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Elevated mazes as animal models of anxiety: effects of serotonergic agents

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ABSTRACT

This article reviews reported results about the effects of drugs that act upon the serotonergic neurotransmission measured in three elevated mazes that are animal models of anxiety. A bibliographic search has been performed in MEDLINE using different combinations of the key words X-maze, plus-maze, T-maze, serotonin and 5-HT, present in the title and/or the abstract, with no time limit. From the obtained abstracts, several publications were excluded on the basis of the following criteria: review articles that did not report original results, species other than the rat, intracerebral drug administration alone, genetically manipulated rats, and animals having any kind of experimental pathology. The reported results indicate that the effect of drugs on the inhibitory avoidance task performed in the elevated T-maze and on the spatio temporal indexes of anxiety measured in the X and plus mazes correlate with their effect in patients diagnosed with generalized anxiety disorder. In contrast, the drug effects on the one-way escape task in the elevated T-maze predict the drug response of panic disorder patients. Overall, the drug effects assessed with the avoidance task in the T-maze are more consistent than those measured through the anxiety indexes of the X and plus mazes. Therefore, the elevated T-maze is a promising animal model of generalized anxiety and panic disorder.

Key words: animal model, elevated maze, anxiety, panic, serotonin.

INTRODUCTION

Early animal models of anxiety have been developed on the basis of paradigms taken from the experimental psychology of the 1950s and 60s, before the modern classification of psychiatric disorders had split anxiety disorders in different diagnostic categories. As a consequence, these animal models refer to normal and pathological anxiety, in general. On the whole, they rely on either inhibition of ongoing behavior elicited by conditioned stimuli that predict unavoidable electric shock or on suppression of rewarded behavior by concurrent electric-shock punishment. The first paradigm is based on associative or Pavlovian conditioning, comprising the so-called conditional emotional response (CER) and the conditioned suppression of lever pressing (Estes and Skinner 1941, Millenson and Leslie 1974). The second, is based on instrumental or operant conditioning, and involves an approach-avoidance conflict. For this reason, the latter models have been called punishment or conflict tests.

Early pharmacological analysis has shown that conflict tests predict clinical drug response better than conditioned suppression (Kelleher and Morse 1968). As a consequence, conflict tests have become widely used for assaying anti-anxiety agents, either for screening purposes or for studying their mechanisms of action. How-

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ever, with the discovery of new anxiolytics, such as buspirone and ritanserin, which act primarily on the neurotransmission mediated by serotonin (5-HT), it became clear that the available conflict tests failed to consistently detect their anxiolytic properties (Handley et al. 1993, Griebel 1995). Therefore, conflict tests seem to be fit only for detecting anxiolytics that act through the neurotransmission mediated by γ-aminobutyric acid (GABA), such as barbiturates or benzodiazepine agonists. Punishment tests have also been criticized because of their artificiality and the confounding influence of appetitive drives, such as hunger and thirst, as well as of pain (Treit 1985).

As these developments were taking place in basic research, classifications of anxiety disorders based on symptom clusters, time course and therapeutic response have been elaborated. Nowadays, the most widely used is part of the DSM IV classification of psychiatric disorders (American Psychiatric Association 1994), comprising the following primary anxiety disorders:

1. Generalized anxiety disorder (GAD), a state of excessive anxiety or apprehension lasting for more than six months. Neurovegetative symptoms are often present, but are relatively minor.

2. Panic disorder (PD), characterized by recurrent panic attacks, either unexpected or associated with particular situations. Panic attacks are sudden surges of intense fear or terror, desire of fleeing and feeling of imminent death, going crazy or loosing control. These subjective symptoms are accompanied by major neurovegetative changes, such as palpitation, hypertension, difficulty in breathing, sweating, urge to void the bladder and increased peristalsis. This leads to worry about the next attack or anticipatory anxiety, and avoidance of places where a panic attack would be embarrassing. Ultimately, generalized avoidance or agoraphobia may ensue. Nevertheless, agoraphobia sometimes occurs without panic attacks.

3. Obsessive-compulsive disorder characterized by intrusive, distressing thoughts (obsessions) and/or stereotyped or ritualized behavior (compulsions) that must be performed in order to alleviate intense anxiety.

4. Specific phobias, which are irrational fears of either objects (animals, blood, pointed instruments) or situations (heights, closed environments).

5. Social phobia (also called social anxiety disorder), or marked anxiety experienced in social situations, such as speaking in public, going to parties or being in a classroom.

The following discussion will refer to the first two categories, GAD and PD. The distinction between GAD and PD stemmed from the original observation by the North-American psychiatrist Donald Klein (Klein 1964) that chronic administration of the antidepressant imipramine improved PD, which was resistant to benzodiazepine anxiolytics at the dose regimens that improve GAD. This pharmacological distinction between the two categories has been further qualified, since it has been shown that chronic imipramine also improves GAD (Kahn et al. 1986) and high-potency benzodiazepines, such as alpralozam, are effective on PD when chronically administered (Schweizer et al. 1993). In spite of this, Donald Klein's postulation spurred research that revealed clear differences in the neurobiology of GAD and PD (for a recent review, see Ninan and Dunlop 2005).

The verification that each anxiety disorder engages a particular set of neurobiological systems led to the conclusion that animal models should address anxiety disorders specifically. Therefore, a given model is expected to have a pharmacological profile that correlates with the clinical drug response of the disorder it is intended to represent. Among the most important models of anxiety disorders that have been developed to replace the classical punishment tests are several types of elevated mazes, three of which are analyzed in the present article.

Elevated Mazes

The most widely used animal models of anxiety in the last two decades have been the elevated "X" and "plus" mazes. Both the elevated X-maze (Handley and Mithani 1984) and the elevated plus-maze (Pellow et al. 1985) consist of two opposed arms enclosed by walls except at the central end. These closed arms are perpendicular to two open arms of equal dimensions, which are devoid of any wall. The whole apparatus is elevated above the

floor. Since rats have innate fear of elevated open places, they enter less and stay for a shorter time in the open arms as compared to the closed arms when allowed to freely explore the maze. Typically, benzodiazepine anxiolytics increase the number of entries into and the time spent on the open arms. Countless drugs have been assayed in this model, and the results obtained summarized in several comprehensive reviews (e.g., Handley et al. 1993, Griebel 1995). In order to improve the capacity of the elevated plus-maze for detecting non-benzodiazepine anxiolytics, ethological analysis of behavioral items shown by the animals while exploring the elevated plus-maze (e.g., nose poking out of the enclosed arm and exploration of the open arm end) has been added to the former, spatio temporal measures (Cruz et al. 1994, Rodgers and Johnson 1995). However, this modification takes away one of the main advantages of the test, which is simplicity.

The British psychologist Sheila Handley, who conceived the idea of the crossed elevated maze, has argued that many of the inconsistencies found with drugs that alter 5-HT neurotransmission in the elevated X and plus mazes may be explained by the fact that these are mixed models, in the sense that the rat displays different strategies of defense while exploring them, which could be influenced in opposite directions by 5-HT (Handley et al. 1993). At least two strategies may be easily seen: 1) avoidance of open arms when the rat is in one of the closed arms, and 2) escape from an open arm to enter a safer, closed arm. This observation led to the idea of separating these behavioral tasks: inhibitory avoidance and one-way escape, a goal that has been achieved by building the elevated T-maze (Graeff et al. 1993).

The elevated T-maze results from shutting off the entrance to one of the closed arms of the elevated plus-maze. For the inhibitory avoidance task, the rat is placed at the end of the remaining closed arm and the latency to withdraw from this arm with the four paws is recorded in three successive trials made at 30-s intervals. Learning is indicated by the increase of the withdrawal latency along the trials. For the escape task, which initiates 30 s after the completion of the avoidance training, the rat is placed at the end of one of the open arms and the withdrawal latency from this arm is similarly recorded. In the studies performed so far, the number of trials of this task has varied from one to three. Pre-exposure to the open arm for 30 min, 24 h before the test has been found to decrease de first withdrawal latency from the open arm and increase the drug sensitivity of the escape task (Teixeira et al. 2000). The resemblance of the motor performance in both tasks serves as a control for non-specific drug effects on motor activity when the latencies to withdraw from the enclosed arm and from the open arm are changed to opposite directions by the drug treatment. However, whenever the latencies are similarly increased or decreased, there is need for independent assessment of motor drug effects. Measuring motor activity inside an arena fulfils this requirement, albeit adding more complexity to the test.

Behavioral and pharmacological validation of the elevated T-maze (Graeff et al. 1998, Zangrossi Jr and Graeff 1997) has shown that the increase in avoidance latency is a function of the aversive character of the open arm, and that the pharmacological profile of the avoidance task is similar to that of GAD. In contrast, the escape task is insensitive to doses of benzodiazepines that have an anxiolytic effect (decrease of withdrawal latency) in the avoidance task, and is impaired by chronic, but not acute administration of imipramine (Teixeira et al. 2000, Zanoveli et al. 2005), clomipramine and fluoxetine (Poltronieri et al. 2003), drugs that are used to treat PD. Moreover, avoidance has been shown to be enhanced by a CCK receptor agonist (Zanoveli et al. 2004), a drug class that has been shown to induce panic attack in PD patients (Graeff et al. 2005). As a result, one-way escape in the elevated T-maze may be viewed as an animal model of PD.

OBJECTIVE

A critical issue is whether the introduction of the T-maze represents an improvement over the preceding X and plus mazes in terms of consistency and predictive value of pharmacological effects of drugs that act on 5-HT-mediated neurotransmission. To address this question, we compare the results reported in the literature describing the effects of such drugs on the behavioral indexes of anxiety and panic measured in the three above types of elevated mazes.

MATERIALS AND METHODS

A MEDLINE search has been made using different combinations of the key words X-maze, plus-maze, T-maze, serotonin and 5-HT present in the title and/or the abstract with no time limit. From the obtained abstracts, several publications were excluded on the basis of the following criteria: review articles that did not report original results, species other than the rat, intracerebral drug administration alone, genetically manipulated rats, and animals having any kind of experimental pathology. As a result, we selected 20 articles for reviewing, which are quoted in Tables I and II. Further references have been added for the sake of introduction and discussion.

REPORTED RESULTS AND DISCUSSION

To improve clarity of analysis, the reviewed results are divided into two sets; the first comprises results obtained with 5-HT reuptake inhibitors and the second, with drugs that act as agonists or antagonists on different subtypes of 5-HT receptors.

5-HT Reuptake Inhibitors

Nowadays, 5-HT reuptake inhibitors are the most widely used class of drugs in the treatment of anxiety disorders. They are first-choice medication for both GAD and PD, in addition to obsessive-compulsive disorder and social anxiety disorder (Nutt 2005).

The reported results on 5-HT reuptake inhibitors that we found in the literature survey are summarized in Table I.

Meaningful clinical correlations have been found in regard to the effects of imipramine, clomipramine and paroxetine on the two tasks of the elevated T-maze. Both imipramine and clomipramine enhanced avoidance after single administration, and this anxiogenic effect has been related to the initial worsening that has been observed during the first week of medication with these drugs in PD patients (Gentil et al. 1993). On the other hand, the anxiolytic effect of imipramine on the same task following chronic administration has been correlated with amelioration of GAD, which has been observed following repeated administration of the same drug for several weeks (Kahn et al. 1986). More important, the impairment of escape in the elevated T-maze caused by these three drugs correlates with their antipanic effect documented in clinical assays (Klein 1964, Gentil et al. 1993, Ballenger et al. 1998).

In contrast, not a single drug correlation has been found between the effect of these drugs on the special indexes of anxiety measured in the elevated plus-maze, although it is arguable that the contrast between the anxiogenic effect of single administration and the anxiolytic effect of chronic administration of cianopramine reported in the elevated plus maze (Griebel et al. 1994a) may correlate with the effect of this drug on GAD. Nevertheless, such clinical effects have not yet been reported.

The inconsistency of the effect of single administration of fluoxetine on the elevated plus maze is particularly remarkable: anxiolytic in one study (Griebel et al. 1999), anxiogenic in three (Silva et al. 1999, Silva and Brandão 2000) and unchanged in another one (Griebel et al. 1997). The same occurs following chronic administration: unchanged (Griebel et al. 1999, Silva and Brandão 2000), versus anxiogenic (Silva et al. 1999).

5-HT Receptor Ligands

The reported results on the effects of agonist and antagonist agents act, more or less selectively, on different subtypes of 5-HT receptors are summarized in Table II.

Among the drugs listed in Table II, only buspirone has been used in clinical practice, and has been tested in the three types of elevated mazes. In the two documented studies performed in the elevated T-maze, single administration of buspirone had an anxiolytic effect in the avoidance task, while not affecting escape performance (Graeff et al. 1998, Poltronieri et al. 2003). This correlates with the beneficial effect of this drug reported in GAD patients (Pecknold 1997). Nevertheless, the clinical effect requires several weeks of repeated drug administration to become apparent. So far, the results with chronic administration of buspirone on the avoidance task in the T-maze are inconsistent, since both no change (Poltronieri et al. 2003) and impairment – an anxiolytic effect (Zanoveli et al. 2005) have been reported. As clinical evidence shows that the drug fails to improve PD when given chronically (Pecknold 1997), the absence of effect on escape reported both by Poltronieri et al. (2003) and Zanoveli et al. (2005) may be viewed as a positive clinical correlation.

In only one out of three studies carried out in the

TABLE I
Comparative effects of reuptake inhibitors on rat behavior in two elevated mazes.

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Imipramine $NA > 5\text{-}HT$	Teixeira et al. (2000)	Single (5-15, IP)	**Enhanced** (Anxiogenic)	Unchanged	Not tested
		21 days (5-15, IP)	**Impaired** (Anxiolytic)	**Impaired** (Antipanic)	Not tested
	Zanoveli et al. (2005)	3 days (15, IP)	Unchanged	Unchanged	Not tested
		21 days (15, IP)	**Impaired** (Anxiolytic)	**Impaired** (Antipanic)	Not tested
	Griebel et al. (1997)	Single (1-10, SC)	Not tested	Not tested	Unchanged
Clomipramine $5\text{-}HT > NA$	Graeff et al. (1998)	Single (10, IP)	**Enhanced** (Anxiogenic)	Unchanged	Not tested
	Poltronieri et al. (2003)	Single (3-30, IP)	Unchanged	Unchanged	Not tested
		21 days (3-30, IP)	Unchanged	**Impaired** (Antipanic)	Not tested
Subutramine $NA/5\text{-}HT$[1]	Jorge et al. (2004)	Single (5-20, IP)	Decrease (Anxiolytic)	Decrease (Antipanic)	Unchanged
Fluoxetine $5\text{-}HT$[1]	Poltronieri et al. (2003)	Single (5-15, IP)	Unchanged	Unchanged	Not tested
		21 days (5-15, IP)	Unchanged	**Impaired** (Antipanic)	Not tested
	Griebel et al. (1997)	Single (1-10, SC)	Not tested	Not tested	Unchanged
	Griebel et al. (1999)	Single (20, IP)	Not tested	Not tested	Increased (Anxiolytic)
		23-24 days (20, IP)	Not tested	Not tested	Unchanged
	Silva et al. (1999)	Single (5, IP)	Not tested	Not tested	Decreased (Anxiogenic)
		21 days (5, IP)	Not tested	Not tested	Decreased (Anxiogenic)

TABLE I (continuation)

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Fluoxetine 5-HT^1	Silva and Brandão (2000)	Single (10, IP)	Not tested	Not tested	Decreased (Anxiogenic)
		14 days (10, IP)	Not tested	Not tested	Unchanged
Paroxetine 5-HT^1	Beijamini and Andreatini (2003)	3 times in 24 h (5, VO)	Impaired (Anxiolytic)	Unchanged	Not tested
		7 days (5, VO)	**Impaired**[3] (Anxiolytic)	**Impaired**[3] (Antipanic)	Not tested
	Koks et al. (2001)	Single (0.5, IP)	Not tested	Not tested	Decreased (Anxiogenic)
Zimelidine 5-HT^1	Griebel et al. (1997)	Single (1-10, SC)	Not tested	Not tested	Unchanged
Fluvoxamine 5-HT^1	Griebel et al. (1997)	Single (1-10, SC)	Not tested	Not tested	Unchanged
Citalopram 5-HT^1	Griebel et al. (1994a)	Single (10,30, IP)	Not tested	Not tested	Decreased (Anxiogenic)
Cianopramine 5-HT^1	Griebel et al. (1994a)	Single (10, SC)	Not tested	Not tested	Decreased (Anxiogenic)
		21 days (10, SC)	Not tested	Not tested	Increased (Anxiolytic)
Fenfluramine 5-HT^2	Graeff et al. (1996)	Single (0.03-0.3, IP)	Enhanced[4]	**Impaired** (Antipanic)	Not tested

5-HT: serotonin; NA: noradrenaline; IP: intraperitoneal; SC: subcutaneous; VO: oral. Bold character words indicate known clinical correlation. [1]Selective agent, not affecting neurotransmitter receptors; [2]also a 5-HT releaser; [3]clinical improvement only after several weeks; [4]nearly significant trend.

elevated plus-maze, buspirone had an anxiolytic effect (Griebel et al. 1997). In the remaining two (Moser et al. 1990, Collinson and Dawson 1997) as well as in the only study reported in the elevated X-maze (Critchley et al. 1992), an anxiogenic effect has been observed. Since chronic administration has not been explored, clinical correlation cannot be appropriately discussed.

Although the buspirone analog ipsapirone has not been marketed, experimental clinical trials have demonstrated its efficacy in GAD patients (Cutler et al. 1993). Like buspirone, ipsapirone had an anxiolytic effect in the avoidance task in the elevated T-maze after single administration (Graeff et al. 1998). An anxiolytic effect of the same drug has also been documented in the X-maze (Critchley et al. 1992), but not in the plus-maze (Setem et al. 1999).

Experimental clinical assays have been carried out with gepirone, another buspirone analog, showing a therapeutic action in GAD patients (Rickels et al. 1997). So far, this drug has not been tested in the elevated T-maze, but interesting results have been reported in the elevated plus maze, namely an anxiogenic effect after single ad-

TABLE II
Comparative effects of 5-HT receptor ligands on rat behavior in two elevated mazes.

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
5-MeODMT 5-$HT_{1/2}$ *agonist*	Critchley and Handley (1987)[2]	Single (0.25-2.5, IP)	Not tested	Not tested	Decreased (Anxiogenic)
RU 24969 5-$HT_{1/2}$ *agonist*	Critchley and Handley (1987)[2]	Single (0.1-3, IP)	Not tested	Not tested	Decreased (Anxiogenic)
	Critchley et al. (1992)[2]	Single (0.5-2, IP)	Not tested	Not tested	Decreased (Anxiogenic)
8-OH-DPAT 5-HT_{1A} *agonist*	Critchley and Handley (1987)[2]	Single (0.015-1, IP)	Not tested	Not tested	Decreased (Anxiogenic)
	Moser et al. (1990)	Single (12.5-200, SC)	Not tested	Not tested	Decreased (Anxiogenic)
	Critchley et al. (1992)[2]	Single (0.05-0.2, IP)	Not tested	Not tested	Decreased (Anxiogenic)
	McBlane and Handley (1994)[2]	Single (0.1, 0.2, IP)	Not tested	Not tested	Decreased[3] (Anxiogenic)
	Collinson and Dawson (1997)	Single (0.01-0.3, SC)	Not tested	Not tested	Increased (Anxiolytic)
	Griebel et al. (1997)	Single (0.1-1, SC)	Not tested	Not tested	Unchanged
BAY R 1521 5-HT_{1A} *agonist*	Critchley et al. (1992)[2]	Single (0.1-1.2, IP)	Not tested	Not tested	Decreased (Anxiogenic)
Flesinoxan 5-HT_{1A} *agonist*	Griebel et al. (1997)	Single (0.1-1, SC)	Not tested	Not tested	Unchanged
Buspirone 5-HT_{1A} *partial agonist*	Graeff et al. (1998)	Single (0.3, IP)	**Impaired**[1] (Anxiolytic)	Unchanged	Not tested
	Poltronieri et al. (2003)	Single (0.3-3, IP)	**Impaired**[1] (Anxiolytic)	Unchanged	Not tested

TABLE II (continuation)

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Buspirone 5-HT_{1A} *partial agonist*	Poltronieri et al. (2003)	21 days (0.3-3, IP)	Unchanged	**Unchanged**	Not tested
	Zanoveli et al. (2005)	21 days (0.3, IP)	**Impaired** (Anxiolytic)	**Unchanged**	Not tested
	Moser et al. (1990)	Single (0.5-2, SC)	Not tested	Not tested	Decreased (Anxiogenic)
	Critchley et al. (1992)[2]	Single (0.1-5, IP)	Not tested	Not tested	Decreased (Anxiogenic)
	Collinson and Dawson (1997)	Single (0.3-4, IP)	Not tested	Not tested	Decreased (Anxiolytic)
	Griebel et al. (1997)	Single (0.1-1, SC)	Not tested	Not tested	**Increased**[1] (Anxiolytic)
Isapirone 5-HT_{1A} *partial agonist*	Graeff et al. (1998)	Single (0.25-2, IP)	**Impaired**[1] (Anxiolytic)	Unchanged	Not tested
	Critchley et al. (1992)[2]	Single (0.25-5, IP)	Not tested	Not tested	**Increased**[1] (Anxiolytic)
	Setem et al. (1999)	Single (0.25-2.25)	Not tested	Not tested	Unchanged
Gepirone 5-HT_{1A} *partial agonist*	Critchley et al. (1992)[2]	Single (0.1-5, IP)	Not tested	Not tested	Unchanged
	Motta et al. (1992)	Single (1-10, IP)	Not tested	Not tested	Decreased (Anxiogenic)
		14 days (1-10, IP)	Not tested	Not tested	**Increased**[1] (Anxiolytic)
	Silva and Brandão (2000)	Single (1-10, IP)	Not tested	Not tested	Decreased (Anxiogenic)
		14 days (10, IP)	Not tested	Not tested	**Increased**[1] (Anxiolytic)

TABLE II (continuation)

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Yohimbine 5-HT_{1A} *partial agonist*/ 5-HT_{1B} *antagonist*	Graeff et al. (1998)	Single (3, IP)	**Enhanced** (Anxiolytic)	Unchanged	Not tested
	Critchley et al. (1992)[2]	Single (0.5, IP)	Not tested	Not tested	**Decreased** (Anxiogenic)
Propanolol 5-$HT_{1A/B}$ *antagonist*	Gibson et al. (1994)	Single (5, IP)	Not tested	Not tested	Unchanged
Pindolol 5-$HT_{1A/B}$ *antagonist*	Critchley and Handley (1987)[2]	Single (0.05, IP)	Not tested	Not tested	Increased[4] (Anxiolytic) Decreased[5] (Anxiogenic)
WAY 1006352 5-HT_{1A} *antagonist*	Collinson and Dawson (1997)	Single (0.3-1, IP)	Not tested	Not tested	Unchanged
MDL 73005EF 5-HT_{1A} *antagonist*	Moser et al. (1990)	Single (0.03-0.25, SC)	Not tested	Not tested	Increased (Anxiolytic)
mCPP 5-HT_{1A} *agonist*	Graeff et al. (1998)	Single (0.1-0.8, IP)	**Enhanced** (Anxiogenic)	Impaired (Antipanic)	Not tested
	Gibson et al. (1994)	Single (0.125-1, IP)	Not tested	Not tested	**Decreased** (Anxiogenic)
	Griebel (et al. (1994b)	Single (1, SC)	Not tested	Not tested	**Decreased** (Anxiogenic)
		21 days (1, SC)	Not tested	Not tested	Unchanged
TFMPP 5-$HT_{1B/2C}$ *agonist*	Graeff et al. (1998)	Single (0.4, IP)	**Enhanced** (Anxiogenic)	Impaired (Antipanic)	Not tested
	Setem et al. (1999)	Single (0.1-0.4, IP)	Not tested	Not tested	**Decreased** (Anxiogenic)

TABLE II (continuation)

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Mianserin 5-$HT_{2A/C}$ *antagonist*	Griebel et al. (1997)	Single (0.3-3, SC)	Not tested	Not tested	Increased (Anxiolytic)
Ritanserin 5-$HT_{2A/C}$ *antagonist*	Critchley and Handley (1987)[2]	Single (0.025-5, IP)	Not tested	Not tested	**Increased**[1] (Anxiolytic)
Ketanserin 5-$HT_{2A/C}$ *antagonist*	Critchley and Handley (1987)[2]	Single (0.1-1, IP)	Not tested	Not tested	Increased (Anxiolytic)
	Griebel et al. (1997)	Single (0.1-1, SC)	Not tested	Not tested	Unchanged
	Motta et al. (1992)	Single (0.5, 1, IP)	Not tested	Not tested	Increased (Anxiolytic)
					Decreased[5] (Anxiogenic)
Seganserin 5-$HT_{2A/C}$ *antagonist*	Critchley and Handley (1987)[2]	Single (0.1-1, IP)	Not tested	Not tested	Increased (Anxiolytic)
Pirenperone 5-$HT_{2A/C}$ *antagonist*	Griebel et al. (1997)	Single (0.01-0.1, SC)	Not tested	Not tested	Decreased (Anxiolytic)
SR 46349B 5-HT_{2A} *antagonist*	Graeff et al. (1998)	Single (1-10, IP)	Impaired (Anxiolytic)	Unchanged	Not tested
RP 62203 5-HT_{2A} *antagonist*	Graeff et al. (1998)	Single (0.25-4, IP)	Unchanged	Unchanged	Not tested
SB 200646A 5-$HT_{2B/C}$ *antagonist*	Graeff et al. (1998)	Single (3-30, IP)	Impaired (Anxiolytic)	Unchanged	Not tested
SER 082 5-$HT_{2B/C}$ *antagonist*	Graeff et al. (1998)	Single (0.1-1, IP)	Impaired (Anxiolytic)	Unchanged	Not tested
SB-242084 5-HT_{2C} *antagonist*	Martin et al. (2002)	Single (0.1-3, IP)	Not tested	Not tested	Increased (Anxiolytic)

ministration and an anxiolytic effect following repeated administration for three weeks (Motta et al. 1992, Silva and Brandão 2000). One reported study in the X-maze has failed to show any effect of gepirone on the spatio temporal indexes of anxiety (Critchley et al. 1992). Like buspirone and isapirone, the clinical anxiolytic effect of gepirone becomes patent only after chronic administration. Therefore, the results reported in plus-maze bear at

TABLE II (continuation)

Drug *Action*	Reference	Administration (mg/kg, route)	T-maze		Plus-maze
			Inhibitory avoidance	One-way escape	Open-arm entries/time
Ondansetron Zacopride ICS 205-9307 MDL 72222 5-HT_3 *antagonists*	Griebel et al. (1997)	Single (0.01-0.1, SC)	Not tested	Not tested	Unchanged
BRL 4670 5-HT_3 *antagonist*	Setem et al. (1999)	Single (0.001-0.1, IP)	Not tested	Not tested	Unchanged

IP: intraperitoneal; SC: subcutaneous. [1]Clinical improvement only after several weeks; [2]X-maze; [3]abolished by restraint for 1 h immediately before the test; [4]0.05-0.5; [5]1mg/kg.

least partial correlation with the clinical evidence.

Overall, the results with the above three 5-HT_{1A} partial agonists suggest that the elevated T-maze can detect an anxiolytic effect of single administration. Although this does not strictly correlate with the time course of their clinical action, it may be useful for the laboratory screening of new anxiolytic agents.

With the exception of piremperone (Griebel et al. 1997) and of the 1 mg/kg dose of ketanserin (Motta et al. 1992), which enhanced anxiety in the elevated plus-maze, both selective and mixed antagonists of the 5-HT_{2A} and 5-HT_{2C} receptor subtypes had anxiolytic effects either in the avoidance task of the T-maze or in the spatio temporal measures of the X or the plus maze. However, a direct comparison among mazes is prevented by the fact that each of these compounds has been tested in only one type of maze. Among the drugs studied, only ritanserin has been used in clinical assays, limiting the assessment of clinical correlations. In any case, the correlation found for ritanserin is positive, since this drug has been reported to improve GAD, although the therapeutic effect became significant only after three weeks of repeated administration (Ceulemans et al. 1985). Mianserin has been used clinically in depressed patients, but we have found no study focused on anxiety disorders. In addition, the actions of mianserin are not restricted to 5-HT mechanisms, since this drug behaves as an antagonist on α_2-adrenergic receptors.

The compounds designed in Table II by character and figure codes have been developed by drug companies as putative anxiolytic agents. With the exception of RP 62203 (Graeff et al. 1998), all of them had anxiolytic effects in the three elevated mazes, highlighting the usefulness of these animal models for screening new therapeutic agents. In addition, the consistent results reported with 5-HT_{2A} and 5-HT_{2C} receptor antagonists support an important role played by these subtypes of 5-HT receptors in the neurobiology of anxiety (Wood 2003, Cohen 2005).

Three agents that increase anxiety in normal humans and/or aggravate symptoms of different anxiety disorders, namely the alkaloid yohimbine and the synthetic drugs mCPP and TFMPP, have been investigated in at least one of the elevated mazes. The mode of action of yohimbine is complex, not only because it affects more than one subtype of 5-HT receptors, but also because it behaves as an antagonist on α_2-adrenergic receptors. In turn, mCPP and TFMPP act as preferential agonists on the 5-HT_{2C} receptor. In agreement with their effect in human beings, the three agents facilitated the avoidance

task in the T-maze (Graeff et al. 1998), and increased anxiety indexes in the plus-maze (Gibson et al. 1994, Griebel et al. 1994b) or in the X-maze (Critchley et al. 1992) after single administration. Interestingly, the anxiogenic effect of mCPP has no longer been detected after 21 days of repeated administration, indicating the development of tolerance (Griebel et al. 1994b).

The remaining 5-HT receptor agonists studied have not yet been investigated in human beings, so that correlation with human anxiety cannot be made. Among these drugs, the selective 5-HT_{1A} receptor agonist 8-OH-DPAT has been the most frequently explored. The majority of the results obtained in either the X or the plus-maze show an anxiogenic effect. This is somewhat puzzling, since experimental evidence indicates that stimulation of 5-HT_{1A} receptors in forebrain limbic structures leads to anxiolytic effects (e.g., Zangrossi et al. 1999). An anxiolytic role of this receptor subtype is strongly supported by recent genetic evidence showing that mice devoid of 5-HT_{1A} receptors (knockout mice) look overanxious in several laboratory tests. Anxiety in mice is defined as a high level of avoidance of novel and unfamiliar environment and an increased fear reaction. Other aspects of anxiety such as autonomic activation, increased stress responsiveness, and neuroendocrine abnormalities have also been described in receptor knockout mice (Toth 2003).

A possible explanation is that systemically administered 8-OH-DPAT preferentially acts on pre-synaptic 5-HT_{1A} receptors localized in the cell bodies of serotonergic neurons, the stimulation of which results in decrease of neuronal firing and of the resulting 5-HT release from 5-HT terminals (Adell et al. 2002). In this way, post-synaptic 5-HT_{1A} receptors would actually be less stimulated than normally following systemic injection of 8-OH-DPAT. Supporting this hypothesis, opposite effects on flight induced by PAG stimulation have been reported by intracerebral administration of 8-OH-DPAT either into the dorsal raphe nucleus or into the PAG, the former reproducing the effect of its systemic administration (Beckett and Marsden 1997). Partial 5-HT_{1A} agonists, such as buspirone, have also been shown to reduce 5-HT neuron firing initially, but tolerance gradually develops along three weeks of repeated administration, testifying for the desensitization of pre-synaptic 5-HT_{1A} receptors (Blier and de Montigny 1990). It has even been suggested that this phenomenon would explain the delayed clinical action of the drug, which would be due, at least in part, to the overstimulation of post-synaptic 5-HT_{1A} receptors (Mongeau et al. 1997).

CONCLUSION

From the evidence reviewed above, a comparative account among the mazes can be made.

The advantages of the elevated T-maze over the elevated X and plus mazes are:

1. The T-maze measures two behavioral tasks, namely inhibitory avoidance and one-way escape that represent two types of defense, respectively related to the emotions of anxiety and fear (panic).
2. There is good correlation between the effects of 5-HT reuptake inhibitors on the avoidance task and on GAD, as well as between those on the escape task and on PD.
3. Like the spatio temporal indexes of anxiety in the X and plus mazes, the avoidance task in the T-maze is sensitive to anxiolytic and anxiogenic drugs that act on 5-HT receptors, but the results obtained in the T-maze tend to be more consistent than in the other mazes.

The disadvantages are:

1. TFMPP and mCPP had an antipanic effect in the avoidance task, which is negatively correlated with the panicogenic action of these compounds that has been evidenced in clinical assays (Graeff et al. 2005).
2. An independent assessment of motor activity is necessary whenever the changes in avoidance and escape latency are in the same direction.
3. The tasks performed in the elevated T-maze cannot be automated, in contrast to the exploratory behavior displayed on the X and plus mazes.

It may be concluded that in terms of predictive value for drug response, the elevated T-maze is a promising model of GAD and PD. In addition, the reported results on the effects of intracerebral injection serotonergic

drugs measured in the two tasks of the elevated T-maze support the proposal by Deakin and Graeff (1991) that 5-HT enhances anxiety by acting on forebrain limbic structures, whereas it inhibits panic by acting on the midbrain periaqueductal gray matter (for a recent review of the empirical evidence, see Graeff 2004). This highlights the usefulness of the elevated T-maze for testing hypothesis on the role of 5-HT in the pathophysiology of these disorders. Therefore, the model may also have some degree of theoretical or construct validity.

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RESUMO

No presente artigo, revisamos resultados publicados relatando efeitos de drogas que atuam na neurotransmissão serotonérgica medidos em três labirintos elevados, que são modelos animais de ansiedade. Realizamos uma busca bibliográfica no MEDLINE, usando diferentes combinações das palavras-chave: *X-maze*, *plus-maze*, *T-maze*, *serotonin* e *5-HT*, presentes no título ou no resumo, sem limite de tempo. Dos resumos obtidos, vários foram excluídos com base nos seguintes critérios: artigos de revisão que não continham resultados originais, espécies diferentes do rato, apenas injeções intracerebrais, ratos geneticamente manipulados, animais com algum tipo de patologia experimental. Os resultados relatados indicam que o efeito de drogas na tarefa de esquiva inibitória desempenhada no labirinto em T elevado, bem como nos índices espaciais de ansiedade nos labirintos em X ou em forma de cruz se correlacionam com os efeitos em pacientes diagnosticados com o transtorno de ansiedade generalizada. Por outro lado, os efeitos de drogas na tarefa de fuga unidirecional do labirinto em T predizem a resposta a drogas dos pacientes com o transtorno de pânico. De modo geral, os efeitos de drogas sobre a tarefa de esquiva no labirinto em T são mais consistentes que os medidos pelos índices de ansiedade calculados nos labirintos em X e em forma de cruz. Portanto, o labirinto em T-elevado é um modelo promissor dos transtornos de ansiedade generalizada e de pânico.

Palavras-chave: modelo animal, labirinto elevado, ansiedade, pânico, serotonina.

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