesis, but parallels the aggravation of panic disorder verified in clinical trials [(29,52), Guimarães, Mabaya, and Deakin, unpublished results]. However, SR 46349B was ineffective on public speaking anxiety.

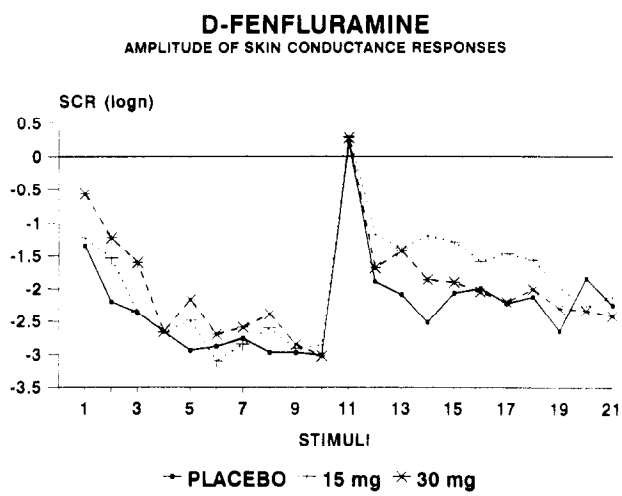

FIG. 7. Effect of D-fenfluramine on the amplitude of skin conductance responses of healthy volunteers to a tone presented 10 times before pairing with aversive white noise (US)—habituation—and 10 times after one pairing (CS-US)—extinction. MANOVA showed a significant effect of conditioning, $F(1, 36) = 11.45$, $p = 0.002$, reflecting the fact that SCR were greater after the CS-UCS presentation. Also, there was a significant effect of time, $F(7, 256) = 13.54$, $p < 0.001$, indicating a decrease of SCR along time in both the habituation and extinction periods. However, no main drug effect was found, although the dose of 15 mg of D-fenfluramine tended to increase SCR after conditioning. SCR: skin conductance amplitude in natural log mmho. $n = 16$ for placebo, 12 for 15 mg, and 14 for 30 mg D-fenfluramine. D-Fenfluramine or placebo were administered in identical gelatin capsules 2:20 h before the test.

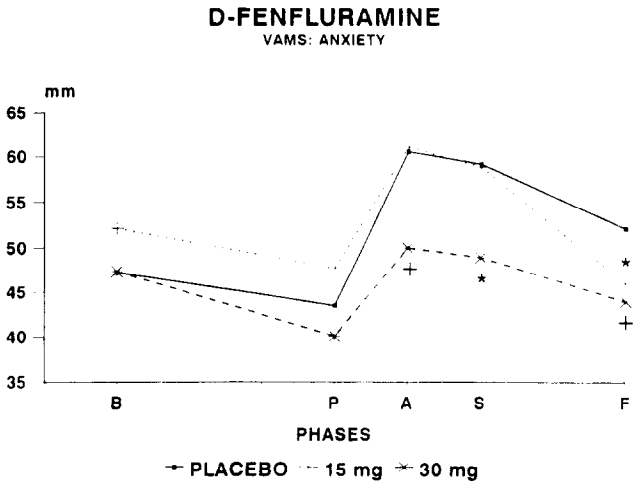

FIG. 6. Effect of D-fenfluramine on anxiety generated by simulated public speaking in healthy volunteers. The Y axis represents the anxiety factor of the Visual Analog Mood Scale (VAMS) in mm. Measurements were taken at baseline (B), prestress (P), just before speech (A), during speech (S), and at poststress (F). MANCOVA showed that the procedure (phase factor) induced a significant increase in subjective anxiety in both groups, $F(2, 76) = 19.92$, $p < 0.001$. MANCOVA with contrast analysis showed that the dose of 30 mg significantly decreased anxiety along the session, $F(1, 39) = 4.40$, $p = 0.043$. Significant ($^{*}p < 0.05$) or nearly significant ($^{+}p < 0.10$) differences from placebo were also detected by ANOVAs plus contrast analysis performed at each phase. $n = 14$ for placebo, 15 for 15 mg, and 14 for 30 mg D-fenfluramine. D-Fenfluramine or placebo were administered in identical gelatin capsules 15 min after B and 2:45 h before P.

DISCUSSION

The preceding results are summarized in Table 1. Of 16 drug experiments performed so far by our groups to test the dual 5-HT-fear hypothesis, 11 showed significant changes as predicted, and 1 revealed a nonsignificant tendency towards the expected direction. None was contrary to the hypothesis, although in four instances negative results were obtained.

Additional tests of the dual 5-HT-fear hypothesis were made by Maier and co-workers (66,67) with the animal model of depression known as learned helplessness. In this experimental paradigm animals are exposed to unavoidable electric shocks and, as a consequence, become less able to escape from controllable shock. Nevertheless, escape deficit is not the only consequence of this form of stress, because rats exposed to inescapable shock acquire fear conditioning more easily than control rats, that is, exhibit enhanced freezing behavior in response to an environment where they had been shocked before. In conformity with the dual 5-HT-fear hypothesis, Maier et al. assumed that both the deficit in escape viewed as an innate defense response and the facilitation of fear conditioning could be due to sensitization of DRN 5-HT neurons projecting to the DPAG and the amygdala, respectively. To test

TABLE 1

EFFECT OF DRUGS ACTING ON 5-HT NEUROTRANSMISSION IN MODELS OF ANXIETY

	Inhibitory Avoidance	One-Way Escape	Aversive Conditioning	Simulated Public Speaking
8-OH-DPAT i.r.	↓	0	–	–
Kainate i.r.	↑	↓	–	–
FG 7142 i.r.	↑	0	–	–
D-Fenfluramine	↑	↓	(↑)	↓
mCPP	–	–	↑	0
Ritanserin	–	–	↓	↑
SR 46349B	–	–	↓	0

i.r., intradorsal raphe nucleus; ↑, increase; ↓, decrease; (↑), nonsignificant tendency to increase; 0, no change.

this prediction, they performed electrolytic lesion of either the amygdala or the DRN. Both lesions abolished enhanced freezing, but only the DRN lesion prevented the escape deficit determined by the inescapable shocks (67), presumably because the projection to the DPAG was also affected. In a subsequent study, the same workers microinjected chlordiazepoxide in the region of the DRN to enhance GABAergic inhibition of 5-HT neurons. As expected, this treatment blocked both enhanced fear conditioning and escape deficit, regardless of the drug being administered before the inescapable shocks or before testing (66).

Clinical implications of the dual 5-HT-fear hypothesis have been discussed in previous publications (23–25,27,34–36,40). In spite of that, the results recently obtained with D-fenfluramine in the elevated T-maze and in healthy volunteers described above deserve comment. According to Deakin and Graeff's model, conditioned fear relates to GAD and unconditioned fear to panic disorder (23–25,35). Since D-fenfluramine reduced one-way escape in the T-maze as well as public-speaking anxiety in healthy volunteers, this drug is expected to improve panic disorder. Indeed, the results of an open study recently reported by Solyom (99) showed that chronic administration of D,L-fenfluramine (60 to 180 mg/day for 3 months) reduced the frequency of panic attacks from 16.9 to 0.9 per month in a group of 18 female patients with a history of panic disorder of 22–33 years of duration. If the therapeutic efficacy of fenfluramine in panic disorder is confirmed by controlled studies, the suggested mechanism, according to the dual 5-HT-fear hypothesis presently discussed, is an enhanced inhibition of neurons that command proximal defense in the DPAG by the drug-induced release of 5-HT from DRN terminals. In seeming contrast, however, the results of a challenge study carried out by Targum and Marshall (100) showed that acute administration of fenfluramine induced anxiety in panic disorder patients. Yet, the anxiety induced by fenfluramine was slow in onset and came in waves of long duration, in contrast to the sudden surge characteristic of panic attacks. Therefore, fenfluramine may have increased anticipatory anxiety rather than induced panic attacks in these patients.

THE MRN-HIPPOCAMPUS 5-HT SYSTEM IN STRESS AND DEPRESSION

Stressful events have been recognized by several studies to be related to the development of affective disorders (58,74,88) and stressful stimuli are present in most of the currently employed animal models of depression (110). According to theoretical model under analysis (23), the 5-HT pathway that initiates in the MRN and innervates the dorsal hippocampus increases resistance or tolerance to stress. The experiments described in the following were performed to investigate this assumption.

Median Raphe Nucleus and Stress

Hoshino and Sugizaki (48) have shown that electrolytic lesion of the MRN, but not of the DRN, causes ulcers in fasted rats. Like stress-induced ulcers, they are localized in the glandular portion of the stomach. In contrast, ulcers caused by fasting alone are mainly localized in the rumen. As a consequence, the same authors suggested that MRN lesion removes a protective mechanism that attenuates the effect of stress, in this case caused by fasting. If gastric ulcers are a manifestation of increased sensitivity to stress, related changes should also be present in animals with MRN lesion. It is well known that stress mobilizes neural mechanisms that affect the immune system. This outcome is mediated by the release of several hormones, among which are catecholamines, glucocorticoids, opioid peptides, growth hormone, and prolactin [for a review, see (2)]. Therefore, we measured both ulcer formation and the immune response of rats to a mitogen, concanavalin A, after electrolytic lesion of the MRN.

Electrolytic lesion (3 mA, 10 s) was made in male Wistar rats under pentobarbital anesthesia. Measurements were taken either 24 h (acute lesion) or 7 days after the surgery (chronic lesion). A group of chronically lesioned rats were submitted to 2-h restraint (see next session) in the sixth day after the operation and the stomach was examined on the seventh day (chronic lesion + restraint). Sham-operated rats were used as control for each procedure.

The effect on MRN lesion on gastric ulcers is shown in Table 2. Both acute and chronic MRN lesion significantly increased the incidence of ulcers. The two proportion comparison test revealed significant differences between sham and experimental groups in every procedure. Concerning the number of ulcers, two-factor ANOVA showed a significant effect of group, $F(1, 126) = 32.08, p < 0.001$, but no effect of procedure and no group × procedure interaction. With regards to size, two-factor ANOVA evidenced a significant influence of procedure, $F(2, 126) = 5.85, p = 0.017$, of group, $F(1, 126) = 7.33, p < 0.001$, and a significant procedure × group interaction, $F(2, 126) = 3.97, p = 0.021$. Multiple comparisons with the Tukey test showed that lesions were bigger in every experimental group as compared to respective sham, as well as in the restraint group as compared to other lesioned animals. In addition, fresh blood was detected in the

TABLE 2

EFFECT OF ELECTROLYTIC LESION OF THE MEDIAN RAPHE NUCLEUS AND RESTRAINT ON GASTRIC ULCERS

Procedure	Group	Incidence %	Number mean ± SEM	Length (cm) mean ± SEM	n
Acute lesion	Sham	11.76	0.35 ± 0.26	0.06 ± 0.04	17
	Experimental	93.10***	6.55 ± 1.28	0.94 ± 0.19***	29
Chronic lesion	Sham	6.67	0.07 ± 0.07	0.01 ± 0.01	15
	Experimental	80.95***	2.19 ± 0.45	0.33 ± 0.08**	21
Chronic lesion	Sham	20.00	0.56 ± 0.26	1.24 ± 0.65	25
plus restraint	Experimental	72.00**	5.36 ± 1.22	8.56 ± 2.93*†	25

Asterisks indicate significant difference as compared to sham: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. †$p < 0.001$ in comparison with the two other lesioned groups.

feces of rats submitted to chronic MRN lesion + restraint, probably due to bleeding gastrointestinal ulcers.

Therefore, the preceding results support the notion that MRN-lesioned animals become more sensitive to stressors, represented by the brain surgery in the acute lesion and restraint in the chronic lesion. This view is supported by the higher mortality of lesioned (25.74%, $n = 101$) as compared to sham-operated rats (8.89%, $n = 45$) during the 24-h period after brain surgery. The difference was significant ($p = 0.034$) according to the two proportion comparison test. All dead rats showed bleeding gastric ulcers. In addition, the surviving animals lost more weight when lesioned (26.10 ± 1.65 g) than sham operated (9.92 ± 5.83 g). This difference was also statistically significant, $t(53) = 3.72, p < 0.001$.

For assessing the immune response, the spleen of rats was removed under aseptic conditions, ground, and centrifuged. A suspension of spleen cells was distributed in a multiwell plate at a concentration of 5×10^6 cells/ml. Concanavalin A (100 μl) and culture medium were added. Following a 48-h period of incubation (100% humidity, 37°C, 5% CO_2), a pulse of [^{3}H]-thymidine (1 μCi in 50 μl per well) was added. After 72 h, the cell cultures were filtered and the cells resuspended in a scintillation liquid. Radioactivity was measured with a scintillation counter and expressed as counts per min (cpm). The stimulation index was the ratio between the radioactivity of cells stimulated by concanavalin A and the radioactivity of nonstimulated cells of the same spleen.

Table 3 shows that the immune response to concanavalin A was depressed after acute MRN lesion, indicating once more that MRN-lesioned rats were more sensitive to surgical stress.

Further experiments are required to verify whether such stress-related changes are really due to loss of 5-HT neurons in the MRN. Nevertheless, the compatibility of these initial results with the hypothesis that the MRN-hippocampal 5-HT pathway exerts a protective action against stressors encourages investigation to proceed along this line.

TABLE 3

EFFECT OF ACUTE ELECTROLYTIC LESION OF THE MEDIAN RAPHE NUCLEUS ON INCORPORATION OF [^{3}H]-THYMIDINE IN SPLENIC CELLS

Procedure	Group	Stimulation Index	n
Unoperated	Control	9.26 ± 1.34	18
Acute lesion	Sham	7.44 ± 1.41	9
	Experimental	3.71 ± 0.54*	17

The asterisk indicates significant difference from sham, $t(24) = 2.99, p = 0.006$.

Hippocampus and Depression

The involvement of 5-HT_{1A} receptors in long-term adaptive or coping responses to stress is suggested by a series of experiments by Kennett et al. (60–62). They showed that 24 h after having been immobilized for 2 h rats display marked reductions in open-field exploration. This effect disappeared after 1 week of daily restraint periods. The animals that became tolerant to repeated stress showed a potentiated 5-HT behavioral syndrome following systemic injection of the nonselective 5-HT agonist 5-MeO-DMT. A systemic injection of 8-OH-DPAT after a single period of restraint also led to normal exploration of the open field 24 h later. Recently, similar results with 8-OH-DPAT were observed in the elevated plus-maze by MacBlane and Handley (70). These results, therefore, suggest that adaptation to stress involves augmentation of 5-HT_{1A} receptor function.

Concerning localization, either autosomic receptors in raphe 5-HT neurons or postsynaptic 5-HT_{1A} receptors that are highly concentrated in the hippocampal formation (50) may be involved. According to the theoretical model under examination (23), the most likely possibility is that postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus mediate the acquisition of tolerance to stress. Results obtained in one of our laboratories with in situ hybridization showed that 30-min restraint induces c-*fos* mRNA expression, a possible marker of neuronal activity (79), in the following brain regions: amygdala, neocortex, paraventricular nucleus of the hypothalamus, piriform cortex, habenula, and the dentate gyrus and CA1–CA3 regions of the hippocampal formation of the rat (41,103). The last observation supports the view that the hippocampus is activated by stress. In addition, microdialysis studies performed in other laboratories indicate that different stressors, including handling, tail pitch, and exposure to the elevated plus-maze increase 5-HT release in the hippocampus (7,57).

To investigate the participation of hippocampal 5-HT_{1A} receptors in the development of tolerance to stress, we used a modification of the restraint model described by Kennett et al. (62). Rats were restrained in a wire chamber for 2 h and 24 h later placed for 5 min on the elevated plus-maze (43,91). Bilateral microinjections into the dorsal hippocampus were performed immediately after the immobilization period. The re-

sults showed that previously immobilized rats display a decrease in percentage of entries and time spent on open arms of the elevated plus-maze, that is, are more anxious than nonrestrained controls. Poststress microinjections into the dorsal hippocampus of the selective 5-HT reuptake blocker, zimelidine, or of the 5-HT_{1A} agonist, 8-OH-DPAT, reversed the deficits of open arm exploration induced by restraint (41). The effect of zimelidine was blocked by previous microinjection of DL-propranolol, a nonselective 5-$HT_{1A/1B}$ antagonist (86). More recently, Mendonça Netto and Guimarães (77) found that the effects of 5-MeO-DMT, a nonselective 5-$HT_{1A/1B}$ agonist, microinjected into the dorsal hippocampus after the restraint stress, were blocked by previous local microinjection of (+)WAY-100135, a selective 5-HT_{1A} receptor antagonist (Fig. 8). Similar results were found with zimelidine (Padovan and Guimarães, unpublished). These results are in agreement with the evidence obtained with systemic drug administration (62,70), and suggest that facilitation of hippocampal 5-HT_{1A}-mediated neurotransmission attenuates the behavioral consequences of stress (23,26).

DISCUSSION

The reversal of restraint effects by 5-HT_{1A} agonists described above, suggests that facilitation of hippocampal 5-HT_{1A}-mediated neurotransmission attenuates the behavioral consequences of stress. The mechanism responsible for such effect is not yet clear, but it may involve the disconnection, mediated by the MRN-hippocampus 5-HT pathway, of aversive events from their behavioral consequences. A recent study of chronic stress after the Three Mile Island nuclear accident suggests a relation between consolidation of stressful memories and stress responding. Frequent experience of intrusive memories about the accident was related to persistent stress responses several years after the event (6). Also, adrenal corticosteroids, proposed to play an etiological role in affective disturbances (see below), facilitate consolidation of stressful memories (89,95)

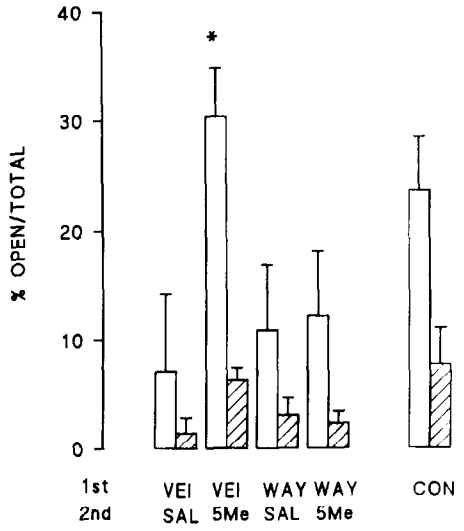

FIG. 8. Hippocampal 5-HT_{1A} receptors in the reversal of the anxiogenic effect of a 2-h restraint period in rats tested 24 h later on the elevated plus-maze. Columns represent mean and vertical bars the SEM of six to nine rats. Open columns refer to % of entries onto and hatched columns to % of time spent on the open arms of the maze. Immediately after stress animals received a first bilateral microinjection into the dorsal hippocampus of either vehicle (VEI, 0.5 μl) or (+)WAY-100135 (WAY, 40 nmol) followed 5 min later by a second injection of either saline (SAL) or 5-MeO-DMT (5 Me, 20 nmol). For graphical comparison, a control, nonimmobilized group (CON) was included. This group received only one bilateral microinjection of saline into the dorsal hippocampus 24 h before the test and, therefore, was not included in the statistical analysis. For % of entries, one-way ANOVA showed a significant overall effect, $F(3, 25) = 3.5$, $p = 0.028$. The asterisk indicates significant difference (Duncan test, $p < 0.05$) from all other restraint groups. For % of time ANOVA showed a nearly significant overall effect, $F(3, 25) = 2.6$, $p = 0.07$.

Supporting a role of MRN-hippocampus 5-HT pathway in disconnection of aversive events, a number of studies have shown that modification of 5-HT neurotransmission interferes with cognitive function. In general, potentiating 5-HT neurotransmission disrupts acquisition of new behaviors (33), an effect demonstrated with selective 5-HT_{1A} ligands after both systemic (12,13) and hippocampal (14,85) administration. Moreover, 5-HT interferes with hippocampal electrical activity (19,69), and inhibits induction of long-term potentiation (109) through 5-HT_{1A} receptors (21,94). Microinjection of 5-HT into the hippocampus or systemic injection of buspirone reduces the amplitude and increases the latency of the P28 and N55 peaks of hippocampal auditory evoked responses in awake rats, suggesting that 5-HT_{1A} receptors inhibit information processing (83).

Is there any evidence that a reduction in 5-HT_{1A} function is associated with depression? The answer seems to be yes. A reduced number of hippocampal 5-HT_1 receptors in postmortem brains of depressed suicides have been reported (17), and depressed patients have an attenuation of prolactin and growth hormone responses to 5-HT challenge tests, such as intravenous infusions of tryptophan, that is thought to indicate impaired 5-HT_1 function (26). Because the abnormality is state dependent, being reversed in nonsymptomatic antidepressant treated patients (97), impaired 5-HT_1 function may cause the state of depression. This possibility is supported by the finding that effective antidepressant treatments progressively enhance 5-HT_{1A} function in the hippocampus (9,11,22) and that acute tryptophan depletion, reducing brain 5-HT synthesis, produces a return in depressive symptoms in about 80% of nonsymptomatic, treated depressive patients (28).

Factors leading to a failure in hippocampal 5-HT_{1A} function would be related to events known to predispose the individual to develop clinical depression. One example would be lack of social support. In Kennett's experiments it has been shown that, if the stressed animals remain in a cage with other rats after the restraint period, they will explore normally the open field 24 h later (32). Therefore, social interaction may protect the individual against the behavioral consequences of stress. In agreement with this proposition, Popova and Petkov (93) showed that 3 months of social isolation decrease the number of hippocampal 5-HT_1 receptors. More recently, using microdialysis to measure 5-HT in the hippocampal formation, Bickerdike et al. (7) showed that isolation-reared rats did not display the increase in 5-HT release after exposure to the elevated plus-maze, as compared to socially reared animals. Social isolation, therefore, seems to be able to undermine hippocampal 5-HT_{1A} neurotransmission by both pre- and postsynaptic mechanisms.

Another factor that has been associated with depression is glucocorticoid secretion. Abnormalities in this secretion have been the most consistent biological marker of depression (31), and it has been shown recently by computed tomography that many patients with major depression present an enlargement

of adrenal glands (81). In addition, drugs that inhibit cortisol synthesis have therapeutic effects on patients with major depression (84,101).

Adrenal steroids are secreted in response to stress, and brain corticoid receptors are mainly located in the hippocampus (71). Moreover, glucocorticoid hormones have major effects on behavior, hippocampal morphology, and 5-HT neurotransmission (30,54,76,80,90,112), downregulating hippocampal 5-HT_{1A} receptors at the level of receptor mRNA expression (15). The latter effect was also observed after chronic unpredictable stress and prevented by concomitant imipramine administration. Mild stress exposure did not change 5-HT_{1A} binding (65). In another study, however, Papp et al. (87) found that chronic exposure to mild stress increased 5-HT_{1A} binding in the hippocampus, an effect also observed after chronic imipramine administration. Moreover, a neuroendocrine functional study showed the development of supersensitivity of 5-HT_{1A} receptors in animals submitted to stressful situations where escape responses cannot be accomplished (63). Therefore, adrenal steroids may have biphasic effects on 5-HT systems. By facilitating 5-HT_{1A}-mediated neurotransmission they may be necessary for development of stress tolerance. Chronic exposure to high levels of glucocorticoids, however, may lead to a downregulation of 5-HT_{1A} receptors and consequent failure of the system (16).

Finally, depressive states are often associated with anxiety. We suggest that overactivity in DRN-5-HT_2 anxiety systems may also undermine 5-HT_{1A} neurotransmission (23). In many situations stimulation of 5-HT_2 receptors opposes 5-HT_{1A} receptor functioning (5), and an increased number of 5-HT_2 receptors has been found in brains of suicide victims and depressive patients who died of natural causes (51). In addition, acute and chronic stress, as well as glucocorticoids, increase 5-HT_2 number in the cortex (64,72,104).

CONCLUSION

The present article summarized the results of several experiments that were aimed at testing the theoretical model proposed by Deakin and Graeff (23) on the role of three different ascending 5-HT pathways in anxiety, panic,and depression, respectively. The model allowed clear predictions to be made about the effect of drugs affecting the functioning of 5-HT neurotransmission on behavior of laboratory rats and healthy human beings under experimental models of anxiety or depression. This indicates that the theoretical model is amenable to experimental verification. In most cases the predictions derived from the model were fulfilled by the obtained results, and in no case were they contradicted. So, in spite of some negative results, it may be concluded that so far the model stood empirical test and, therefore, merits further examination. The last suggestion is reinforced by the potential of the model for improving our understanding of the pathophysiology of anxiety and depressive disorders and the mode of action of psychotherapeutic drugs.

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REFERENCES

1. Aghajanian, G. K. Central 5-HT receptor subtypes: Physiological responses and signal transduction mechanisms. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychoptropic drugs. Oxford: Blackwell Scientific Publications; 1992: 39-55.
2. Anisman, H.; Zalcman, S.; Zacharko, M. The impact of stressors on immune and central neurotransmitter activity: Bidirectional communication. Rev. Neurosci. 4:147-180; 1993.
3. Artigas, F. 5-HT and antidepressants: New views from microdialysis studies. Trends Pharmacol. Sci. 14:262; 1993.
4. Azmitia, E. C. The serotonin-producing neurons of the midbrain median and dorsal raphe nuclei. In: Iversen, L. L.; Iversen, S. D.; Snyder, S. H., eds. Handbook of psychopharmacology. Vol. 9: Chemical pathways in the brain. New York: Plenum Press; 1978:233-314.
5. Backus, L. I.; Sharp, T.; Grahame-Smith, D. G. Behavioral evidence for a functional interaction between central 5-HT_2 and 5-HT_{1A} receptors. Br. J. Pharmacol. 100:793-799; 1990.
6. Baum, A.; Cohen, L.; Hall, N. M. Control of intrusive memories as possible determinants of chronic stress. Psychosom. Med. 55:274-286; 1993.
7. Bickerdike, M. J.; Wright, I. K.; Marsden, C. A. Social isolation attenuates rat forebrain 5-HT release induced by KCl stimulation and exposure to novel environment. Behav. Pharmacol. 4:231-236; 1993.
8. Blanchard, R. J.; Blanchard, D. C.; Hori, K. Ethoexperimental approaches to the study of defensive behavior. In: Blanchard, R. J.; Brain, P. F.; Blanchard, D.C.; Parmigiani, S., eds. Ethoexperimental approaches to the study of behavior. Dordrecht: Kluwer Academic Publishers; 1989:114-136.
9. Blier, P.; de Montigny, C.; Chaput, Y. Modification of the serotonin system by the antidepressant treatments: Implications for the therapeutic response in major depression. J. Clin. Psychopharmacol. 7:24S-35S; 1987.
10. Bonvento, G.; Scatton, B.; Claustre, Y.; Rouquier, L. Effects of local injection of 8-OH-DPAT into the dorsal or median raphe nuclei on extracellular levels of serotonin in serotonergic projection areas in the rat brain. Neurosci. Lett. 132:101-104; 1992.
11. Briley, M.; Moret, C. Neurobiological mechanisms involved in antidepressant therapies. Clin. Neuropharmacol. 5:387-400; 1993.
12. Carli, M.; Samanin, R. 8-Hydroxy-2-(di-n-propylamino)tetralin impairs spatial learning in a water maze: Role of postsynaptic 5-HT_{1A} receptors. Br. J. Pharmacol. 105:720-726; 1992.
13. Carli, M.; Tranchina, S.; Samanin, R. 8-Hydroxy-2-(di-n-propylamino)tetralin,a 5-HT_{1A} agonist, impairs performance in a passive avoidance task. Eur. J. Pharmacol. 211:227-234; 1992.
14. Carli, M.; Lazarova, M.; Tatarczynska, E.; Samanin, R. Stimulation of 5-HT_{1A} receptors in the dorsal hippocampus impairs acquisition and performance of a spatial task in a water maze. Brain Res. 595:50-56; 1992.
15. Chalmers, D. T.; Kwak, S. P.; Mansour, A.; Akil, H.; Watson, S. J. Corticosteroids regulate brain hippocampal 5-HT_{1A} receptor mRNA expression. J. Neurosci. 13:914-923; 1993.
16. Chalmers, D. T.; López, J. F.; Vázquez, D. M.; Akil, H.; Watson, S. J. Regulation of hippocampal 5-HT_{1A} receptor gene expression by dexamethasone. Neuropsychopharmacology 10:215-222; 1994.
17. Cheetham, S. C.; Crompton, M. R.; Katona, C. L. E.; Horton, R. W. Brain 5-HT_1 binding sites in depressed suicides. Psychopharmacology (Berlin) 102:544-548; 1990.
18. Connell, J.; Graeff, F.; Guimarães, F.; Hellewell; Hetem, L. A.; Deakin, J. F. W. 5-HT_2 receptors and anxiety. Behav. Pharmacol. 6(Suppl. 1):35; 1995.

19. Coop, C. F.; McNaughton, N. Buspirone affects hippocampal rhythmical slow activity through serotonin 1_A rather than dopamine D_2 receptors. Neuroscience 40:169-174; 1991.
20. Coppen, A. The biochemistry of affective disorders. Br. J. Psychiatry 113:1237-1264; 1967.
21. Corradetti, R.; Ballerini, L; Pugliesi, A. M.; Pepei, G. Serotonin blocks the long-term potentiation induced by primed burst stimulation in the CA1 region of the rat hippocamppal slices. Neuroscience 46:511-518; 1992.
22. De Montigny, C.; Blier, P. Effects of antidepressant treatment on 5-HT neurotransmission. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychotropic drugs. Oxford: Blackwell Scientific Publications; 1992:223-238.
23. Deakin, J. W. F.; Graeff, F. G. 5-HT and mechanisms of defence. J. Psychopharmacol. 5:305-315; 1991.
24. Deakin, J. F. W.; Graeff, F. G.; Guimarães, F. S. 5-HT receptor subtypes and the modulation of aversion. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychotropic drugs. Oxford: Blackwell Scientific Publications; 1992:147-174.
25. Deakin, J. F. W.; Guimarães, F. S.; Graeff, F. G. Testing theories of anxiety in normal volunteers. In: Palomo, T.; Archer, T., eds. Strategies for studying brain disorders. Vol. 1. Depressive, anxiety and drug abuse disorders. Madrid: Editorial Complutense; 1994:211-238.
26. Deakin, J. F. W.; Pennel, I.; Upadhyaya, A. K.; Lofthouse, R. A neuroendocrine study of 5-HT function in depression: Evidence for biological mechanisms of endogenous and psychosocial causation. Psychopharmacology (Berlin) 101:85-92; 1990.
27. Deakin, J. F. W.; Guimarães, F. S.; Wang, M.; Hellelwell, J.; Hensman, R. 5-HT receptor mechanisms in human anxiety. In: Briley, M.; File, S. E., eds. New concepts in anxiety. London: The Macmillan Press Ltd.; 1991:74-93.
28. Delgado, P. L.; Charney, D. S.; Price, L. H.; Aghajanian, G. K.; Landis, H.; Heninger, G. R. Serotonin function and the mechanism of antidepressant action. Arch. Gen. Psychiatry 47: 411-418; 1990.
29. Den Boer, J. A.; Westenberg, H. G. M. Serotonin function in panic disorder: A double blind placebo controlled study with fluvoxamine and ritanserin. Psychopharmacology (Berlin) 102: 85-94; 1990.
30. Dickinson, S. L.; Kennett, G. A.; Curzon, G. Reduced 5-hydroxytryptamine dependent behaviour in rats following chronic corticosterone treatment. Brain Res. 345:10-18; 1985.
31. Dinan, T. G. Glucocorticoids and the genesis of depressive illness. A psychobiological model. Br. J. Psychiatry 164:365-371; 1994.
32. Dourish, C. T.; Gorka, Z.; Williams, A. R.; Iversen, S. D. Potential influence of social support in a rodent model of depression. J. Psychopharmacol. 3:38P; 1989.
33. Gower, A. J. 5-HT receptors and cognitive functions. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychotropic drugs. Oxford: Blackwell Scientific Publications; 1992:239-259.
34. Graeff, F. G. Brain defense systems and anxiety. In: Roth, M.; Burrows, G. D.; Noyes, R., eds. Handbook of anxiety. Vol. 3: The neurobiology of anxiety. Amsterdam: Elsevier Science Publishers; 1990:307-354.
35. Graeff, F. G. Neurotransmitters in the dorsal periaqueductal gray and animal models of panic anxiety. In: Briley, M.; File, S. E., eds. New concepts in anxiety. London: Macmillan Press; 1991:288-312.
36. Graeff, F. G. Role of 5-HT in defensive behavior and anxiety. Rev. Neurosci. 4:181-211; 1993.
37. Graeff, F. G.; Viana, M. B.; Mora, P. O. Opposed regulation of dorsal raphe nucleus 5-HT pathways of two types of fear in the elevated T-maze. Pharmacol. Biochem. Behav. (in press).
38. Graeff, F. G.; Viana, M. B.; Mora, P. O. Dual role of 5-HT in defense and anxiety. Neurosci. Biobehav. Rev. (in press).
39. Graeff, F. G.; Viana, M. B.; Tomaz; C. The elevated T-maze, a new experimental model of anxiety and memory: Effect of diazepam. Braz. J. Med. Biol. Res. 26:67-70; 1993.
40. Graeff, F. G.; Silveira, M. C. L.; Nogueira, R. L.; Audi, E. A.; de Oliveira, R. M. W. Role of the amygdala and periaqueductal gray in anxiety and panic. Behav. Brain Res. 58:123-131; 1993.
41. Guimarães, F. S.; Del Bel, E. A.; Padovan, C. M.; Mendonça Netto, S.; Titze de Almeida, R. Hippocampal 5-HT receptors and consolidation of stressful memories. Behav. Brain Res. 58: 133-139; 1993.
42. Guimarães, F. S.; Hellewell, J.; Hensman, R.; Wang, M.; Deakin, J. F. W. Characterization of a psychophysiological model of classical fear conditioning in healthy volunteers: Influence of gender, instructions, personality and placebo. Psychopharmacology (Berlin) 104:231-236; 1991.
43. Handley, S. L.; Mithani, S. Effects of alpha-adrenoceptor agonists in a maze-exploration model of "fear"-motivated behaviour. Naunyn Schmiedebergs Arch. Pharmacol. 327:1-5; 1984.
44. Hensman, R.; Guimarães, F. S.; Wang, M.; Deakin, J. F. W. The effects of ritanserin on aversive classical conditioning in humans. Psychopharmacology (Berlin) 104:220-224; 1991.
45. Hetem, L. A. B.; De Souza, C. J.; Guimarães, F. S. G.; Zuardi, A. W; Graeff, F. G. D-Fenfluramine reduces anxiety induced by simulated public speaking. Braz. J. Med. Biol. Res. 26:971-974; 1993.
46. Higgins, S.; Jones, B. J.; Oakley, N. R. Effect of 5-HT_{1A} receptor agonists in two models of anxiety after dorsal raphe injection. Psychopharmacology (Berlin) 106:261-267; 1992.
47. Hodges, H.; Green, S.; Glenn, B. Evidence that the amygdala is involved in benzodiazepine and serotonergic effects on punished responding but not in discrimination. Psychopharmacology (Berlin) 92:491-504; 1987.
48. Hoshino, K.; Sugizaki, M. Ulcerogenic effect of the lesion of the median raphe nucleus in fasted rats. Braz. J. Med. Biol. Res. 19:123-130; 1986.
49. Hoyer, D. The 5-HT receptor family: Ligands, distribution and receptor-effector coupling. In: Rodgers, R. J.; Cooper, S. J., eds. 5-HT_{1A} agonists, 5-HT_3 antagonists and benzodiazepines. Chichester, UK: John Wiley & Sons; 1991:31-57.
50. Hoyer, D.; Palacios, J. M.; Mengod, G. 5-HT receptor distribution in human brain: Autoradiographic studies. In: Marsden, C. A.; Heal, D. J., eds. Central serotonin receptors and psychotropic drugs. Oxford: Blackewll Scientific Publications; 1992: 100-125.
51. Hrdina, P. D.; Demeter, E.; Vu, T. B.; Sótónyi, P.; Palkovits, M. 5-HT uptake sites and 5-HT_2 receptors in brain of antidepressant free suicide victims/depressives: Increase 5-HT_2 sites in cortex and amygdala. Brain Res. 614:37-44; 1993.
52. Humble, M.; Aberg-Wistedt, B. Bertilson, L. A pilot study of ritanserin (a selective serotonin-2 receptor antagonist) in panic disorder and agoraphobia. Clinical and biochemical effects. 5th CINP Congress, San Juan, Puerto Rico, Abstract P-165; 1986.
53. Iversen, S. D. 5-HT and anxiety. Neuropharmacology 23:1553-1560; 1984.
54. Joels, M.; Hesen, W.; de Kloet, E. R. Mineralocorticoid hormones suppress serotonin-induced hyperpolarization of rat hippocampus CA1 neurons. J. Neurosci. 11:2288-2294; 1991.
55. Jones, B. J.; Paterson, I. A.; Roberts, M. H. T. Microinjections of methyl-β-carboline-3-carboxylate into the dorsal raphe nucleus: Behavioural consequences. Pharmacol. Biochem. Behav. 24:1487-1489; 1986.
56. Kahn, R. S.; van Praag, H. M.; Wetzler, S.; Asnis, G. M.; Barr, G. Serotonin and anxiety revisited. Biol. Psychiatry 23:189-208; 1988.
57. Kálen, P.; Rosegren, E.; Lindvall, O.; Bjorklund, A. Hippocampal noradrenaline and serotonin release over 24 hours as measured by the dyalisis technique in freely moving rats: Correlation to behavioural activity state, effect of handling and tail pitch. Eur. J. Neurosci. 1:181-188; 1989.
58. Kendler, K. S.; Kessler, R. C.; Neale, M. C.; Heath, A. C.; Eaves, L. J. The prediction of major depression in women: Toward an integrated etiological model. Am. J. Psychiatry 150: 1139-1148; 1993.

59. Kennett, G. A. 5-HT_{1C} receptors and their therapeutic relevance. Curr. Opin. Invest. Drugs 2:317–362; 1993.
60. Kennett, G. A.; Dickinson, S. L.; Curzon, G. Central serotonergic responses and behavioral adaptation to repeated immobilisation: The effect of the corticosterone synthesis inhibitor metyrapone. Eur. J. Pharmacol. 119:143–152; 1985.
61. Kennett, G. A.; Dickinson, S. L.; Curzon, G. Enhancement of some 5-HT-dependent behavioral responses following repeated immobilization in rats. Brain Res. 330:253–263; 1985.
62. Kennett, G. A.; Dourish, C. T.; Curzon, G. Antidepressant-like action of 5-HT_{1A} agonists and conventional antidepressants in an animal model of depression. Eur. J. Pharmacol. 134:265–274; 1987.
63. Korte, S. M.; Bouws, G. A. H.; Bohus, B. Adrenal hormones in rats before and after stress-experience effects of ipsapirone. Physiol. Behav. 51:1129–1133; 1992.
64. Kuroda, Y.; Mikuni, M.; Nomura, N.; Takahashi, K. Differential effect of subchronic dexamethasone treatment on serotonin-2 and beta-adrenergic receptors in rat cerebral cortex and hippocampus. Neurosci. Lett. 155:195–198; 1993.
65. López, L. F.; Chalmers, D. T.; Vázquez, D. M.; Akil, H.; Watson, S. L. Chronic unpredictable stress downregulates serotonin-1A receptor in the hippocampus. Soc. Neurosci. Abstr. 19: 216; 1993.
66. Maier, S. F.; Kalman, B. A.; Grahn, R. E. Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding produced by inescapable shock whether administered before inescapable shock or escape testing. Behav. Neurosci. 108:121–130; 1994.
67. Maier, S. F.; Grahn, R. E.; Kalman, B. A.; Sutton, L. C.; Wiertelak, E. P.; Watkins, L. R. The role of the amygdala and dorsal raphe nucleus in mediating the behavioral consequences of inescapable shock. Behav. Neurosci. 107:377–388; 1993.
68. Mamounas, L. A.; Mullen, C. A.; O'Hearn, E.; Molliver, M. E. Dual serotonergic projections to forebrain in the rat: Morphologically distinct 5-HT axon terminals exhibit differential vulnerability to neurotoxic amphetamine derivatives. J. Comp. Neurol. 314:558–586; 1991.
69. Maru, E.; Takahashi, L. K.; Iwahara, S. Effects of median raphe nucleus lesions on hippocampal EEG in the freely moving rat. Brain Res. 163:223–234; 1979.
70. McBlane, J. W.; Handley, S. L. Effects of two stressors on behavior in the elevated X-maze: Preliminary investigation of their interaction with 8-OH-DPAT. Psychopharmacology (Berlin) 116:173–182; 1994.
71. McEwen, B.; Gould, E. A.; Sakai, R. R. The vulnerability of the hippocampus to protective and destructive effects of glucorticoids in relation to stress. Br. J. Psychiatry 160(Suppl. 15):18–24; 1992.
72. McKittrick, C. R.; Blanchard, D. C.; Blanchard, R. J.; McEwen, B. S.; Sakai, R. R. Serotonin receptor binding in a colony model of chronic stress. Biol. Psychiatry 37:383–393; 1995.
73. McNair, D. M.; Frankenthaler, L. M.; Czerlinsky, T.; White, T. W.; Sasson, S.; Fischer, S. Simulated public speaking as a model of clinical anxiety. Psychopharmacology (Berlin) 77:7–10; 1982.
74. McNaughton, M. E.; Patterson, T. L.; Irwin, M. R.; Grant, I. The relationship of life adversity, social support, and coping to hospitalization with major depression. J. Nerv. Mental Dis. 180: 491–497; 1992.
75. McTavish, D.; Heel, R. C. Dexfenfluramine: A review of its pharmacological properties and therapeutic potential in obesity. Drugs 43:713–733; 1992.
76. Mendelson, S. D.; McEwen, B. S. Autoradiographic analyses of the effects of adrenalectomy and corticosterone on 5-HT_{1A} and 5-HT_{1B} receptors in the dorsal hippocampus and cortex of rats. Neuroendocrinology 55:444–450; 1992.
77. Mendonça Netto, S.; Guimarães, F. S. Role of hippocampal 5-HT_{1A} receptors on elevated plus maze exploration after single restraint experience. Behav. Brain Res. (in press).
78. Montgomery, K. C. The relationship between fear induced by novel stimulation and exploratory behaviour. J. Comp. Physiol. Psychol. 48:254–260; 1955.
79. Morgan, J. T.; Curran. T. Stimulus-transcription coupling in the nervous system: Involvement of the inducible proto-oncogene *fos* and *jun*. Annu. Rev. Neurosci. 14:421–451; 1991.
80. Nausieda, P. A.; Carve, P. M.; Weiner, W. J. Modification of central serotonergic and dopaminergic behaviours in the course of chronic corticosteroid administration. Eur. J. Pharmacol. 78: 335–343; 1982.
81. Nemeroff, C. B.; Krishnan, K. R. R.; Reed, D.; Leder, R.; Beam, C.; Dunnick, R. Adrenal gland enlargement in major depression. Arch. Gen. Psychiatry 49:384–387; 1992.
82. Nutt, D. J. Anxiety and its therapy: Today and tomorrow. In: Briley, M.; File, S. E., eds. New concepts in anxiety. London: Macmillan Press; 1991:1–12.
83. O'Connor, J. J.; Rowan, M. J.; Anwyl, R. Serotoninergic depression of auditory evoked responses recorded in the rat hippocampus: Effect of repeated buspirone treatment. Brain Res. 573: 190–196; 1992.
84. Odwyer, A. M.; Lightman, S. L.; Marks, M. N.; Checkley, S. A. Treatment of major depression with metyrapone and hydrocortisone. J. Affect. Dis. 33:123–128; 1995.
85. Ohno, M.; Yamamoto, T.; Watanabe, S. Working memory deficits induced by intrahippocampal administration of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, in the rat. Eur. J. Pharmacol. 234:29–34; 1993.
86. Padovan, C. M.; Guimar&atiles;es, F. S. Attenuation of behavioural consequences of immobilization stress by intra-hippocampal microinjection of zimelidine. Braz. J. Med. Biol. Res. 26: 1085–1089; 1993.
87. Papp, M.; Klimek, V.; Willner, P. Effects of imipramine on serotonergic and beta-adrenergic receptor binding in a realistic animal model of depression. Psychopharmacology (Berlin) 114: 309–314; 1994.
88. Paykel, E. S. Psychosocial influences on the curse of depression. In: Swinkels, J. A.; Blijleven, W., eds. Depression, anxiety and aggression: Factors that influence the course. Houten: Mediadact; 1988:55–62.
89. Peeters, B. W. M. M.; Broekkamp, C. L. E. Involvement of corticosteroids in the processing of stressful life-events. A possible implication for the development of depression. J. Steroid. Biochem. Mol. Biol. 49:417–427; 1994.
90. Peeters, B. W. M. M.; Smets, R. J. M.; Broekkamp, C. L. E. The involvement of glucocorticoids in acquired immobility response is dependent on the water temperature. Physiol. Behav. 51:127–129; 1992.
91. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open : closed arm entries in the elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
92. Petersen, E. N.; Scheel-Krüger, J. Anticonflict effects of 5-HT antagonists by intraamygdaloid injection. Abstr. of the 14th CINP Cong.; 1984:654.
93. Popova, J. S.; Petkov, V. V. Changes in 5-HT_1 receptors in different brain structures of rats with isolation syndrome. Gen. Pharmacol. 21:223–225; 1990.
94. Sakai, N.; Tanaka, C. Inhibitory modulation of long-term potentiation via the 5-HT_{1A} receptor in slices of the rat hippocampal dentate gyrus. Brain Res. 613:326–330; 1993.
95. Sandi, C.; Rose, S. P. R. Corticosterone enhances long-term retention in one-day-old chicks trained in a weak passive avoidance learning paradigm. Brain Res. 647:106–112; 1994.
96. Series, H. G.; Cowen, P. J.; Sharp, T. *p*-Chloroamphetamine (PCA), 3,4-methylenedioxy-methamphetamine (MDMA) and *d*-fenfluramine pretreatment attenuates *d*-fenfluramine-evoked release of 5-HT in vivo. Psychopharmacology (Berlin) 116:508–514; 1994.
97. Shapira, B.; Cohen, J.; Newman, N. E.; Lerer, B. Prolactin response to fenfluramine and placebo. Challenge following maintenance pharmacotherapy withdrawal in remitted depressed. Biol. Psychiatry 33:531–535; 1993.

98. Söderpalm, B.; Hjorth, S.; Engel, J. A. Effects of 5-HT_{1A} agonists and L-5-HTP in Montgomery's conflict test. Pharmacol. Biochem. Behav. 32:259–265; 1989.
99. Solyom, L. Controlling panic attacks with fenfluramine. Am. J. Psychiatry 151:621–622; 1994.
100. Targum, S. D.; Marshall, L. E. Fenfluramine provocation of anxiety in patients with panic disorder. Psychiatry Res. 28:295–306; 1989.
101. Thakore, J. H.; Dinan, T. G. Cortisol synthesis inhibition: A new treatment strategy for the clinical and endocrine manifestations of depression. Biol. Psychiatry 37:364–368; 1995.
102. Thiébot, M. H.; Jobert, A.; Soubrié, P. Chlordiazepoxide and GABA injected into the raphe dorsalis release the conditioned behavioural suppression induced in rats by a conflict procedure without nociceptive component. Neuropharmacology 19:633–641; 1980.
103. Titze de Almeida, R.; Shida, H.; Guimarães, F. S.; Del Bel, E. A. Stress-induced expression of the c-*fos* proto-oncogene in the hippocampal formation. Braz. J. Med. Biol. Res. 27:1083–1088; 1994.
104. Torda, T.; Murgas, K.; Cechova, E.; Kiss, A.; Saavedra, J. M. Adrenergic regulation of [^{3}H]ketanserin binding sites during immobilization stress in the rat frontal cortex. Brain Res. 527: 198–203; 1990.
105. Treit, D.; Menard, J.; Royan, C. Anxiogenic stimuli in the elevated plus-maze. Pharmacol. Biochem. Behav. 44:463–469; 1993.
106. Tye, N. C.; Everitt, B. J.; Iversen, S. D. 5-Hydroxytryptamine and punishment. Nature 268:741–742; 1977.
107. Viana, M. B.; Silveira, R.; Graeff, F. G. D-Fenfluramine releases 5-HT from terminals of the dorsal raphe nucleus. Braz. J. Med. Biol. Res. (in press).
108. Viana, M. B.; Tomaz, C.; Graeff, F. G. The elevated T-maze: A new animal model of anxiety and memory. Pharmacol. Biochem. Behav. 49:549–554; 1994.
109. Villani, F.; Johnston, D. Serotonin inhibits induction of long-term potentiation at commissural synapses in hippocampus. Brain Res. 606:304–308; 1993.
110. Willner, P. Animal models of depression: An overview. Pharmacol. Ther. 45:425–455; 1990.
111. Wise, C. D.; Berger, B. D.; Stein, L. Benzodiazepines: Anxiety-reducing activity by reduction of serotonin turnover in the brain. Science 177:180–183; 1972.
112. Wooley, C. S.; Gould, E.; McEwen, B. S. Exposure to excess glucocorticoids alters dendrite morphology of adult hippocampal pyramidal neurons. Brain Res. 531:225–231; 1990.