

Possible role of hippocampal GPR55 in spatial learning and memory in rats

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Endocannabinoids (eCBs) are involved in the hippocampal mechanisms of spatial learning and memory in rats. Although eCBs exert many of their actions on spatial learning and memory via CB₁ receptors, the putative cannabinoid receptor GPR55 (expressed in the hippocampus, cortex, forebrain, cerebellum and striatum) seems to be also involved. To investigate the potential role of GPR55 in spatial learning and memory, Wistar rats received bilateral infusions of lysophosphatidylinositol (LPI, GPR55-agonist) into the hippocampus 5-minutes before training-phase in the Barnes-maze (BM). This manipulation increased the use of serial navigation while preventing the learning of spatial navigation strategy and decreasing the use of random activity to find the escape-tunnel in the BM. In contrast, CID16020046 (GPR55-antagonist) increased the use of random activity at the expense of spatial and serial navigation strategies. Finally, CID16020046 significantly reduced the time spent in the target zone during a retention test. Our results suggest: (i) a potential role of GPR55 in developing navigation strategies; (ii) a prospective function for LPI acting in hippocampal CA1 (probably via GPR55) to perform a serial navigation strategy; and (iii) a potential role of GPR55 in the mechanisms involved in spatial memory (object placement memory).

Key words: GPR55, lysophosphatidylinositol, CA1, Barnes-maze, spatial memory

INTRODUCTION

Apart from the well-characterized cannabinoid type 1 and type 2 receptors, there is evidence that endocannabinoids (eCBs) exert their actions via putative cannabinoid G-protein receptor 55 (GPR55) (Lauckner et al. 2008, Marichal-Cancino et al. 2013, 2016, 2017, Yang et al. 2016). Cannabinoids affect behaviours related with hippocampal activity via CB₁ receptors (e.g., long-term potentiation; Basavarajappa et al., 2014), but few studies have investigated the physiological role of hippocampal GPR55 (Hurst et al. 2017, Kramar et al. 2017, Rojo et al. 2012, Sylantiev et al. 2013). There is GPR55 mRNA in the hippocampus and other brain areas (Ry-

berg et al. 2007, Wu et al. 2013). According with Kramar et al. (2017), injections of palmitoylethanolamide into ventral hippocampus affected spatial memory probably via GPR55. Hurst et al. (2017) recently reported that hippocampal GPR55 stimulation with lysophosphatidylinositol improved synaptic plasticity.

In contrast, systemic augments of anandamide (which activates both CB₁ and GPR55) by fatty acid amide hydrolase (FAAH) inhibitors (enzyme that participates importantly in endocannabinoids clearance) impaired LTP, learning and memory. In addition, infusions of anandamide into the hippocampal area CA1 modified spatial navigation (Rueda-Orozco et al. 2008). It is important to note (Table I) that several cannabinoids exert actions on both CB₁ and GPR55

Table I. Affinity of Noladin-ether, AM251 and CID16020046 for CB₁ and GPR55.

Compound	CB ₁	GPR55
Lysophosphatidylinositol	<4.5 ¹ pEC ₅₀	5.9 ¹ pEC ₅₀
AM251	7.7 ² pIC ₅₀	6.2 ³ pEC ₅₀
CID16020046	Not detected ⁴	>6.2 ⁴ pIC ₅₀
Δ9-tetrahydrocannabinol	8.1 ⁵ pEC ₅₀	8.2 ⁵ pEC ₅₀

Data taken from: ¹Kapur et al. 2009, ²Brigthon et al. 2009, ³Henstridge et al. 2010, ⁴Kargl et al. 2013, ⁵Ryberg et al. 2007.

receptors (Henstridge et al. 2010). At this respect, we reported that stimulation of GPR55 in dorsal striatum seemed to improve procedural memory, whereas CB₁ stimulation seemed to impair it (Marichal-Cancino et al. 2016).

As few data are available for the role of hippocampal GPR55, this study investigated the effects of its pharmacological manipulation on spatial navigation strategies to solve the Barnes-maze (BM) in Wistar rats.

METHODS

Subjects

Wistar male rats weighing 250–300 g at the beginning of the experiments were used (N=32). Animals were housed individually under controlled conditions, temperature 21±1°C, 52% humidity, a reverse 12-h light/dark cycle; lights on at 20:00 h with *ad libitum* access to water and food. Experiments started at 08:00 h.

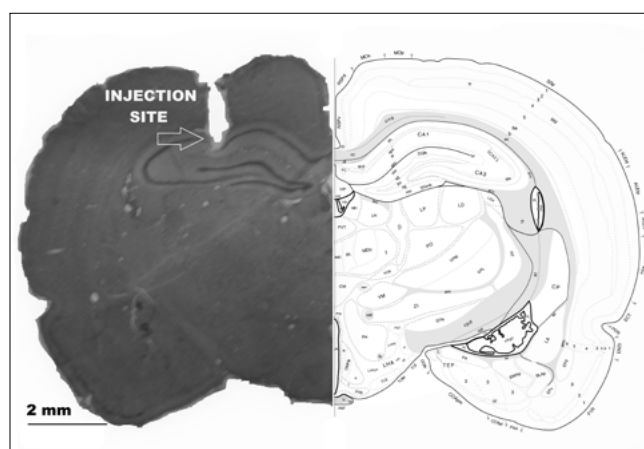


Fig. 1. Photomicrographic and schematic representation (taken from Swanson, 2004) of the injection sites in the hippocampus.

Every protocol adhered to the provisions of the Official Mexican Regulation on “Technical specifications for the production, care and use of laboratory animals” (NOM-062-ZOO-1999). The Research and Ethics Committee of the School of Medicine (UNAM) accepted this study. This study was constructed in accordance with the Guidelines of the U.S. Public Health Service and NIH regarding the care and use of animals for experimentation and with the ARRIVE guidelines (McGrat et al. 2010).

Experimental groups

Animals were randomly divided in two main sets (set 1 n=8; set 2 n=24). In set 1 (n=8) rats received no manipulation or surgery before training (intact group). After surgery recovery (see below), set 2 was divided in three groups (n=8 each group), which received CA1 hippocampal injections (0.5μl) of: (i) DMSO 100% (vehicle group); (ii) 10 nM of LPI; or (iii) 10 nM of CID16020046.

Surgery and cannulae placement verification

Rats were implanted bilaterally in the hippocampal area CA1 under an anaesthesia cocktail (ketamine 66 mg/kg plus xylazine 0.26 mg/kg plus acepromazine 1.3 mg/kg). A couple of guide cannulae (23-gauge, 0.6 mm of outer diameter) were affixed to the skull with dental cement, according to Paxinos and Watson (2007) coordinates (P=0.4, L=2.5, V=2.2). Animals were allowed to recover from surgery for ten days.

Once the experiments ended, rats were sacrificed with an overdose of sodium pentobarbital and transcardially perfused with 200 ml of PBS and 200 ml of 4% of paraformaldehyde to prepare the brains for histological analysis with cresyl violet staining to verify the correct placement of the injector (Fig. 1), as previously described (Rueda-Orozco et al. 2008).

Intra-hippocampal administrations of DMSO, LPI and CID16020046

Animals from set 1 and set 2 were kindly handled 2 h during 2 days before any experimental manipulation to diminish stress as previously reported (Soria-Gómez et al. 2007). 15 minutes before each training day (i.e., session 1–4; see Fig. 2) in the Barnes-maze (BZ) animals from set 1 were handled for 5 min and allowed to stay at home cages during other 10 min. Animals from set 2 were hippocampal infused with DMSO, LPI

or CID16020046 0.5 μ l in 5 min each side (final volume 1 μ l). Then, animals stayed at cage homes for 10 min before training to allowed distribution of treatments as previously reported (Marichal-Cancino et al. 2016).

Barnes maze and strategy criteria

Our BM was an adaptation from the original apparatus published by Barnes (1979) consisting of a circular wooden disk (150 cm of diameter; 2.0 cm thick, 90 cm height). At 4.5 cm from the edge, forty holes (7 cm diameter) were equidistantly located throughout the disk (Fig. 2). A black wooden box was used as an escape tunnel (10 \times 10 \times 30 cm) randomly placed beneath one hole which was different for each rat. From a speaker affixed to the room's ceiling and located 1 m over the center of the maze, a white noise (90 dB) was delivered. Although the escape tunnel was always at the same spatial position, the BM was rotated for each trial to prevent use of clues on the apparatus surface. The BM was thoroughly cleaned with a 5% chlorine solution after every trial. Three types of navigation were evaluated in each trial:

Serial navigation strategy

To be considered as a serial strategy, once rats visited a hole, they had to explore adjacent holes sequentially and follow one direction until finding the hole with the escape tunnel (Fig. 2A).

Spatial navigation strategy

In this case, rats visit the hole with the escape tunnel directly or at most two adjacent holes to the left or to the right (target zone) with no visits to holes outside of this zone until they escape (Fig. 2B).

Random navigation escape

Any procedure used to escape which did not fit the criteria for serial or spatial strategy was automatically considered as a random escape (Fig. 2C).

Latency to escape and errors

In addition to the navigation strategies, latency to escape and errors were measured. An error was con-

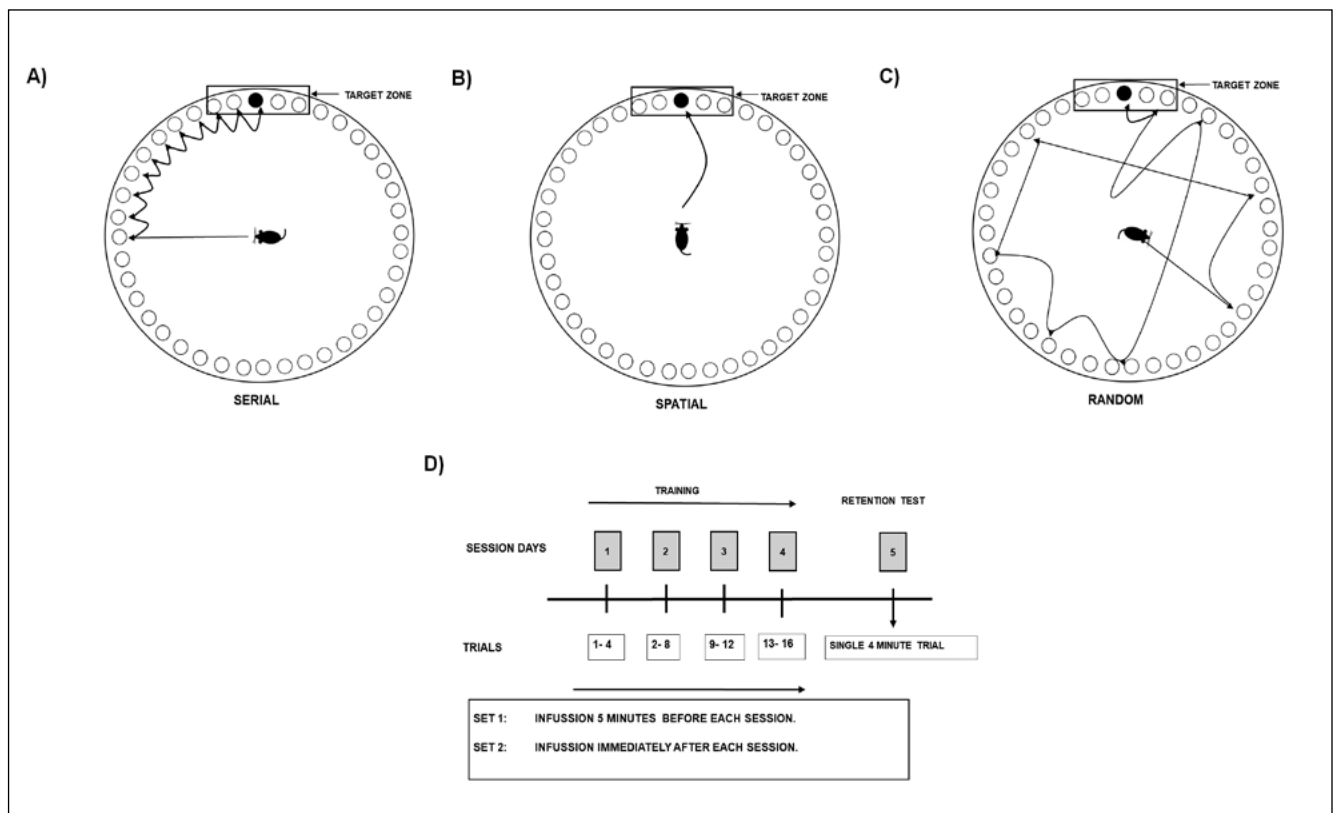


Fig. 2. Schematic example of navigation strategies to solve the Barnes-maze and protocol time line. In serial navigation, rats visit subsequent holes following one direction until they find the escape tunnel (A), in spatial navigation, rats go directly to the escape tunnel with no visits outside of the target zone (B), all navigation different to serial or spatial was considered as random (C). Session days are shown (D).

sidered to any visit to holes different from the escape tunnel. A daily session consisted of 4 trials; each trial ended, when the rat entered the escape tunnel or once 4 min elapsed. If after 4 min, the rat failed to enter the escape tunnel, they were gently guided to it, and the trial was considered as an omission, as previously described (Rueda-Orozco et al. 2008). According with our criteria, if an animal did not learn to locate the escape tunnel after 2 trials, it was discarded. However, none of the rats met this criterion. A learning curve was obtained at the end of the training phase, depicting the reduction of the time required to find the escape tunnel.

Behavioural training and treatment infusions

Ten days after the surgery, rats were trained to solve the BM for 4 consecutive days (S1–S4). Every training session day was video recorded. Before the beginning of the first trial, rats were placed in the escape tunnel for 1 min to make them familiar with it, as we have previously described (Rueda-Orozco et al. 2008). Upon completion of this time, animals were placed in a removable cylindrical chamber (15 cm diameter) situated in the centre of the BM, while a white noise (90 dB) was delivered from a speaker located in the ceiling of the room (1.5 m above the BM). Once rats spent 10 seconds in this condition they were let free and allowed to explore until they escaped or 4 min elapsed. When rats entered the escape tunnel, the white noise went off. Between each trial the BM was thoroughly cleaned with a 5% chlorine solution and rotated to prevent rats from using non-controlled BM clues, albeit the escape tunnel remained in the same spatial place per rat. The location of the escape tunnel was randomly assigned per rat. Once the location of the escape tunnel was determined, it remained in the same spatial location for the entire study. Rats were randomly assigned to a group during all experiments. Animals from set 2 received either vehicle (DMSO 100%), LPI (10 nM) or CID16020046 (10 nM). Each group were infused 5 min before the beginning of each training session (S1–S4).

Retention test

The day after the last training session, rats were evaluated in a retention test (a 4-min trial per rat, just once). In this session (S5), rats were placed in the BM following the same procedure as in the training sessions, but no escape box was placed under any of the holes. The total time spent in the target zone (see

above) was estimated (Fig. 2). It is noteworthy to mention that no treatment was infused before or during the retention test.

Drugs

In addition to anaesthetics (ketamine, xylazine, acepromazine), the compounds used in this study were Lysophosphatidylinositol (LPI, Sigma–Aldrich) and CID16020046 (Sigma–Aldrich). LPI and CID16020046 were dissolved in DMSO 100%. The dose used for LPI was selected based on its affinity for GPR55 (Table 1) and pilot experiments. Whereas the dose used for CID16020046 was considered high enough to block GPR55 receptors (Marichal-Cancino et al. 2016). The final volume for all injections was 0.5 µl per side.

Statistical analyses

Results were analyzed as follows: latency to enter the escape tunnel (learning curve), errors, progression in the strategies to solve the BM from session 1 to session 4 and seconds per minute spent in the target zone during the retention test were analyzed by means of a two-way mixed ANOVA test (treatment x session; and treatment x seconds in target zone). In addition, strategies to solve the BM during the 16 trials and total time in the target zone during the retention test in different groups were analyzed by means of a one-way ANOVA test. Differences between treatments for each session were evaluated by Bonferroni *post-hoc* test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Latency (learning curve) and errors

Fig. 3A shows the latency to find the escape tunnel in each session and Fig. 3B shows errors (visits to holes without the escape tunnel) in intact rats or those infused with vehicle (DMSO 100%), LPI (GPR55 agonist) or CID16020046 (GPR55 antagonist) before each training session. No main differences among treatments (latency to escape) or interactions (treatments vs. sessions; learning curve) were detected ($P > 0.05$). None of the treatments interfered with the latency to escape during sessions. However, differences between sessions (regardless of treatment) [$F(3,84)=37.38$, $P < 0.05$] were detected. Session 1 was significantly different from session 4 ($P < 0.05$; Fig. 3A).

Therefore, rats under all treatments displayed a normal learning curve.

On the other hand, main differences in the quantity of errors for treatments [$F(3,28)=3.319$, $P<0.05$], sessions [$F(3,84)=6.563$, $P<0.05$] and interactions [$F(9,84)=3.289$, $P<0.05$] were detected. Rats infused with LPI committed more errors (particularly in the training session 2) than others (Fig. 3B). CID16020046, but not LPI exhibited a quantity of errors like intact or vehicle groups throughout sessions (i.e., S1 vs. S4; $P<0.05$; Fig. 3B).

Strategies used to solve the Barnes-maze

Fig. 4 shows the use of different navigation strategies during the training phase (S1–S4, 16 trials in total) to solve the BM in intact rats, or when groups receiving vehicle (DMSO 100%), LPI or CID16020046 infusions, respectively. Main differences among groups were detected for serial strategy [$F(3,28)=7.197$, $P<0.05$] and random strategy [$F(3,28)=14.280$, $P<0.05$], but not for spatial strategy [$F(3,28)=0.475$, $P>0.05$]. Animals infused with LPI showed a high increase in the use of serial strategy compared with intact rats, vehicle and CID16020046 ($P<0.05$; Fig. 4A). No differences were detected in the use of spatial strategy (Fig. 4B). Animals treated with LPI showed a decrease in the use of random navigation compared with vehicle or CID16020046

(Fig. 4C), while CID16020046 induced an increased in the use of the random navigation when compared with intact rats ($P<0.05$).

Fig. 5 shows the progression in the strategy used by rats between first (assays 1–4) and last training days (assays 13–16) in intact rats or treatment groups. Main differences for treatments [$F(3,56)=6.773$, $P<0.05$] and differences among sessions [$F(1,56)=5.120$, $P<0.05$], but not interaction (treatments vs. sessions) [$F(3,56)=2.613$, $P>0.05$] were detected in the serial strategy. Rats infused with LPI highly increased the use of serial strategy from session 1 to session 4 ($P<0.05$). Intact rats, vehicle or CID16020046 induced no change in this strategy throughout the sessions (Fig. 5A). Regarding the spatial strategy, differences only among sessions were detected [$F(1,56)=11.570$, $P<0.05$]. Intact rats and those infused with vehicle increased the use of spatial strategy from session 1 to session 4 ($P<0.05$); whereas rats infused with LPI or CID16020046 did not increase the use of this strategy throughout the sessions (Fig. 5B). For random strategy, main differences for treatments [$F(3,56)=7.925$, $P<0.05$], differences among sessions [$F(1,56)=4.226$, $P<0.05$], but not interactions [$F(3,56)=2.201$, $P>0.05$] were detected. Intact rats and those infused with vehicle decreased the use of random strategy from S1 to S4 ($P<0.05$). LPI dramatically reduced the use of random strategy from session 1 to session 4 compared with intact rats, vehicle or CID16020046. Finally, CID16020046 group showed

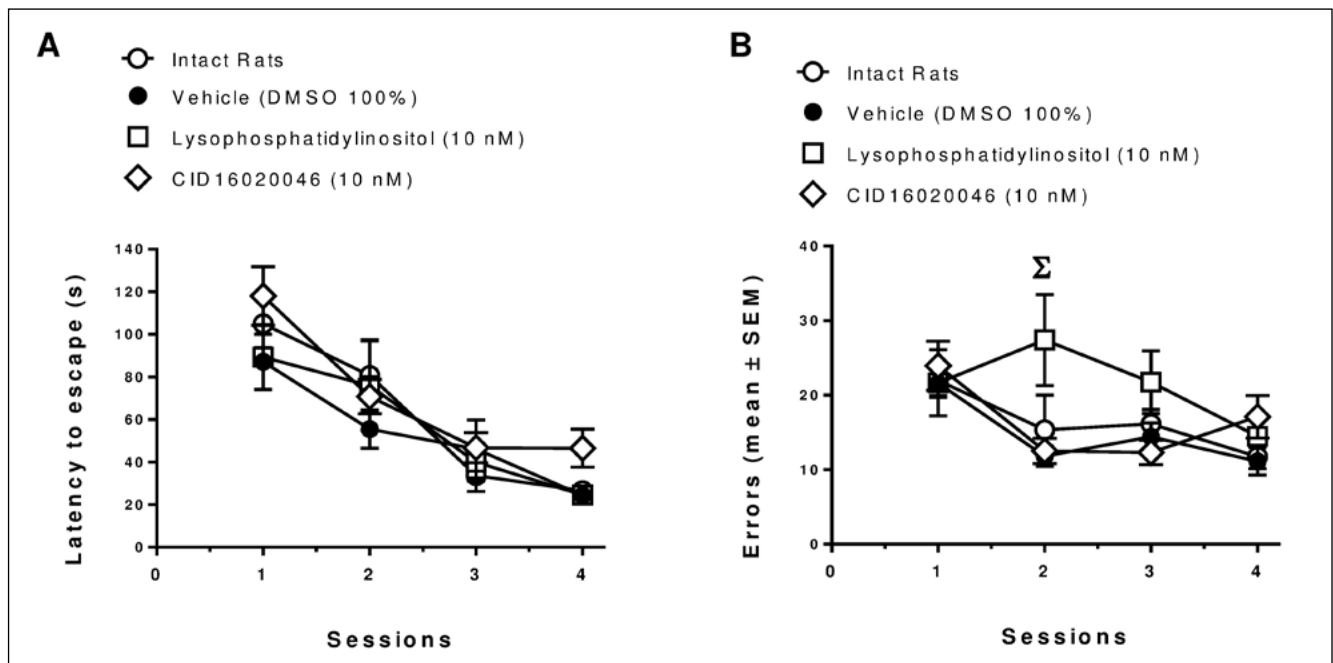


Fig. 3. Effects of lysophosphatidylinositol and CID16020046 on latency and errors during training in Barnes-maze. Apart from intact rats, other groups ($n=8$ each group) received infusions into the dorsal hippocampus of vehicle (DMSO 100%), lysophosphatidylinositol (10 nM) or CID16020046 (10 nM) before training sessions. (A) latency and learning curve. (B) errors. $^{\#}$, $P<0.05$ vs. any other group.

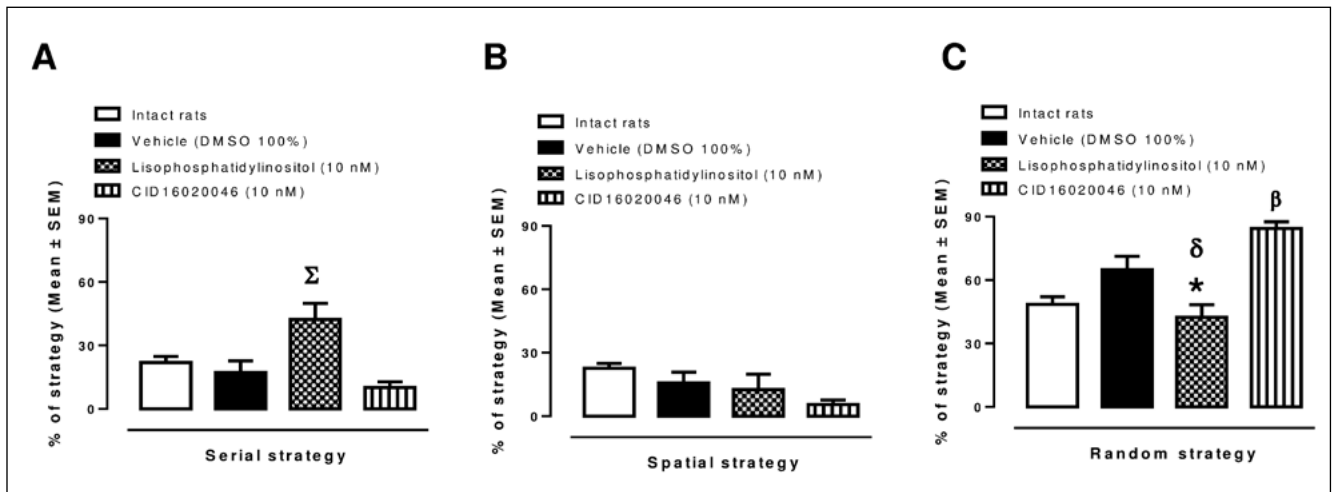


Fig. 4. Effect of lysophosphatidylinositol and CID16020046 on the navigation strategy used during the training phase. Data represent the mean of the strategy used during four training sessions (16 trials). Apart from intact rats, other groups (n=8 each group) received infusions into the dorsal hippocampus of vehicle (DMSO 100%), lysophosphatidylinositol (10 nM) or CID16020046 (10 nM) before each training session. (A) serial strategy, (B) spatial strategy and (C) random strategy. Σ, P<0.05 vs. any other group; *, P<0.05 vs. vehicle group; δ, P<0.05 vs. CID16020046 and β, P<0.05 vs. intact rats.

no reduction in random activity during sessions and induced more random activity compared with intact rats (Fig. 5C).

total time than intact rats in the target zone. Moreover, CID1602046-infused rats spent less time than vehicle group (P<0.05).

Retention test

Fig. 6 illustrates time spent in the target zone during the retention test (S5; a single 4-min trial). Main differences among treatments [F (3, 29)=7.536, P<0.05] were detected. LPI and CID16020046-infused rats spent less

DISCUSSION

General

Hippocampal activity (among several other functions) allows us to locate specific places or objects in

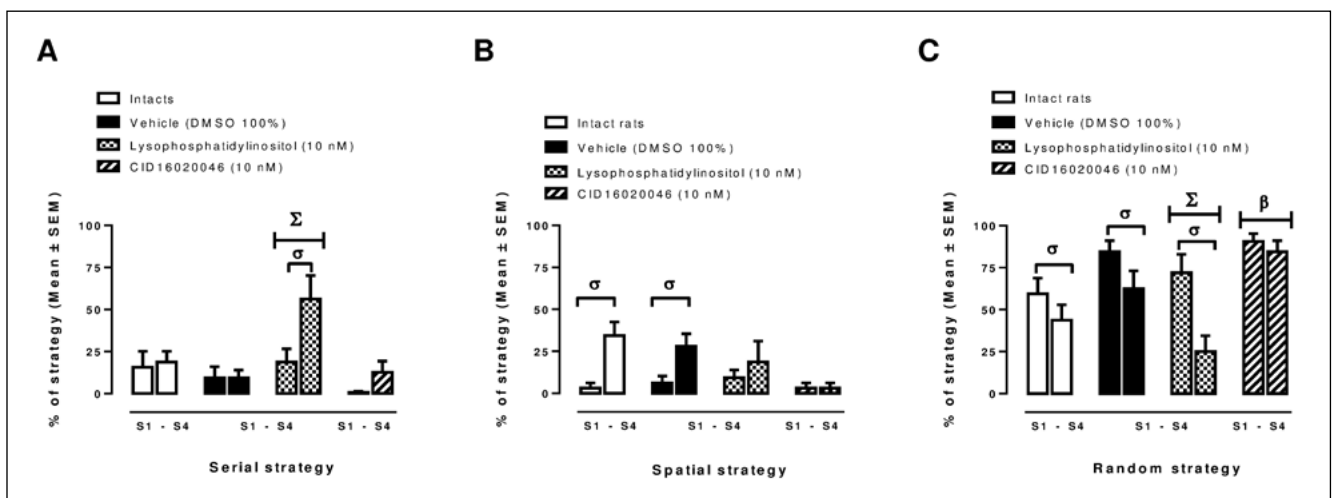


Fig. 5. Progression in the use of navigation strategies. Rats (n=8 each group) received infusions into the dorsal hippocampus of vehicle (DMSO 100%), lysophosphatidylinositol (10 nM) or CID16020046 (10 nM) before 4 training sessions. Main effects of treatments, sessions (S1 vs. S4) and interaction (treatments vs. sessions) were analyzed for navigation strategies among groups. (A) Serial strategy; (B) spatial strategy; (C) random strategy. *, P<0.05 main differences vs. vehicle group; p, P<0.05 main differences vs. CID16020046 group. σ, P<0.05 S1 vs. S4; Σ, P<0.05 vs. any other group; *, P<0.05 vs. vehicle group and β, P<0.05 vs. intact rats.

space and it is involved in choosing the trajectory to be used in a goal-directed behaviour (Evensmoen et al. 2013, Ito et al. 2015, Manns and Eichenbaum 2009). In the present study, we found that hippocampal injections of LPI (GPR55 agonist) increased the use of the serial navigation strategy.

Fouquet et al. (2013) reported that lesions in the dorsal hippocampus increased the use of serial navigation. Nevertheless, lesions in the dorsal striatum did not decrease the use of the serial strategy and abolished the use of a direct (spatial) strategy, suggesting potential communication between hippocampus and striatum in establishing a spatial navigation strategy. Under certain experimental conditions, spatial navigation involves cooperative activity between different brain structures such as the dorsal hippocampus and the dorsal striatum (Chersi and Burgess 2015, Doeller et al. 2008, Fouquet et al. 2013). Interestingly, stimulation of GPR55 in the dorsal striatum increased the learning of procedural memories (Marichal-Cancino et al. 2016). In addition, mutant mice that lack GPR55 showed a high impairment in motor coordination (Wu et al. 2013). Thus, this receptor may be important in coordinating sequential motor actions at different levels (Marichal-Cancino et al. 2017).

Our results suggest a potential role for LPI, acting on hippocampal neurons (probably via GPR55) in the neural-circuitry involved in coordinating spatial navigation strategies. Supporting the above, blockade of GPR55 before training increased random navigation activity. The above indicates that integrity of GPR55 pathway may be required to establish a specific navigation strategy. Finally, blockade of GPR55 during the training phase decreased time spent in the escape tunnel placement. This result may point out a role of GPR55 in the formation of spatial memory. However, more experiments need to be developed to clarify this possibility.

Latency, learning curve and errors

In these experiments, we used an aversive version of the Barnes maze (Harloe et al. 2008). The rodents were motivated to find the escape tunnel to avoid exposure to light and noise (90 dB) as previously described (Harloe et al. 2008, Rueda-Orozco et al. 2008). In this context, learning in solving the task was inferred from the reduction in latency to find the escape tunnel, thus, eluding these aversive conditions. Interestingly, GPR55 pharmacological activation or blockade before training induced no changes in the latency to escape. Therefore, the learning curve across training-sessions is not different from the control. Thus, hippocampal GPR55 could not be involved in the regulation of the motivation to escape from this aversive condition. This result

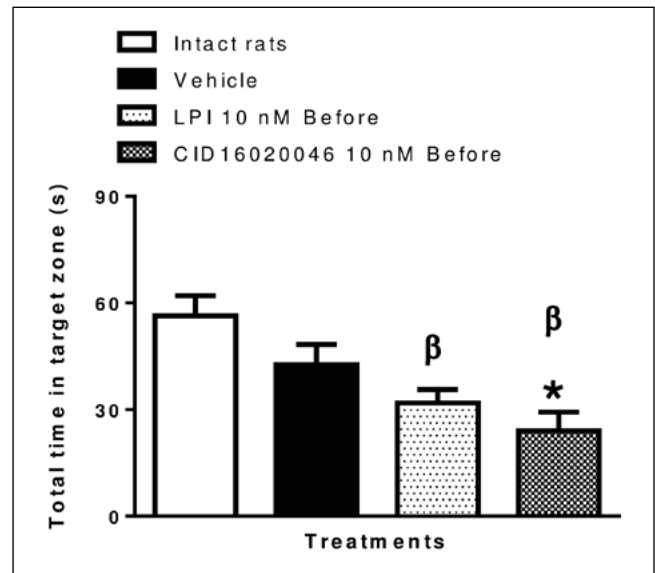


Fig. 6. Effect of lysophosphatidylinositol and CID16020046 on the retention test. Apart from intact rats, other groups ($n=8$ each group) received infusions of vehicle (DMSO 100%), lysophosphatidylinositol (10 nM) or CID16020046 (10 nM) during the training phase; no treatment was infused on the retention test day. Data represent total time spent in target zone in the single 4-minute trial. *, $P<0.05$ vs. vehicle group and β , $P<0.05$ vs. intact rats.

agrees with Kramar et al. (2017), which concluded no participation of GPR55 in context fear conditioning test under their experimental conditions.

On the other hand, animals receiving LPI committed a higher number of errors across the training sessions. As LPI increased the use of the serial strategy (which requires subsequent visits to non-target holes), this higher number of errors may reflect the navigation strategy used (see below).

Possible mechanisms involved in the effects of LPI on the navigation strategies to solve the BM

We have previously reported (Rueda-Orozco et al. 2008) that anandamide infusions in CA1 significantly modified the use of the spatial or serial navigation strategy. The actions of anandamide were resistant to blockade with AM251 (CB₁ inverse agonist/antagonist) suggesting the potential participation of other cannabinoid target receptors. As GPR55 is expressed in the hippocampus (Ryberg et al. 2007) and other brain structures where the actions have been only partially identified (Marichal-Cancino et al. 2017), it was logical to assume that this receptor may be involved.

Our data show that CA1-hippocampus infusions of a GPR55 endogenous agonist (LPI) promoted the use of the serial strategy, while preventing learning of the spa-

tial strategy. The above suggests that LPI may be altering hippocampal activity related with goal-directed navigation behaviours. In *in vitro* models, hippocampal GPR55 activation increases the probability of glutamate and dopamine release (Kramar et al. 2017, Sylantsev et al. 2013). If GPR55 enhances hippocampal activity *in vivo* also, there are at least four speculative possibilities to explain our results: (i) after GPR55 stimulation and the subsequent glutamate/dopamine induction, other neurotransmitters may be recruited to decrease goal-directed behaviours (e.g. endocannabinoids; see below); (ii) hippocampal GPR55 expressing-neurons may be involved in serial navigation (or in actions that alter spatial navigation); (iii) LPI may be activating other receptors (different or aside from GPR55); and (iv) hippocampal GPR55 may be involved in the exploratory behaviour.

Supporting the first speculation, GPR55 activation seems to increase the release of glutamate (Sylantsev et al. 2013) that may open NMDA receptors facilitating Ca^{2+} currents into the postsynaptic neuron. In addition, GPR55 may raise intracellular Ca^{2+} (Henstridge et al. 2010). In other studies, elevation in levels of intracellular Ca^{2+} increased the synthesis of anandamide and the activation of phospholipase C; which in turn, induced synthesis of 2-arachidonoyl glycerol (2-AG) (Jung et al. 2007, Hashimoto et al. 2008). These endocannabinoids might interact with hippocampal cannabinoid receptors. On the other hand, increase in dopaminergic activity induced by hippocampal injections of palmitoylethanolamide (non-selective GPR55 endogenous agonist) were prevented in presence of CID160120046 (GPR55 antagonist) or MK801 (NMDA receptor antagonist) suggesting glutamate participation in the mentioned effects (Kramar et al. 2017).

To support the second possibility, several authors have reported non-spatial behaviours involving hippocampal neurons (Cohen et al. 2013, Hampson et al. 1999, Wood et al. 1999, Yi et al. 2016). Some evidence suggests a cooperative action of dorsal hippocampus with striatal areas to allow spatial navigation in the Morris-maze (Miyoshi et al. 2012) signifying simultaneous activity in both structures as reported by others (Regier et al. 2015). Thus, hippocampal GPR55 expressing-neurons may be involved in the expression of the serial navigation strategy via interaction with striatal neurons. In addition, Rahimi et al. (2015) reported that central GPR55 stimulation induced an anxiolytic effect that could be influencing the navigation behaviour in rats. At this respect, Morena et al. (2015) established a relationship between stress levels and cannabinoid activity modulating spatial memory.

On the other hand, LPI seems to induce effects via other non-GPR55. Soga et al. (2005) reported that LPI (*in vitro*) induced insulin secretion by GPR119. Monet et al. (2009) reported induction of cell migration after

LPI by TRPV2. mRNA for GPR119 (Bonini et al. 2002) and TRPV2 (Pan et al. 2011) have been reported in hippocampal tissue, but their functions (if any) remain obscure. Lastly, hippocampal GPR55 could be involved in controlling exploratory behaviour which may be responsible for changes seen during the tests (Good and Honey 1997, Hernández-Tristán et al. 2000). Nevertheless, exploratory behaviour during retention test was decreased in rats infused with LPI and no differences in latency to escape from Barnes-maze were detected.

Finally, blockade of GPR55 with a selective antagonist (CID16020046; Kargl et al. 2013) increased the use of random navigation (Fig. 4C and Fig. 5C) suggesting that GPR55 signalling pathway integrity may be a requirement to establish a specific navigation strategy.

The retention test: spatial memory

GPR55 blockade during acquisition in the BM decreased the time spent in the target zone during the retention test. Since animals received no drugs during the retention test day, this test involved effects on learning and/or memory formation during the training phase. Nevertheless, one may argue that the state during retention test session was different from that during training phase (since rats received treatment only during training phase); thus, other effects (not tested in this study) could have interfered with behavior of rats at retention test session. At any case, these preliminary results should motivate the advent or further experimental protocols directed to analyze the specific functionality of hippocampal GPR55 in spatial learning and memory.

CONCLUSIONS

Taken together, our findings suggest that LPI interacts with CA1 hippocampal cells that may be involved in the expression of serial navigation. On the other hand, GPR55 signaling integrity seems to be necessary to integrate a specific navigation strategy. Finally, our data suggest the existence of a potential participation of hippocampal GPR55 during formation/acquisition of spatial memory (specifically, object placement memory).

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REFERENCES

- Barnes CA (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J Comp Physiol Psychol* 93: 74–104.
- Basavarajappa BS, Nagre NN, Xie S, Subbanna S (2014) Elevation of endogenous anandamide impairs LTP, learning, and memory through CB1 receptor signaling in mice. *Hippocampus* 24: 808–818.
- Brighton PJ, McDonald J, Taylor AH, Challiss RA, Lambert DG, Konje JC et al. (2009) Characterization of anandamide-stimulated cannabinoid receptor signaling in human ULTR myometrial smooth muscle cells. *Mol Endocrinol* 23: 1415–1427.
- Bonini JA, Borowsky BE, Adham N, Boyle N, Thompson TO (2002) Methods of identifying compounds that bind to SNORF25 receptors. U.S. Patent 6,468,756.
- Chersi F, Burgess N (2015) The Cognitive Architecture of Spatial Navigation: Hippocampal and Striatal Contributions. *Neuron* 88: 64–77.
- Cohen SJ, Munchow AH, Rios LM, Zhang G, Asgeirsdóttir HN, Stackman RW Jr (2013) The rodent hippocampus is essential for nonspatial object memory. *Curr Biol* 23: 1685–1690.
- Doeller CF, King JA, Burgess N (2008) Parallel striatal and hippocampal systems for landmarks and boundaries in spatial memory. *Proc Natl Acad Sci U S A* 105: 5915–5920.
- Evensmoen HR, Lehn H, Xu J, Witter MP, Nadel L, Håberg AK (2013) The anterior hippocampus supports a coarse, global environmental representation and the posterior hippocampus supports fine-grained, local environmental representations. *J Cogn Neurosci* 25: 1908–1925.
- Fouquet C, Babayan BM, Watilliaux A, Bontempi B, Tobin C, Rondi-Reig L (2013) Complementary Roles of the Hippocampus and the Dorsomedial Striatum during Spatial and Sequence-Based Navigation Behavior. *PLoS One* 8: e67232.
- Good M, Honey RC (1997) Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning. *Behav Neurosci* 111: 487–493.
- Hampson RE, Simeral JD, Deadwyler SA (1999) Distribution of spatial and nonspatial information in dorsal hippocampus. *Nature* 402: 610–614.
- Harloe JP, Thorpe AJ, Lichtman AH (2008) Differential endocannabinoid regulation of extinction in appetitive and aversive Barnes maze tasks. *Learn Mem* 15: 806–809.
- Hashimoto-dani Y, Ohno-Shosaku T, Maejima T, Fukami K, Kano M (2008) Pharmacological evidence for the involvement of diacylglycerol lipase in depolarization-induced endocannabinoid release. *Neuropharmacol* 54: 58–67.
- Henstridge CM, Balenga NA, Schröder R, Kargl JK, Platzer W, Martini L, Arthur S et al. (2010) GPR55 ligands promote receptor coupling to multiple signalling pathways. *Br J Pharmacol* 160: 604–14.
- Hernández-Tristán R, Arévalo C, Canals S, Leret ML (2000) The effects of acute treatment with delta9-THC on exploratory behaviour and memory in the rat. *J Physiol Biochem* 56: 17–24.
- Hurst K, Badgley C, Ellsworth T, Bell S, Friend L, Prince B, Welch J, Cowan Z, Willaimson R, Lyon C, Anderson B, Poole B, Christensen M, McNeil M, Call J, Edwards JG (2017) A putative lysophosphatidylinositol receptor GPR55 modulates hippocampal synaptic plasticity. *Hippocampus* 27: 985–998.
- Ito HT, Zhang SJ, Witter MP, Moser EI, Moser MB (2015) A prefrontal-thalamo-hippocampal circuit for goal-directed spatial navigation. *Nature* 522: 50–55.
- Jung KM, Astarita G, Zhu C, Wallace M, Mackie K, Piomelli D (2007) A key role for diacylglycerol lipase- α in metabotropic glutamate receptor-dependent endocannabinoid mobilization. *Mol Pharmacol* 72: 612–621.
- Kapur A, Zhao P, Sharif H, Bai Y, Caron MG, Barak LS, et al. (2009) Atypical responsiveness of the orphan receptor GPR55 to cannabinoid ligands. *J Biol Chem* 284: 29817–29827.
- Kargl J, Brown AJ, Andersen L, Dorn G, Schicho R, Waldhoer M, Heinemann A (2013) A selective antagonist reveals a potential role of G protein-coupled receptor 55 in platelet and endothelial cell function. *J Pharmacol Exp Ther* 346: 54–66.
- Kramar C, Loureiro M, Renard J, Steven RL (2017) Palmitoylethanolamide Modulates GPR55 Receptor Signaling in the Ventral Hippocampus to Regulate Mesolimbic Dopamine Activity, Social Interaction, and Memory Processing. *Cannabis and Cannabinoid Res* 2: 8–20.
- Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B, Mackie K (2008). GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *Proc Natl Acad Sci U S A* 105: 2699–2704.
- Manns JR, Eichenbaum H (2009) A cognitive map for object memory in the hippocampus *Learn Mem* 16: 616–624.
- Marichal-Cancino BA, Fajardo-Valdes A, Ruiz-Contreras AE, Méndez-Díaz M, Prospéro-García O (2017) Advances in the Physiology of GPR55 in the Central Nervous System. *Curr Neuropharmacol* 15: 771–778.
- Marichal-Cancino BA, Manrique-Maldonado G, Altamirano-Espinoza AH, Ruiz-Salinas I, González-Hernández A, Maassenvandenbrink A, Villalón CM (2013) Analysis of anandamide- and lysophosphatidylinositol-induced inhibition of the vasopressor responses produced by sympathetic stimulation or noradrenaline in pithed rats. *Eur J Pharmacol* 721: 168–177.
- Marichal-Cancino BA, Sánchez-Fuentes A, Méndez-Díaz M, Ruiz-Contreras AE, Prospéro-García O (2016) Blockade of GPR55 in the dorsolateral striatum impairs performance of rats in a T-maze paradigm. *Behav Pharmacol* 27: 393–396.
- McGrath JC, Drummond GB, McLachlan EM, Kilkenny C, Wainwright CL (2010) Guidelines for reporting experiments involving animals: the ARRIVE guidelines. *Br J Pharmacol* 160: 1573–1576.
- Miyoshi E, Wietzikoski EC, Bortolanza M, Boschen SL, Canteras NS, Izquierdo I, Da Cunha C (2012) Both the dorsal hippocampus and the dorsolateral striatum are needed for rat navigation in the Morris water maze. *Behav Brain Res* 226: 171–178.
- Monet M, Gkika D, Lehen'kyi V, Pourtier A, Vanden Abeele F, Bidaux G, Juvin V, Rassendren F, Humez S, Prevarskaya N (2009) Lysophospholipids stimulate prostate cancer cell migration via TRPV2 channel activation. *Biochim Biophys Acta* 1793: 528–539.
- Morena M, De Castro V, Gray JM, Palmery M, Trezza V, Roozendaal B, Hill MN, Campolongo P (2015) Training-associated emotional arousal shapes endocannabinoid modulation of spatial memory retrieval in rats. *J Neurosci* 35: 13962–13974.
- Pan JP, Zhang HQ, Wei-Wang, Guo YF, Na-Xiao, Cao XH, Liu LJ (2011) Some subtypes of endocannabinoid/endovanilloid receptors mediate docosahexaenoic acid-induced enhanced spatial memory in rats. *Brain Res* 1412: 18–27.
- Paxinos G, Watson C (2007) The rat brain in stereotaxic coordinates. 6th ed. San Diego: Elsevier Academic Press.
- Rahimi A, Hajizadeh-Moghaddam A, Roohbakhsh A (2015) Central administration of GPR55 receptor agonist and antagonist modulates anxiety-related behaviors in rats. *Fundam Clin Pharmacol* 29: 185–190.
- Regier PS, Amemiya S, Redish AD (2015) Hippocampus and subregions of the dorsal striatum respond differently to a behavioral strategy change on a spatial navigation task. *J Neurophysiol* 114: 1399–1416.
- Rojo ML, Rodriguez-Gaztelumendi A, Fowler CJ (2012) Lysophosphatidylinositol stimulates [35 S]GTP γ S binding in the rat prefrontal cortex and hippocampus. *Neurochem Res* 37: 1037–1042.
- Rueda-Orozco PE, Soria-Gomez E, Montes-Rodríguez CJ, Martínez-Vargas M, Galicia O, Navarro L, Prospero-García O (2008) A potential function of endocannabinoids in the selection of a navigation strategy by rats. *Psychopharmacology (Berl)* 198: 565–576.
- Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J, Elebring T, Nilsson K, Drmota T, Greasley PJ (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 152: 1092–1101.

- Soria-Gómez E, Matias I, Rueda-Orozco PE, Cisneros M, Petrosino S, Navarro L, Di Marzo V, Prospéro-García O (2007) Pharmacological enhancement of the endocannabinoid system in the nucleus accumbens shell stimulates food intake and increases c-Fos expression in the hypothalamus. *Br J Pharmacol*. 151: 1109–1116.
- Swanson LW (2004). *Brain Maps: Structure of the rat brain*. 3rd Edition. San Diego: CA, Elsevier Academic Press. At http://larryswanson.com/?page_id=164.
- Sylantsev S, Jensen TP, Ross RA, Rusakov DA (2013) Cannabinoid- and lysophosphatidylinositol-sensitive receptor GPR55 boosts neurotransmitter release at central synapses. *Proc Natl Acad Sci USA* 110: 5193–5198.
- Wood ER, Dudchenko PA, Eichenbaum H (1999) The global record of memory in hippocampal neuronal activity. *Nature* 397: 613–616.
- Wu CS, Chen H, Sun H, Zhu J, Jew CP, Wager-Miller J, Straiker A, Spencer C, Bradshaw H, Mackie K, Lu HC (2013) GPR55, a G-protein coupled receptor for lysophosphatidylinositol, plays a role in motor coordination. *PLoS One* 8: e60314.
- Yang H, Zhou J, Lehmann C (2016) GPR55 – a putative “type 3” cannabinoid receptor in inflammation. *J Basic Clin Physiol Pharmacol* 27: 297–302.
- Yi JH, Park HJ, Kim BC, Kim DH, Ryu JH (2016) Evidences of the role of the rodent hippocampus in the non-spatial recognition memory. *Behav Brain Res* 297: 141–149.