TITLE:

INTRACRANIAL SELF-STIMULATION FACILITATES MEMORY CONSOLIDATION, BUT NOT RETRIEVAL: ITS EFFECTS ARE MORE EFFECTIVE THAN INCREASED TRAINING.

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ABSTRACT

To evaluate possible differential effects of lateral hypothalamic intracranial self-stimulation (ICSS) on memory consolidation and retrieval, independent groups of Wistar rats were trained in a single session of two-way active avoidance task (acquisition session) and tested 24 hours later (retention session). The Post-ICSS groups received an ICSS treatment immediately after the acquisition session, and the Pre-ICSS groups received the same treatment immediately before the retention session. Because the ICSS effects on memory seem to be dependant on the initial performance level shown by the subjects, the possible influence of initial training (number of trials) on ICSS effects was also studied. Therefore, we used different control and experimental groups which received either 30 or 50 trials in the acquisition session. Post-training ICSS facilitated the 24-hr retention in both training conditions (30 and 50 trials). In contrast, pre-retention ICSS treatment did not facilitate performance in the retention test. We also observed that post-training ICSS was more effective for improving the 24-hr retention than increasing the initial training from 30 to 50 trials. This findings confirm that ICSS treatment improves memory consolidation and suggest that it might not affect memory retrieval mechanisms.

INTRODUCTION

Brain rewarding stimulation is a reliable way of facilitating learning and memory processes. Evidence from several studies indicates that post-training intracranial self-stimulation at the lateral hypothalamus (ICSS) can improve learning in a wide variety of paradigms (aversive and appetitive classical conditioning [7, 8]; sensory preconditioning [9]; appetitive and aversive operant conditioning [12, 13, 14, 15]). Experiments from our laboratory have verified that post-training ICSS enhances the acquisition of two-way active avoidance conditioning in a distributed paradigm, in both adult [25, 26] and old rats [1]. Improving effects after post-training ICSS have also been observed on the retention of the same task. In this study, ICSS treatment facilitated the 24-hr retention of conditioning in such a way that the treated rats reached the same level of performance than that achieved by controls (without ICSS) after a period of 7 days of memory consolidation. This effect was observed mainly in the subjects with an initial low level of performance [2]. These results suggest that post-training ICSS may accelerate memory consolidation.

Some experimental data support the hypothesis that the improving effect of ICSS on learning could be due to the artificial increase of a general central nervous system activation during the critical period of information processing [11]: (1) cortical and subcortical desynchronization (arousal) are neural correlates of the reinforcing effect underlying ICSS responses [20], (2) there is a linear and positive relation between the number of ICSS trains administered and the level of the learning improvement produced by ICSS [25], (3) there are evidences supporting the view that specific hormonal and brain systems activated by emotional or non emotional arousal regulate long-term memory storage [3, 4, 5, 6, 18, 19],

and (4) the reinforcing component of brain stimulation is not necessary for ICSS to facilitate learning, since post-trial LH stimulation at sub-threshold intensities, which do not produce ICSS behaviour, is also able to improve long-term memory formation [11].

Memory is an active and complex process that covers different stages such as acquisition, consolidation or retrieval. Acquisition of information through the senses is necessarily the first step, which is followed by consolidation, an active process for storing the information just acquired. The retrieval could reactive these stored memories which then can be used to guide behaviour [27]. It has been demonstrated that the facilitative effects of several activatory treatments on different learning and memory stages depend on the time of administration with regard to training. Up to now, in most experiments that have studied the effects of ICSS on specific conditioning tasks, the ICSS treatment has been administered post-training, and its modulatory effect on retention has been said to affect consolidation. Only in a previous work, the effects of ICSS on acquisition were studied, showing that ICSS, administered immediately before each of the training sessions in a distributed paradigm (one daily session for 5 consecutive days), improved two-way active avoidance [25]. However, the features of a distributed paradigm make it methodologically difficult to discern if this facilitative effect is related to the acquisition process, the ongoing consolidation or the retrieval of stored information. Furthermore, ICSS has also been shown to be effective to facilitate several learning tasks performed later (between 2 and 6 weeks), in both neonatal [28, 29] and adult rats [30]. However, none of these previous experiments have applied the ICSS treatment immediately before the retention session, testing its direct effect on retrieval. The present experiment was aimed at evaluating the possible differential effects of ICSS on consolidation (immediately post-training ICSS treatment) and retrieval (immediately

pre-retention ICSS treatment) of a single session of two way active avoidance (massed training). Because previous experiments from our laboratory have also verified that the facilitatory effects of ICSS are stronger in those subjects with a low level of conditioning and weaker in those with a high level of conditioning [2], the subjects of the present experiment were also submitted to either 30 or 50 trials in order to evaluate the influence of the initial level of training on the ICSS effect.

MATERIALS AND METHODS

Subjects

Eighty naive male Wistar rats, obtained from our laboratory breeding stock, with a mean age of 94.08 days (S.D.= 3.40) at the beginning of the experiment and mean weight of 482.69 g (S.D.= 47.41) at the time of surgery, were used. All rats were singly housed, always kept under conditions of controlled temperature (20-24 ºC) and humidity (40-70%), and subjected to an artificial light/darkness cycle of 12/12 h (lights on at 08:00h). Food and water were available *ad libitum*. The rats were tested during the first half of the light cycle. The experiments were carried out in compliance with the European Community Council Directive for care and use of laboratory animals (CEE 86/609) and the *Generalitat de Catalunya* Decret (DOGC 2073 10/7/1995, DARP protocol number 1221).

Stereotaxic Surgery

Under general anaesthesia (110 mg/Kg Ketolar® *Ketamine chlorhydrate* and 0.09ml/100g Rompun® *Xylazin 23 mg/ml*), all rats were implanted with a monopolar stainless steel electrode (150 μm in diameter) aimed at the LH, into the fibers of the MFB, with the incisor bar set at -2.7 mm below the interaural line and according to coordinates from the stereotaxic atlas of Paxinos and Watson [21]: AP= -2.3 mm from bregma, L= 1.8 mm (right hemisphere) and P= -8.8 mm, with the cranium surface as dorsal reference. ICSS electrodes were anchored to the skull with jeweller's screws and dental cement.

Procedure

Before surgery, the animals were given one-daily handling sessions on three consecutive days. In each of those sessions, the rats were weighed and manipulated for about 10 min. Once the rats had recovered from surgery (9 days), they were randomly distributed into the following four groups: Post-ICSS, Pre-ICSS, Control-ICSS and Control-SHAM. Rats in the Post-ICSS, Pre-ICSS and Control-ICSS groups were taught to self-stimulate by pressing a lever in a conventional Skinner box (25 x 20 x 20-cm) constructed of Plexiglas. Electrical brain stimulation consisted of 0.3-s trains of 50 Hz sinusoidal waves at intensities ranging from 10 and 250 μA. The ICSS behaviour was shaped for each subject to establish the range of current intensities that would support responding on a continuous reinforcement schedule. On three consecutive days, the animals were trained in ICSS to establish the individual optimum current intensity of ICSS (as described in [25]). The mean of the two current intensities that gave rise to the highest response rate in each of the last two days was considered as the optimum intensity (OI) of ICSS for each rat.

Before the conditioning phase, each group was randomly distributed into two subgroups according to the independent variable *number of trials* (30 or 50), resulting in the following final eight experimental groups (n=10/group): Post-ICSS-30, Post-ICSS-50, Pre-ICSS-30, Pre-ICSS-50, Control-ICSS-30, Control-ICSS-50, Control-SHAM-30 and Control-SHAM-50. Then, all the rats were trained in a massed session (30 or 50 trials) of a two-way active avoidance task (acquisition session). Active avoidance testing was conducted in a 50 x 24 x 23-cm two-way automated shuttle-box (Letica LI-916), enclosed in a sound-attenuating box ventilated by an extractor fan. The conditioned stimulus (CS) was a 60-dB and 1-kHz tone of 3s duration. The unconditioned stimulus (US) was a 0.5-mA

electrical footshock, presented for 30 s at maximum. The trials followed a variable interval schedule of 1 min (+/- 10 s). Just before this acquisition session, the rats were submitted to one adaptation session (10 min) consisting of free ambulation in the shuttle-box so as to familiriarize them with the learning environment. Besides the number of avoidance responses (considered as the level of performance of the task), intertrial crossings and crossings during the habituation session (considered as an index of locomotor activity) were also scored. Immediately after this training session, Post-ICSS (30 and 50 trials) rats were placed in the ICSS box and received an ICSS treatment session (2500 trains at the 100 % of their IO).

To test the retention level of the learned response, all the animals received an additional avoidance session identical to the acquisition one (10 min of free ambulation in the shuttle-box followed by either 30 or 50 trials), 24 hours after the acquisition session. Immediately before this retention session, Pre-ICSS (30 and 50 trials) rats were placed in the ICSS box and received an ICSS treatment session (2500 trains at the 100 % of their IO). Rats in Control-ICSS (30 and 50 trials) and Control-SHAM (30 and 50 trials) groups did not receive ICSS treatment either before nor after training sessions, but were placed in the ICSS box for 40 min.

Histology

At the end of the experiment, histological analyses were performed to verify the location of the electrode tip. The animals were anaesthetized with an overdose of sodium pentobarbital (150 mg/Kg, i.p.) and transcardially perfused with 0.9% physiological saline followed by 10% formalin (water and 37-40% formaldehide). The brains were removed and placed in a 30% sucrose solution before being cut into 40 μm sections on a freezing stage

microtome (Cryocut 1800 with microtome 2020, JUNG). The tissue sections were stained with cresyl violet and examined for electrode tip placement under a negatoscopy and an amplifier (SONY SSC-C35OP model). Electrode track locations were reconstructed on standardized sections of the rat brain from the atlas of Paxinos and Watson [21].

Data analyses

To process the statistical data, statistical computer package program SPSS 10 was used. The main analyses were carried out considering the independent variables as qualitative (*treatment*: four categories; *trials*: two categories) and the dependent variables as quantitative (performance in acquisition and retention sessions, ICSS parameters, and locomotor activity). The two independent variables were mixed to obtain eight experimental groups. Thus, mixed analyses of variance were performed with their corresponding contrast analyses ('simple' or 'special' for the between-group effect, and 'polynomial' for the within-groups effect). A survival analyses was also made to analyse and compare the mean number of trials required by each experimental group to reach a preestablished learning criterion (five consecutive avoidance responses in retention session).

RESULTS

The final sample consisted of seventy-eight rats (two rats were eliminated because of technical problems in the shuttle-box, one in Control-ICSS-50 group and another in Control-SHAM-50 group). There were no statistical differences between groups in the evolution of body weight along the experiment according to a multivariate analysis of variance.

Two-way Active Avoidance Conditioning

Acquisition: As expected, an analysis of variance for the entire sample did not show significant differences among groups, either in the 30-trial (Fig.1a) or in the 50-trial (Fig.2a) conditions, in the total number of avoidance responses on the acquisition session. All the groups, in both conditions, reached the same learning level on the acquisition session, a critical condition to study the effects of the ICSS treatment on retention.

In order to study more accurately the evolution of conditioning throughout the acquisition session and to compare 30-trial with 50-trial conditions, the acquisition session was subdivided into blocks of ten trials each (either three or five blocks, depending on the total number of trials). Thus, the study of learning evolution pointed out that in the 30-trial-condition (Fig.1b) all groups showed a significant upward linear tendency [*polynomial contrast* (1^{st} degree): $F(1,72)=86.01$, $p<0.0005$], whereas in the 50-trial-condition (Fig.2b) all the groups also showed a significant upward linear tendency but with an inflection [*polynomial contrast* (1^{st} degree): $F(1,136)= 76.89$, $p<0.0005$, (2^{nd} degree): $F(1,136)= 53.72$, $p<0.0005$]. This shape, in the 50-trial-condition, can be explained by the significant increase observed in

the number of avoidances from the 1st to the 3rd block [F(2,68)= 57.9, p<0.0005], similar to that observed in the 30-trial-condition, and by a stabilization of performance on the last two blocks. In consequence, there were not significant differences between the groups submitted to 30 trials and those subjected to 50 trials in the number of avoidance responses performed on the last block of trials. Thus, contrary to what it was expected, the number of trials did not seem to affect the final acquisition performance level of the subjects.

Retention. In general, and as it can be observed in Fig.1 and Fig. 2, the ICSS treatment facilitated the 24-hr retention of avoidance conditioning in both 30- and 50-trial conditions, but only when it was administered after the acquisition session (Post-ICSS groups), and not when it was administered pre-retention (Pre-ICSS groups). These results were confirmed with a MANOVA, where the *group by session* interaction factor showed a tendency towards statistical significance for the 30-trial condition [F(3,36)= 2.4, p=0.08] or was statistically significant for the 50-trial condition [F(3,34)= 4.47, p=0.01]. Thus, contrast analyses showed that the total increase in the number of avoidances from the acquisition to the retention session (Fig.1a and Fig.2a) was higher in Post-ICSS groups compared to both, Control-ICSS and Control-SHAM groups, in both training conditions [*30-trial-condition*: F(1,36)= 3.9, p<0.0005; F(1,36)= 6.6, p=0.01; and *50-trial-condition*: F(1,34)= 12.43, p=0.0015; F(1,34)=4.7, p=0.03, respectively]. In addition, significant differences among groups in the total number of avoidances performed in the retention session were found. Specifically, Post-ICSS group made a significant higher number of avoidances than control groups in both 30-trial and 50-trial conditions [*special contrast Post-ICSS vs. Control-ICSS-Control-SHAM; 30-trial condition*: F(1,36)= 6.91, p=0.012; *50-trial condition*: F(1,34)= 11.01, p=0.002].

The study of the evolution of the performance in the retention session showed that in

the 30-trial-condition the facilitatory effects of Post-ICSS were maintained throughout all the retention session. All the groups showed parallel evolutions, but the Post-ICSS group showed a higher retention level than both control groups in all the three retention blocks *[special contrast Post-ICSS vs. Control-ICSS-Control-SHAM*: $F(1,36)=5$, $p=0.03$]. The performance evolutions showed a significant upward linear tendency with an inflection in all the experimental groups [*polynomial contrast* (1^{st} degree): $F(1,72)= 66$, $p<0.0005$, (2^{nd} degree): $F(1,72)= 3.4$, $p=0.0014$]. Thus, the within-groups MANOVA pointed out that, in all the groups, the more important improvement in performance in the 30-trial-condition retention session were observed between the first two blocks [$F(1,36)=36.8$, $p<0.0005$], and that there was a performance stabilization between the last two blocks.

In the 50-trial-condition, the facilitatory effect was not maintained over the session (*block by group factor*: $F(12,136)=3.11$, $p=0.001$]. The analyses of simple effects pointed out significant differences among groups on the first block [$F(3,34)= 5.31$, $p=0.004$] and a tendency towards statistical significance on the second block [$F(3,34)= 2.58$, $p=0.07$]. Thus, the contrast analyses showed that the retention level was higher in Post-ICSS group than in Control-ICSS and Control-SHAM groups only on the first two blocks or only in the first block of trials, respectively *[Control-ICSS*: $F(1,34)=12.07$, $p=0.001$; $F(1,34)=6.07$, $p=0.018$; *Control-SHAM*: $F(1,34)= 11.36$, $p=0.01$], but no differences were found among groups in the last three blocks. This result can be explained by the fact that, while the Post-ICSS group maintained the performance level on the five blocks (the treatment caused an asymptotic level of performance from the first trials of the session), evolution of learning in the control groups showed a significant upward linear tendency with an inflection [*polynomial contrast* (1^{st} degree): $F(1,136)=40.32$, $p<0.0005$, (2^{nd} degree): $F(1,136)=13.03$, $p=0.001$], the more

important improvements in performance taking place in the first three blocks and a plateau on the last two blocks. Thus, in the 50-trial-condition, while Post-ICSS group reached a maximal performance level in the first block of trials, the control groups needed much more training to reach the same performance level than the Post-ICSS group.

To distinguish between retrieval and additional learning in the retention session, the last block of the acquisition session and the first block of the retention session were compared. In the 30-trial-condition, the only group that did not show a decrease in the number of avoidances between the last block of acquisition session and the first block of retention session was the Post-ICSS group (absolutes values), although this decrease was only statistically significant in the Control-SHAM group [$F(1,36)=11.6$, $p=0.002$]. In the 50-trial-condition, similar but more effective effects were observed. While control groups showed a significant decrease in the number of avoidances *[Control-ICSS*: $F(1,34)= 18.92$, $p<0.0005$, *Control-SHAM*: $F(1,34)=10.34$, $p=0.003$], a significant increase [$F(1,34)= 14.2$, $p=0.0001$] was shown by the Post-ICSS group.

As shown in Fig. 3, the facilitatory effect of the Post-ICSS treatment on the 24-hr retention session was also confirmed by survival analyses, which indicated significant differences between Post-ICSS and Control-SHAM groups, in both the 30-trial and the 50-trial conditions [$\chi^2=8.34$, $df=1$, $p=0.0039$; $\chi^2= 0.93$, $df=1$, $p=0.0009$, respectively]. So, all the rats in the Post-ICSS groups achieved an established learning criterion (five or more consecutive avoidance responses), whereas only 70% of subjects in Control-SHAM-30 group and 77.8% of animals in Control-SHAM-50 group reached it. Moreover, the Post-ICSS groups achieved the learning criterion faster than the controls, as shown by the fact that 90% of ICSS rats reached the criterion in a mean of only15 trials (30-trial condition) or 10 trials (50-trial condition),

whereas only 29.9% of the control rats in the 30-trial condition reached it in trial 15, and 22% of the control rats in the 50-trial condition reached it in trial 10.

Did the Pre-ICSS treatment have any effect on performance in retention session?. In general, the performance level shown by the Pre-ICSS group was similar to the one observed in control groups, in both the 30-trial and the 50-trial conditions. Furthermore, contrast analyses showed that in the 50-trial condition the Pre-ICSS group performance level, besides not differing from the controls, was also lower than that of the Post-ICSS group in the first two blocks of trials [$F(1,34)=5.81$, $p=0.021$; $F(1,34)=5.38$, $p=0.02$, respectively].

Did the number of trials in the acquisition session affect performance in the 24-hr retention session? In general, in spite that the number of trials did not affect acquisition, the addition of 20 extra trials improved the 24-hr retention of conditioning [*treatment by trials by block interaction factor*: $F(6,140)= 2.09$, $p=0.05$]. Specifically, the more important improvements in learning were observed on the first block [$F(1,70)= 3.70$, $p=0.05$] of the retention session, in which the performance level shown by the groups submitted to 50 trials was higher than that of the 30-trial condition groups, regardless of treatment.

Thus, both the post-training ICSS treatment and the higher number of acquisition trials seem to facilitate the 24-hr retention of conditioning. But, are those two factors similarly effective?. As shown in Fig.4, a survival analysis revealed significant differences [$\chi^2=6.07$, $df=1$, $p=0.0138$] between the Post-ICSS-30 and Control-SHAM-50 (without treatment but with the additional extra trials) groups. All rats in the Post-ICSS-30 group achieved the established learning criterion, whereas only 77,8% of the animals in the Control-SHAM-50 group reached it. Moreover, the Post-ICSS-30 group achieved this established criterion faster than the control group. The analysis of variance confirmed this result, showing that

Post-ICSS-30 group made a significant higher number of avoidance responses than Control-SHAM-50 group on the first block of the retention session [F(1,18)=22.05, p=0.006]. Therefore, post-training ICSS seems to be more effective than a higher number of acquisition trials for improving the retention of a massed two-way active avoidance.

ICSS behaviour and shuttle-box locomotor activity

No differences were found between groups in any of the different ICSS parameters evaluated (OI values, ICSS rates in the three sessions performed to establish the OI and in the treatment sessions, and duration of the treatment sessions). Moreover, correlation analysis showed that none of these variables was related to the level of conditioning achieved by the rats on the acquisition or the retention sessions. The mean values (and standard deviations) of these ICSS variables are summarized in Table 1.

Concerning locomotor activity in the shuttle-box during the habituation sessions (10 min before both the acquisition and the retention sessions), an analysis of variance did not detect any statistical differences among groups in the number of crossings performed in these sessions. Statistical differences were not found among the different groups with reference to the number of inter-trial crossings made either in the acquisition and retention sessions. There were no significant correlations between locomotor activity in the shuttle-box and the level of conditioning.

Histology

As shown in Fig. 5, all the ICSS electrodes were implanted into brain sites between -1.30 mm and -2.56mm AP coordinates with reference to bregma, in the medial forebrain

bundle region of the LH. Statistical analyses showed that there does not seem to exist any relationship between the histological location of the electrode tip and the activity in the shuttle-box (both during habituation and conditioning sessions), the ICSS variables, or the number of avoidances performed during conditioning.

DISCUSSION

Effects of Post-training ICSS on two-way active avoidance retention

The results of the present experiment showed that lateral hypothalamic ICSS administrated immediately after the acquisition session improves the 24-hr retention of two-way active avoidance conditioning. The facilitation of retention observed seems to be attributable to the effects of the ICSS treatment, and not to other variables such as the specific electrode location, OI values, ICSS rates, or locomotor activity of the subjects, since none of these variables were related to the performance in the shuttle-box on any conditioning session. The observed 24-hr facilitatory effect confirms our previous results with the same task under different training conditions [2, 17]. They also agree with other results showing a facilitatory effect on the 24-hr retention of other kinds of conditioning tasks [7, 8, 9, 12, 13, 16]. All these results show that post-training ICSS is able to improve retention in a wide variety of conditioning paradigms.

We think that the present findings agree with the hypothesis that post-training ICSS acts on the process of memory consolidation activated by the initial experience. This hypothesis is also supported by the experiments showing that the efficacy of the treatment depends on temporal continuity to the training episode. Thus, the ICSS treatment loses its facilitative effect on 24-hr retention when it is delayed between 1 and 4 hours, depending on the particular kind of conditioning, [7, 8, 9, 16]. The present results, as well as other ones from

our laboratory [2], showing that control groups are able to reach the same performance level than the ICSS-treated groups but after a higher amount of trials and/or sessions, led us to suggest that the post-training ICSS facilitative effects could lie on an acceleration of the memory consolidation process.

With regard to the effects of the number of trials, and contrarily to what we had expected, both training conditions (30 and 50 trials) allowed the subjects to reach the same performance level in the acquisition session. However, the level of performance achieved on the 24-hr retention session by the 50-trial groups was higher than that of the 30-trial groups. So, it can be suggested that more intensive training could facilitate memory consolidation and, then, improve subsequent remembering. Did the ICSS treatment equally facilitate the retention in both training conditions? In the 30-trial-condition, the control groups never reached the level of performance of the Post-ICSS group, that is, the facilitative effects of Post-ICSS were clearly maintained throughout the retention session. In contrast, in the 50-trial-condition, the control groups reached the same retention level achieved by the Post-ICSS group in the third block of trials. It seems, therefore, that the ICSS treatment was more effective in the 30-trial-condition. These findings agree with previous ones showing a greater effect of post-training ICSS in animals with a low basic level of conditioning [2].

Is the retention improvement caused by increased training similar to that found after post-training ICSS treatment? Evidences from some studies indicate that both ICSS and increased experience can modulate learning and memory consolidation processes in a similar way [7]. In the present experiment, the facilitative effects caused by both variables on two-way active avoidance retention seem to be additive. Thus, rats in the Post-ICSS-50 group showed a better retention than the ones in the Post-ICSS-30 group. However, the fact that the ICSS

treated rats in the 30-trial condition achieved a higher retention level than the control rats in the 50-trial-condition, suggests that post-training ICSS treatment had a stronger effect on memory consolidation than the addition of 20 trials.

Effects of Pre-retention ICSS on two-way active avoidance retrieval

In the present experiment, the Pre-ICSS treatment was administrated immediately before the retention session, approximately 23 hours after the acquisition session. This methodological approach is useful for determining if ICSS can also have some effect on retrieval of the previously acquired information. In the 30-trial-condition, the performance level obtained by the Pre-ICSS rats was similar to that of the control rats. Although the Pre-ICSS group in the 50-trial condition did not show the performance decrease observed in the control groups between the last acquisition block and the first retention block of trials, the fact that the number of avoidance responses achieved by Pre-ICSS group was similar to that obtained by the control groups suggests that the pre-retention ICSS treatment did not affect the retention performance. We have no knowledge of previous experiments studying the effects of pre-retention ICSS specifically on retrieval.

A first explanation of the lack of effects of the Pre-ICSS treatment could be a "state-dependent" phenomenon. Here, the physiological state of organism is supposedly incorporated into the conglomerate of memory attributes and exerts control over retrieval of the memory [15]. So, in the present experiment, ICSS treatment was administered only before the retention session (decoding), but not before the acquisition one (codification of information). Nevertheless this does not seem to be a compelling account of the present results because of two main reasons. First, contrary to what it would be then expected, Pre-ICSS groups did not have impaired retrieval of the previously acquired information, since

they performed similar to the controls. Second, pre-retention treatments that generate arousal, such as electrical stimulation of the mesencephalic reticular formation or the administration of hormones released during stress, do no seem to generate state dependence, because they could reinstate the internal context of initial training that facilitated access to target memory [15, 24]. Similarly, the fact that ICSS also increases arousal [20], even though by different mechanisms, makes it unlikely that ICSS cause state dependence.

A second possibility is that ICSS could actually facilitate retrieval but that, in the present experiment, no effects were observed because the Pre-ICSS treatment was administered on an already consolidated or inactive memory. According to the reconsolidation hypothesis, only the reactivated memories can be facilitated by treatments that enhance memory consolidation [22], such as ICSS. In fact, there are reports of marked improvement of memory in the rat when retrieval is accompanied by arousal, but only when memory is "primed" or reactivated by exposure to the context in which the training had taken place [10, 23]. Thus, in the present conditions, it would be expected that if the memory were previously reactivated by appropriate reminders, the pre-retention ICSS treatment then would be able to facilitate retrieval.

Another possibility is that ICSS treatment does not have effects on retrieval of the information previously acquired. This hypothesis does not agree with the results obtained in a previous experiment from our group [25], showing facilitation of the same avoidance response when a similar ICSS treatment was administrated immediately before each of the 5 training sessions (10 trials/session; one daily). In that experiment, pre- and post-training ICSS induced a very similar evolution of learning throughout the training sessions, showing the facilitative effects since the third session. Taking into account that brain activation induced by ICSS may

persist for some time after treatment, a possible explanation for the discrepance between the previous results and the present ones could be that the pre-ICSS treatment could exert an anterograde effect on those memory processes that are active in the consecutive conditioning session. Because it can be assumed that every retrieval operation should trigger a reconsolidation process [23], pre-retention ICSS could facilitate consolidation (or reconsolidation) of information in the retention session, in a similar way that when administered post-training. If this were the case, it would be expected that ICSS treatment did not have prompt effects on performance in the current retention session but it may have had an effect on later tests, as it happened in the previous experiment [25].

In summary, the present results agree with the hypothesis that ICSS facilitates consolidation processes, but more studies are necessary to clarify its effects on retrieval of the previously acquired information.

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FIGURE AND TABLE LEGENDS

Figure 1. 30-trial condition: effects of Pre and Post-ICSS treatments on 24-hr retention of active avoidance. (A) Mean avoidance responses (and standard errors) on the acquisition and retention sessions. (B) Mean avoidance response (and standard errors) in each of the three blocks of trials that composed the acquisition and retention sessions.

Figure 2. 50-trial condition: effects of Pre and Post-ICSS treatments on 24-hr retention of active avoidance. (A) Mean avoidance responses (and standard errors) on the acquisition and retention sessions. (B) Mean avoidance responses (and standard errors) in each of the five blocks of trials that composed the acquisition and retention sessions.

Figure 3. Survival curve of the 24-hour retention session comparing Post-ICSS and Control-SHAM groups in the 30-trial and 50-trial conditions.

Figure 4. Survival curve of the 24-hour retention session comparing Post-ICSS-30 and Control-ICSS-50 groups.

Figure 5: Electrode tip locations for each animal in the four experimental groups. Control-ICSS, Pre-ICSS and Post-ICSS received ICSS during their corresponding experimental sessions, but Control-SHAM (with electrode implantation) never received ICSS. Sections correspond to brain sites between -1.40 mm and -2.56 mm antero-posterior coordinates with reference to bregma (Paxinos and Watson, 1986).

Table I: Mean values (and standard deviations) of ICSS variables. *OI:* mean optimum intensity of ICSS used in the treatment sessions; *Rate*: mean of the ICSS maximum rate (R/min) achieved during the sessions carried out to establish the individual OI; *Treatment Duration:* duration of the ICSS treatment session.