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The effects of dorsal-hippocampal administration of GPR55 Selective agonist and antagonist on anxiety-like behaviors in male rats

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Abstract: The recognition of G-protein coupled receptor 55 (GPR55) as a potential cannabinoid receptor has been created an important interest. The hippocampus is a prominent brain site in the action of modulating of anxiety. In the present study, we examined the effects of dorsal-hippocampal administration of GPR55 agents on anxiety-like behaviors in rats using the elevated plus-maze test of anxiety. The dorsal-hippocampal administration of O-1602 (GPR55 receptor agonist) at the doses of (0.2, 1 and 5 µg/rat) increased %open arm time and % open arm entries, but not locomotor activity, showing an anxiolytic-like response. Dorsal-hippocampal administration of ML193 (GPR55 receptor antagonist; 0.01, 0.1 and 0.5 µg/rat) decreased %OAT ، % OAE and locomotor activity, showing an anxiogenic-like effect. In another series of experiments, the ineffective doses of ML193 (0.01 µg/rat) on anxiety-like behaviors were injected with O-1602 (0.2, 1 and 5 µg/rat). The obtained data showed that ML193 could reverse the anxiolytic-like effect at the doses of O-1602 (0.2 and 1 µg/rat). The results suggest that cannabinoid system into the dorsal-hippocampal may modulate anxiety-like behaviors with the GPR55 receptor.

Key words: Anxiety, Elevated plus-maze; GPR55; Rat

1. Introduction

The hippocampus organization is a cortical region in frontal lobe which has been expanded along with the ventral posterior axis. This structure is composed of the three parts: dorsal (CA 1), intermediate (CA 2) and ventral (CA3) parts which have played a significant role in neurobiology of anxiety. Hippocampus, which has been located in limbic system, has connection with many emotional centers in the brain and its pyramidal cells act like a keyboard (Enjin and Treit, 2007).

The compounds which have been extracted from the plant leaves of Indian hemp with scientific name of Cannabis sativa contain more than 60 chemical substances called cannabinoid. These unique compounds, which have been found in no plant, are terpenophenolic compounds and the most active form of these materials are Δ9 -THC and cannabidiol (Begg et al., 2005). These substances have been lipophilic and easily pass through blood- brain barrier (Mechoulam, et al., 1970).

When an individual uses the Cannabis for the first time, often feels psychological arousal and the most common its symptoms are paranoia and anxiety (Roohbakhsh, Hajizadeh Moghaddam, Massoudi, Zarrindast, 2007). The receptors of cannabinoids are a kind of G- protein coupled receptors and recently are classified in two groups of CB1 and CB2 receptors (Pertwee, 2008). The CB1 receptor is the first receptor which is coding with gene CNR 1.signaling ofCB1 receptor is basically a regulator for controlling and releasing of the neurotransmitters (Idris and Ralston, 2010).

The CB2 receptor with gene of CNR2 is expressed mainly in macrophages, the marginal zone of the spleen and in the other tissues such as bone and joints, unlike to the previous thought it is also expressed in a number of areas of the central nervous system (Nunez et al., 2004). the human GPR55 was discovered for the first time in the year 1999 in the area of the brain called striatum with high expression as a new receptor of GPCR (Sawzdargo et al., 1999).

The Gene coding the human GPR55 is located on the long arm of human chromosomes number 2 and in the man nervous system is expressed dominantly in striatum, caudate nuclei and putamen. Today it has been cleared that the amount of expression of the GPR55 in the body of mice in the order of increasing is as the following: Adrenal> frontal lobe> striatum= ileum= jejunum> hypothalamus>hippocampus> spinal cord> long, liver, uterus, urinary bladder, stomach, kidney > esophagus> adipose tissue (Wu, Chen, sun, Zhu, Jew, 2013). Lysophosphatidylinositol (LPI), in all conducted studies regardless to the type of cell and its function, has a high potential agonistic effect on GPR55 (Bondarenko et al., 2010).

The activation of GPR55 inhibits the potassium channels type M; this finding proposes that GPR55 may increase neuronal excitability (Henstridge et al., 2010). The GPR55 can balance synaptic strength in the CA1 area of hippocampus

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during periods of severe synaptic activity (Jensen et al., 2011).

The GPR55 is expressed in the basal ganglia nuclei and cerebellum, these are the important centers for coordinating the actions of movement and GPR55 antagonists have influences to some extent on the behaviors of the central nervous system, because that might lead disorder in coordination of movement (Wu, et al, 2013), the specific agonist of this receptor (O- 1602) when is injected into the brain ventricle causes an increase in taking food in the mice (Dı´az- Arteaga et al., 2011). Since, still the effects of injecting agonist and antagonist of cannabinoid GPR55 receptors in posterior nucleus of hippocampus has not been investigated, thereby this research has been attempted to study it.

2. Method

2.1. Subjects

Male Wistar rats from the Pasteur Institute (Amol, Iran), with weight of 200-250 g at the time of surgery, were used. Animals were housed five per cage, in a room with a 12:12 h light/dark cycle (lights on at 07.00 h) and controlled temperature (23±2C). Animals had free access to food and water and they were permitted to adapt to the laboratory conditions for at least 1 week before the surgery. Rats were handled about 5 min each day earlier to behavioral testing. All experiments were performed between 09:00 and 12:00 am and each rat was tested only once. Seven animals were used in each experimental group. The study was conducted based on the national guidelines for animal care and use.

2.2. Stereotaxic surgery microinjection

Rats were anaesthetized intraperitoneally with ketamin hydrochloride (50mg/kg) and xylazine (4mg/kg) and placed in a stoelting stereotaxic instrument (stoelting Co., Illinois, USA). A stainless-steel guide cannula(22-gauge, Supa; Osve Tehran, Iran) was embedded bilaterally in the right and left dorsal-hippocampal according to Paxinos and watson (1998) Stereotaxic coordinates for this region were as follwes: -2.2mm (depending on body weight) posterior to bregma, ±1.7mm lateral to the midline and -2.6mm ventral of the dorsal appearance of the skull. It was then fixed to the skull with acrylic dental cement. The animals were authorized 6 days to recover before the test. Dorsal-hippocampal injections were administered with means of an internal cannula (27-gauge, supa; Iran), terminating 1mm below the tip of the guides, connected by polyethylene tubing to a 1-µl Hamilton syringe. Animals received bilateral injection of 0.5 µl of each solution over a 60-s period (1µl/rat). The inner cannula was left in place for an additional 60s to permit distribution of the solution and to reduce the possibility of reflux. At the end of the study, 0.5µl/rat of 1% methylene blue solution was injected, and determination of the injected dye in the right and left dorsal hippocampal identified and verified the injected site.

2.3. Drugs

O-1602 and ML193 were obtained from Tocris and Glixxlabs. O-1602 (5-Methyl-4-[(1R, 6R) -3-methyl-6-(1-cyclohexen-1-yl]-1, 3-benzenediol was dissolved in saline. ML193 (4-[4,6-Dihydro-4-(3-hydroxyphenyl) -3-(4-methylphenyl)-6-oxopyrrolo [3, 4-c] pyrazol-5(1H)-yl] benzoic acid) was dissolved in 100%DMSO. The drugs were injected in dorsal-hippocampus.

2.4. Drug treatments

Experiment 1: effects of O-1602 on anxiety in this experiment, four groups of rats received saline (1 µl/rat) or three different doses of O-1602 (0.2, 1 and 5 µg/rat).

Experiment 2: effects of ML193 on anxiety four groups of rats received %100 DMSO (1 µl/rat) or three different doses of ML193 (0.01, 0.1 and 0.5 µg/rat).

Experiment 3: effects of ML193 with O-1602 on anxiety in this experiment, four groups of rats received saline (1 µl/rat) or three different doses of O-1602 (0.2, 1 and 5 µg/rat) 5min after dorsal-hippocampal injection of ML193 (0.01 µg/rat).

2.5. Behavioral test (elevated plus-maze)

The elevated plus-maze was a wooden, cross-shaped maze, consisting of four arms arranged in the shape of a cross sign. Two of the arms had no side or end walls (open arms; 50× 10 cm). The other two arms had side walls and end walls but were open on the top (closed arms; 50×10× 40 cm). Where the four arms intersected, there was a square platform of 10×10 cm. The maze was elevated to a height of 50 cm. In order to elevate total arm entries on the maze, rats were placed in a wooden test arena (50×50×35 cm) for 5 min earlier to maze testing. Seven days after implantation, the effects of dorsal-hippocampal injection of drugs were tested in the elevated plus-maze. At least 5min before testing, rats were lay in the experimental room. Animals were randomly allotted to treatment conditions and tested in a counterbalanced order. The rats were individually placed in the center of the maze facing a closed arm and allowed 5 min of free exploration. The number of entries into open arms, the number of entries into closed arms and the total time spent in the open arms and total time spent in the closed arms were measured.

Information were computed as following: (A) the ratio of times spent in the open arms to total times spent in any arms×100 (%OAT); (B) the ratio of entries into open arms to total entries×100 (%OAE);

(C) total closed arm entries were measured as a relative pure index of locomotor activity.

3. Statistical analysis

A one-way ANOVA was used for the comparison between the effects of different doses of O-1602 with its solution. A two-way ANOVA was used for evaluating of interactions between drugs. Following a significant F-value, post-hoc analysis (Tukey test) was applied for assessing specific group comparisons. Differences with $P<0.05$ among experimental groups at each point were considered as statistically significant.

3.1. Effects of ML193 on anxiety

Figure 1 shows the effects of ML193 on anxiety-like parameters in the elevated plus-maze. A one-way ANOVA exhibit that ML193 decreased %OAT [$F(3, 24) = 21.23$, $P<0.001$] and %OAE [$F(3,24)=13.32$, $P<0.001$] indicating an anxiogenic-like response by ML193. Significant change in locomotors activity was observed following injection of ML193 [$F(3, 24)=9.14$, $P<0.001$].

3.2. Effects of O-1602 alone or with ML193 on anxiety

Figure 2 shows the effects of O-1602 on anxiety-like parameters in the elevated plus-maze. A one-way ANOVA shows that O-1602 increased %OAT [$F(3, 24) = 22.27$, $P<0.001$] and %OAE [$F(3, 24) =12.23$, $P<0.01$] indicating an anxiolytic-like response with O-1602. No significant change in locomotor activity was observed following injection of O-1602 [$F(3, 24) =0.43$, NS], and the effects of O-1602 with ML193 on anxiety-like parameters in the elevated plus-maze. A two-way ANOVA, using post-hoc analysis, showed that ML193 could significantly reverse the increase in %OAT [$F(3, 56) =21.74$, $P<0.001$] and %OAE [$F(3, 56) =13.66$, $P<0.01$] induced by O-1602. No interaction was found between the effects of ML193 on locomotor activity and those induced by O-1602 [$F(3, 56) =3.44$, NS]. Post-hoc analysis showed that O-1602 increased %OAT and %OAE at the doses of 0.2, 1 and 5 µg/0.5 µl bilateral, showing a decrease in the anxiolytic effect of O-1602 in the presence with ML193(0.01µg/rat) in doses (0.2 , 1 and 5 µg/0.5µl bilateral)the dorsal-hippocampal.

3.3. Substantiation of cannula appointment

Figure 3 demonstrates the come near point of the drug injections in the central amygdala. The histological results were scheme on characteristic sections taken from the rat brain atlas of Paxinos and Watson (1998). After completion of the experimental term, rats received a 0.5 µl/side of methylene blue. Nearly 5–10 min after the injection, the animals was beheaded and their brains were removed, obstructed and cut coronal through both cannula appointment.

4. Discussion

Many neurotransmitters and various regulators play a role in the excited behaviors, anxiety- like and anxiety behaviors. The most important neural mediators regarding to behaviors corresponding with anxiety which can be listed are: glutamate, Gamma amino butyric acid (GABA), dopamine, serotonin, histamine, acetylcholine, opioid system.

In addition to these, cannabinoid system, which is wide spread throughout the body and central nervous system, is one of the important nervous systems that interferes in the adjustment of behaviors corresponding with anxiety and creates its biological effects with activating or deactivating of the cannabinoid classical and non-classical receptors (Moreira, 2012).

In this study influences of the injecting specific agonist and antagonist of GPR55 in dorsal hippocampal nucleus on anxiety with help of behavioral model elevated plus maze (EPM) have been investigated.

In the first phase of this study, bilateral injection of agonist in dorsal hippocampal nucleus has increased the percent of open arm time (OAT %) and percent of open arm entries (OAE %), which indicates the occurrence of anxiolytic-like behaviors in studied animals. Due to the lack of significant change in locomotor activity, the anxiolytic-like behaviors of animals are independent of this last factor and have been specifically occurred.

Consistent with our findings, in several separate studies brain intraventricular injection of win5512-2, ACEA, CP55940 agonist receptors of the CB1 in lower doses have increased the percent time of open arm entries, which anti-anxiety effects of them are inhibited via AM251 (Moreira, 2012) and the injection of win5512-2 in the area of CA1 of dorsal hippocampus has anti-anxiety effect (Roohbakhsh et al., 2007).

While in previous studies anxiolytic effect of SR141617 has been reported, in another study, with acute injection of it anti-anxiety effect was reported (Griebel et al., 2005). In other study on mice without CB1 receptor also had anti-anxiety influence, these findings show that this effect is introduced through non- CB1 receptors (Haller et al., 2002).

Also following studies on impact of cannabinoids on anxiety, in the year 2008 have been reported that injecting of Met- Anandamide (natural analog of Anandamide) in frontal cortex caused to develop responses regarding to anti-anxiety behaviors (Rubino et al., 2008).

Additionally injecting of 2-Arachidonoyl glycerol to the gray matter around aqueduct of Sylvius to mice in behavioral test of elevated plus maze showed anti-anxiety effects (Almeida-Santos et al., 2013). The interesting and similar finding to the above results is the injection of Win55212-2 in

Marmoset monkey (little monkey in South America) which caused anti-anxiety behaviors in the open-field test.

Following the experiments, injection 0.1 and 0.50 µg/rat of ML193 (specific antagonist of GPR55 receptor) in dorsal hippocampus nucleus, meaningfully decreased percent of open arm time (OAT %) and percent of open arm entries (OAE %) and also caused a decrease in locomotor activities of animals that shows occurring of the anxiety- like behavior in mice.

At the same direction Rodgers and colleagues in 2003 showed injection of SR141716A (CB1 antagonist) in ventral hippocampus creates of anxiety- like effects.

Also it has been reported that intraperitoneal injection of SR141716A (CB 1 antagonist) not only reverseanti-anxiety effects of CP55940 and AM40 but also when it is injected alone induces anxiogenic effects (Patel et al., 2005).

The results of studies on the CB1 receptors within central amygdala using AM251 (specific antagonist of GPR55 receptor) showed a decrease in locomotor activities but without alteration in anxiety factors (Zarrindast et al., 2008).

The injection of Win55212-2 agonist of cannabinoid receptors in low doses in lateral septum, part of the limbic system causes a reduction in percent of open arm time and percent of open arm entries. This indicates anti- anxiety influence of it. In addition to that, Arevalo et al in the year 2001 showed that injecting of Win55212-2 inside of peritoneum in rats in Cross-shaped maze testcauses anxiety- like behavior (Miguel and Tristan, 2001).

Also different results which have been obtained from different studies are in consistency with these findings, for instance in 2001, Arnold et al reported that injection of the CP55940, an agonist of CB1 receptor intraperitoneally to the rats, caused an enhancement of the anxiety- like behaviors (Arnold, Topple, Maller, Hunt, McGregor, 2001).

Despite of anti-anxiety effects of low doses of Win55212-2 in lateral septum, high doses didn't produced any impact on the behaviors associated with anxiety in EPM test in Wistar rats of the race, which this contradiction is related to the effects of cannabinoids on the non- CB1 receptors for example TRPV1 (Fogaça, Aguiar, Moreira, Guimarães, 2012). Because in previous studies it has been determined the Anandamide and Win5512-2 inhibit theTRPV1 channel. since inhibition of vanilloid channel TRPV1 has a direct connection with anti-anxiety behaviors, therefor this shows that cannabinoids in different doses has interaction with the non-classical cannabinoid receptors, which itself is interesting (Hakimizadeh, Oryan, Hajizadeh moghaddam, Shamsizadeh, Roohbakhsh, 2012).

Of course dual effects of cannabinoids may depend on different areas of brain, expression of the various cannabinoid receptors in different cells, environmental conditions and different animal species (mouse or rat), pharmacological properties, the specificity of the ligand to the receptor, the amount and the level of stress applied to the animal (Cagni Barros, 2013).

Different sections of the brain like limbic system, Mid brain and diencephalon are involved in behavioral and psychosomatic situation (Sheehan et al., 2004).

In the continuation of investigation of these two drug interactions, ML193 specific antagonist of GPR55 was injected five minutes before injecting different doses of O- 1602. Comparison of these groups with those which received only O- 1602 showed that ML193 has been significantly able to reduce anti-anxiety effects of O- 1602 in doses of 1 and 2 with a decrease in OAE % and OAT%. Due to lack of numerous ligands and a scale of dosimeter of cannabinoids, there is no reported clear picture from the effect of cannabinoids on the anxiety. The results of this study suggested that probably GPR55 receptor has a role in modulating of anxiety behaviors.

The recent pharmacological data report the presence of signaling of endogenous cannabinoids non- CB 1 receptor that identification of these compounds, their receptors and their signaling will provide a new step in treating diseases regarding with anxiety, from ligands of thesenewreceptors are Anandamide and 2-Arachidonoyl glycerol of which are made in the neurons through an independent route in a cascade by certain enzymes are broken down by enzymes of monoacylglycerol lipase and fatty acid hydrolase. The presence of these receptors and enzymes in various parts of brain such as amygdala, frontal cortex, Accumbens, hypothalamus which are involved in emotional behaviors is indicating the importance of role of these compounds in controlling excited behaviors (Piomelli, 2003).

Additionally using of the AM404 as an inhibitor of end cannabinoids reuptake in synaptic space in low doses causes an increase in percent of open arm entries in the EPM test however in the high doses is ineffective, while the same tissues in the case of SR141716, antagonist of CB 1 with the increase in the dose has been shown revers influence. On the other hand, THC in low doses hasanti-anxiety effects and in high doses produces anxiety effects (Viveros, Marco, and File, 2005).

The results of studies showed that GPR cannabinoid receptors probably play a role in modulating anxious behaviors and in the future is counted as a new goal in the treatment of anxiety disorders.

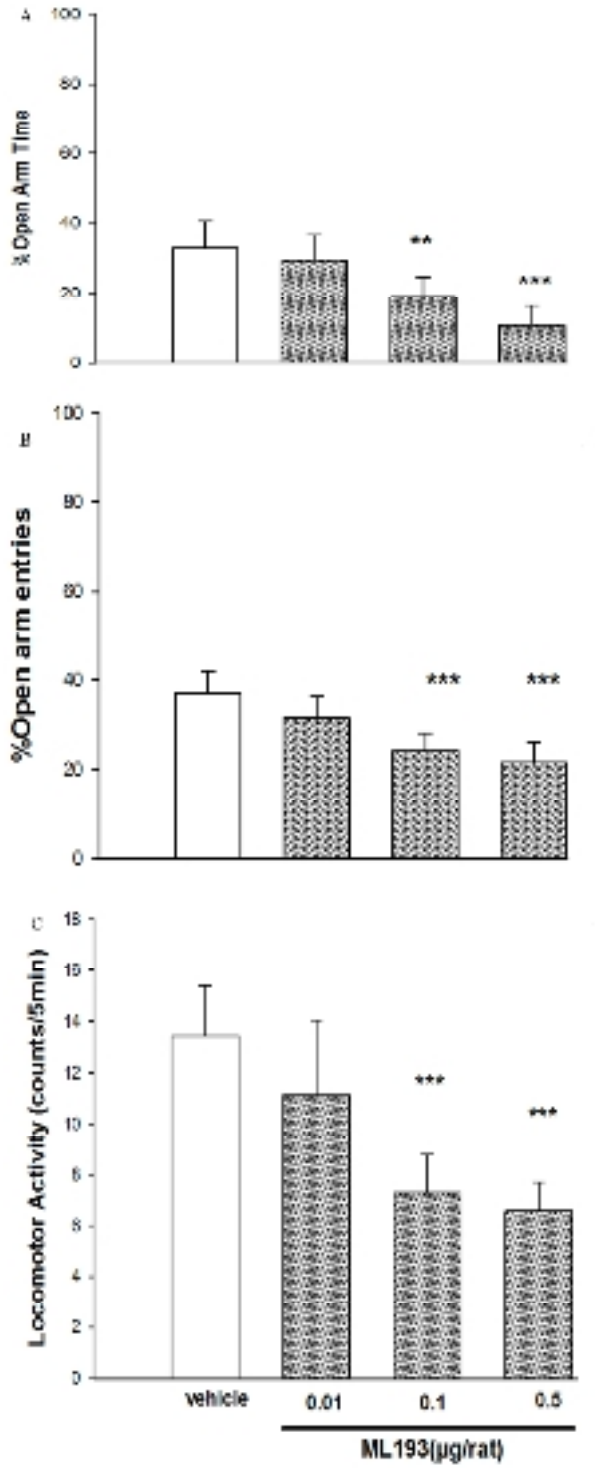

Fig. 1: Effects of dorsal-hippocampal injection of ML193 on anxiety

The below written is for the antagonist figure alone. Rats were injected with DMSO (1 µl/rat) or ML193 (0.01, 0.1 and 0.5 µg/rat). The test was performed 20 min after dorsal hippocampal injections. Each bar represents mean±SEM of the ratio of times spent in the open arms to total times spent in any arms×100(%OAT) (A), the ratio of entries into open arms to total entries×100(%OAE) (B) or locomotor activity (C). n=7. **P<0.01 and***P<0.001, when compared with the DMSO-treated rat

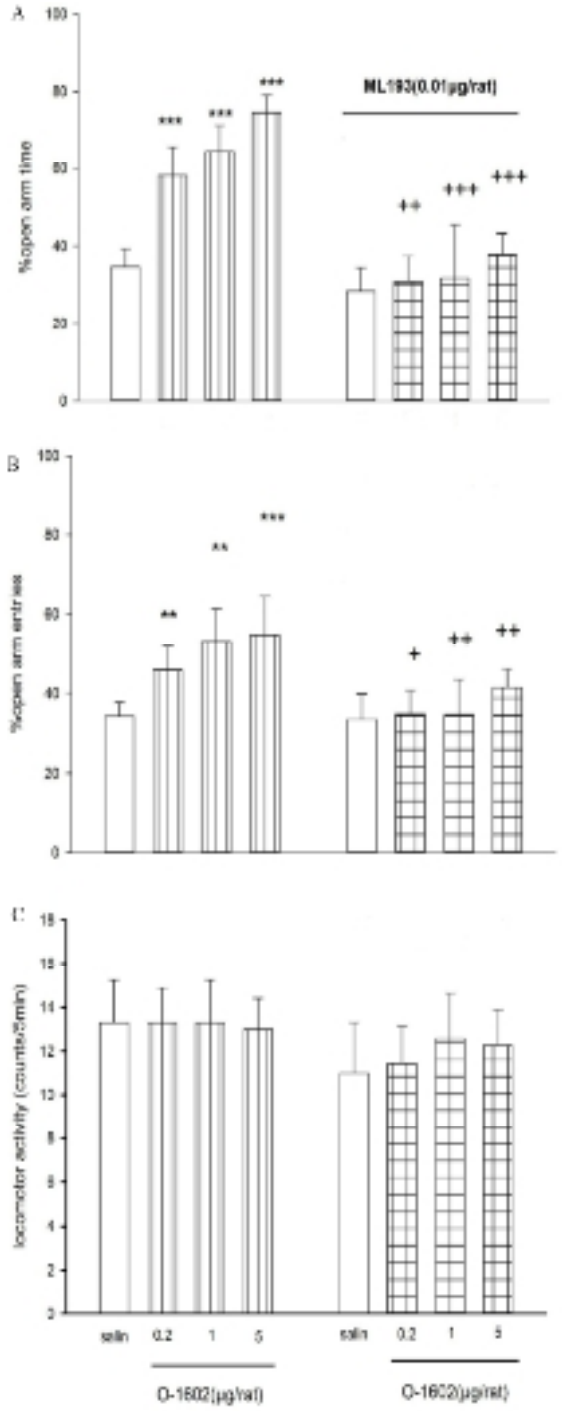

Fig. 2: Effects of dorsal-hippocampal injection of O-1602 alone or with ML193 on anxiety

The below written is forfigure of the agonist alone or along with antagonist. Rats were injected with saline (1 µl/rat) or O-1602 (0.2, 1 and 5 µg/rat) alone or 5 min after injection of ML193 (0.01 µg/rat). The tests were performed 20 min after dorsal-hippocampal injections. Each bar represents the mean±SEM of the ratio of times spent in the open arms to total times spent in any arms×100 (%OAT) (A), the ratio of entries into open arms to total entries×100 (%OAE) (B) or locomotor activity (C). n=7. **P<0.01 and ***P<0.001 when compared with the saline-treated rats. ++P<0.01 and+++P<0.001 when compared with the O-1602-treated rats.

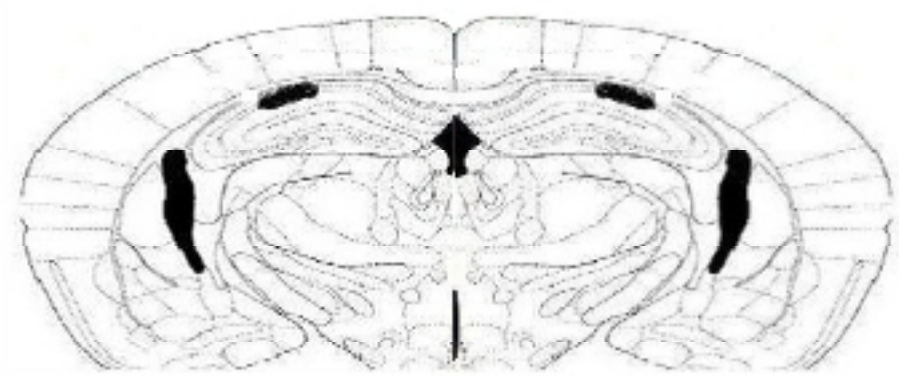

Fig. 3: Characteristic of dorsal hippocampus

This figure has been shown the characteristic of dorsal hippocampus according to PaxinosAtlas. The location is determined by the color

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