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Abstract: Intracranial self-stimulation (ICSS) of the medial forebrain bundle is an effective treatment to facilitate memory. Performance in both explicit and implicit memory tasks has been improved by ICSS, and this treatment has even been capable of recovering loss of memory function due to lesions or old age. Several neurochemical systems have been studied in regard to their role in ICSS effects on memory, however the possible involvement of the orexinergic system in this facilitation has yet to be explored. The present study aims to examine the relationship between the OX1R and the facilitative effects of ICSS on two different types of memory tasks, both carried out in the Morris Water Maze: spatial and visual discrimination. Results show that the OX1R blockade, by intraventricular administration of SB-334867, partially negates the facilitating effect of ICSS on spatial memory, whereas it hinders ICSS facilitation of the discrimination task. However, ICSS treatment was capable of compensating for the severe detrimental effects of OX1R blockade on both memory paradigms. These results suggest different levels of involvement of the orexinergic system in the facilitation of memory by ICSS, depending on the memory task.

T. Abel
University of Pennsylvania, Philadelphia, Pennsylvania, USA
Neurobiology of Learning and Memory
Editor-in-Chief

Enclosed is a copy of the revised manuscript entitled: **Orexin-1 receptor blockade differentially affects spatial and visual discrimination memory facilitation by intracranial self-stimulation** (NLM-19-203R1) that we submit for consideration for publication after careful revision in *Neurobiology of Learning and Memory*.

We would like to thank the editor as well as the reviewers for their valuable time and useful contribution. Their inputs are appreciated and will definitely help improve the manuscript. You will find the response to the reviewers in a separate document attached in the submission.

The article is a research paper that evaluates whether the orexinergic system is involved in the facilitation on memory by intracranial self-stimulation (ICSS). The results show that blocking the Orexin-1 receptor by SB-334867 intracerebroventricular microinfusion hinders the facilitative effects of ICSS on memory at different levels, depending on the type of memory task. Our results also suggest that ICSS can still compensate for the detrimental effects blocking the OX1R have on both spatial and discrimination memory.

Overall, the outcome of the research paper suggests that the orexinergic system is one of the neurochemical systems involved in the facilitation of memory by ICSS. Furthermore, it confirms that ICSS is a treatment capable of recovering performance in memory tasks after impairment of normal function.

The paper has been seen and approved by all the listed authors.

Yours sincerely,

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***Response to Reviews**

Reviewers' comments:

Note: While submitting the revised manuscript, please double check the author names provided in the submission so that authorship related changes are made in the revision stage. If your manuscript is accepted, any authorship change will involve approval from co-authors and respective editor handling the submission and this may cause a significant delay in publishing your manuscript.

Reviewer #1: I am largely satisfied by the author's response to reviewer comments, as outlined in their rebuttal. But on several points this has not translated into actual changes in the manuscript. I appreciate that the authors have made changes such as reframing their paper around the correct molecular target (OX1R rather than OX-A) and adding some definitions, but what's the point of telling me about how Blokland et al. found differences in the accuracy and performance during MWM if this is not included in the manuscript body? I have therefore gone through my previous comments and outline how the authors have modified their manuscript and whether I think this is sufficient.

Major Concerns
1. Framing. The authors have generally reframed the paper around the signalling component they are actually targeting (i.e. OX1 receptors, as opposed to OX-A). However in the last paragraph of the introduction where they describe studying "the relationship between orexin-A via OX1R activation". In my opinion they can drop the mention of orexin-A from this sentence.

This has been modified in the manuscript.

2. Statistical reporting. a) Thank you for providing more complete reporting of statistical results. I'm not sure about the reporting of post-hocs for the non-significant effect of group in section 3.2.2, while I don't want to be dogmatic about $p = 0.05$ and it looks to me like there is something going on here (especially given the significant results on the other measures), I think it needs to be clear in figure 3A that these post-hocs follow a non-significant main effect. Given that other measures in this figure have significant ANOVA results and posthocs, perhaps the best solution is to simply remove the asterisks from figure 3A.

We agree with the reviewer and the asterisks have been removed from figure 3A. In addition, we have specified that the shown differences by # and ## are between groups and chance level in the caption of Figure 3A.

b) Thank you for providing clearer information about sphericity corrections.
c) Thank you for correcting and clarifying the use of Welch F.
d) The authors provide further information about why they chose the 30 s analysis in the rebuttal, but have chosen not to amend their manuscript. I am puzzled by this decision on behalf of the authors. I still believe the analysis should be labelled as exploratory and the justification for breaking down their analysis of the session in this way should be provided in the manuscript.

The according justification was added to the "Statistical analysis" section (2.11.)

> "Given that the accuracy and level of performance could change throughout the probe trial in the MWM (Blokland, Geraerts, & Been, 2004), results for the retention test were analyzed with a one-way ANOVA for the totality of the trial (60s) and also for the first half of the trial (30s)."

e) Thank you for reporting that figure 5F (now 6F) was associated with no significant difference. However, it should be made clear that the same procedure was followed in this experiment, where an ANOVA was used to examine between group differences and one-sample t-tests were used to check the result against chance. While this is detailed in the methods and the figure legend, it should be reported consistently in the results - i.e. the full t-test results should be reported instead of just p values. Ideally, I think the full ANOVA should be reported too.

The one-sample t-test analysis for both experiments is detailed in the "statistical analysis" section (2.11). We have thoroughly checked the manuscript to make sure all results were appropriately reported. You will find that all ANOVAs and *t*-test results are now fully reported.

3. I think it is helpful that consolidation has been added to the discussion.

Minor concerns

1a) Messina et al. (2014) is a very short paper and there may be better options, but makes the point the authors want to in this sentence.

b) OK.

c) OK.

2. OK.

3. Thank you for these helpful definitions.

4. Thank you for these helpful figures.

5. My comment here was a rather long way of asking the authors to remove the word "completely" from "completely hindered". Severely? Maybe, but I think "hindered" or "impaired" would be sufficient on its own.

Our main concern with removing the modifier "severely" is that it could lead to an underappreciation of the differences in hinderance of ICSS facilitation by SB-334867 in the two types of memory tasks. However, after further revision of the overall content and tone of the manuscript, we have come to agree with the reviewer on this matter.

6. Please add the note about buprenorphine analgesia to the manuscript. Telling me has little benefit if the final manuscript doesn't include this detail.

This has been corrected in the manuscript.

"The animals were weighed and handled daily during the post-surgery recovery period (7 days), and analgesia (0.03mg/kg subcutaneous buprenorphine) was administered every 8-12 hours for the first 48h of post-surgical care."

Additional notes

I know that reviewer 2 asked for the SB-334867 concentration to be given in M, but given the number of papers which give doses in ug/ul, perhaps both measurements can be given in the first instance to facilitate easy comparison between studies. Moreover, the authors can cite McElhinny Jr et al. (2012; doi:10.1016/j.bmcl.2012.08.109) with respect to the aqueous insolubility of SB-

334867 and instability of SB-334867 in acidic solutions (thus necessitating DMSO).

We thank the reviewer for the detailed comments and the additional citation.

"The selective OX1R antagonist SB-334867 (Tocris Bioscience, Bristol, UK) was dissolved in dimethyl sulfoxide (DMSO) (Tocris Bioscience, Bristol, UK) due to its aqueous insolubility and instability in acidic solutions (McElhinny et al., 2012). It was then aliquoted in tightly sealed vials and stored at -20ºC for up to 5 days."

Reviewer #2: The authors have largely done a good job of addressing my previous concerns. I have a couple of minor comments on their revisions:

1. I think it would be good for the authors to clarify their statement around the potential therapeutic use of ICSS. A statement along the lines of the one they suggest would suffice.

We have added the clarification to the appropriate section of the discussion.

"Therefore, the MFB-ICSS treatment could compensate for some of the memory detriment patients may suffer, of both implicit and explicit learning and memory, as a result of a dysfunction in the orexinergic system, found in AD, among others."

2. The body weight data are useful - thank you for including. It is interesting that the controls appear to largely maintain a stable weight, whereas there is a trend towards some weight loss in all other treatment groups, with this being most pronounced in the ICSS+SB group. Please indicate what statistical test was used to show no significant differences in weight gain across the experiment (as reported in the Results). Also, please indicate in Table 1 what the numbers in the first column refer to - I assume 'session'? Also, please replace the label 'weight' with 'body weight'.

We have included the analysis model for the weight evolution in the statistical analysis section of the manuscript (2.11).

"The evolution of weight throughout the experiments was also analyzed using a 5×4 mixed ANOVA (SESSION WEIGHT×GROUP)"

In addition, the table has been modified.

3. I urge the authors to again consider adding some discussion - if only 1-2 sentences - regarding the lack of effect of SB on ICSS OI and duration parameters. These data have potentially important implications for the hypothesis that the orexin system is important for motivational processes only when augmented by conditioned stimuli. This finding would be of significant interest to motivational researchers (and thus might increase interest in the current manuscript) - I worry that it could be lost in the manuscript otherwise.

We completely agree with the reviewer, and we have included a paragraph addressing the implications of our finding regarding ICSS threshold in the discussion (4.3).

"As we explored the involvement of the orexinergic system in the facilitation of memory by ICSS, it is important to note that we did not find that ICSS threshold was affected by the blockade of OX1R. This suggests that motivational states, which could play a part in memory processing (Kennedy & Shapiro, 2009), were not affected by the SB-334867 infusion. Orexin-A has been said to play a complex role in reward and other motivational processes (Mahler et al., 2014), but evidence of its specific role is very sparse and sometimes contradictory; for example, intra-VTA and icv administration of orexin-A have increased ICSS threshold (Boutrel et al., 2005; Hata et al., 2011), while intra-insular and intraperitoneal administrations have not (Hollander, et al., 2008; Riday et al., 2012).

Highlights

- SB-334867 impairs both spatial memory and visual discrimination in the MWM.
- ICSS compensates for the detrimental effects of SB-334867 on both memory paradigms.
- OX1R blockade partially negates the facilitating effect of ICSS on spatial memory.
- OX1R blockade severely hinders ICSS facilitation of the discrimination task.
- The orexinergic system’s involvement in ICSS facilitative effect is task-dependent.

TITLE:

Orexin-1 receptor blockade differentially affects spatial and visual discrimination memory facilitation by intracranial self-stimulation

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***Abbreviations*:** ICSS, intracranial self-stimulation; OX1R, orexin-A selective receptor; DMSO, dimethyl sulfoxide; MWM, Morris Water Maze; SVD, simultaneous visual discrimination.

***Keywords*:** Intracranial self-stimulation; Spatial memory; Simultaneous visual discrimination memory; Morris Water Maze; OX1R; SB-334867.

ABSTRACT

Intracranial self-stimulation (ICSS) of the medial forebrain bundle is an effective treatment to facilitate memory. Performance in both explicit and implicit memory tasks has been improved by ICSS, and this treatment has even been capable of recovering loss of memory function due to lesions or old age. Several neurochemical systems have been studied in regard to their role in ICSS effects on memory, however the possible involvement of the orexinergic system in this facilitation has yet to be explored. The present study aims to examine the relationship between the OX1R and the facilitative effects of ICSS on two different types of memory tasks, both carried out in the Morris Water Maze: spatial and visual discrimination. Results show that the OX1R blockade, by intraventricular administration of SB-334867, partially negates the facilitating effect of ICSS on spatial memory, whereas it hinders ICSS facilitation of the discrimination task. However, ICSS treatment was capable of compensating for the severe detrimental effects of OX1R blockade on both memory paradigms. These results suggest different levels of involvement of the orexinergic system in the facilitation of memory by ICSS, depending on the memory task.

1. Introduction

Intracranial self-stimulation (ICSS) is an operant response in which subjects self-administer electrical stimulation to brain areas belonging to the reward system. As a treatment, ICSS to the medial forebrain bundle (MFB) in the lateral hypothalamus (LH) is capable of facilitating the acquisition and retention of implicit (Huston et al., 1977; Huston & Mueller, 1978; Redolar-Ripoll et al., 2002; Ruiz-Medina et al., 2008; García-Brito et al., 2017) and explicit (Soriano-Mas et al., 2005; Chamorro-López et al., 2015) memory tasks in rats, even managing to recover lost memory function caused by electrolytic brain lesions (Segura-Torres et al., 2010; Kádár et al., 2014) and old age (Aldavert-Vera et al., 1997).

Several mechanisms to explain ICSS' facilitative effects on learning and memory have been proposed. For instance, this treatment has been linked to the structural plasticity of very specific memory-related areas, such as the pyramidal dendrites in CA3 (Shankaranarayana Rao et al., 1993) and CA1 after training in a spatial memory task (Chamorro-López et al., 2015). Additionally, ICSS has been found to be capable of activating general arousal systems (Newman & Feldman, 1964; Wise, 2005) through dopaminergic, cholinergic, noradrenergic and serotoninergic ascendant fibers (Nieuwenhuys et al., 1982; Shankaranarayana Rao et al., 1998). These as well as other neurotransmission systems have been extensively studied in relation to the reinforcing effects of ICSS (Rolls, 1974; Fibiger et al., 1987; Negus & Miller, 2014; Murakami et al., 2015) and its facilitating effects on learning and memory processes (Owesson-White et al., 2008; Ramkumar et al., 2008; Vega-Flores et al., 2014). However, research exploring the involvement of the orexinergic system, more specifically orexin-A, on the facilitation of memory by ICSS has yet to be carried out.

Since its discovery, orexin-A has been studied due to its implication in homeostatic functions and food intake (Sakurai et al., 1998; for review see Messina. et al., 2014) and arousal (Sakurai, 2007; Li et al., 2014). Additionally, researchers have looked into the key role it plays in reward processes (Borgland et al., 2009; Aston-Jones et al., 2010; Arias-Carrión et al., 2014; Muschamp et al., 2014). Both arousal and reward are of paramount importance when considering ICSS effects. Importantly, this neurochemical system has recently gained relevance in relation to learning and memory processes. Orexin-A binds with high affinity to OX1R (Sakurai et al., 1998), which is expressed widely throughout memory-related areas in the brain (Trivedi et al., 1998; Hervieu et al., 2001; Marcus et al., 2001), including CA1, DG and CA2 of the hippocampus (HPC), the prefrontal cortex (PFC) and retrosplenial cortex (RSC), and the administration of SB-334867 can result in reduced c-Fos expression in the above-mentioned areas (García-Brito et al., 2018). In addition, several studies have described how the selective blockade of OX1R can impair a spatial task in the Morris Water Maze (MWM) (Akbari et al., 2006; Akbari, et al., 2007; García-Brito et al., 2018), while orexin-A administration to rodents has been reported to have facilitative effects on passive avoidance (Jaeger et al., 2002; Telegdy & Adamik, 2002) and spatial (Zhao et al., 2014) memory tasks. Furthermore, an increase in perseverative errors has been observed in discrimination tasks after the blockade of OX1R in the basal forebrain (BF) (Piantadosi et al., 2015).

The mechanisms through which orexin-A seems to be affecting spatial memory processes are somewhat comparable to those of ICSS. An increase in levels of orexin-A has been linked to a rise in phosphorylation of MAPK proteins in vitro (Ammoun et al., 2006; Kukkonen & Leonard, 2014) and more specifically in hippocampal cells in rodents in vivo (Selbach et al., 2010; Yang et al., 2013; Zhao et al., 2014); these

proteins are closely related to plasticity signaling (Thomas & Huganir, 2004; Giese & Mizuno, 2013). Similarly, ICSS has been reported to increase the expression of learning and memory-related genes in the HPC (Kádár et al., 2013), as well as the expression of Nurr1, c-Fos and Arc protein in HPC, LH and RSC (Huguet et al., 2009; Aldavert-Vera et al., 2013; Kádár et al., 2016). Another important correlate is an increase in neurogenesis in the DG, which has been reported for both orexin-A (Ito et al., 2008) and ICSS (Takahashi et al., 2009) administration in rats. This parallel can also be drawn in regards to implicit memory; both ICSS (Shankaranarayana Rao et al., 1998) and orexin-A (Telegdy & Adamik, 2002) enhance the activity of necessary neurotransmission pathways for the acquisition and consolidation of implicit memory tasks (Winters et al., 2010; Yao et al., 2016). Considering that the orexin-A-producing neurons originate in the LH (de Lecea et al., 1998; Peyron et al., 1998; Sakurai et al., 1998), ICSS on this site could be activating orexinergic pathways which could, in turn, participate in the modulation of memory facilitation by ICSS.

Therefore, we set out to study the relationship between OX1R activation and the facilitative effect of ICSS on two memory tasks that have previously been shown to be facilitated by ICSS. In order to achieve this objective, we evaluated how post-training intracerebroventricular (icv) microinfusions of the OX1R selective antagonist SB-334867 affects the facilitative effect of ICSS on the acquisition and retention of a spatial task (Experiment 1), as well as a simultaneous visual discrimination (SVD) task (Experiment 2), both carried out using the MWM.

2. Materials and Methods

2.2. Experimental subjects

Seventy-six male Wistar rats from our laboratory's breeding stock were used: forty-eight rats in Experiment 1 (mean age = 94.79±3.17 days; mean weight = 408.50±6.14) and twenty-eight rats in Experiment 2 (mean age = 92.65±3.27 days; mean weight = 412.72±6.23g). Three days before the stereotaxic procedure they were isolated and kept in individual cages (50×22×14cm, plastic bottomed and sawdust-bedded). The animals were kept under conditions of controlled temperature and humidity, and subjected to an artificial 12-hour light/dark cycle (light on at 08:00). All behavioral tests took place during the first 6 hours of light. All subjects were kept in an ad libitum regime of food and water. All procedures were carried out in compliance with the Directive 2010/63/EU and were approved by the institutional animal care committee.

2.3. Stereotaxic surgery

Previous to the surgery, two sessions of handling took place in order to diminish the animals' emotional reactivity towards experimental manipulation. Under general anesthesia using 150 mg/kg Imalgène® ketamine chlorhydrate (Merial, Lyon, France) and 0.08 mg/kg Rompun® xylazine (Bayer, Barcelona, Spain); i.p.) all rats were chronically implanted with a 7.5mm infusion guide cannula (Plastics One®, Raonoke, VA, US, purchased through Bilaney consultants, Düsseldorf, Germany; ref: C315G/PK/Spc) into the left lateral ventricle (LV), according to coordinates from the stereotaxic atlas of Paxinos and Watson (2007), anterior: -0.7mm from bregma, lateral: 1.6mm and ventral: -4.0mm. A dummy cannula (Plastics One®; ref: C315DCN/Spc), filled the infusion guide cannula and expanded 0.5mm into the brain. The cannula was fixed in position using an auto-polymerizing acrylic resin (Vertex

self-curing, Dentimex, Netherland). All animals were chronically implanted with a monopolar stailess steel electrode (150µm in diameter) aimed at the right lateral hypothalamus (LH) into the fibers of the medial forebrain bundle (MFB), according to coordinates from the stereotaxic atlas of Paxinos and Watson (2007), anterior: -2.3mm from bregma, lateral: 2.00mm and ventral: -8.5mm. Both the cannulae and the electrodes were anchored to the skull with jeweler's screws and dental cement. The animals were weighed and handled daily during the post-surgery recovery period (7 days), and analgesia (0.03mg/kg subcutaneous buprenorphine) was administered every 8-12 hours for the first 48h of post-surgical care.

2.4. Experimental groups

Once rats had recovered from surgery (7 days), they were randomly distributed into four groups, following a 2×2 (SB×ICSS treatment) experimental design for each of the experiments: SB, Control, ICSS and SB+ICSS.

2.5. Intracranial self-stimulation shaping procedure.

Subjects in the ICSS and SB+ICSS groups were trained to self-stimulate by pressing a lever in a conventional Skinner box (25×20×20cm). Electrical brain stimulation consisted of 0.3s trains of 50Hz sinusoidal waves at intensities ranging from 5µA to 250µA. The ICSS behavior was shaped by progressive approximations, and established in one further session of search for the optimal intensity (OI). OI is defined as the lowest intensity that would lead to a stable rate of about 250 responses in 5 min.

2.6. Morris Water Maze Apparatus.

The MWM consisted of an elevated circular pool (2m diameter; 60cm above the pool floor) filled with water (45cm height) maintained at 22 ± 2ºC. The pool was in

the middle of a semi-dark room and surrounded by black curtains hanging from a false ceiling to the base of the pool forming a circular enclosure 2.4m in diameter. A clear Plexiglas platform (11cm diameter) was placed centrally in one of the four equal quadrants in which the tank was virtually divided, with its top 2cm below the surface of the water. The different cues were placed inside the enclosure and suspended from a false ceiling. For experiment 1, the cues surrounding the pool were: a plastic beach ball with alternate blue, white, yellow, white, orange, and white vertical segments, a white box with horizontal black stripes, a brown teddy bear and a white box with a light inside and a cross form window. For experiment 2, two mobile cues rested in the middle of the virtual quadrant in the tank, 45cm above the water level, and consisted of identical squares ($40cm^2$) with a vertical or horizontal black and white stripes pattern of 1cm wide stripes. All swim paths were recorded using a closed-circuit video camera (Smart Video Tracking System, Version 2.5, Panlab) with a wide-angle lens mounted 1.75m above the center of the pool embedded in the false ceiling.

2.7. Behavioral procedure.

All animals were given one habituation session in the MWM in order to reduce emotional reactivity 72 hours prior to the first acquisition session. Similarly, all animals underwent two habituation sessions with the microinfusion apparatus in order to reduce initial emotional and physical reactivity.

2.7.1. Experiment 1 – Spatial memory

The acquisition phase consisted of two daily trials for five consecutive days. At the beginning of each trial, the animal was placed into the pool at one of four different cardinal points (N, E, S and W) in a pseudorandom schedule. The position of the distal cues did not vary between trials or sessions. When an animal failed to find the

platform after 120s, it was manually directed to mount it for 15s and then removed from the tank. The average intertrial interval (ITI) was 120s.

Seventy-two hours after the last acquisition session each animal performed a probe test, which consisted of removing the platform and placing the animal in the pool from the E starting position and allowing it to search for the platform for 60s.

2.7.2. Experiment 2 – Simultaneous visual discrimination

All subjects were given six daily trials for five consecutive days. Starting from one of four different cardinal points (N, E, S and W) in a pseudorandom schedule each water-maze trial consisted of one swim from the edge of the pool to the platform. The correct cue (1) was associated with the escape platform (*escape area*), while the incorrect cue (2) was associated with the area of no escape (*area of error*). The position of the two cues was manipulated so that every ten trials the correct cue was closer, farther or at the same distance than the incorrect cue in relation to the starting point. When a rat failed to find the platform within 90s, it was manually guided to the platform for 15s and then removed from the tank. When a rat found the platform it was left on it for 15s and then removed from the tank. The average intertrial interval (ITI) was 120s. A detailed protocol of the cues' manipulation can be found in a previous report (García-Brito et al., 2017).

The retention test took place 72 hours after the last acquisition session. It consisted of removing the platform and placing the animal in the pool from the East (E) starting position and allowing it to approach the cues in search for the escape platform during 60s.

2.8. Post-training microinfusion procedure.

Immediately after each acquisition session, the animals were gently restrained while its dummy was removed and replaced with a 26-gauge injector (PlasticOne®, Roanoke, VA, USA; ref.: C313CT) extending 0.5mm below the cannula tip. The injectors were connected by polyethylene tubing (PlasticOne®, Roanoke, VA, USA; ref.: C315I/PK/Spc) to two 10µL syringes (SGE Analytical Science, Cromlab S.L. Barcelona, Spain) that were placed in the infusion pump (11 Plus Syringe Pump, Harvard Apparatus Inc., Holliston, Massachusetts, USA). The selective OX1R antagonist SB-334867 (Tocris Bioscience, Bristol, UK) was dissolved in dimethyl sulfoxide (DMSO) (Tocris Bioscience, Bristol, UK) due to its aqueous insolubility and instability in acidic solutions (McElhinny et al., 2012). It was then aliquoted in tightly sealed vials and stored at -20ºC for up to 5 days. The animals in SB and SB+ICSS groups were administered SB-334867 (5µg/2µL, 7.8mM), and animals in Control and ICSS groups received a total volume of 2µL of DMSO. Microinfusions were administered into the left LV at a rate of 1µL/min. The injectors were left in place for an additional 60s to allow for the diffusion of the solution away from the tip.

2.9. Intracranial self-stimulation procedure.

Immediately after microinfusion, the ICSS and SB+ICSS rats were placed in the self-stimulation box and received the ICSS treatment, consisting of 2500 trains of stimulation at the OI established during the shaping phase for each rat. Rats in the Control and SB groups underwent sham treatment, by being placed in the self-stimulation box for 45min without receiving any stimulation.

2.10. Tissue collection.

Ninety minutes after the retention test animals received a pentobarbital overdose (150mg/Kg, i.p.) and were transcardially perfused with a solution of 0.1M of

phosphate buffer saline (PBS), pH 7.4, followed by a solution of 4% paraformaldehyde in PBS. Brains were removed and post-fixed in 4% paraformaldehyde in PBS, then cryoprotected in 15% and 30% sucrose in PBS and stored at -80ºC. Localized coronal sections (40µm), between the coordinates -0.6mm and -1.20mm, and -2.28mm and -3.12mm of Bregma, were mounted onto a gelatin-coated slide, stained with cresyl violet and examined for cannula and electrode placement, respectively.

2.11. Statistical analysis

Statistical analysis was performed using R (R Core Team, 2015). Analysis of the acquisition phase of both tasks in the MWM was conducted using a 5×4 mixed ANOVA (SESSION×GROUP). When the effect of the interaction factor was statistically significant, simple effect analysis were performed to explore group differences in each. When the effect of SESSION was statistically significant, polynomial contrasts were applied to explore the presence of linear and/or quadratic trends in performance across sessions. A multiple comparison analysis (Tukey HSD) was performed to assess differences between specific groups across each session. For Experiment 1, the main outcome variable for acquisition sessions in the MWM was the *Escape latency* or time (s) needed to find and climb onto the platform. For Experiment 2, the main outcome variables for acquisition in the SVD were *Escape latency* and the *Number of errors* or the number of contacts with the area associated with the incorrect cue (no escape). The evolution of weight throughout the experiments was also analyzed using a 5×4 mixed ANOVA (SESSION WEIGHT×GROUP). Given that the accuracy and level of performance could change throughout the probe trial in the MWM (Blokland, Geraerts, & Been, 2004), results for the retention test were analyzed with a one-way ANOVA for the totality of the

trial (60s) and also for the first half of the trial (30s). The following retention variables were analyzed in both experiments: (1) *Percentage of time spent in the target quadrant*, (2) *Percentage of time spent in the target annulus*, (3) *Number of target crossings*, (4) *Proximity to target*, and (5) *Whishaw's error* (percentage of time an animal swims inside a virtual 30cm wide corridor from the starting point to the platform); additionally, (6) *Number of errors* was also analyzed in Experiment 2. A one-sample *t*-test against a constant was used for each group to determine whether the *Percentage of time spent in the target quadrant* was different from chance level (25% in Experiment 1, four quadrants; 33% in Experiment 2: target quadrant, error quadrant or the remaining two quadrants). Moreover, the *percentage of time spent near the walls* (measure of thigmotaxis - anxiety), *length* (total distance in cm) and *speed* (motor activity measure) were analyzed and considered as control variables for each group. A Greenhouse-Geisser correction was used when sphericity was violated and a Welch's *F* test and Post Hoc Games-Howell correction was applied when homogeneity was not reached. A Chi-square test for independence was performed to determine the relation between the group and strategy used to find the platform in both memory tasks. For the spatial task, retention test trajectories were categorized into: *Focalized search* (as determined by observation and a minimum 40% of time in the target quadrant), *Non-focalized search* and *Thigmotaxis* (as determined by observation and a minimum 40% of time in the wall zone). For the visual discrimination task, trajectories in the last acquisition trial were categorized into: *Direct*, *Trial and Error*, *Thigmotaxis* and *N/A* (non-applicable: random navigation). The strength of the association was evaluated by means of Cramer's V value, and Post Hoc comparisons adjusted using a Bonferroni correction were used to assess differences in group counts from expected values. The α level for all tests was 0.05.

3. Results

3.2. Experiment 1

One subject was excluded from the analysis due to a misplaced cannula. The final sample consisted of 47 subjects (SB: n=12; Control: n=12; ICSS: n=11; SB+ICSS: n=12). There was no statistical difference between groups in age at the start of the experiment or in weight evolution throughout the experiment (see Table 1). Similarly, the infusion of SB-334867 had no significant effect on the average OI of stimulation or rate of the ICSS treatment between groups (see Table 2).

3.2.1. Acquisition phase

A mixed ANOVA of the escape latencies for the acquisition showed an interaction GROUP×SESSION [Greenhouse-Geisser (ε: 0.790): $F_{9.476,135.829}$=3.376, P=0.001], indicating a difference between the groups depending on the session (Figure 2). Main effects analysis also show significant effects of the factors GROUP [$F_{3,43}$=12.457, P<0.001] and SESSION [$F_{3.159,135.829}$=84.332, P<0.001]. The simple effects analysis detected differences between groups in all sessions except for session 1, previous to receiving any treatment, indicating a similar starting point for all groups [session 1: $F_{3,43}$=0.37, P=0.778; session 2: $F_{3,43}$=3.68, P=0.019; session 3: $F_{3,43}$=5.43, P=0.003; session 4: $F_{3,43}$=10.79, P<0.001; and session 5: $F_{3,43}$=17.35, P<0.001]. A multiple comparison (Tukey HSD) within each session showed that the SB group's latencies were higher than the ICSS group (session 2: P=0.015; session 3: P=0.005; session 4: P<0.001; session 5: P<0.001), the Control group (session 3: P=0.006; session 4: P=0.001; session 5: P<0.001) and the SB+ICSS group (session 4: P<0.001; session 5: P<0.001).

The within group analysis of latencies throughout acquisition sessions showed that although all groups adjust to a significant downward linear function [SB: $F_{1,43}$=12.47, P=0.001; Control: $F_{1,43}$=89.85, P<0.001; ICSS: $F_{1,43}$=95.93, P<0.001; SB+ICSS: $F_{1,43}$=88.13, P<0.001], the SB group's slope was significantly less pronounced when compared to each of the other groups [Control: $F_{1,43}$=16.84, P<0.001; ICSS: $F_{1,43}$=22.15, P<0.001; SB+ICSS: $F_{1,43}$=14.44, P<0.001]. In this sense, the SB group was the only one to show no significant decrease in the latency to target across the first three sessions of acquisition [SB: $F_{1,43}$=0.01, P=0.925; Control: $F_{1,43}$=6.69, P=0.013; ICSS: $F_{1,43}$=19.82, P<0.001; SB+ICSS: $F_{1,43}$=5.71, P=0.021]. In addition, the ICSS group was the only group to also adjust to a quadratic function [$F_{1,43}$=6.12, P=0.018], indicating a sharper decline in the latencies after the first ICSS treatment.

3.2.2. Retention test

All groups performed above chance level (25%) except for the SB group in both the first 30 seconds [SB: t_{11}=1.377, P=0.198; Control: t_{11}=3.822, P=0.002; ICSS: t_{10}=2.968, P=0.016; SB+ICSS: t_{11}= 4.096, P=0.001] and the totality of the trial [SB: t_{11}=0.775, P=0.456; Control: t_{11}=3.607, P=0.004; ICSS: t_{10}=3.113, P=0.012; SB+ICSS: t_{11}=3.107, P=0.009]. Although the GROUP factor did not reach significance for percentage of time in target quadrant [$F_{3,43}$=2.307, P=0.090], a Post Hoc revealed that the SB group performed worse than the other groups (Control: P=0.043; ICSS: P=0.029; SB+ICSS: P=0.038) (Figure 3A).

The proximity to target was significantly different between groups for the first 30s [$F_{3,43}$=2.945, P=0.043] and totality of the trial [$F_{3,43}$=3.249, P=0.031] (Figure 3B). A Post Hoc analysis for first half of the trial showed a shorter distance to target for the ICSS group compared to the Control (P=0.038) and SB (P<0.001) groups. The

SB+ICSS group also achieved a shorter distance than the SB group for both the first half (P=0.009) and the totality of the test (P=0.012).

Differences between groups were also observed for Whishaw's error in the first 30s of the trial [Welch: $F_{3,21.969}$=4.092, P=0.019], for which a Post Hoc showed that ICSS had higher values than the Control group (P=0.003) and the SB group (P=0.006) (Figure 3C).

3.2.3. Swimming trajectories in retention test of spatial memory

The qualitative analysis of the swimming trajectories revealed that rats followed two defined strategies to find the platform, focalized and non-focalized. In addition, a group of animals displayed a thigmotactic behavior. A chi-squared test confirmed that there were differences amongst the 4 groups of subjects ($\chi^2_{6,47}$ = 13.898, P=0.031, ϕc=0.385). A Post Hoc test revealed that the SB group's lack of focalized swimming was significantly different from the expected value (P=0.047). Further analysis showed that the ICSS treatment favored the execution of a focalized strategy ($\chi^2_{2,47}$ = 11.305, P=0.004, ϕc=0.490), regardless of the infusion. Specifically, animals receiving the ICSS treatment applied a focalized strategy at a higher rate than expected (P=0.009), while non-ICSS animals did so at a lower rate than expected (P=0.009). A contingency table (Table 2), displays the counts and percentages for each group and strategy, while Figure 4 depicts images of the strategies used by the animals in experiment 1.

3.3. Experiment 2

The final sample consisted of 28 subjects (SB: n=7; Control: n=7; ICSS: n=7; SB+ICSS: n=7). There was no statistical difference between groups in weight change

across the experimental procedure (Table 1). Furthermore, no differences in rate or OI of ICSS were found among groups (Table 2).

3.3.1. Acquisition phase

A significant effect of interaction GROUP×SESSIONS [$F_{12,96}$=5.622, P<0.001], for the escape latency was observed (Figure 5A). Main effects analysis also show significant effects of the factors GROUP [$F_{3,24}$=13.498, P<0.001] and SESSION [$F_{4,96}$=123.004, P<0.001]. All groups had equal escape latencies in the first session [$F_{3,24}$=0.857, P=0.477], and they started to differ after the first infusion/ICSS session [session 2: $F_{3,24}$=6.656, P=0.002; session 3: $F_{3,24}$=18.610, P<0.001; session 4: $F_{3,24}$=10.315, P<0.001; session 5: $F_{3,24}$=9.464, P<0.001]. A Post Hoc (Tukey HSD) within each session showed that the SB group latencies were higher than ICSS group (session 2: P=0.001; session 3: P<0.001; session 4: P<0.001; session 5: P<0.001), the Control group (session 2: P=0.046; session 3: P=0.009; session 4: P=0.039; session 5: P<0.001) and the SB+ICSS group (session 3: P=0.008; session 4: P=0.044). In addition, latencies for the ICSS group were lower than the Control and SB+ICSS groups in the third (Control: P=0.004; SB+ICSS: P=0.010) and fifth (Control: P=0.034; SB+ICSS: P=0.050) sessions of the training phase.

In relation to the evolution of escape latency, all groups showed a significant decrease across sessions, revealed by a significant downward linear function [SB: $F_{1,24}$=88.86, P<0.001; Control: $F_{1,24}$=170.63, P<0.001; ICSS: $F_{1,24}$=399.74, P<0.001; SB+ICSS: $F_{1,24}$=149.06, P<0.001]. However, only the ICSS group adjusted to a quadratic function [$F_{1,24}$=27.36, P<0.001], showing a sharper decline in the first three sessions compared to the rest of the groups [SB: $F_{1,24}$=0.01, P=0.925; Control: $F_{1,24}$=6.69, P=0.013; ICSS: $F_{1,24}$=19.82, P<0.001; SB+ICSS: $F_{1,24}$=5.71, P=0.021].

Regarding the number of errors, there was no interaction GROUP×SESSIONS [$F_{12,96}$=0.765, P=0.685], but the main effect of GROUP and SESSIONS was shown to be significant [$F_{3,24}$=3.680, P=0.049 and $F_{12,96}$=4.458, P=0.002, respectively] (Figure 5B). Post Hoc analysis did not discriminate differences between groups. Further analysis of the intragroup error evolution across sessions showed that the ICSS animals significantly and consistently committed fewer errors in later sessions compared to the first one (S3: P=0.026, S4: P=0.044, S5: P=0.018), while the Control group only showed differences between the first and fifth sessions (P=0.045) and the SB+ICSS group between the second and fourth sessions (P=0.047). The SB group was the only one that did not reduce the number of errors committed across sessions (S1 vs all sessions: P>0.05).

3.3.2. *Retention test*

The time the animals spent in the target annulus was different between groups in the first 30 seconds [$F_{3,24}$=3.418, P=0.033] and in the totality of the trial [Welch: $F_{3,12.438}$=15.210, P<0.001] (Figure 6A). For the first 30 seconds of the trial, the ICSS group outperformed the rest of the groups (SB: P=0.014; Control: P=0.018; SB+ICSS: P=0.022). ICSS group also outscored the Control and SB groups for the totality of the trial (P=0.017 and P<0.001, respectively), whereas the SB+ICSS group only surpassed the SB group (P=0.045). Group differences were also found for the proximity to target [Welch: $F_{3,11.528}$=32.430, P<0.001] (Figure 6B). A Post Hoc revealed that the ICSS group swam closer to the platform (correct cue) than the Control (P=0.028), while the SB group swam farther from the platform than the rest of the groups (Control: P=0.006; SB+ICSS: P=0.020; ICSS: P<0.001).

Whishaw's error was also different between groups for the first 30 seconds [$F_{3,24}$=6.810, P=0.002] and the totality of the trial [$F_{3,24}$=15.072, P<0.001] (Figure 6C). During the first half of the trial, the ICSS group's navigation was more accurate than the SB+ICSS and SB groups' (P=0.025, P<0.001, respectively), while the SB group performed worse than the Control group (P=0.026). For the totality of the trial, the ICSS group's performance was better than the rest (Control: P<0.001; SB+ICSS: P<0.001; SB: P<0.001). The number of target crossings showed a significant difference between groups [$F_{3,24}$=5.551, P=0.005], where once again the ICSS performed better than the other groups (SB: P=0.007; Control: P=0.023; SB+ICSS: P=0.044) (Figure 6D). Regarding the number of errors, there is a tendency towards a significant difference among groups [$F_{3,24}$=2.081, P=0.064], and the ICSS group committed less errors than the SB group (P=0.045) (Figure 6E). No differences regarding percentage of time spent in the target quadrant were found among groups (Figure 6F). Only the ICSS and Control groups spent enough time in the target quadrant to differ from chance level (ICSS: t_6=4.787, P=0.002; Control: t_6=3.207, P=0.018; SB+ICSS: t_6=1.161, P=0.298; SB: t_6=1.246, P=0.259).

3.3.3. Swimming trajectories in last trial of SVD acquisition session

The qualitative analysis of the swimming trajectories revealed that while some animals displayed a direct strategy, others seemed to have used a trial and error approach to finding the platform. In addition, a group of animals displayed a thigmotactic behavior, and some animals seemed to execute a random search for the platform (labelled as N/A). A chi-squared test revealed no differences amongst the 4 groups ($\chi^2_{9,28}$ = 7.530, P=0.582). A contingency table (Table 2) displays the counts and percentages for each group and strategy.

4. Discussion

4.1. Intracranial self-stimulation facilitates implicit and explicit memory

Results obtained in this report indicate that ICSS is a treatment capable of facilitating both spatial and visual discrimination in the MWM, confirming the general boosting effects of ICSS on different types of memory (Ruiz-Medina et al., 2008; Chamorro-López et al., 2015; García-Brito et al., 2017). The facilitating effects of ICSS on the acquisition phase of the SVD task confirm previous findings (García-Brito et al., 2017), which reported that ICSS-treated animals solve the SVD task faster than the controls. Such a clear difference between groups was not observed for the spatial task. Yet, the ICSS group displayed a rapid decline in latencies of the two sessions following the first administration of the ICSS treatment. This suggests that the facilitative effect of ICSS might consist of the acceleration of learning and, considering that the treatment is administered post-training, the facilitation of the consolidation of its memory. In support of this idea, previous studies have found a similar effect of ICSS on the consolidation of other learning tasks (Redolar-Ripoll et al., 2002).

Moreover, ICSS treatment has consistently improved long-term retention in both tasks, which further indicates a strengthening of the consolidation of both explicit and implicit memory by ICSS. This especially interesting when considering that the outcome regarding comparable criteria between memory tasks was somewhat similar in this study. For example, the value of the variable "proximity to target" was consistently improved by ICSS across both memory tasks. Although this measurement had already been identified as the most sensitive for detecting differences between groups in the probe test of a spatial memory task (Gallagher et al., 1993; Maei et al., 2009; Pereira & Burwell, 2015), this is the first time, to our

knowledge, that this measurement has been assessed and found to be an accurate measurement of memory in an SVD task.

Finally, all groups start out at similar values of escape latencies, and they begin to differentiate after the first administration of ICSS. This indicates that any differences observed are linked to the treatment. Further analysis of control variables rule out that divergence could be related to locomotor activity or anxiety.

4.2. OX1R blockade impairs memory

It is important to note that our experimental procedure included numerous registers designed to control for any side effect that the OX1R blockade may have on the execution of the memory task, especially considering the wide implication of orexins in physiological functions (Li et al., 2014), such as food intake (Sakurai et al., 1998), locomotor activity (Hagan et al., 1999) and reward (Hata et al., 2011; Patyal et al., 2012). Since no side effects were observed, our results suggest that the post-training infusion of SB-334867 into the lateral ventricle resulted in a marked impairment to the acquisition and retention of both the spatial and SVD memory tasks, possibly through interfering in the consolidation of the memory. This outcome corroborates what previous studies have also reported regarding the detrimental effect of OX1R blockade on the performance in explicit (Akbari et al., 2006; Akbari et al., 2007; Yang et al., 2013; Zhao et al., 2014; García-Brito et al., 2018) and implicit (Jaeger et al., 2002; Akbari et al., 2008; Mavanji et al., 2017) memory tasks.

The impaired performance of animals infused with SB-334867 during the acquisition sessions was maintained in the long-term. Specifically, it was reflected in the lack of accuracy and proximity to target during memory retrieval of the SVD task.

As stated above, these variables are especially sensitive to detection of memory deficits in the probe session of a spatial memory task (Maei et al., 2009; Pereira & Burwell, 2015). However, this is the first time, to our knowledge, that proximity to target variable has revealed deficits in an SVD task in the MWM.

Overall, our results corroborate the impairing effects of OX1R blockade on different types of memory. This detrimental effect could take place through several mechanisms, including the regulation of activity in specific memory-related areas as shown by a c-Fos expression reduction in prelimbic and retrosplenial cortices, as well as in some thalamic nuclei and hippocampal areas of spatial-memory-impaired rats after receiving the same OX1R antagonist (García-Brito et al., 2018).

4.3. Effects of OX1R blockade on memory facilitation by ICSS

Our results suggest that the orexinergic system is involved in the facilitating effects of ICSS on memory. Yet, the degree to which blocking OX1R interrupts memory facilitation by ICSS seems to differ between the spatial and SVD tasks. Despite obtaining similar results in both tasks, OX1R blockade appears to partially negate the facilitating effect of ICSS on spatial memory, whereas it hinders ICSS facilitation of the SVD task. Given that the infusion of the OX1R antagonist took place after each acquisition session, and before the administration of ICSS, it could be suggested that the blockade interferes with ICSS's ability to boost consolidation of the SVD, but not the spatial version of the task. Previous studies have reported that ICSS facilitates the consolidation of spatial memory (Chamorro-Lopez et al., 2015) and emotional memory (Ruiz-Medina et al., 2008). In addition, there is evidence of impairment of spatial memory consolidation after hippocampal administration of SB-334867 (Akbari et al., 2006; 2007) and of fear memory consolidation in $OX1R^{-/-}$ mice

(Soya et al., 2013). Although OX1R blockade does not seem to impair ICSS's boosting effect on consolidation in the spatial task in our study, this could be due to the higher level of training, which has been shown to improve spatial memory in SB-334867 infused rats (García-Brito et al., 2018).

In addition, the differential effects on ICSS facilitation of two types of memory is especially noticeable when we look at specific variables analyzed for both paradigms in the retention test. The analysis of Whishaw's error or navigation accuracy, which assesses the precision of animals in their approach to the goal (Whishaw, 1995), reveals that despite the blockade of OX1R, the facilitative effect of ICSS can be maintained in the spatial memory task, but not in the SVD task.

Furthermore, the study of the swimming trajectories supports our findings regarding the spatial task. ICSS treatment promotes a focalized swimming strategy regardless of the infused substance. Thus, OX1R blockade does not prevent animals from adopting this strategy which is known to be the most adequate strategy to solve this task (Rogers et al., 2017). Conversely, no such conclusive results were obtained from the trajectory study in the SVD task. Nevertheless, the prevalence of SB+ICSS subjects implementing a direct strategy is exactly the same as the controls. In addition, this strategy was followed by 57.1% of ICSS-treated rats, while 0% of the SB infused rats displayed such trajectory. Consistently, other studies suggest that ICSS is not only capable of improving latencies in this task but also promotes a direct swim toward the correct cue (García-Brito et al., 2017). The lack of significant effects of either the SB-334867 infusion or ICSS on the swimming strategies in Experiment 2 could lie on the size of the sample, as well as the number of compared categories.

A valuable consideration regarding the differential effects of OX1R blockade on the facilitating effects of ICSS lies in the importance of object saliency for each of the tasks. Orexins contribute to the perception of relevant and salient contextual cues (Mileykovskiy et al., 2005, Petrovich et al., 2012), which indicates that they play a big part in the control of the selective attention needed to perform simultaneous discrimination between visual stimuli (Teng et al., 2015). Perhaps the facilitating effect of ICSS on an SVD task is dependent on the detection of salient individual stimuli, while a greater reliance on richer and more complex configurations may be required in spatial tasks (Lopez et al., 2008). Thus, the richness of the context could help compensate for the loss of orexinergic function and still allow ICSS to improve spatial learning and memory.

As we explored the involvement of the orexinergic system in the facilitation of memory by ICSS, it is important to note that we did not find that ICSS threshold was affected by the blockade of OX1R. This suggests that motivational states, which could play a part in memory processing (Kennedy & Shapiro, 2009), were not affected by the SB-334867 infusion. Orexin-A has been said to play a complex role in reward and other motivational processes (Mahler et al., 2014), but evidence of its specific role is very sparse and sometimes contradictory; for example, intra-VTA and icv administration of orexin-A have increased ICSS threshold (Boutrel et al., 2005; Hata et al., 2011), while intra-insular and intraperitoneal administrations have not (Hollander, et al., 2008; Riday et al., 2012).

Despite the more profound affectation of discrimination learning by OXR1 blockade, the ICSS treatment was still capable of compensating deficits in the SB+ICSS group to the point at which their performance was equal to the Controls'.

The capability of ICSS to recover loss of memory function has already been reported in other implicit memory task. Specifically, ICSS has been shown to ameliorate active avoidance memory impairments due to localized lesions in the parafascicular nucleus of the thalamus (Redolar-Ripoll et al., 2003) or amygdala (Segura-Torres et al., 2010; Kádár et al., 2014), as well as normal aging (Aldavert-Vera et al., 1997). However, this is the first time, to our knowledge, that ICSS has been shown to be capable of recovering the loss of visual discriminative memory in animals infused with SB-334867. The rescue of memory by ICSS in animals with OX1R blockade is especially relevant considering that Alzheimer's disease (AD) patients suffer from significant losses of orexin-A neurons (Fronczek et al., 2012) and low levels of orexin-A in cerebrospinal fluid (Slats et al., 2012). Moreover, although explicit memory deficits are broadly accepted to be part of early onset AD, damage to areas related to perceptual learning only becomes evident in the later stages (Manzanero, 2007). More specifically, AD patients show deficiencies in visuo-perceptive priming (Boccia et al., 2014) as well as visual discrimination (Harnish et al., 2010). Therefore, the MFB-ICSS treatment could compensate for some of the memory detriment patients may suffer, of both implicit and explicit learning and memory, as a result of a dysfunction in the orexinergic system, found in AD, among others.

The study of the mechanisms for ICSS memory facilitation is a highly complex one, particularly due to the variety of neurochemical systems and functional connections between areas involved in memory processing which are also affected by ICSS. In order to further explore the role of the orexinergic system in the facilitating effect of ICSS on different types of memory, future studies should evaluate how ICSS impacts the function of the orexinergic system, by examining whether or not the expression of OX1R is altered by ICSS. Overall, our results suggest that ICSS

facilitation of memory is mediated by multiple neurochemical systems, including the orexinergic system. This could help explain the impressive capacity of ICSS to compensate for memory deficits, such as those caused by cerebral lesions or the blockade of specific receptors.

Conflicts of interest

None of the authors have conflicts of interest.

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FIGURE CAPTIONS AND TABLE HEADERS

Figure 1. Histological evaluation of the implantation of cannula into the left LV and electrode into the right LV of the rat. Figure 1a depicts placement of the cannula according to anterior and lateral coordinates in all groups, in experiment 1 and experiment 2. The removal of one of the subjects in experiment 1 resulted from the misplacement of cannula be seen in coordinate -0.84. Figure 1b depicts placement of the electrode according to anterior, lateral and ventral coordinates in subjects from self-stimulation groups (ICSS and SB+ICSS) in experiment 1 and experiment 2. Group legends: ICSS (diamond), SB+ICSS (circle), Control (square) and SB (triangle). Abbreviations: LV, lateral ventricle; cc, corpus callosum; mfb-LH, medial forebrain bundle in the lateral hypothalamus.

Figure 2. Effects of ICSS and SB-334867 on the acquisition of spatial memory in the MWM. Mean *Escape latencies* (±SE) for the five sessions of the training phase. Arrows show the start of daily post-training ICSS and SB-334867 microinfusions. Factor interaction significance is depicted as #P<0.05. Significant differences between groups in simple effects analysis are shown as *P<0.05, **P<0.01, ***P<0.001.

Figure 3. Effects of ICSS and SB-334867 on the retention of spatial memory in the MWM. Retention test variables (means+SE): (A) Percentage of time spent in the target quadrant in the fist 30 s and totality of the test, (B) Proximity to target in the fist 30 s ad totality of the test, (C) Whishaw's error in the first 30 s and totality of the test. Significant differences in group contrasts are shown with *P<0.05, **P<0.01. Dotted line in (A) represents chance level (25%) and significant differences for each group and chance level are depicted with #P<0.05, ##P<0.01.

Figure 4. Swimming trajectories of all subjects in retention test. Platform is located in the lower-right (southeast) quadrant of the circular tank. Swimming trajectories of the subjects are separated by group.

Figure 5. Effects of ICSS and SB-334867 on the acquisition of an SVD task in the MWM. (A) Mean *Escape latencies* (±SE) for the five sessions of the training phase. (B) Mean *Number of errors* (±SE) committed during the five acquisition sessions by each group. Arrows show the start of daily post-training ICSS and SB-334867 microinfusions. Factor interaction significance is depicted as #P<0.05, ###P<0.001. Significant differences between groups in simple effects analysis are shown as **P<0.01, ***P<0.001.

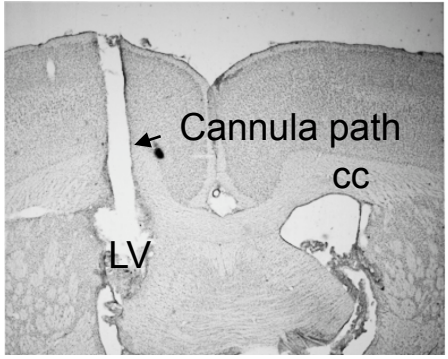
Figure 6. Effects of ICSS and SB-334867 on the retention of an SVD task in the MWM. Retention test variables (means+SE): (A) Percentage of time spent in the target annulus in the fist 30 s and totality of the test, (B) Proximity to target in the fist 30 s ad totality of the test, (C) Whishaw's error in the first 30 s and totality of the test. (D) Target crossings, (E) Number of errors committed by each group, (F) Percentage of time spent in the target quadrant in the fist 30 s and totality of the test; no differences between groups were found for this variable. Significant differences in group contrasts are shown with *P<0.05, **P<0.01, ***P<0.001. Dotted line in (F) represents chance level (33%) and significant differences from chance level for each group are depicted with #P<0.05, ##P<0.01.

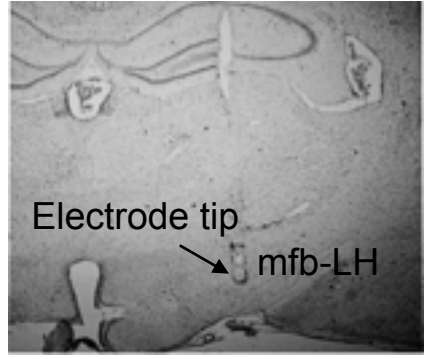
Table 1. Weight throughout the SB-334867 administration phase. Means (±SD) of weights (in grams) by group.

Table 2. ICSS parameters. Means (±SE) of optimal intensity (µA) and duration of treatment (min:sec).

Table 3. Percentages of subjects adopting different swimming strategies for each group in the retention test. Significant differences in the frequency of subjects in the SB group adopting a non-focalized swimming strategy, *P<0,05, and the frequency of ICSS-treated animals adopting a focalized strategy, $^{\#\#}$P<0.01 in experiment 1. No differences were found in the swimming strategy of subjects in experiment 2. Frequency of subjects is shown in brackets next to percentages. N/A: non-applicable.

Figure 1





Cannula Placement

Experiment 1 | Experiment 2

-0.60

-0.72

-0.84

Electrode Placement

Experiment 1 | Experiment 2

-2.40

-2.64

Figure 2

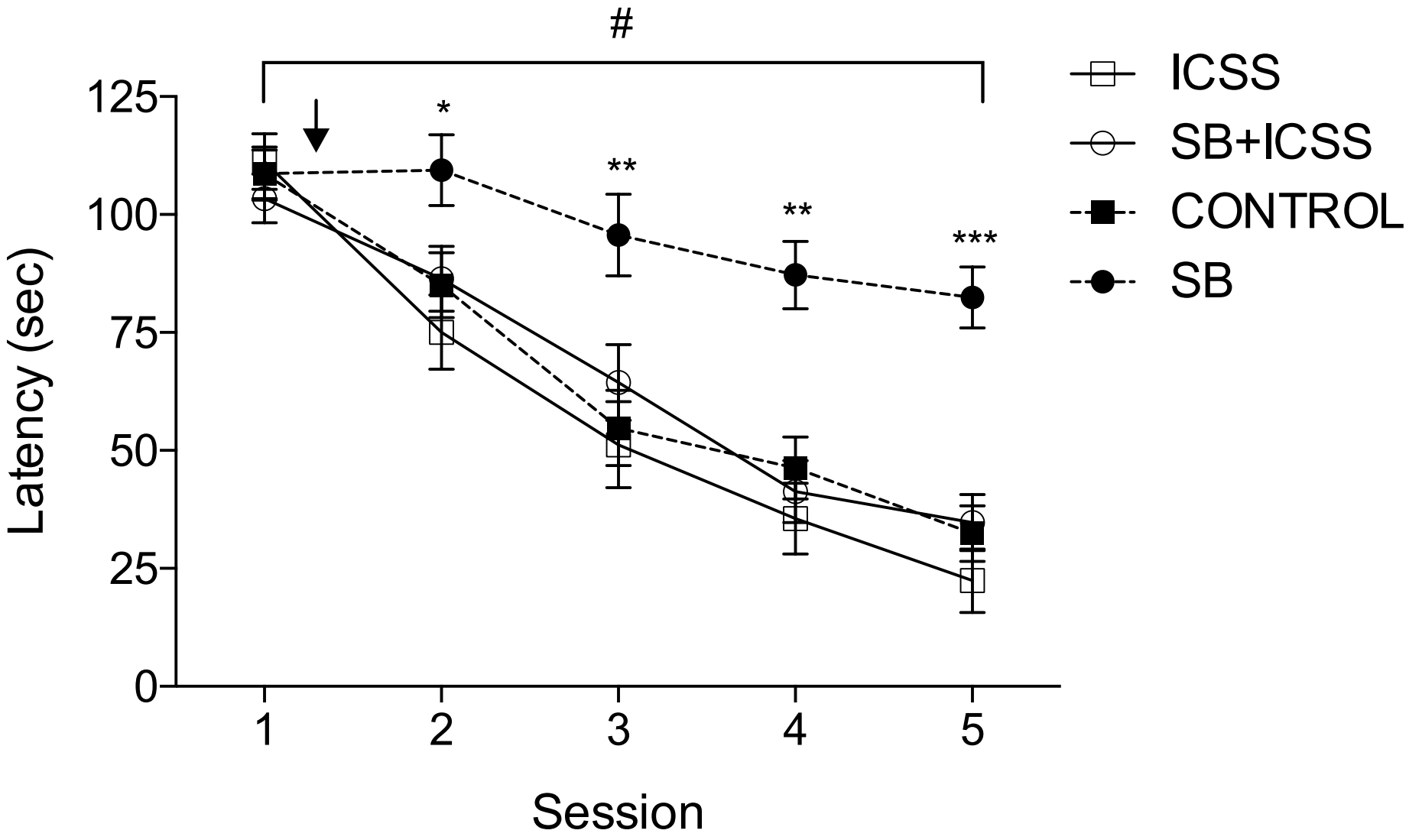

#
*
**
**
***
ICSS
SB+ICSS
CONTROL
SB
Latency (sec)
125
100
75
50
25
0
1
2
3
4
5
Session

Figure 3

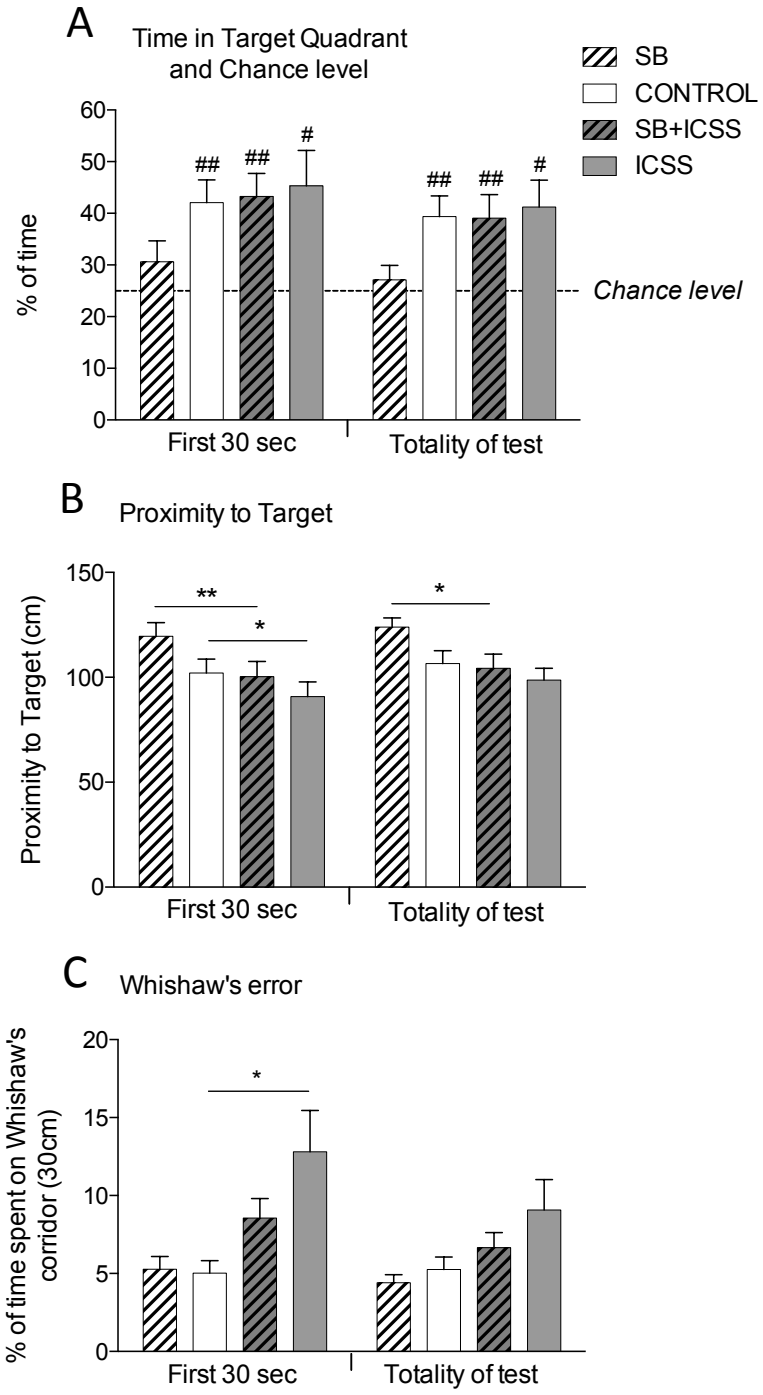

A
Time in Target Quadrant
and Chance level
SB
CONTROL
SB+ICSS
ICSS
% of time
60
50
40
30
20
10
0
##
##
#
##
##
#
Chance level
First 30 sec
Totality of test
B
Proximity to Target
Proximity to Target (cm)
150
100
50
0
**
*
*
First 30 sec
Totality of test
C
Whishaw's error
% of time spent on Whishaw's corridor (30cm)
20
15
10
5
0
*
First 30 sec
Totality of test

Figure 4

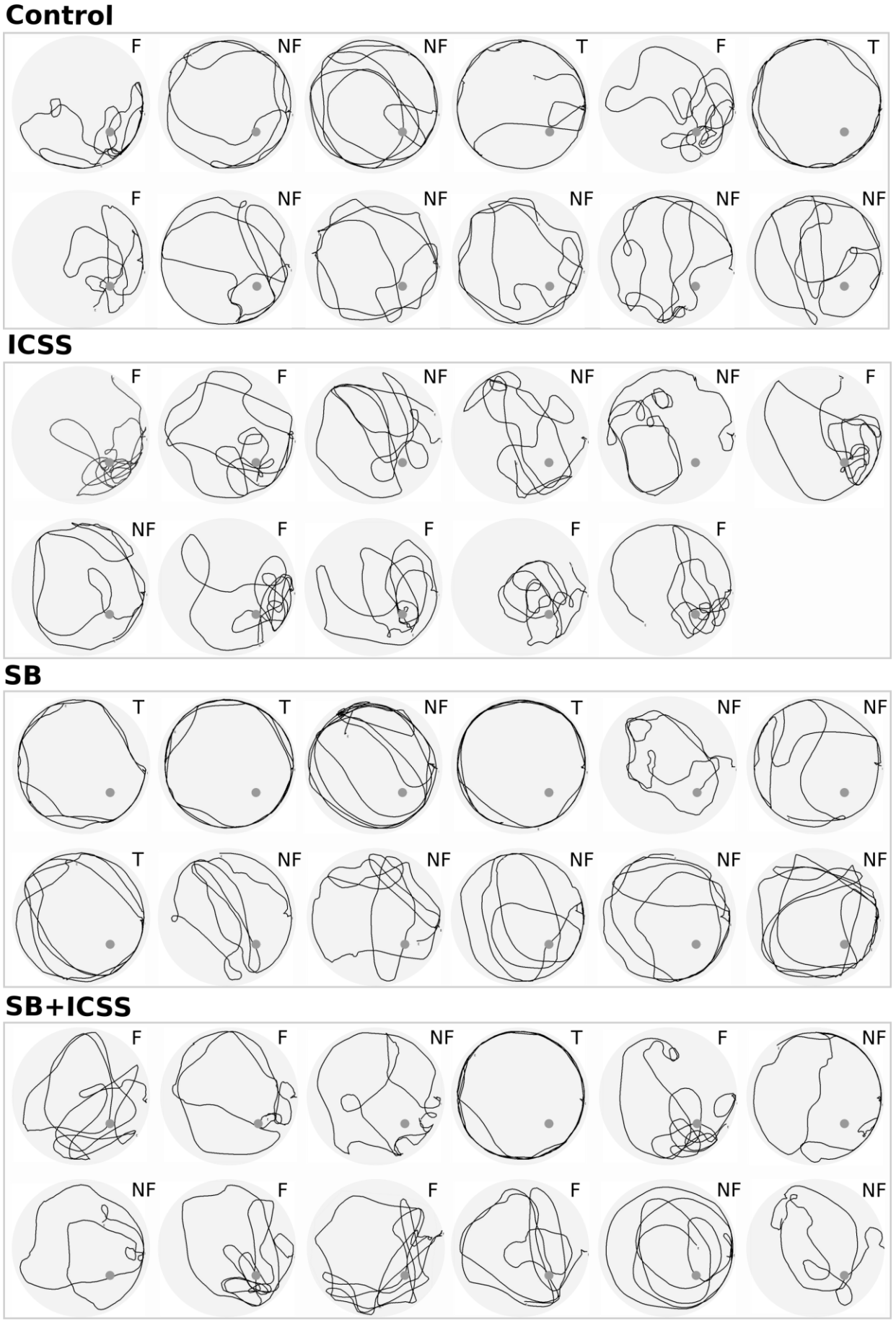

Control
F
NF
NF
T
F
T
F
NF
NF
NF
NF
NF
ICSS
F
F
NF
NF
NF
F
NF
F
F
F
F
SB
T
T
NF
T
NF
NF
T
NF
NF
NF
NF
NF
SB+ICSS
F
F
NF
T
F
NF
NF
F
F
F
NF
NF

Figure 5

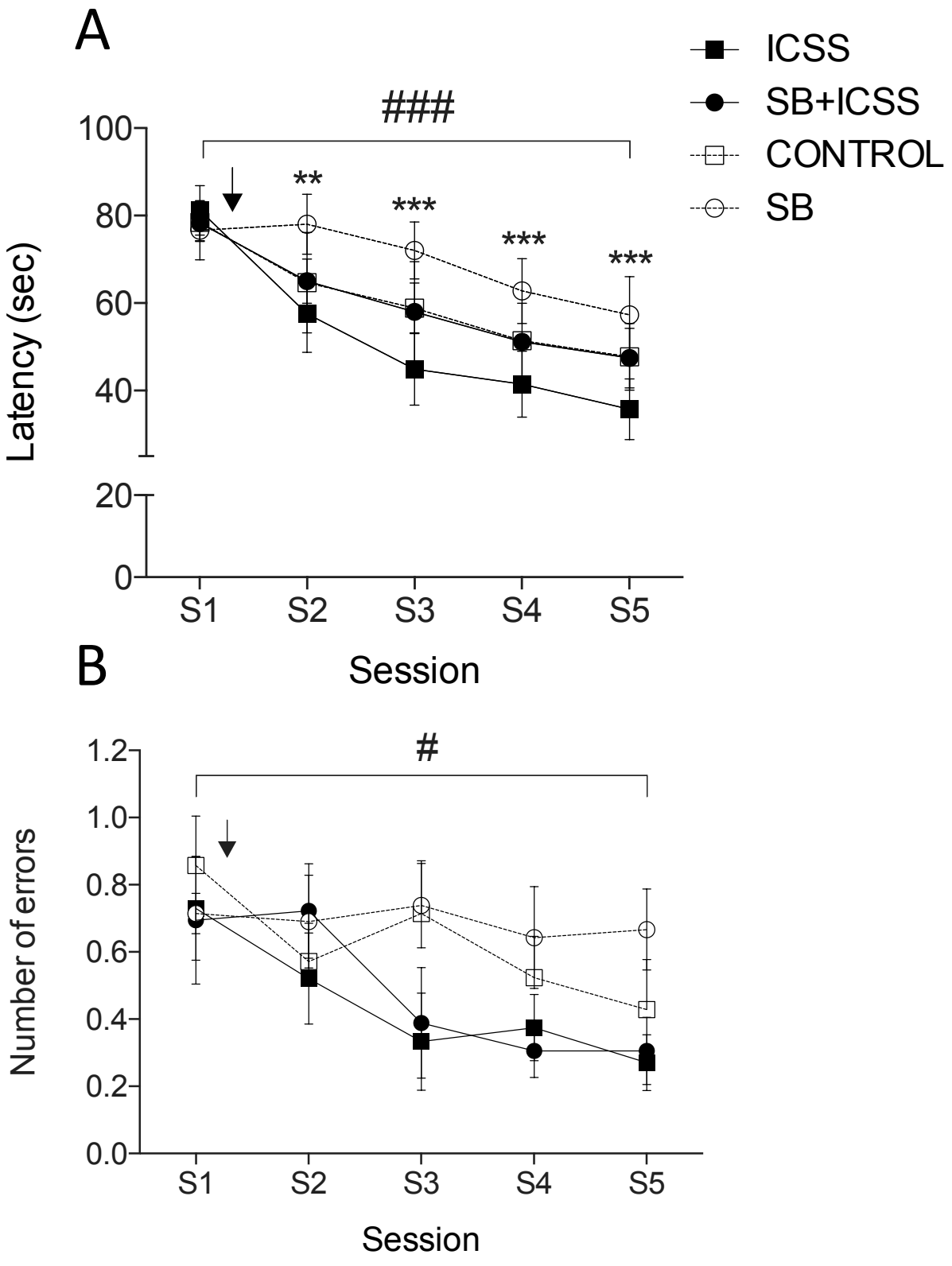

A
ICSS
SB+ICSS
CONTROL
SB
###
**
***
***
***
100
80
60
40
20
0
Latency (sec)
S1
S2
S3
S4
S5
Session
B
#
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Number of errors
S1
S2
S3
S4
S5
Session

Figure 6

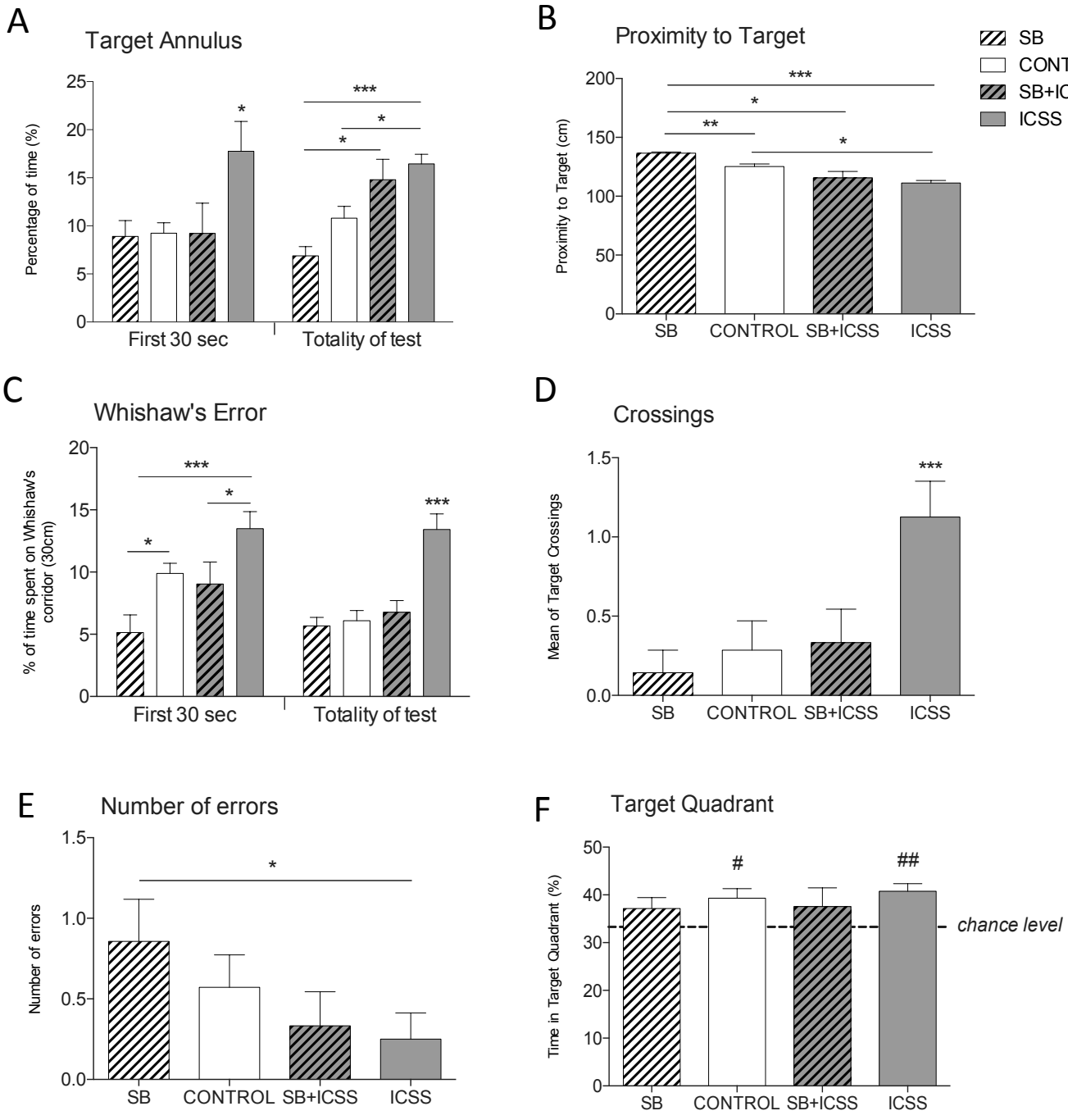

A
Target Annulus
Percentage of time (%)
25
20
15
10
5
0
*
*
***
*
First 30 sec
Totality of test
B
Proximity to Target
Proximity to Target (cm)
200
150
100
50
0
***
*
**
*
SB
CONTROL
SB+ICSS
ICSS
C
Whishaw's Error
% of time spent on Whishaw's corridor (30cm)
20
15
10
5
0
***
*
*
***
First 30 sec
Totality of test
D
Crossings
Mean of Target Crossings
1.5
1.0
0.5
0.0
***
SB
CONTROL
SB+ICSS
ICSS
E
Number of errors
Number of errors
1.5
1.0
0.5
0.0
*
SB
CONTROL
SB+ICSS
ICSS
F
Target Quadrant
Time in Target Quadrant (%)
50
40
30
20
10
0
#
##
chance level
SB
CONTROL
SB+ICSS
ICSS

Table 1

		BODY WEIGHT			
	SESSION	Control (12)	ICSS (12)	SB (12)	SB+ICSS (12)
EXP1	1	391.12±47.23	412.52±27.65	418.59±51.06	414.73±38.82
	2	384.72±49.01	405.09±27.47	402.46±45.64	396.43±40.22
	3	386.02±47.34	406.28±26.63	407.47±44.81	398.46±35.30
	4	386.69±46.59	406.36±27.99	408.70±45.26	400.40±32.46
	5	388.14±48.38	406.19±28.50	408.58±43.94	395.16±41.58
		Control (7)	ICSS (7)	SB (7)	SB+ICSS (7)
EXP2	1	387.08±44.14	421.63±54.12	395.97±15.69	416.81±71.54
	2	379.74±43.45	404.86±52.83	389.72±12.09	407.80±69.53
	3	379.57±47.39	402.51±50.25	388.24±12.27	400.96±71.56
	4	395.75±47.30	406.77±51.46	389.38±12.00	404.31±71.70
	5	395.15±47.11	408.88±50.08	389.92±10.37	405.58±69.10

Table 2

	EXPERIMENT 1		EXPERIMENT 2	
	ICSS (N=11)	SB+ICSS (N=12)	ICSS (N=7)	SB+ICSS (N=7)
Optimal intensity	69.38µA (±8.63)	75.75µA (±13.48)	71.25µA (±9.54)	72.50µA (±11.726)
Duration of treatment	51:00 (±11:22)	48:30 (±05:13)	55:00 (±11:01)	58:20 (±09:18)

Table 3

		SWIMMING TRAJECTORIES			
		Control	*ICSS*	*SB*	*SB+ICSS*
EXP1	Focalized	25% (3)	63.64% (7)$^{\#\#}$	0% (0)	50% (6)$^{\#\#}$
	Non-focalized	58.33% (7)	36.36% (4)	66.67% (8)*	41.67% (5)
	Thigmotaxis	16.67% (2)	0% (0)	33.33% (4)	8.33% (1)
	TOTAL	**100% (12)**	**100% (11)**	**100% (12)**	**100% (12)**
EXP2	Direct	28.57% (2)	57.14% (4)	0% (0)	28.57% (2)
	Trial and Error	28.57% (2)	28.57% (2)	42.86% (3)	42.86% (3)
	Thigmotaxis	14.28% (1)	0% (0)	14.28% (1)	28.57% (2)
	N/A	28.57% (2)	14.28% (1)	42.86% (3)	0% (0)
	TOTAL	**≈100% (7)**	**≈100% (7)**	**100% (7)**	**100% (7)**