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5-HT1A receptors of the lateral septum regulate inhibitory avoidance but not escape behavior in rats

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Abstract

Serotonin in the lateral septum (LS) has been implicated in the modulation of defensive behaviors and in anxiety. However, it is currently unknown whether changes in 5-HT mechanisms in this brain area may selectively affect defensive responses associated with specific subtypes of anxiety disorders recognized in clinical settings. To address this question, we evaluated the effect of the intra-LS injection of the 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT (0.6, 3.0, 15.0 nmol) in male Wistar rats exposed to the elevated T-maze animal model of anxiety. This test allows the measurement of two behavioral defensive responses in the same rat: inhibitory avoidance and escape behavior. In clinical terms, these responses have been respectively related to generalized anxiety and panic disorder. The effects of 8-OH-DPAT were compared to those caused by a standard anxiolytic compound, the benzodiazepine receptor agonist midazolam (MDZ, 20 nmol). We also investigated whether the intra-LS injection of the 5-HT_{1A} receptor antagonist WAY-100635 (0.37 nmol) was able to block the effects of 8-OH-DPAT. All animals were also tested in an open field for locomotor activity assessments. Results showed that whereas intra-LS administration of MDZ decreased avoidance latencies, suggesting an anxiolytic action, 8-OH-DPAT caused the opposite effect. Neither drug affected the escape performance. Intra-LS administration of WAY-100635 blocked the anxiogenic effect caused by 8-OH-DPAT. No changes to locomotion were detected in the open field. The data suggests that LS 5-HT_{1A} receptors are involved in the control of inhibitory avoidance behavior and that a failure in this regulatory mechanism may be of importance to the physiopathology of generalized anxiety disorder.

Keywords: Anxiety; Panic; Lateral septum; 5-HT_{1A} receptors; Elevated T-maze

1. Introduction

Recently obtained evidence indicates that the serotonergic pathway, connecting the dorsal raphe nucleus (DRN) to the lateral septum (LS), is involved in the mediation of defensive behavior to threatening/stressful stimuli. For instance, exposure of mice to a predator results in behavioral activation (with predominant risk assessment behavior) and an increase in serotonin (5-HT) release in the LS (Beekman et al., 2005). Moreover, stimulation of the DRN 5-HT neurons by corticotrophin-releasing factor (CRF), a neurohormone involved in the mediation of stress responses (Boorse and Denver, 2006), resulted in an increase of 5-HT release in the LS (Price and Lucki, 2001). Facilitation of 5-HT-mediated neurotransmission in the LS, by local administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT, has also been shown to increase the expression of defensive behavior in rats exposed to two widely used animal models of anxiety, the elevated plus-maze and the social interaction test (Cheeta et al., 2000a,b). Altogether, these evidences suggest that 5-HT mechanisms in the LS may be implicated in the neurobiology of anxiety.

In the last years, a growing number of evidence has led to the idea that 5-HT exerts a distinctive role in the physiopathology of different types of anxiety recognized in clinical settings (Graeff, 2002, Domschke et al., 2006; Maron and Shlik, 2006; Lanzenberger et al., 2007). This can be attested, for instance, from clinical data showing that 5-HT-acting drugs such as buspirone is effective in the treatment of generalized anxiety,

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but not of panic or post-traumatic stress disorders (Gorman, 2003; Perugi et al., 2007; Bisson, 2007).

In animal studies, it has also been increasingly documented that the interference with brain 5-HT neurotransmission has distinctive consequences on the expression of defensive behaviors thought to be associated with specific anxiety pathologies. Accordingly, it has been observed (Sena et al., 2003; Pobbe and Zangrossi, 2005) that the stimulation of 5-HT neurons in the DRN facilitates the acquisition of inhibitory avoidance, indicating a pro-aversive effect. On the other hand, it impairs the expression of escape behavior, an antiaversive-like action. Based on an extensive series of pharmacological studies conducted in different laboratories, inhibitory avoidance has been related to generalized anxiety, whereas escape is to panic disorder (Blanchard et al., 2001; Mongeau and Marsden, 1997; Jenck et al., 1995; Gray and McNaughton, 2001; Graeff and Zangrossi, 2002; McNaughton and Corr, 2004).

The aforementioned results from DRN pharmacological manipulation have been interpreted in terms of the existence of different DRN-5-HT pathways innervating anxiety- and panic-related structures in the brain. More specifically, it has been proposed (Deakin and Graeff, 1991; Graeff, 2002) that this indolamine, by acting in forebrain structures such as the amygdala, hippocampus and frontal cortex, would facilitate the expression of anxiety-related responses, whereas in the dorsal periaqueductal gray matter (DPAG), it would inhibit panic-like behaviors.

Although there is evidence from animal studies that 5-HT mechanisms in the LS are involved in anxiety modulation, it is presently unknown whether this involvement is restricted to a particular set of defensive behaviors which are distinctively associated with clinical anxiety disorders. For this reason, this study evaluated whether the activation of 5-HT_{1A} receptors of the LS, which are densely found in the region (Pazos and Palacios, 1985; Hall et al., 1997), causes differential effects on the acquisition of inhibitory avoidance and escape performance of rats submitted to the elevated T-maze test of anxiety. This model allows the measurement of these two responses in the same animal and it has been extensively validated (Graeff et al., 1993; Viana et al., 1994; Zangrossi and Graeff, 1997; Teixeira et al., 2000; Poltronieri et al., 2003). The results of the elevated T-maze pharmacological validation have shown that compounds representative of two classes of anxiolytics – namely the agonist of benzodiazepine receptors diazepam and the 5-HT_{1A} agonist buspirone – selectively impair inhibitory avoidance while leaving escape performance unchanged (Graeff et al., 1993; Viana et al., 1994; Graeff et al., 1998a,b). These results are compatible with the idea that inhibitory avoidance relates to generalized anxiety disorder. In contrast, escape performance is impaired by chronic, but not acute administration of imipramine (Teixeira et al., 2000), clomipramine and fluoxetine (Poltronieri et al., 2003), drugs that are used to treat panic disorder. As a result, escape in the elevated T-maze has been used as an animal model of panic.

The role of 5-HT_{1A} receptors of the LS was assessed by local microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT. Since it has been previously shown that the drug has also moderate affinity for 5-HT_7 receptors (Thomas et al., 1998), we investigated whether local pre-administration of the selective 5-HT_{1A} receptor antagonist WAY-100635 counteracts its behavioral effects. The effects of 8-OH-DPAT were compared to those caused by a standard anxiolytic compound, the benzodiazepine receptor agonist midazolam.

In order to assess the effect of the drugs on locomotion, all animals were also tested in an open field immediately after being exposed to the elevated T-maze.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) were housed in groups of 4–6 *per* cage (50 × 60 × 22 cm) until surgery. After surgery, animals were housed in pairs (cages measuring 30 × 19 × 13 cm). Room temperature was maintained at 22 ± 1 °C with lights on from 0700 to 1900 h. Food and water were freely available throughout the experiments. All procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior Guidelines for Care and Use of Laboratory Animals, which are also in compliance with international laws and policies. All efforts were made to minimize animal suffering.

2.2. Apparatus

2.2.1. Elevated T-maze

The elevated T-maze was made of wood and had three arms of equal dimensions (50 × 12 cm). One arm, enclosed by walls of 40 cm high, was perpendicular to two opposed open arms. To avoid falls, the open arms were surrounded by a 1 cm high Plexiglas rim. The whole apparatus was elevated 50 cm above the floor.

2.2.2. Open field

The apparatus used to measure locomotion was a wooden square box (60 × 60 cm), with walls 30 cm high and the floor divided into 9 squares of 20 × 20 cm.

Luminosity at the level of the maze arms or the open field center was 60 lx.

2.3. Drugs

Midazolam maleate and WAY-100635 (Sigma, USA) were dissolved in sterile saline 0.9%. 8-OH-DPAT (Sigma, USA) was dissolved in a saline-Tween 80 2% solution. Control animals were administered with either saline or saline-Tween 80 2% solution.

2.4. Surgery

Rats were anaesthetized with 2,2,2 tribromoethanol (250 mg/kg, i.p.) associated with local anesthesia (2% lidocaine with a vasoconstrictor; Harvey, Brazil) and fixed in a stereotaxic frame (David Kopf, USA). Guide cannulae were made in our laboratory, from stainless steel (0.6 mm outer diameter, 0.4 mm inner diameter), and

were bilaterally implanted 2 mm above the LS, to minimize damage to the injection site. The following coordinates from Bregma were used: posterior: +0.5 mm, lateral: ±1.9 mm, ventral: −3.3 mm. The guide cannulae were inserted at an angle of 14° with the sagittal plane and were fixed to the skull with acrylic resin and two stainless steel screws. At the end of surgery, cannulae were sealed with stainless steel wires to protect them from obstruction. To prevent infections, at the end of the surgery, all animals were injected (IM) with a 0.2 ml of a pentabiotic preparation (Pentabiotico Veterinário Pequeno Porte; Forte Dodge, Brazil).

2.5. Procedure

On the fourth and fifth day after surgery, the rats were gently handled by the experimenter for 5 min. On the sixth day, they were exposed to one of the open arms of the elevated T-maze for 30 min. A wooden barrier mounted on the border of the open arm, between the maze's central area and the arm's proximal end, isolated this arm from the rest of the maze. It has been shown that this pre-exposure procedure shortens escape latencies. Thus, it renders the escape task more sensitive to the effects of antipanic drugs (Teixeira et al., 2000; Poltronieri et al., 2003).

On the seventh day, animals were tested in the elevated T-maze and in the open field. For each experiment, independent group of animals were used.

In experiment 1, animals were injected into the LS (0.2 μl) with midazolam (20 nmol) or saline (n=8–9).

In experiment 2, animals were microinjected (0.2 μl) with 8-OH-DPAT (0.6, 3.0 ou 15.0 nmol) or vehicle (n=12–13).

In experiment 3, rats were injected (0.2 μl) into the LS with WAY-100635 (0.37 nmol), saline or vehicle. Ten minutes later, animals of each group were injected into the LS either with vehicle, saline or with 8-OH-DPAT (3.0 nmol, n=10–11). Thus, the following groups were formed: saline/vehicle, WAY/saline, vehicle/8-OH-DPAT and WAY/8-OH-DPAT. Doses were selected on the basis of previous studies (de Paula Soares and Zangrossi, 2004; Zanoveli et al., 2005; de Bortoli et al., 2006).

For drug microinjection, a needle (0.3 mm outer diameter) was introduced through the guide cannula until its tip was 2 mm below the end of the cannula. The drugs were injected over a period of 120 s using a 10 μl microsyringe (Hamilton 701-RN, USA) attached to a microinfusion pump (KD Scientific, USA). The displacement of an air bubble inside the polyethylene catheter connecting the syringe needle to the intracerebral needle was used to monitor the microinjection. The intracerebral needle was removed 60 s after the injection.

Ten minutes after drug administration, animals were tested in the elevated T-maze for inhibitory avoidance measurements. Each rat was placed at the end of the enclosed arm, and the time taken to exit this arm with all four paws was recorded (baseline latency). The same measurement was repeated in two subsequent trials (avoidance 1 and 2) at 30 s intervals. The time untreated rats remain in the enclosed arm, in a second and third exposure to this arm, usually increases due to these animals' innate fear to height and openness (Treit et al., 1993). Following avoidance training (30 s), rats were placed at the end of the same previously experienced open arm and the latency to leave this arm with the four paws was recorded for three consecutive times (escape 1, 2 and 3), again at 30 s intertrial intervals. A cutoff time of 300 s was established for the avoidance and escape latencies.

Immediately after being tested in the elevated T-maze, each animal was placed for 5 min in the open field for the evaluation of locomotor activity.

2.6. Histology

After the behavioral tests, the animals were anesthetized with urethane (3 g/kg; Sigma, USA) and injected through the guide cannulae with 0.2 μl of Evans Blue 1% before their brains were perfused, through the left ventricle of the heart, with isotonic saline (0.9%) followed by a 10% formalin solution. The brains were then removed and immersed in a 10% formalin solution for a minimum period of 2 days until frozen sections of 60 μm were obtained on a cryostat. The microinjection sites were localized in diagrams from Paxinos and Watson's (1998) rat brain atlas. Only animals with injection sites located inside the LS were included in the statistical analysis.

2.7. Statistical analysis

For experiments 1 and 2, two-way analysis of variance (ANOVA) with repeated measures was used to analyze avoidance and escape data in the elevated T-maze, with treatment as the independent factor and trials (baseline, avoidance 1 and 2, or escape 1 to 3) as the dependent factor. A three-factor design was used to analyze the T-maze results from experiment 3, with the two treatments as the independent factors and trials as the dependent factor. Significant effects of the independent factors or of the interaction between the independent and dependent factors were analyzed by one-way ANOVA followed by the Duncan *post-hoc* test. Locomotor activity in the open field was also analyzed by one-way ANOVA followed by the Duncan *post-hoc* test.

3. Results

The diagrams of Fig. 1 show microinjection sites of rats treated intra-LS with midazolam, 8-OH-DPAT, WAY-100635 and their respective control groups.

3.1. Experiment 1: effects of midazolam

As shown in Fig. 2 (upper panel), treatment with midazolam significantly altered inhibitory avoidance acquisition. Two-way ANOVA showed a significant effect of treatment ($F(1,15)$= 9.22, $p<0.01$), trials ($F(2,30)=34.19$, $p<0.001$), and of treatment by trials interaction ($F(2,30)=8.69$, $p=0.001$). The Duncan *post-hoc* test showed that avoidance 1 and 2 latencies were significantly ($p<0.05$) shorter in the groups treated with midazolam when compared to control animals.

As shown in Fig. 2 (lower panel), treatment with midazolam did not interfere with escape behavior. Two-way ANOVA showed

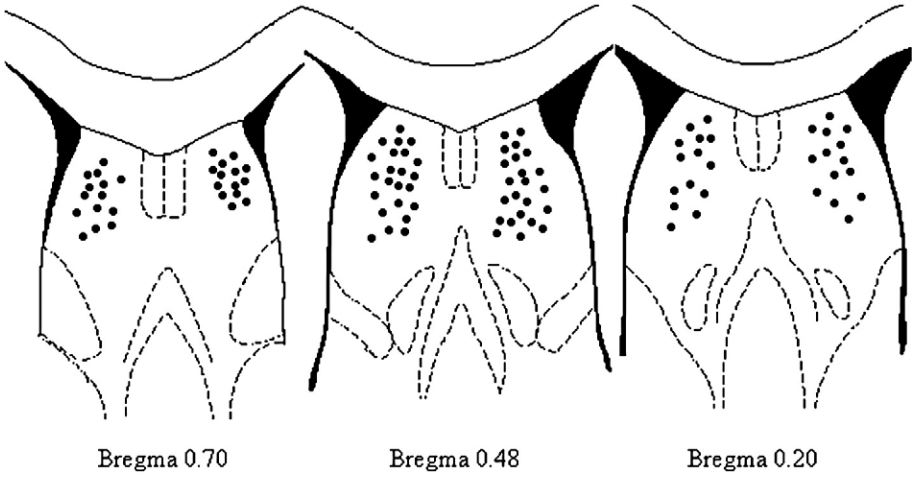

Fig. 1. Localization of injection sites inside (dots) the lateral septum. Figures represent coordinates from Paxinos and Watson (1998) rat brain atlas, with respect to Bregma. The number of points in the figure is fewer than the total number of rats used because of several overlaps.

a significant effect of trials ($F(2,30)=9.06$, $p=0.001$), but not of treatment ($F(1,15)=1.11$, $p=0.31$) nor of treatment by trials interaction ($F(2,30)=0.70$, $p=0.51$).

One-way ANOVA revealed no significant differences between animals treated with midazolam or saline in the open field. Neither the number of crossings ($F(1,15)=0.41$, $p=0.84$) nor the number of rearings ($F(1,15)=0.20$, $p=0.65$) were altered by drug administration (see Table 1).

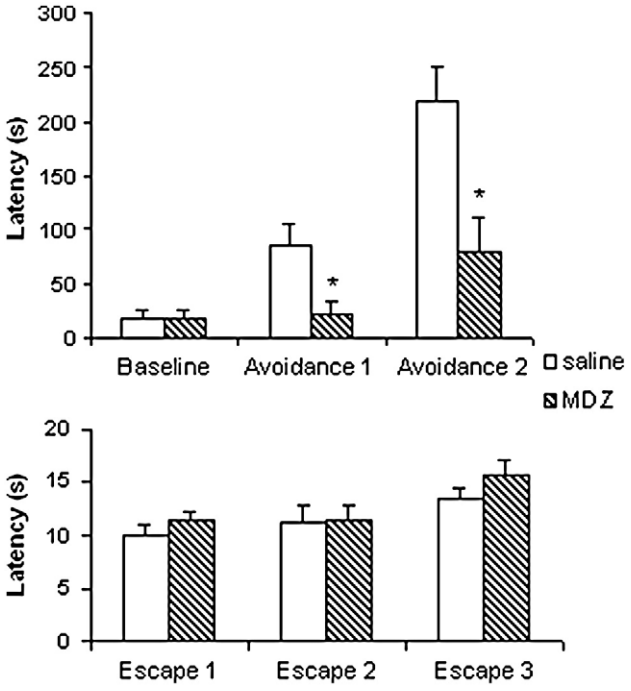

Fig. 2. Effects (mean+S.E.M.) of intra-lateral septum injection of midazolam (MDZ, 20 nmol/0.2 μl) or saline (0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. The latencies to leave the enclosed arm (baseline, avoidance 1 and avoidance 2) or one of the open arms (escape 1–3) were measured sequentially at 30 s intervals beginning 10 min after the injections. Twenty-four hours before the test, all animals were exposed to one of the open arms for 30 min. *$p<0.05$ with respect to control in the same trial (one-way ANOVA followed by Duncan *post-hoc* test). $n=8$–9.

3.2. Experiment 2: effects of 8-OH-DPAT

Contrarily to midazolam, 8-OH-DPAT facilitated inhibitory avoidance acquisition (Fig. 3, upper panel). Two-way ANOVA revealed a significant effect of treatment ($F(3,47)=3.99$, $p=0.01$), trials ($F(2,94)=35.46$, $p<0.001$) and of treatment by trials interaction ($F(6,94)=3.03$, $p<0.01$). The Duncan *post-hoc* test showed that the three groups treated with the drug had avoidance 2 latencies significantly ($p<0.05$) higher than the control group.

Treatment with 8-OH-DPAT did not interfere with escape behavior (Fig. 3, lower panel). Two-way ANOVA showed a significant effect of trials ($F(2,94)=17.73$, $p<0.001$), but not of treatment ($F(3,47)=1.54$, $p=0.22$) nor of treatment by trials interaction ($F(6,94)=1.31$, $p=0.80$).

One-way ANOVA revealed no significant differences between animals treated with 8-OH-DPAT or vehicle in the open field. Neither the number of crossings ($F(3,47)=1.59$,

Table 1
Locomotor activity in the open field after drug injection into the lateral septum

Drugs (nmol)	Number	
	Crossings	Rearings
Saline	53.56±3.50	15.33±2.36
Midazolam (10)	55.00±6.41	13.75±2.53
Saline	51.69±3.31	15.54±1.69
8-OH-DPAT (0.6)	51.62±4.84	12.85±2.20
8-OH-DPAT (3.0)	48.69±4.06	15.77±1.70
8-OH-DPAT (15.0)	39.67±5.32	11.50±2.40
Saline/Vehicle	45.67±2.88	16.00±1.44
WAY/Saline	41.83±5.44	12.00±2.07
Vehicle/8-OH-DPAT	42.44±6.70	11.44±2.01
WAY/8-OH-DPAT	48.56±4.62	13.56±1.21

$p=0.20$] nor the number of rearings ($F(3,47)=1.06$, $p=0.37$] were altered by drug administration (see Table 1).

3.3. Experiment 3: effects of WAY-100635

Fig. 4 (upper panel) shows that WAY-100635 was able to block the facilitatory effect induced by 8-OH-DPAT on inhibitory avoidance. Three-factor ANOVA showed a significant effect of trials ($F(2,78)=40.20$, $p<0.001$) and of 8-OH-DPAT ($F(1,39)=8.53$, $p=0.006$). There was no effect of WAY ($F(1,39)=0.73$, $p=0.40$) or of the interaction between factors (treatment by treatment: $F(1,39)=0.18$, $p=0.67$); WAY by trials: ($F(2,78)=1.85$, $p=0.16$); 8-OH-DPAT by trials: ($F(2,78)=2.09$, $p=0.13$); WAY by 8-OH-DPAT by trials: ($F(2,78)=1.04$, $p=0.36$)). The Duncan *post-hoc* test showed that in avoidance 2 the group treated with vehicle/8-OH-DPAT was significantly different ($p<0.05$) from the control group.

Fig. 4 (lower panel) shows that no effects were obtained with WAY-100635 on escape. Three-factor ANOVA showed a significant effect of trials ($F(2,78)=4.59$, $p=0.013$) and of the interaction between treatments ($F(1,39)=4.41$, $p=0.04$), but no effect of 8-0H-DPAT ($F(1,39)=1.11$, $p=0.30$), WAY ($F(1,39)=0.23$, $p=0.63$) or of the interaction between treatment and trials (WAY by trials: ($F(2,78)=1.27$, $p=0.29$); 8-OH-DPAT by trials: ($F(2,78)=0.98$, $p=0.38$); WAY by 8-OH-DPAT by trials: ($F(2,78)=1.44$, $p=0.24$)).

One-way ANOVA revealed no significant differences between the different groups of treatment in the open field. Neither the number of crossings ($F(3,39)=1.43$, $p=0.73$) nor the number of rearings ($F(3,39)=0.44$, $p=0.72$) were altered by drug administration (see Table 1).

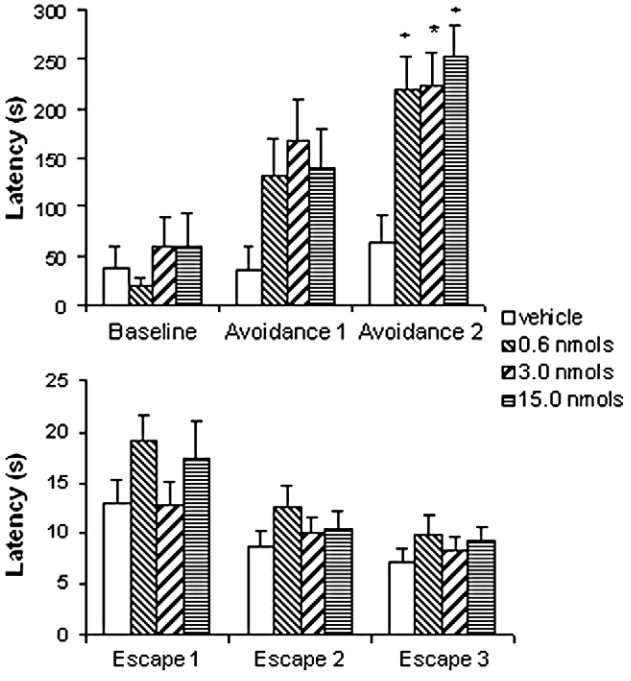

Fig. 3. Effects (mean+S.E.M.) of intra-lateral septum injection of 8-OH-DPAT (0.6, 3.0 and 15.0 nmol/0.2 μl) or vehicle (0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. For further specifications, refer to Fig. 2. $n=12–13$.

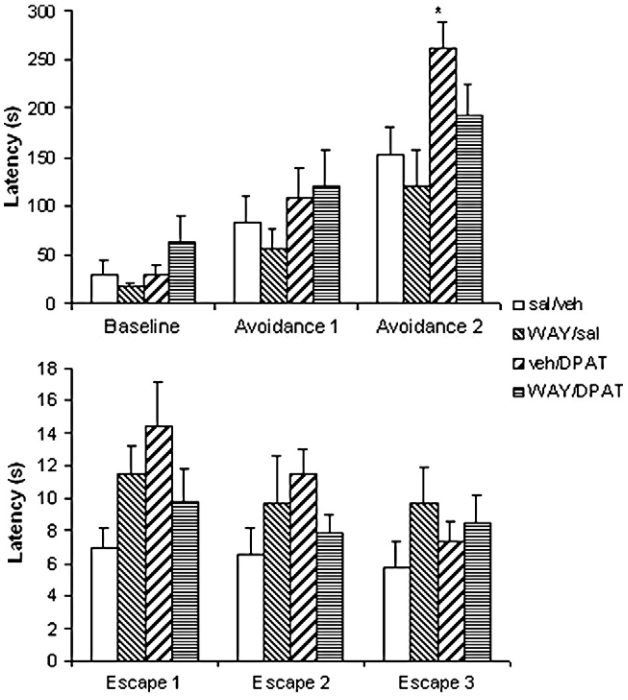

Fig. 4. Effects (mean+S.E.M.) of intra-lateral septum injection of WAY-100635 (WAY, 0.37 nmol/0.2 μl), vehicle (veh, 0.2 μl) or saline (sal, 0.2 μl), previous to the administration of either saline (sal, 0.2 μl), vehicle (veh, 0.2 μl) or 8-OH-DPAT (DPAT, 3.0 nmol/0.2 μl) on inhibitory avoidance (upper panel) and escape (lower panel) latencies measured in the elevated T-maze. For further specifications, refer to Fig. 2. $n=10–11$.

4. Discussion

The present study showed that intra-LS administration of midazolam decreased inhibitory avoidance latencies, an anxiolytic effect, whereas microinjection of 8-OH-DPAT had the opposite effect. Neither drug affected escape performance. Intra-LS administration of WAY-100635 blocked the effect induced by 8-OH-DPAT. Since no significant effects were observed with any of the drugs tested in the open field, it is reasonable to affirm that the results obtained in the elevated T-maze are not due to motor alterations.

Corroborating our present results with the elevated T-maze inhibitory avoidance task, it has been previously shown that intra-LS injection of midazolam decreases the expression of anxiety-like behaviors in two other animal models of anxiety, the elevated plus-maze and the shock-probe burying test (Pesold and Treit, 1996). This effect is akin to that reported in the same tests with the systemic administration of this drug and other clinically effective benzodiazepine anxiolytics (Hogg, 1996; Rodgers et al., 1997; Graeff and Zangrossi, 2002). This is indicative that the LS may be part of the neural substrate recruited for the anxiolytic action of benzodiazepines. In support of this idea, it has been shown that peripheral administration of midazolam in rats not only attenuates the expression of defensive responses to an object impregnated with cat odor (e.g.

inhibitory avoidance), but also causes a strong inhibitory effect on the expression of Fos protein evoked by this stimulus in the LS and in areas associated with pheromone transduction (McGregor et al., 2004). There is also evidence that both the elicitation of anxiety and its inhibition by benzodiazepine anxiolytics can result from plasticity-related changes in LS neurons. For instance, an electrophysiological study performed by Thomas et al. (2005) has revealed that the intraperitoneal administration of the benzodiazepine chlordiazepoxide blocks the conditioned suppression of unit activity evoked in the LS during Pavlovian aversive conditioning.

Contrarily to the results obtained with midazolam, intra-LS administration of the 5-$HT_{1A/7}$ receptor agonist 8-OH-DPAT facilitated the acquisition of inhibitory avoidance in the elevated T-maze. This anxiogenic effect is due to the activation of 5-HT_{1A} receptors, since it could be blocked by microinjection of the selective 5-HT_{1A} antagonist WAY-100635. It is noteworthy to mention that the dose of WAY-100635 used did not significantly affect the acquisition of inhibitory avoidance. This result goes against previous evidence, showing that 5-HT release in the LS is increased during exposure to aversive stimuli such as a predator (Beekman et al., 2005). If 5-HT release in the LS is a general consequence of exposure to different types of aversive/stressful stimuli, one would expect an anxiolytic effect of WAY-100635 in the elevated T-maze. To better evaluate this possibility, a study using a broad-range of doses of this antagonist is still warranted.

Previous anxiogenic effects of intra-LS 8-OH-DPAT administration have also been found in the elevated plus-maze and in the social interaction test (Cheeta et al., 2000a,b; Menard and Treit, 1998). In a way similar to our results, these anxiogenic effects were reversed by the administration of WAY-100635 (Cheeta et al., 2000a,b). Also, in the study by Cheeta et al. (2000a) no significant differences were found between the drug-treated group and the control animals in a second exposure to the elevated plus-maze. Contrarily to the social interaction test, which is sensitive to generalized anxiety-effective anxiolytics (File, 1995; Graeff and Zangrossi, 2002; File and Seth, 2003), trial 2 in the elevated plus-maze is resistant to these compounds and has been described as a model of specific phobia (File et al., 1993; Cheeta et al., 2000b; Graeff and Zangrossi, 2002). Thus, these results add to our observations suggesting that the activation of 5-HT_{1A} and GABA/benzodiazepines receptors in the LS modulates defensive responses which have been specifically associated with generalized anxiety disorder.

Along this line of evidence, a recent study performed using intra-dorsal hippocampus administration of 8-OH-DPAT showed that the drug was also capable of facilitating elevated T-maze inhibitory avoidance, without affecting escape behavior (dos Santos et al., in press). In this particular study, WAY-100635 administered into the same region was also without effect (dos Santos et al., in press). The similarity between these results and ours deserves attention since the LS and the hippocampus are highly interconnected structures (Swanson, 1977; Thomas et al., 1991), which have both been implicated in the control of anxiety (Gray, 1987; Graeff, 1994; Gray and McNaughton, 2001; McNaugthon and Corr, 2004). Conceptually, the septum (including its lateral and medial aspects) and the hippocampus have been considered as the core structures of the so-called "Behavioral Inhibition System" (Gray and McNaughton, 2001; McNaughton and Corr, 2004). According to Gray and McNaughton (2001), this system is activated when the animal is faced with a threat which involves approach behavior (e.g. search for food in an area previously visited by a predator), the major outputs of which are inhibition of prepotent responses, risk assessment, and increased arousal or vigilance. In such approach-avoidance conflict situations, risk assessment functions to reduce the level of perceived threat, while behavioral inhibition prevents competition between risk assessment and non-defensive behavior and reduces risk, in cases of actual threat. Based on evidence that all these manifestations are sensitive to anxiolytic drugs, such as diazepam and buspirone, they have been associated to generalized anxiety disorder (Gray and McNaughton, 2001; McNaughton and Corr, 2004).

We have previously shown (Zangrossi et al., 1999) that the administration of 8-OH-DPAT into the basolateral nucleus of the amygdala, another limbic area widely associated with anxiety modulation (LeDoux, 2003), inhibits the acquisition of inhibitory avoidance in the T-maze, an anxiolytic effect. As with the septo-hippocampal system, the drug did not affect escape performance. On the other hand, when 8-OH-DPAT is administered into the dorsal periaqueductal gray (Zanoveli et al., 2005; de Paula Soares and Zangrossi, 2004), a key structure associated with panic (Deakin and Graeff, 1991; Jenck et al., 1995; Schenberg et al., 2001; Mobbs et al., 2007), it interferes with both elevated T-maze tasks. In this case, the drug inhibits the acquisition of inhibitory avoidance and the expression of escape behavior. Results such as these highlight the intricate mechanisms by which serotonin regulates defensive responses and open new pathways to understand the multifaceted role played by this neurotransmitter in the neurobiology of anxiety disorders.

In conclusion, our data suggest that 5-HT_{1A} receptors in the LS are involved in the control of inhibitory avoidance behavior and that a failure in this regulatory mechanism may be of importance to the physiopathology of generalized anxiety disorder.

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